THE DEGRADATION OF REFRACTORY MINERAL OIL RESIDUES USING BIOREACTORS

VOLUME I OF II

by

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ABSTRACT

This thesis is concerned with the biodegradation of oily sludges typically found at refinery sites as waste residues from the refining of mineral oil. Currently these type of wastes represent significant environmental risk. Current technology that addresses containment during treatment to regulate the release of volatile organic carbons and reduction of the polynuclear aromatic hydrocarbons in the sludge, is incineration. Incineration is a costly option and this research has shown that the same treatment standards as incineration is achievable through bioreactor treatment with the correct process and reactor design.

Prior to investigating reactor and process design, batch flask culture tests were carried out to screen the most important environmental and loading parameters. The results were used to set up the continuous bioreactor trials.

A novel Airlift bioreactor was designed to improve the mixing of the oily sludges compared to standard stirred tank systems. The results showed that the Airlift bioreactor was successful in reducing the polynuclear aromatic hydrocarbons to incineration limits through the promotion of biosurfactant production which led to improved mass transfer of the oily solids into mixed liquor. The Airlift bioreactor achieved a 61 % loss for oil and grease and a 68 % loss of polynuclear aromatic hydrocarbon compared to a 45 % loss of oil and grease and a 46 % loss of polynuclear aromatic hydrocarbon in the standard stirred tank.

This research also investigated the benefits of surfactants as an amendment in continuous trials to increase the fraction of soluble oil in the mixed liquor. The addition of surfactant was successful in improving the extent of oil degradation to 60 %. Mass transfer of the oil associated solids were shown to be higher with the addition of the surfactant and oil was also seen to be desorbed into the mixed liquor.

Reaction rate coefficients for oil degradation, fitted to zero rate orders, from the three reactors showed the best rate was from the standard stirred tank design at $k = 0.87$ day $^{-1}$ while the Airlift and surfactant amended bioreactors gave $k = 0.6$ day $^{-1}$ and 0.45 day $^{-1}$ respectively. The higher reaction rate coefficient in the standard stirred tank has been attributed to the lower amounts of oil in the mixed liquor. The higher amounts of oil desorbed into the mixed liquor in the other two reactors possibly resulted in toxic inhibition. The extent of degradation was however not affected by the lowered rates.

The results of this study show that reactor and process design to improve mass transfer of oil into the mixed liquor can have a significant impact in the treatment limits to make aerobic bioreactor treatments of oily sludges a viable choice.

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This work is dedicated to the memory of my late father, Dr Osman Mohammad, my mother, Sakina Babjee, and to Jaizan and Emeel.

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COMMON ABBREVIATIONS USED IN THE TEXT, TABLES AND FIGURES

APHA	American Public Health Authorities
API	American Petroleum Institute
BOD	biological oxygen demand
BBH	Bushnell Haas
BBHO	Bushnell Haas with oil
BBHN	Bushnell Haas with naphthalene
BPEO	Best Practicable Environmental Option
BATNEEC	Best Available Technique Not Entailing Excessive Cost
BDAT	Best Demonstrated Available Technique
BSTR	batch stirred tank reactor
BTEX	benzene, toluene, ethylene, xylene
CMC	critical micellar concentration
C:N	carbon is to nitrogen ratio
C:P	carbon is to phosphorus ratio
COD	chemical oxygen demand
CO_2	carbon dioxide
Cm	concentration of tracer measured
Ct	theoretical concentration of tracer
Ca	concentration of reactant/product (a) at time = t
Cao	concentration of reactant/product (a) at time = 0
O&G	oil and grease
δ	standard deviation
DCM	dichloromethane
DOE	Department of Environment, Malaysia
DoE	Department of Environment, United Kingdom
DOUR	dissolved oxygen uptake rate
dsf	dry solids fraction
EEC	European Economic Council
EPA	Environmental Protection Agency
GC	gas chromatograph
HC	hydrocarbon
HOC	hydrophobic organic compounds
HPLC	high pressure liquid chromatography

LIST OF ABBREVIATIONS

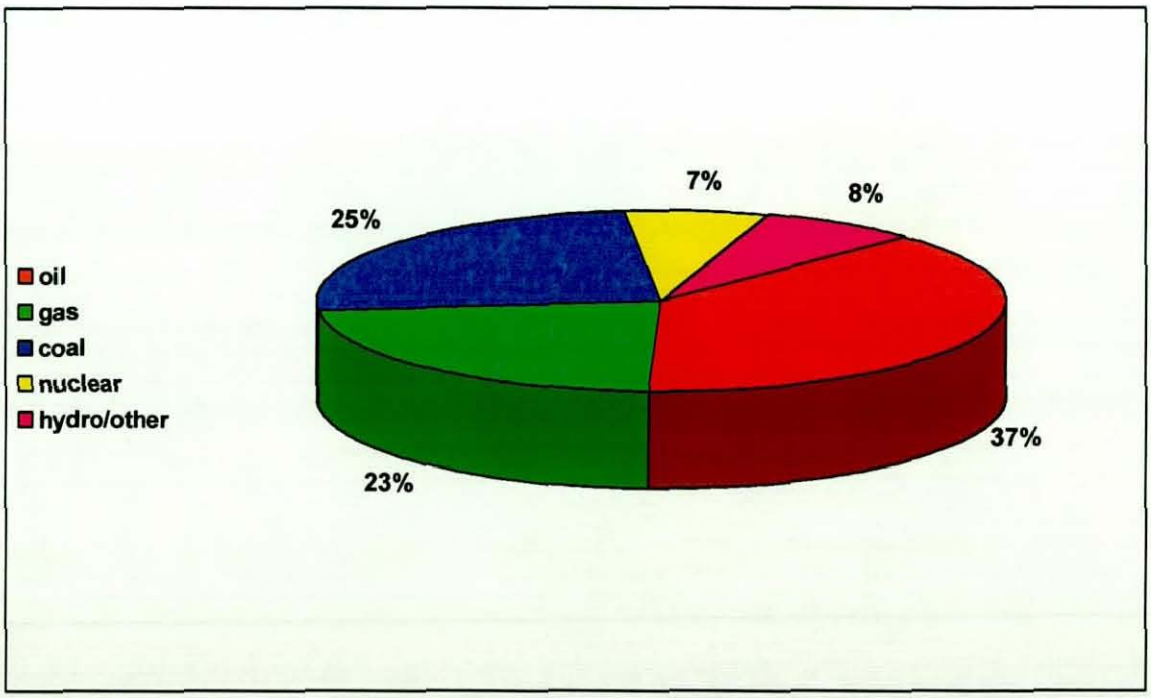
HMSO	Her Majesties Stationary Office
IARC	International Agency for Research on Cancer
LSC	liquid-solid contact reactors
ML	mixed liquor
NA	not available/analyzed
N	nitrates
OUR	oxygen uptake rate
P	phosphates
PAH	polynuclear aromatic hydrocarbon
PCA	plate count agar
ppm	part per million
RT	retention time
TCTFE	trichlorotrifluoroethane
TOC	total organic carbon
TS	total solids
TSS	total suspended solids
TVS	total volatile solids
TVSS	tolal volatile suspended solids
TFS	total fixed solids
TFSS	total fixed suspended solids
UK	United Kingdom
USA	United States of America
UTS	universal treatment standards
USEPA	United States Environmental Protection Agency
VOC	volatile organic carbon
WWTP	waste water treatment plant

CHAPTER ONE
BACKGROUND

1.1 INTRODUCTION

Petroleum is the largest source of energy throughout the world (World Energy Yearbook 1996). Petroleum achieved this status due to: its stable and competitive price; versatility in producing a range of products; relative cleanliness and ease of handling. In addition to its use as an energy source, petroleum is also the principle source of lubricants and solvents as well as a variety of chemical feedstock for the synthesis of fertilisers, plastics, fibre, detergents, pharmaceutical and cosmetics. The scale of operations from the mining of the crude to the processing of the varied products and the widespread use, makes it inevitable there will be risks to the air, water and soil pollution worldwide.

Figure 1: World energy production 1996 (Source from World Energy Yearbook,1996)



Notwithstanding pollution sources such as those that arise through accidental spills, transportation and pipe leakage etc., the industrial processing of the crude into its

products generates oily wastes which will cause severe pollution if not strictly controlled and monitored. In the USA, a 1981 Environmental Protection Agency (EPA) survey of hazardous waste indicated that out of manufacturing operations which accounted for 90% of the hazardous waste generated, 70% of this came from the chemical and petroleum industry (Wentz 1989). It is estimated that the USA petroleum refining industry annually produces more than 2.5 million tons of wet hazardous sludges (Abrishamian *et al.*, 1992). This is an area where pollution can be prevented from entering the environment and methods to improve effluent quality are continuously being developed to meet the ever increasingly stringent environmental laws and regulations.

1.2 WASTES FROM THE PETROLEUM INDUSTRY

Wastes from the activities of the petroleum industry are generated in both upstream and downstream operations. Upstream covers the exploration and production of the oil whereas the downstream include the processing of the crude and marketing/distribution of the products. A complete review of the industry's activity can be read from.;

- Petroleum Handbook (1983) by Shell
- Fundamentals of Petroleum (1986) by University of Texas at Austin
- Modern Petroleum (1978) by Berger and Anderson

Typical activities and type of wastes arising from these activities are summarised in Table 1.1.

Wastes arising from the activities of the petroleum industry are varied in characteristic and quality. Typical waste sources, characteristics, and quantities arising from oil exploration, mining and refining are covered comprehensively in several texts and reviews.

Industrial and Hazardous Waste Treatment by Nemerov and Dasgupta (1991) is a general text that gives a concise description of the origins and characteristics of oil field and refinery waste as well as its treatment. A publication by CONCAWE (1989); Report no. 5/89 entitled "Oil refineries waste survey-disposal methods, quantities and costs", gives statistical data on several waste types.

Table 1.1 : Petroleum industry waste source and types

	ACTIVITY	WASTES
UPSTREAM		
EXPLORATION	well development	drilling mud residues & cuttings
PRODUCTION	mining	oily waste water (produced water) sludge brine
DOWNSTREAM	refining	oily waste water sour water oily sludge spent catalysts waste caustics acid sludge condensate water
	product storage	oily sludge

The subject of this research is specifically the treatment of oily sludges such as those arising from tank bottom cleanings and refinery wastewater treatment plant (WWTP) solids. Refinery operations generally yield sludge as the unprocessable part of the crude, which accumulates in the WWTP. Readers are referred to *Appendix B* (p. 244) for a detailed review of a Refinery WWTP where these sludges are generated. The review covers the principles of the unit processes with diagrams and efficiencies for oil removal per unit process.

1.3 LAWS AND REGULATIONS ON THE DISPOSAL OF OILY SLUDGE

One of the objectives of this research is to determine an environmentally safe method of treating oily sludges. The impetus and direction of research for waste treatment has always been driven by policy decisions and consequent regulatory and legal considerations. Industry needs to control costs. The key in defining treatment standards

as well as costs and potential savings lie in understanding the regulations in place at present as well as being able to predict future trends. Reviewing the history of these laws gives a clearer picture of what the future holds and what the industry should expect and prepare for. Industry has traditionally viewed waste treatment as an added cost to production and expense in this area is the last on the budget list. This view is changing fast due to public attitudes and perception, stricter laws and regulations governing waste disposal as well as a general increase in environmental awareness. Industries are seen as having a moral, legal and economic responsibility to consider waste management as an integral part of production cost.

There is a worldwide movement towards integrated pollution management through such programmes as ISO 14000 and life cycle analysis. The practice of clean technology and waste reduction through such methods as prevention, recycling, recovery and reuse are common place and these are usually driven by economic incentives as well as regulated by law. Nevertheless, even with these measures, there will still be residual wastes requiring disposal.

In the past, because of the implications for drinking water, laws on waste have concentrated on the aqueous effluent discharges. This waste stream is usually regulated by laws that require a license that specifies discharge limits for particular components of the waste streams. Traditional analysis has been used to assess organic matter for example BOD, COD, TSS, pH and *O & G*. These are being supplemented by newer parameters including toxicity assays, heavy metals, ammonia-nitrogen as well as other substances such as pesticides, organic halogens, mercury etc. Emphasis in the protection of human health and environment has shifted concern from acute effects to chronic effects and this has effected an implementation of increasingly stringent limitations on potential toxics (Englande Jr. and Guarino 1992).

Current treatment processes designed with meeting aqueous effluent discharge limits concentrate the hazardous waste component in the solid/sludge waste stream. For example, better separation of the suspended solids have concentrated the heavy metals in the sludges that are collected in the wastewater treatment units. More recently, the disposal of these more toxic sludges, has come under the scrutiny of regulation.

Disposal of hazardous wastes is usually limited to specialised facilities and the waste

has to be treated to remove the hazardous components or reduce its toxicity or migration potential before disposal. The following review of the laws in place governing oily sludge disposal is to indicate the present and future disposal limitation or standards for oily sludges and the necessity for innovative technology to meet these standards.

1.3.1 REGULATED SUBSTANCES

In order to regulate 'hazardous wastes', the first step is to define the wastes in question. Wastes are usually categorised as hazardous if they exhibit certain criteria such as toxicity, flammability, corrositivity, reactivity, radioactivity, persistence or accumulation (Wentz 1989). The criteria, such as the above are used by the USA and more recently adopted by UK. Comprehensive waste lists of substances that would be regulated are usually supplemented to simplify the identification and enforcement procedures for all parties e.g. *Priority Pollutants* in USA, *Scheduled Waste* list in Malaysia and *Special Wastes* List in UK. Identification of hazardous waste by listing has limitations as they may be too defined and become limiting and inflexible when meeting the needs presented by new understanding and technology. Malaysian laws currently cover wastes that are listed only in its *Scheduled Waste* list but it is foreseeable that Malaysia will follow the USA and UK in defining hazardous waste by its hazardous criteria to be more comprehensive in categorising wastes. The classification may be by type of industry or activity that would produce the waste; e.g. Petroleum Refining and Paper and Pulp or; by the chemical or parameter in the waste stream such as benzene, xylenes or use both system.

Wastes from refinery operations usually fall under the list of regulated substances. In the USA, these sludges are characterised as *toxic* due to their heavy metal and polynuclear aromatic hydrocarbon content (PAH) (Bonner and Autentrieth 1989). i.e. PAH and heavy metals (barium, cadmium, lead, nickel, chromium, zinc, etc.) are *Priority Pollutants*. The International Agency for Research on Cancer (IARC) has published data that indicate that PAH have toxic, mutagenic and carcinogenic properties (IARC, 1983) and benzo(a)pyrene in particular is one of the most potent chemical carcinogens known (Miller and Miller 1985). The *Special Waste* list of UK regulate carcinogens if they are present at a total concentration of 1 % w/w or more. In contrast, Malaysia does not distinguish PAH as potentially toxic but covers all oily sludges in general.

The following Table 1.2 shows some of the most important laws which regulate oily sludge disposal in the USA, the UK and Malaysia. A description of the waste (based on waste category number) is found in *Appendix C*, p.257-258.

Table 1.2 : Oily sludge disposal laws (from various sources)

COUNTRY	LAW	YEAR	CATEGORY	WASTE CATEGORY NUMBER
USA	RCRA	1976	non-specific source waste	F037&F038
	HSWA	1984	specific sources	K048,K049, K050,K051,K052
			chemical product	U019,U022, U063,U064,U120,U16
MALAYSIA	EQA	1974	non-specific source	N014,N015,N016
	EQ(SW)A	1989	specific source	S011,S014,S021
U.K.	EPA (PP&S)	1991		05,0501,050103,050105, 050108,13,1304,130401, 130402,130403.1305, 130501,130502,130503, 130504,130505,130506, 130601
	Control of Pollution Act	1974		
	Disposal of Controlled Wastes Regulation	1991		
	Special Waste Regulation	1996		

1.3.2 TREATMENT AND DISPOSAL PRACTICES AS SHAPED BY THE LAWS

The history of waste disposal laws are reviewed in these three countries to note some general trends and predict the future attitudes towards treatment and final disposal practice for the waste oily sludges.

In general, laws on air and water pollution have been in place earlier than laws on land pollution. Concerns about the deteriorating conditions of the rivers and streams and air quality have lead to major laws to curb pollution. These laws in effect have shifted the burden of ultimate disposal (pollution) to land hence current concern with integrated

pollution control and practices of Best Practicable Environmental Option (BPEO) and Best Available Technique Not Entailing Excessive Cost (BATNEEC). In order to close the loophole of air, water and land pollution, laws on waste disposal, if not at present then in the future, are likely to support destruction of the hazardous components of the wastes prior to placement in or on land.

American law is quite clear on this as it has already specified limits that need to be achieved for PAH, BTEX and heavy metals, common components of oily sludges. A review of the American laws shows how new laws are triggered by previous laws in order to close loopholes as well as in response to public pressure reacting to certain tragic environmental events. These sequence of cause and effect are common precipitates of new laws. The severity of American laws regarding the limits imposed are reflective of the magnitude of past mismanagement on disposal and accumulation of toxic waste.

Based upon the current laws already in place in these three countries as well as other EEC member states, oily sludge is a regulated waste substance. The UK waste disposal laws as well as its regulatory bodies have had a recent revamp to keep in line with EEC directives and 'mineral oil' has been included as *Special Waste*. The *Special Waste Regulation of 1996* has put the UK more in line with the practices of Europe and USA and the set up of the new Environmental Agency will provide the regulatory framework the required autonomy to ensure compliance.

Despite the fact, that the toxicity and hazard associated with the oily sludges remains almost the same wherever it is produced, what is practised for its treatment and disposal is highly dependent on the minimum treatment limit imposed by the laws in place. At present only USA has specified Universal Treatment Standards (UTS) (Hydrocarbon Processing 1995). Previous USA limits (BDAT) were based on the standard achieved through incineration (Refer to Table 1.3). Similar to BDAT limits, UTS limits are imposed on a particular hazardous constituent but with UTS, wastes are no longer limited to source labels such as API separator solids, slop oil emulsion solids, etc. The uniform set of levels mean that treatment standard for a constituent in waste A will be the same as the constituent in waste B. Once UTS limits are met, the waste can be disposed in an RCRA permitted landfill (Oolman *et al.* 1992).

Malaysia and UK list oily wastes or PAH as regulated substance but treatment limits are not specified. Malaysian law regulators, the Department of Environment, operates on a case by case basis due to the presence of only five refineries that are the main generators of such wastes. However, treatment to approximately 1% (or background levels) *O & G* by dry solids weight meets consent limits for disposal on site i.e. within refinery grounds. (Pers. Comm. DOE Malaysia). In the UK, rather than specify constituent limits, treatment technologies are endorsed and oily wastes have been either incinerated, sent to controlled landfills or were landfarmed, a practice endorsed as BPEO. However, with the public land registry landfarming may be a liability the landowners would want to avoid.

In the USA, the *Clean Water Act* of 1977 and the amended act of 1981 specified discharge of pollutants into surface and ground waters by a permit obtained under the *National Pollutant Discharge Effluent System* (NPDES). Through this act and the *Clean Air Act* of 1970, the entire burden of ultimate disposal was shifted to land. Disposal of hazardous wastes to landfills has been a common practice in the USA as early as 1930 (Wentz 1989). During this time, disposal to landfills were not very highly regulated and since it was the cheapest disposal method, it was the most commonly practised. Initially, these wastes were not treated prior to disposal and the landfill themselves were not well designed or placed. This led to several serious contamination incidents such as the Love Canal (Wentz 1989). The USA then set up the *Resource Conservation and Recovery Act (RCRA)* of 1976 as an amendment to *the Solid Wastes Disposal Act* of 1965. *RCRA* was Congress' response to the increasingly severe threat to human health and the environment posed by the unregulated generation and disposal of hazardous waste and closed a gap in the federal scheme for environmental regulation. *RCRA* mandated a comprehensive national regulatory programme for hazardous waste, embodying a 'cradle-to-grave' approach.

Subsequent *RCRA* amendments, such as the 1984 *Hazardous and Solid Waste Act (HSWA)*, imposed further restrictions and greater protection measures (Oolman *et al.* 1992). Contamination from leaking landfills were a prime issue and the *HSWA* amendment required certain standards to be met for designing landfills i.e. Minimum Technical Requirements set up under Subtitle C requirement of 40 CFR 264 and 265. More recent amendments to *RCRA*, includes treatment of wastes to Best Demonstrated Available Technology (BDAT or Land Ban Standards) standards prior to landfilling.

These limits have been replaced by UTS limits in Rule 59 FR 47982 which also impacted on the refining industry. These standards specify limits for particular chemical constituents that are listed in the hazardous waste list and include PAH and volatile inclusions such as benzene, toluene, ethylene and xylene (BTEX). Inclusion of volatiles has further implications as this dictates complete containerisation of any receptacle accepting such wastes, and oily sludges usually contain some amount of BTEX volatiles.

At present these laws have effectively banned the traditional method of treating oily sludges by landfill and landfarming (Abrishamian *et al.* 1992). Landfarming is a managed application of oily sludges onto the uppermost soil layers. Since the land ban restrictions of *RCRA* in 1984 and its amendment in 1991, landfarm facilities are considered as waste disposal facilities and must meet the MTR of a land disposal facility (double liners, leachate collection and ground water monitoring) (Norman 1991). The *RCRA* has specified oily wastes as hazardous and they must be pre-treated prior to land disposal using a technology that meets or exceeds treatment standards established by UTS. Accordingly, the sludges must meet certain UTS concentration standards for organics; BTEX and PAHS and leachable (Toxicity Characteristic Leachate Procedure (TCLP)) metals prior to land disposal to a landfarm meeting similar MTR as an RCRA landfill or directly to an RCRA landfill (Huesemann *et al.* 1993). Refer to Table 1.3.

Table 1.3 : BDAT vs UTS Limits

Constituents	BDAT	UTS
VOA's	ppm	ppm
Benzene	14 total	10 total
Toluene	14 total	10 total
Ethylbenzene	14 total	10 total
Xylene's	22 total	30 total
SVOA's		
Acenapthene		3.4 total
Anthracene	28	3.4 total
Benzo (a) anthracene	20	3.4 total
Benzo (a) pyrene	12	3.4 total
Chrysene	15	4.8 total
Fluorene		3.4 total
Napthalene	42	5.6 total
Phenanthrene	34	5.6 total
Pyrene	36	8.2 total

European Economic Council Member states, such as Germany, Denmark, France, Sweden and Switzerland have favoured destruction and treatment prior to landfill disposal long before the USA (Wentz 1989; Little *et al.* 1993). This practice has saved these countries from severe public criticism that follow contamination incidences that would have initially led to more pressure to tighten the laws. Although, there are still incidences of contamination, it is not of the same scale as the USA. However, the problems of inadequate treatment and improper containment have still to be addressed.

In the UK, the practice of landfilling untreated wastes in co-disposal landfills is one of the primary methods of disposal. Landfills account for 83 % of total disposal of hazardous wastes in 1986 -7 in the UK (Little *et al.* 1993). Although wastes imported to UK, under the free trade provision of the Treaty of Rome, generally are treated, locally produced wastes are directly landfilled. In the UK, before the enactment of *Special Waste Regulation 1996 (SWR 1996)*, waste disposal was in accordance to the *Control of Pollution Act (COPA)* or more specifically, section 17 of *COPA* on *Special Waste Regulation*. Currently, section 17 of *COPA* has been revoked and replaced with *SWR 1996*. *COPA* specified the needs of a licensed landfill and the *COPA 1980* amendment included a *Special Waste* list. While *COPA* has been revoked, this *Special Waste* list still applies but has been expanded to include, not just the list but also the hazard criteria to determine a waste as *Special Waste*. *COPA* was designed for the management of *Special Wastes* to licensed landfills such as consignment notes, registry, logging etc. However with *COPA* the stand on treatment before disposal and co-disposal were unclear. Under UK's previous legislation and policy, imports of *'Special Wastes'* into the UK increased from 5,000 tonnes in 1984 to 40,000 tonnes in 1986. This is in part due to the tightening of landfills rules in Belgium, France, Germany and Netherlands as well as official British government's policy which was to encourage the import of wastes destined for waste treatment in order to support UK's specialist treatment and incineration plants. However, even then, disposal authorities generally did not accept requests to import hazardous wastes for direct landfills and recently, UK's Management Plan for Exports and Imports of Waste, coming into effect 1st June 1996, specifically bans imports for disposal other than in exceptional cases where environmental considerations apply (IWM 1996). While, imported wastes become destined for waste treatment, UK produced wastes have been directly landfilled. However, the development of the landfill tax may reduce the amount of waste sent directly for landfilling.

Based on a Department of Environment (DoE) report on the behaviour of hazardous wastes in landfill sites, (DoE UK, 1978) it was concluded, provided landfilling was properly controlled, the degree of risk in co-disposal of hazardous wastes was acceptable and an ultra cautious approach was not warranted. This view has been sharply criticised by some members of the community as it was based on a 'dilute and disperse' approach for pollution control which is not in line with most current waste management practice. 'Dilute and disperse' condones the leaching of hazardous substances into unsaturated zones beneath landfills and threatens pollution of groundwater. Further pressures on the 'dilute and disperse' concept has been through the EC directive on the 'protection of groundwater against pollution caused by certain dangerous substances: 80/68/ECC' which declares protection on usable groundwater. This means that future sites for co-disposal will, in all likelihood, be based on the principle of containment, either based on naturally impermeable strata or artificial liners. Neither approach gives permanent protection on geological time scales as has been shown in numerous contamination incidences in the USA and other European countries. High standards in site selection and investigation, in site planning and engineering, in site operation, monitoring and control and in site restoration and reuse will be required for proper control of landfills for co-disposal.

A 1986, Hazardous Waste Inspectorate (HWI) review of co-disposal sites in the UK reflected the Inspectorate's concerns that the various Waste Disposal Authorities mechanism for ensuring proper management of the sites were inadequate. Due to the decentralisation of regulation to separate Waste Disposal/Regulation Authorities, who were responsible for issuing licenses to operate these sites, there were variable standards for the operation of landfills and the Waste Disposal/Regulation Authorities were frequently criticised for being too tolerant of license infringements. Although the HWI sees no objection to co-disposal, if well managed as a Best Practicable Environmental Option (BPEO), it stated that these regulations required continuous review. The new Environment Agency, set up on 1st April 1996, centralises these activities and should finally be able to bring together the work of 83 separate Waste Regulation Authorities into a nationally co-ordinated coherent operation (IWEM, May 1996).

In the UK, industries have to follow the *Integrated Pollution Control Act* that is part of the *Environmental Protection Act* of 1990. The main aims of this act are:

i. " to prevent or minimise the release of prescribed substances and to render harmless any such substances which are released".

ii "to develop an approach to pollution control that considers the releases from industrial processes to all media in the context of the effect on the environment as a whole".

Under this act, BPEO and Best Available Technique Not Entailing Excessive Cost (BATNEEC) apply. BPEO refers to an integrated pollution control that would:

i. direct the release of pollution to the environmental medium where the least environmental damage would be done.

ii. minimise the pollution effects in all three environmental media.

All Integrated Pollution Control processes are subjected to BATNEEC requirements. Her Majesty Inspectorate of Pollution used to be the enforcing and authorizing body that decides what is BATNEEC for each prescribed process. Under the *IPC* Act, the BPEO for oily sludges are treatment by incineration or landfarming on site (IPC,1991) and no limits for treatment have been issued. However, there are new implications to landfarming with the *Environment Act 1995* coming into force as the question of land pollution and liability become more significant (Swannel 1998). Having a public land registry with information of contamination, also impacts landfarming. Emerging new laws show a trend towards imposing self regulation through imposing 'liability' that transcends into the future for pollution. The liability rests when possible, with the polluter, and if not, the land/facility owner. Therefore, it would seem unlikely that refinery plant operators would deliberately contaminate their grounds by practising landfarming.

With the current changes in the UK, it can be seen that a tightening of the controls for deposition of hazardous waste has been set in motion. Mineral oil has been classified as *Special Waste* and disposal by containment has its limitations. Therefore, it is conceivable that landfarming of oily sludges currently endorsed as BPEO, may no longer be viable as it is the deposition of a *Special Waste* to land and it is also probable that treatment of oily sludges before disposal to landfills is required to reduce environmental risks and the associated liabilities. Helen Toft, President of IWM UK 1996 – 1997, reflected the EEC stance in her presidential address, in which she reiterated that most sustainable waste strategy for hazardous waste would be gradually to phase out direct landfilling and for the pretreatment of wastes before landfilling.

Malaysian laws on the disposal of *Scheduled Wastes* has been in place since 1989. The *Environmental Quality (Scheduled Waste) Regulation, 1989* specifies treatment to render waste "innocuous" before disposal to a licensed facility. However, since its promulgation, facilities for treatment of hazardous waste and disposal of its residues are not widely available and the operator is generally responsible to ensure that *Scheduled Waste* are either treated on site and stored in interim facilities till the hazardous waste landfill comes into operation. A National hazardous waste facility is planned for completion by 1998. Malaysia is a newly industrialised country and as yet has few contamination incidences to stir up public protests that would in turn pressure governments to tighten its regulatory controls. Its environmental laws and practices are adopted from USA and EEC. However, in the interim period until the hazardous waste facility is in operation the DOE has allowed a certain latitude to industry in carrying out its own treatment and disposal. At present, oily sludges are allowed to be treated on site in landfarms, which requires a permit under the *Environmental Quality [Prescribed Premises, (Scheduled Waste Treatment and Disposal Facilities Order)] 1989.* Landfarming of oily sludges on lined degradation beds with groundwater monitoring and a leachate collection system is permitted by license. Treatment limits are not specified as yet, however, if the treated residue meets background levels, they may be removed and used on site as soil. The permit to practice landfarming and treated residue removal however has to be continually approved. It is foreseeable that Malaysia may adopt USA law on landfarming should further information become available on incidences of groundwater contamination, leaching, volatilisation, etc. and that this practice may become prohibited or made more stringent. More stringent controls are currently being introduced expressed by the current withdrawal of licenses from small treatment plant operators as well as on site landfill facilities pending the operation of the National hazardous waste facility (Pers. comm. DOE, 1996).

There is a general consensus in these three countries, as surmised by the laws classifying oily sludges, that these sludges because they contain a number of toxic substances are a threat to human health when disposed of indiscriminately. Dumping of these wastes in unlined pits and lagoons, frequently practised at refinery sites, and landfarming causes leaching of these substances into surface and groundwater and gives off volatile organic carbon (VOC) emissions. If these practices are not as yet regulated, it is certain that they will be in the future by the closing of loopholes in the environmental protection Laws. New laws on hazardous waste management are

gaining momentum in most parts of the world and its acceptance has been precipitated by such events as the Love Canal in the USA, the spillage of Amoco Cadiz, etc. Treatment of wastes and contaminated materials prior to final disposal will likely be the only acceptable practice. Complete containerisation for treatment also seems to be the least compromising as all emissions are controlled. Based on current trends, it is likely that in the future, standards for waste treatment, based on specified technology, such as incineration, or similar treatment standards will be imposed. It is imperative that suitable alternative treatment methods capable of achieving similar destructive capability as incineration be investigated. Incineration is an expensive unpopular option for treatment. Refineries could increase cost for waste treatment by 500% if incineration is specified (Loftus *et al.* 1993). In the United States, it is estimated to cost an additional US $1.28 billion per year to treat petrochemical wastes by incineration (Bonner and Autenrieth 1989). On the other hand, biological treatment has been shown to be as effective as incineration, generate harmless residues and costs are very much lower.

CHAPTER 2
REVIEW OF TREATMENT TECHNOLOGIES AND MEASUREMENTS OF BIODEGRADATION

2.1 TREATMENT TECHNOLOGIES

Available technologies to treat oily waste are either physical, chemical or biological. This thesis is concerned with biological methods but the following review includes some discussion of the alternative physical and chemical methods to allow for comparison. The biological treatment of oily sludge review covers the different technologies utilising microbiological methods and will allow comparison to the method chosen in this research. Examples of applications of microbial degradation principles; in landfarming, composting, and liquid-solids contact reactor treatment are presented together with the rates achievable and the limitations or advantages of the treatment technology.

2.2 NON BIOLOGICAL TREATMENT METHODS

Current technologies addressing sludge disposal include solidification and encapsulation; a process involving the addition of a solidifying agent that physically surrounds the contaminant. i.e. cement or lime or chemical fixation process i.e. sorbents. Fixation techniques typically involve the addition of pozzuolanic material (e.g. fly ash, cement kiln dust) to fix or solidify the entire waste. However, sludges with high *O & G* content are difficult to fix. The Hazcon Inc. process, reviewed by Berkowittz *et al.* (1986) contains a preliminary step of microencapsulation of the organic matter which has been shown to improve oil fixation. Encapsulation involves the complete coating or enclosure of a waste with a new impermeable substance. Micro-encapsulation techniques are based on the reduction of surface-to-volume ratio of the waste by the formation of a monolithic, hard mass with a very low permeability (CONCAWE 1990). Macroencapsulation is the enclosing of a relatively large quantity of waste, such as the entire waste container. The disadvantage of solidification and encapsulation methods is the increase in the bulk and weight of the waste for disposal and thereby costing an increase in the charge for landfill disposal.

Solvent extraction using triethylamine (TEA) is a recovery method. TEA and water are miscible at 50^0 F but separate at 120^0 F. Solids are separated from the oil/water phase by combining the sludge with TEA and separating the TEA/water/oil mixture at elevated temperatures and the recovered TEA is recontacted with fresh sludge (Berkowitz *et al.* 1986). Another propriety extraction technology, CF Systems, owned by Morrison Knudsen, uses a liquefied gas solvent such as propane, to extract solids from liquids, sludge and aqueous wastewaters. The recovered organics are separated from the solvent and recycled or sent for disposal whereas the solvent is recycled back into the treatment train (Morrison).

Another recovery method uses the waste stream as feed to the coker unit in a refinery (Loftus *et al.* 1993). Coker units thermally crack heavy petroleum fractions into lighter hydrocarbons and petroleum coke. However, the sludge waste may require treatment to make it suitable for the coker unit and to ensure the fractionating tower remains clear of deposits.

Incineration is commercially available for oily sludges but the costs are as high as Malaysian Ringgit 4,000 per tonne and US $800 per tonne (Kualiti Alam 1996; Abrashamian *et al.* 1992). The residual ash from incineration still has to be chemically fixed before disposal to contain the heavy metals that have become concentrated.

Another treatment method is low temperature thermal treatment. One patented version of this is X*TRAX ™ (Ayen and Swanstrom 1992). This process uses temperatures of 250 - 450 ° C to separate organic compounds from solids. An indirectly heated rotary dryer volatilizes water and organic contaminants in a sealed system. An inert carrier gas transport the volatilized components to a gas treatment train where the entrained particulate solids are removed and the gas stream is condensed to recover the organics. This process reduces the volume of sludge produced by about 40% and increases the heat value of the sludge by one third.

2.3 BIOLOGICAL TREATMENT

Biological breakdown of oils occurs as part of a natural process. Primary mediators of this type of carbon recycling are microorganisms in particular fungi and bacteria. There is significant previous literature covering various aspects of biological degradation of

hydrocarbon and readers are referred to the following for in depth accounts on the different topics:

Table 2.1 : Literature reference

Determination of the effects of environmental parameters on biodegradation rates	"*Effects of Environmental Parameters on the Biodegradation of Oil Sludge*" by Dibble and Bartha 1979 "*Oil Degradation in Soil*" by Raymond *et al.* 1976
Numerical taxonomy of bacteria and fungi associated with HC degradation	"*Enumeration of Petroleum-Degrading Microorganisms*" by Walker and Colwell 1976b. "*Numerical Taxonomy and Ecology of Petroleum-Degrading Bacteria*" by Austin *et al.* 1977.
Elucidation of metabolic pathways/hydrocarbon degradation	"*The Biodegradation of Hydrocarbons*" by Higgins and Gilbert 1977 "*Biodegradation of Polycyclic Aromatic Hydrocarbons*" by Cerniglia 1992.
Examination of the effects of hydrocarbon contamination on micro-organisms and microbial communities	"*Effects of Jet Fuel Spills on the Microbial Community of Soil*" by Hong-Gyu Song and Bartha 1990. "*Effects of Petroleum on Microbial Communities*" by Pfaender and Buckley, 1984.

These literature and others were used in defining some of the operating conditions for the research study which will be discussed in more detail in Chapter 5.

In general, waste-water treatment facilities for the oil industry at present do not adequately address the treatment of sludges. Most sludges are stored in sludge pits or landfarmed. However, as has been reviewed in section 1.3, these practises need to be phased out. Alternative treatment technologies have to be investigated and this is the basis of this research.

2.3.1 LANDFARMING

Land treatment of waste oily sludges has been practised as early as 1954 in the USA (Huddleston 1979). Land treatment, or landfarming, is described by the American Petroleum Institute (API Manual 1983) as "a managed technology that involves the controlled application of a waste on the soil surface and the incorporation of the waste into the upper soil zone". Natural populations of microorganisms living in the soil layer are exploited for their degradative capabilities in this process whereby waste is broken

down into additional microbial mass, carbon dioxide and water. A properly maintained landfarm requires; regular tilling to improve mixing of the waste as well as introducing oxygen; nutrient supplementation when necessary to keep rates high; good drainage to prevent waterlogged and oxygen deficient conditions; recirculation of leachate to minimise surface runoff; proper carbon loading to maintain high rates. Rates achievable using these methods for heavy oils, sludges, and crudes varied between 0.02 and 0.60 g hydrocarbon per kg soil per day (Morgan and Watkinson 1989; Bossert and Bartha 1984). Equivalent technology to landfarming are bioremediation treatment for spilled oils. Similar practices in bioremediation allows for rate comparisons with landfarming. Rates of 0.001 to 60 g/m^3 /day have been reported by Bartha and Atlas (1987) for in situ natural rates.

The rate for biodegradation of oils is usually not constant and is better described by rate orders. Based on this, another useful way of presenting rates is the use of half life calculations. Biodegradation rates are often described by either first or zero order kinetic equations whose application in the study of oil degradation will be discussed in section 2.4.5. Half lifes for first rate orders of between 122 to 38 days have been reported for bioremediation of crude and crude residuals (Bossert and Compeau 1995) in laboratory and field treatment experiments. Brown and Donnelly (1983) have reported half lives, based on CO_2 evolution of refinery sludges at 130 days and 600 days for petrochemical wastes. Residual hydrocarbon extracts indicated a half life of 143 and 264 days for refinery and petrochemical sludges respectively.

The problems associated with a landfarm are due to the difficulties in containing the leaching of oil into surface and groundwater and the accumulation *in situ,* of recalcitrant organic residuals and heavy metals. Laying down fresh sludge on the ground leaves it open to surface run off and volatilisation. In the USA landfarming as a treatment for waste oils, as has been mentioned earlier, is actually limited to waste that has already met the UTS standards and the landfarm itself must meet MTR. The earlier practice by refinery operators have been banned.

2.3.2 COMPOSTING

Composting is similar to landfarming. For composting, the oily sludges need to be mixed with inexpensive and inert bulking agent such as wood chip, straw or rice husks and piled into low mounds (Morgan and Watkinson 1989). The bulking agents serve to

improve soil texture for aeration but care must be taken that the bulking agent is not preferentially utilised as a carbon source. Piling into mounds maintains the heat generated by the respiring microorganisms which helps to improve rates when the outside temperature is low. Composting is best used in areas where temperature is critical to the sustained treatment process. The system may be optimised in a similar way to landfarm for inoccula, nutrients, oxygen, moisture, pH, etc. However, literature on composting experiments are fairly limited to allow a thorough review as a treatment technology for oily sludges. There are mixed accounts on the type of bulking material used. Szabo and co-workers (1987) composted oily sludge with cattle manure and a 50 % reduction in mineral oil was recorded in a 6 months experimental period. Total PAH content decreased by 40 %, however chrysene and benzo(b)fluoranthene remained unchanged. Total number of microorganisms, reached a high of 10^6 - 10^7/g with oil degraders displaying a hundredfold increase from 10^3/g - 10^5/g. The findings of this research were not substantiated with results data or analytical methods but the low rates and extent of degradation do indicate that the cattle manure could have been used as an alternative carbon source. There have been other research that have published findings that if the compost used can provide an alternative carbon source, the degradation rate of the oil is retarded. Dibble (1982) pre-adsorbed oil onto rice hulls and found little degradation of oil due to the preferential utilisation of the rice hulls. In another study by Francis (1988), composting oil with a relatively inert compost; an oil absorbent peat product, commercially available as Oclansorb; increased degradation to 44 % oil loss in three months, almost double the rate achieved by Szabo and co-workers. Composting has similar disadvantages as landfarming in that it accumulates heavy metals and hydrocarbon residuals and leachates have to be monitored (Bossert and Compeau 1995). However the advantages composting has over landfarming is that it uses less space and this allows for enclosing the whole treatment area to collect and treat leachates and volatile emissions. Composting also generates heat that means it could operate at a higher rate over a cold season.

2.3.3 LIQUID - SOLID CONTACT REACTORS

With the existing laws in place and predicted trends as reviewed earlier, research on oily sludge treatment in containerised vessels have become inevitable. Containerised vessels allow for greater emissions control in order to reduce risks to the environment

and in addition allow for better process control for optimisation. Reactor based technology for treating sludges are presently either at laboratory scale or pilot scale testing (Anon. 1996). There is fairly limited literature strictly dealing with the treatment of waste oily sludges in reactors. Due to this, other research work on oil contaminated soil treated in reactors were also reviewed as the nature of the two wastes are similar.

Liquid - Solid Contact (LSC) reactors are increasingly being used to treat soil contaminated from oil spills and it is also looking to replace landfarming type of activities. Reactor based treatments improve the mixing of the wastes, thereby reducing mass transfer limitations, and generally improve rates of reaction. Research by Yare (1991) compared the rates and extent of degradation of oil from contaminated soil that could be achieved in a landfarming type of treatment and a reactor based treatment. He modelled landfarming in a lined treatment bed enclosed by a plastic film green house. The landfarm was operated to achieve the highest rates possible by maintaining a program of tilling (aeration and mixing) and the addition of water, nutrients and inocula. The rates achieved were a half life of 311 days for TOC and the extent of degradation was a 90.8 % removal of PAHs and 19 % TOC removal over a time period of 94 days. The LSC reactor design consisted of a two baffled, flat bottomed 380L tank equipped with a centre mounted 1.74 hp variable speed agitator. The agitator had a six bladed radial flow impeller mounted just above the bottom of the tank and a three bladed axial flow impeller mounted in the middle of the tank. It was operated at 30 % solids slurry and dissolved oxygen was maintained at a minimum of 2.0 mg/l through the introduction of compressed air from perforated pipes at the bottom of the tank. Contaminated soil was added at the start and the nutrients nitrogen and phosphorus were added at C:N:P of 100:5:1. In contrast to the landfarm model the LSC reactor was operated for 10 days and the rates achieved were a half life of 22 days for TOC and a 62.5 % removal of PAHs. This lower extent of removal is probably due to the short duration of treatment (10 days vs 94 days). TOC removal was 22%. As a further comparison, phenanthrene had a half life of 8 days in the LSC reactor and 32 days in the landfarm. A summarised report by Bossert and Compeau (1995) has calculated variable half lives from landfarming activities for: crude oil at 122 days, heavy oil at 60 days, refinery oils at 71 days and crude residuals at 38 days.

LSC reactors offer improved mass transfer rates for biodegradation. However, mixing

characteristics vary according to design and very little research is available correlating design with reaction kinetics. Since most LSC reactors treating sludge waste operate at a higher solid loading, 10 - 30% , mixing does pose a problem and reactor design to ensure an efficient mix is an important consideration. Stirring uses a lot of energy and there has to be a compromise between the degree of improvement in mixing and the energy input. There also has to be a consideration of too much shearing disrupting floc formation of the culture. Reactor design and mixing requirement are thus a complex issue in the design of a reactor to treat oily sludge. The nature of the waste, is heterogenous and mostly immiscible and mixing is very important. Too low a speed will reduce the contact time between the micro-organism and oil droplet, too high a mix may also be detrimental to the degradation rate as it causes the bacterial flocs to be sheared as well as the oily sludge changes consistency to a more 'pasty' state (the particulates become sticky, or agglomerate) (Marks *et al.* 1988a; Stroo *et al.* 1989). Stroo *et al.* (1989) found that high energy mechanical mixing was actually detrimental as it promoted the agglomeration of oily materials and hence reduced the surface area available for dissolution and microbial colonisation. This change in state also reduced the percentage of solids in the mixed liquor where most degradation is taking place and thus reduces overall degradation rate.

Foaming in reactors treating oily wastes is also a common occurrence, frequently due to the presence of organisms such as *Nocardia*. The nature of the waste also plays a part in foaming or frothing. Air bubbles, introduced to aid oxygenation of the mixed liquor, dissolve within the oleophilic interior of oil droplets and cause these to rise to the surface and 'froth'. However the nature of these two foams are characteristically different (Vail 1991). Foams caused by the introduction of air are non-persistent and disappear upon turning off the air source. Bacterial foams on the other hand, are stable, persistent; it can be several feet high and the addition of antifoaming agents may be non-effective. In Vail's (1991) study, the foam was so thick it caused a few of the aerator shafts to break down. Reactor design will also have to take this foaming into consideration.

Reactor based treatments offer quite a few advantages over other biological treatment technologies. These systems are analogous to conventional biological suspended growth processes such as activated sludge. This process has the advantage of lower

land requirements (compared to landfarming and composting), better process control and a reduced risk of off site contamination. Reactors may be equipped for instrumentation and control to optimise the process and parameters such as pH and dissolved oxygen can be continuously monitored with the use of probes. Mixing, which is an important rate liming parameter, may also be optimised by design of the stirrer and vessel. Enhancement of biodegradation rates with the use of nutrients is a simple introduction as opposed to application and tilling as in landfarming. Due to improvements in mass transfer rates, population densities in the order of 10^{10} cells/ ml mixed liquor are achievable in LSC operations compared to 10^{7} to 10^{8} cells / solids during land treatment (Stroo *et al.* 1989:Marks 1988b). Maintenance of LSC reactors may be higher due to the mixing requirements but this is offset by the saving in space, manpower and time. Reactor operations may be automated and reaction rates are higher which means less time is needed to achieve similar results. To be successful, LSC reactors must meet treatment standards set as UTS. Yare (1991), demonstrated in his feasibility study that, slurry phase bioremediation was capable of providing the same level of environmental protection as incineration, which were at that time BDAT standards. Abrishamian *et al.* (1992) also demonstrated treatment levels meeting UTS (most BTEX and PAH treated to < 2 ppm) in a demonstration scale biological liquid/solid treatment system.

2.4 MEASUREMENT AND INDICATORS OF BIODEGRADATION ACTIVITY

A significant amount of this research utilised indicators of biodegradation activities. An understanding of the limitations of each indicator or analysis becomes important when interpreting the results. While the use of standard methods goes some way in assuring consistent and comparable results, an understanding of the chemistry behind the analysis is also essential. For this reason, the following review covers the different methods available for the determination of biodegradation activity and the shortcomings or advantages.

The methods used for the evaluation of biodegradation of organics are reviewed in the following sections and the analytical procedures used are assessed for application in this study. Furthermore, mass transfer and reaction rate kinetics of the biodegradation process are discussed together with its application in other similar research to gain

insight for the study.

Several strategies are available to evaluate biodegradation of hydrocarbons. Measurement of reactants, i.e. HC or their end products, provides a good means for obtaining a mass balance and determining the efficacy of biodegradation. Mass balance studies directly quantify routes of hydrocarbon loss. Other indirect indicators of biodegradation activity include the enumeration of bacteria and/or fungi and measurement of their respirometric activity. It is assumed that these indirect methods would show elevation in the number of hydrocarbon degraders as well as respiration rates should there be any significant biodegradation activity occurring.

2.4.1 HYDROCARBON ANALYSIS

The definitive means for evaluating biodegradation is a direct measure of hydrocarbon losses (Bossert and Compeau 1995; Atlas 1991). The analytical method chosen to verify a treatment process is very important as the inappropriate selection of a method may result in a masking of the true process. The measurement of hydrocarbon loss is commonly measured by the periodic extraction of replicate samples with an organic solvent in either a liquid - liquid extraction procedure, Soxhlet apparatus or sonication. The solvent extract is then either; evaporated in a tared dish and the hydrocarbon is determined gravimetrically as residual weight or; measured using infrared adsorption and calibration charts. Recent development in extraction methods is towards the reduction in use of chlorinated solvents as well as utilising supercritical fluids for extraction (SFE).

If greater resolution of the hydrocarbon components are required, then fractionation into classes, such as saturates, monoaromatics, di + triaromatics and polyaromatics, heterocyclics, are achieved through successive elution in a discontinuous solvent gradient of increasing polarity using thin layer chromatography or column chromatography (silica gel or alumina column) (Jewell *et al.* 1972; Jobson *et al.* 1972; Petrakis *et al.* 1980). Further separation of the extract to characterise or quantify the individual hydrocarbon may be carried out in methods such as GC, GLC, GC/MS or HPLC with detection using FID, ultraviolet or infrared, fluorescence adsorption or diode

detectors .

There are a number of extraction methods involving different solvents either singly or in combination and there are also numerous ways of separating hydrocarbon components. This discussion will be limited to methods that have been in common use in the study of biodegradation of oil in either waste treatment or bioremediation.

The choice of solvent for the extraction of hydrocarbons depends on the material to be extracted. Solvents such as pentane and hexane (the C5 - C8 solvents) have been used to extract aliphatic or straight chain hydrocarbons. If polyaromatics are the compounds of interest, then the solvents commonly used include cyclohexane, TCTFE, carbon tetrachloride and methylene chloride. The efficiency of extraction is very dependent on the choice of solvent, the extraction method, the sample state (matrix) and the total time of extraction. Other factors, such as the presence of surfactants, may introduce variability in the percentage of extractable HC.

The Table 2.2 lists the commonly used standard methods for HC extraction. The choice of method used will depend on the sample. Method 3520 (SW846) is for aqueous samples that form emulsions that cannot be physically broken when combined with solvent. Method 3510 (SW 846) is similar to Method 5520 B (APHA) and Method B (HMSO publication) which are for aqueous samples. Solids and sludges are usually extracted by Method 3540 (SW 846), 3550 (SW 846) and 5520 E (APHA). Further sample clean up to obtain hydrocarbons from '*O & G*' extract use method 5520 F (APHA) or the HMSO publication methods. The complete details of the methods are in the Standard Manuals cited. The final choice of method will depend on the subsequent method used for characterisation, such as HPLC, and the final compounds of interest. Some of the methods require acidification of samples for sample preservation or for concentration of particular compounds (such as phenols), should a base or neutral compounds such as the PAH be required, this acidification step should be omitted or rectified.

Table 2.2 : Standard methods for hydrocarbon extraction

STANDARD METHOD	DESCRIPTION		SAMPLE STATE
SW 846 *- METHOD 3500	Sample preparation		aqueous sample
SW 846 - METHOD 3510	Separator Funnel -Liquid - liquid extraction		aqueous sample
SW 846 - METHOD 3520	Continuous Liquid - liquid extraction		aqueous sample
SW 846 - METHOD 3540	Soxhlet extraction		soils, solids, sludges
SW 846 - METHOD 3550	Sonication		
APHA **- METHOD 5520 B	Partition -Gravimetric		aqueous sample
APHA - METHOD 5520 C	Partition -Infrared		aqueous sample
APHA - METHOD 5520 D	Soxhlet extraction		aqueous sample
APHA - METHOD 5520 E	Soxhlet extraction		sludge sample
APHA - METHOD 5520 F	Hydrocarbons (TPH) (silica gel separation)		O & G sample
***HYDROCARBON OILS IN WATER BY INFRARED ABSORPTION & GRAVIMETRY	Method A Infrared Absorption	Method B Gravimetry	aqueous sample

* Test Methods for Evaluating Solid Wastes - Physical Chemical Methods, USEPA SW 846 (Volume 1B), 3rd Edition 1986

** Standard Methods for the Examination of Waters and Waste Waters by American Public Health Association, 1992

*** The Determination of Hydrocarbon Oils in Waters by Solvent Extraction, Infra Red Absorption and Gravimetry 1983. Methods for the Examination of Waters and Associated Materials. London. HMSO

A study by Fowlie and Bulman (1986), on the efficiency of a Soxhlet extraction showed a 74.5% recovery of PAH. They studied the effects of the extraction method on PAH extractability. This study was essentially comparing two extraction methods with several other variables. The other method used a Polytron homogenizer, which is not commonly used, and the findings of this study on the variables affecting extraction is far more important than the method comparison. The Polytron showed lower recoveries compared to the Soxhlet but both extraction methods showed similar differences in extraction efficiency with the variables, i.e. PAH compound, percentage present in matrix (5 or 50 ug/g), presence of mercuric chloride (frequently used in sterile controls). They found benzo(a)pyrene was extracted in higher amounts than anthracene; the

5 ug/g concentration of the compound in the matrix resulted in lower percentage recovery compared to the 50 ug/g concentration and mercuric chloride increased the amount of unextractable PAH associated with the matrix. Based on their findings, they concluded that the recovery of PAH from environmental samples is influenced by many factors and are likely to be underestimated. This underestimation can be minimised by using the most efficient extraction technique and since this is very matrix dependent it has to be determined each time for a particular sample matrix.

Most studies on the degradation of oils use more definitive analytical procedures such as GC, HPLC and Mass Spectrometry to determine the compositional profile of the oil under investigation. However, gravimetric or infrared spectroscopic determination of either *O & G* or total petroleum hydrocarbons (TPH) which can be determined by the methods in Table 2.2, have remained as workhorse methods in research due to their simplicity and cost effectiveness (Bossert and Compeau 1995). The results obtained by these methods continue to be the accepted regulatory end point for environmental clean ups in a lot of countries. The solvent used in SW 846 methods is methylene chloride. APHA extraction uses TCTFE and the HMSO publication method uses carbon tetrachloride or TCTFE. With these methods, it is not the absolute quantity of a specific substance being measured, rather, groups of substances with similar physical characteristics are determined quantitatively on the basis of their common solubility in the particular solvents. Hence, some analysts prefer the terms such as 'acidified/base neutral, TCTFE extractable material' etc.

Oils and greases are defined by the methods used for their determination (APHA 1992) unlike some other constituents that represent distinct chemical elements, ions, compounds, or groups of compounds. Solvent extracted material may be passed through a florisil column to obtain what is commonly referred to as Total Petroleum Hydrocarbon or TPH as delineated in Method 5520 F and the HMSO publication method. These methods separate hydrocarbons from the total *O & G* on the basis of polarity. Florisil adsorbs polar material, hence fatty acids, common components of natural oils and fats are retained and a 'hydrocarbon' portion can be quantified. Nevertheless these methods are still non specific and care must be taken in the interpretation of results. In the infrared detection method, which measures the carbon-hydrogen stretching in the infrared spectrum, the use of inappropriate analytical

standard may not adequately reflect the composition of hydrocarbons in the test sample (Puttnam 1988). It should also be noted that due to the non selectivity of the solvents, the gravimetric concentrate may contain both the hydrocarbons from oil as well as any metabolic products (Glaser 1991) thus in biodegradation studies, degradation may be underestimated.

As mentioned earlier, more specific indicators are frequently used in assessing the biodegradation of oils. Petroleum is a highly complex mixture of mostly hydrocarbons but with significant representation from other elements including oxygen, sulfur, and smaller amounts of nitrogen, nickel and vanadium. Molecular weights observed easily reach the several thousand mark and therefore the number of chemical compounds present is very great. Thus any chemical and physical characterisation precludes the monitoring of all the individual components (Petrakis *et al.* 1980). Compositional profiles using chromatographic analysis 'subset' these compounds and allow for more meaningful comparisons. Standardisation of analytical methods, terminology and approaches have to a degree allowed for comparison of different studies. However the most common usage of compositional information by environmentalists and microbiologists is comparative in nature; that is, a change is looked for from a baseline feature rather than individual compound identification. This requires consistency, accuracy, and repeatability in data acquisition over a large sample size range.

This research has focused on a group of compounds that are recalcitrant and highly toxic, the PAHs. There are numerous PAH compounds in petroleum but this research has identified 16 PAHs currently referred to as priority pollutants in the USA (refer to Table1. 3). These 16 PAHs are semi volatile, non polar HC compounds and a few of the commonly used standard methods to separate these 16 compounds are as listed in Table 2.3. There are numerous analytical techniques for quantifying PAH and it is impossible to cite all the papers that have been published on this subject. There are several journal reviews as well as books that cover in detail the different approaches, analytical techniques and advantages, disadvantages, method limitation etc. These reviews are not comprehensive enough to cover all aspects of PAH analysis but they describe the complications involved in the analytical techniques and interpretation of results. For more information refer to;

i) Lee *et al.* 1981. Analytical Chemistry of Polycyclic Aromatic Compounds.

ii) Petrakis *et al.* 1980. Petroleum in the Marine Environment.

Table 2.3 : Standard method for PAH determinations

Method	Description
APHA Method 6440	Liquid-liquid extraction chromatographic method
EPA Method 610	Liquid-liquid extraction chromatographic method
SW 846 Method 8310	Determination by HPLC
SW 846 Method 550.1	Determination of PAH by liquid-solid extraction and HPLC with coupled ultraviolet and fluorescence detection

2.4.2 MEASUREMENT OF MICROBIAL NUMBERS, RESPIROMETRIC ACTIVITY AND BIOMASS AS INDICATORS OF BIOLOGICAL DEGRADATION OF OIL/HC

Indirect evidence for biodegradation include the proliferation of hydrocarbon degraders in the presence of oil (Bossert and Bartha 1984). Enhancement in the number of oil degraders relative to total heterotrophic counts have been used to demonstrate biodegradation in landfarming and bioremediation type activities (Mulkin-Phillips and Stewart 1974; Walker and Colwell 1976a; Horowitz and Atlas 1977; Sexstone and Atlas 1977). Based on Monod kinetics, given the assumption that there is a complete availability of food, nutrients, oxygen and the absence of toxicants, the bacteria should multiply exponentially. A plot of the log of the plate count versus time increases linearly over time and as the substrate concentration decreases, so does the growth rate until a period where the substrate or nutrients are exhausted (Lawrence and Mc Carthy 1970). This relationship is clearly demonstrable in pure cultures using easily metabolizable substrates. However studies with mixed cultures utilising petroleum crudes and its products show variations in this plot due to several reasons. The response of a bacterial community is a function of both the nature of the community and the composition and concentration of the hydrocarbons. Hydrocarbons can serve both as a food source or a potential toxicant, thus even when environmental conditions are maximised for growth, it may not proceed exponentially due to inhibition or death within the community. It has been reported by Dibble and Bartha (1979) that stimulation of microbial activity was positively correlated to increasing amounts of hydrocarbons in soil

up to the 5% level. Odu (1972) also reported that at higher hydrocarbon concentrations although, activity or rate may not be enhanced, the soil receiving the largest application (39.2%) of crude oil possessed the highest number of microorganisms. In his study, the population initially declined but within six weeks, the hydrocarbon degraders were selectively enriched beyond background levels. The initial decline may be due to the toxicity of the applied oil to certain susceptible micro-organisms, consequently leading to enrichment of hydrocarbon resistant and hydrocarbon metabolising bacteria.

Hydrocarbons are complex, mostly water insoluble substrates and microorganisms adapt different mechanisms and have different capacities in utilising it (Cooper and Hedrick 1976). Although there may be an increase in numbers of microorganisms the activity of the population may actually have decreased, in other words, activity and numbers are not correlated. Frequently there are shifts in population size and species diversity upon exposure to hydrocarbons. In using enumeration as a yardstick for biodegradation, these and other factors affecting rates of degradation need to be kept in mind. In depth discussion of these factors will be included in the next section on kinetics of rates of reaction and mass transfer. The following discussion is confined to the methods applied for enumeration, biomass calculation and metabolic activity measurements.

Enumeration methods for hydrocarbon biodegradation studies include classic and novel techniques. Culture techniques are routinely used to enumerate total heterotrophs as well as substrate specific microorganisms (Gerhardt *et al.* 1981). A clear disadvantage of culture techniques is in the use of media for growth. All media are selective to a point as only the cultivatable organisms are counted. Number of recoverable cells are estimated at 1 to 10 % of total number of cells present (Stroo *et al.* 1989). The two most widely used methods in the laboratory and field are plate count and most probable number (MPN) using either undefined or selective media (Beliaeff and Mary 1993; Compeau *et al.* 1991). An adaptation of MPN uses microtiter plates in order to screen large number of samples rapidly and reduce the amount of glassware involved in traditional MPN technique (Stieber *et al.* 1994).

A study by Walker and Colwell (1976b) on; *Enumeration of Petroleum Degrading*

Microorganisms, examined a variety of factors to determine their effect on the total counts of microorganisms on oil containing media. Their study found a relationship between hydrocarbon degrading activity and number of petroleum degraders (r=0.86) or the percentage of the total count that are degraders (r=0.99). The use of 0.5% (Vol/Vol) oil and 0.003% phenol red with fungizone (for bacteria isolation) and streptomycin and tetracycline (for isolating yeast and fungi) were recommended for plating hydrocarbon degraders. They also suggested that petroleum degraders be expressed as a percentage of the total population rather than total number of petroleum degraders per se. A lot of researchers have followed this convention of specifying petroleum degraders as a percentage of total heterotrophs in studies involving microbial oil degradation (Rogers *et al.* 1993). Total heterotrophs have been counted on plate count agar (Westlake *et al.* 1978), trypticase soy agar (Sexstone and Atlas 1977) and nutrient agar (Song and Bartha 1990).

Methods for plating hydrocarbon degraders have been continuously developed and researched since the technique was first presented by Bushnell and Haas in the 1940's (Bushnell and Haas 1941). The fact that hydrocarbons are relatively insoluble has made them a difficult substrate to incorporate on solids such as agar or silica gel. Earlier methods used hydrocarbon in vapour form introduced either into chambers (vacuum dessicators) with the plates or were incorporated onto filter papers and kept on the covers of inverted glass plates (Raymond *et al.* 1967; Walker and Colwell 1976b). 'Oil agar' plates incorporated 0.5% crude or crude product directly into minimal salts agar media (Baruah *et al.* 1967; Sexstone and Atlas 1977) or silica gel (Seki 1973). These type of plates have been in common use in microbial oil degradation research with the more recent adaptations using high purity agar or silica gel (Walker and Colwell 1976b). The use of high purity agar or silica gel is necessary as the impurities on agar can support a large number of the zymogenous (opportunistic) community and therefore hydrocarbon degraders are overestimated (Eisman and Dorwin 1991). The drawbacks in these plate methods include; spread plates frequently produced 'spreaders', making counts impossible; very small colonies against a dark oil background are difficult to read; oil droplets and colonies are difficult to differentiate; the two weeks it takes for the plates to develop is long and the gel or agar may become dry.

Interest in studying degradation of specific hydrocarbon compounds, such as the 16

priority pollutant PAH, have resulted in improvements in the analytical procedures for plate counts which have addressed some of the drawbacks mentioned earlier. Specific hydrocarbons e.g. naphthalene or phenanthrene are incorporated into basal salts media by; dissolution in water or acetone and mixed with agar (Grifoll *et al.* 1995); dissolved in acetone or ethanol and sprayed onto solid agar (Shiaris and Cooney 1983); or naphthalene crystals are placed in inverted petri covers (Guerin and Boyd 1995). In a procedure recommended by Bogardt and Hemmingsen (1992), bacteria are added to molten, cooled agarose containing fine particles of phenanthrene. This is poured over an already solidified agar underlayer. This semi pour plate technique eliminates spreaders and swarmers that are quite commonly found with 'spread' oil agar plates. Colonies of bacteria are easily visible as there is a halo of phenanthrene disappearance around it.

A summary of microbial plate methods is included in a review by Atlas (1992).

Direct counts of microorganisms is another frequently used method in oil degradation studies. These methods use fluorescent dyes and microscopy to visualise microorganisms within an abiotic matrix (Hobbie and Daley 1977; Song and Bartha 1990). Interference from the matrix such as organic debris and metals can impede counting. Direct counts provide fast results but will not differentiate between the heterotrophs and the HC degraders. Fluorescent techniques can however, differentiate between live and dead bacteria.

All the methods mentioned above have been used in routine laboratory evaluation for the progress of biodegradation of crude oil or its products in land contamination and bioremediation studies as well as landfarming or waste treatment. Other microbiological tests associated with these studies include confirmatory tests for hydrocarbon degraders. It is normal that oil contamination generates a rise in total counts and a corresponding rise in the hydrocarbon degraders population of the heterotrophs. However, most plate count methods are uncertain about the source of respirable carbon unless tracers are used. Contamination of carbon source could come from the introduced water sample, agar, dead bacteria etc. Methods have been developed to confirm hydrocarbon metabolism. Some of these methods include the use of dyes to

demonstrate the actual metabolism of aromatic hydrocarbon by specific organisms on agar plates (Deziel *et al.*1996) or in liquid culture in microtiter plates (Shiaris and Cooney 1983). Others use the production of radiolabelled carbon dioxide from radiolabeled hydrocarbon substrate by cultures isolated from the initial plate counts (Guerin and Jones 1988; Keuth and Rehm 1991). Colony hybridization procedures have also been used to identify positively the colony forming units with the genetic capacity for degrading specific aromatic hydrocarbon (using gene probes) (Sutherland *et al.* 1995). Isolating pure cultures in broth and measuring hydrocarbon disappearance (Yong *et al.* 1991) is also another method that has been used.

Microbial population counts serve as an indicator of microbial vitality and shifts in population and give an estimate of the in situ microbiota. However, numbers do not always reflect actual microbial activity. Activity measurements may provide additional useful information on the biodegradation activities. Rates of microbial respiration are frequently used as indicators of microbial utilisation of hydrocarbons. Consumption of oxygen or oxygen uptake rate (OUR) and CO_2 production is a standard means to asses biodegradability in the laboratory (Bossert and Compeau 1995). CO_2 production is a widely used measure of 'mineralization' or the complete oxidation of the parent compound. In an ideal environment, mineralization is proportional to the amount of substrate (HC) present and can be used to establish a mass balance for biodegradation. Sharabi and Bartha (1993) tested some assumptions about biodegradability in soil as measured by CO_2 evolution. They found conversion percentage to CO_2 is relatively independent of the chemical composition other than substrate's carbon and CO_2 production is proportional to the amount of added test compound (substrate). However, seemingly controversial to the second finding, they also found that more than half of net CO_2 production did not come directly from the test substance and thus may represent the mineralization of biomass and soil organic matter. In some cases, the net CO_2 evolution exceeded 100% of the carbon added in the form of test substance. Sharabi and Bartha noted that this may be due to the 'priming' effect of adding readily decomposable matter. Other researchers have reported that the addition of readily decomposable organic matter stimulates the mineralization of indigenous soil organic matter (Alexander 1977). Sharabi and Bartha (1993) took this into account and added a positive control to correct for the variable soil quality. To account for the proportionality of substrate carbon to CO_2 evolution the authors noted that it could be a function of

'biomass yield coefficients' which are dependent on the energy content of the substrate. They have assumed, in short term biodegradation studies, that roughly 50 % of substrate carbon is converted to carbon dioxide and in experiments lasting over a month, found that substrate carbon, initially fixed in biomass, is also mineralised in part which together with the priming effect would account for the raised net CO_2 evolution. Generally, in biodegradation studies where mineralization is measured by the CO_2, evolved, a 50% conversion efficiency is assumed (for every oil carbon converted to CO_2, another is incorporated to bacterial biomass) (Bossert and Bartha 1984).

As with carbon dioxide production, oxygen consumption also provides a rapid, simple evaluation of biodegradation as a measure of respirometry. Whereas carbon dioxide measures the ultimate product of biodegradation, OUR reflects overall activity and includes transient uptake due to partial oxidation of substrate. As with carbon dioxide data, metabolic stoichoimetry can be determined. It has been estimated that the theoretical oxygen demand during hydrocarbon degradation is approximately 3.5 mg molecular oxygen per mg hydrocarbon (Miller *et al.* 1991; Gibbs and Davies 1976). However, measurement of OUR may reflect the utilisation of other contaminants. The introduction of oil may result in the death of some organisms and this provides respirable organic input. Thus, the measured OUR will be a function of the contaminants as well as the oil. Dupont and Doucette (1991) found OUR to be more consistent and more sensitive than CO_2 production rates in detecting effects of treatments on microbial activity. CO_2 production rates are more affected by cases of soil drying and moisture additions where the air drying of soil leads to an anomalous increase in CO_2 evolution upon remoistening. This finding has more relevance in field remediation studies or laboratory microcosm studies where soil may be either dried out due to existing environment or purposely dried in the laboratory as the homogeneous application of hydrophobic samples necessitates the air drying of soil (Sharabi and Bartha 1993). The burst of CO_2 comes from the mineralization of soil biomass killed by the drying process.

Waste water treatment facilities routinely measure biomass as an indication of microbial growth which is then linked to substrate utilisation. Biomass of microorganisms may be determined from the measurement of parameters such as volatile suspended solids (VSS), nitrogen, DNA, ATP and protein (Metcalf and Eddy 1991). The most commonly

measured parameter is VSS due to its analytical simplicity. The volatile fraction of total suspended solids is used to estimate the active biological mass. Conversion factors may be used to convert biomass to numbers with the assumption that every bacteria is of the same size and has the same amount of volatile material or protein or DNA or ATP. According to Mc Carthy (1975), 1 bacterial cell = 2×10^{-10} mg dry weight. In most reactor based studies biomass determinations, in mass VSS/unit volume, are used to determine rate equations of the process and in waste water treatment plants VSS is monitored for process design and control. Remediation or waste treatment using reactor tank slurry treatment and activated sludge treatment of oily waste waters can monitor their processes using this parameter (Rogers *et al.* 1993; Castaldi and Ford 1992; Marks *et al.* 1992). VSS measurements in hydrocarbon degradation studies are hampered by the presence of oil adhering to solids in the waste water as this remains on the filter and is measured as part of the volatile suspended solids (Marks *et al.* 1988a). This can be overcome by washing the filter with solvents capable of dissolving the oil prior to drying (Reddy *et al.* 1982). Protein (Michaelsen *et al.* 1992) or ATP determinations (Yare 1991) also overcome this problem. However these methods are more time consuming than VSS determinations.

In summary, there are a lot of methods used to monitor hydrocarbon degradation. Oil loss as determined by *O & G* or total petroleum hydrocarbons determinations are frequently used as the first general indicator of the breakdown of hydrocarbons. These methods are frequently complicated by the different extractibility according to solvent efficiency; reversible and irreversible adsorption of the different components of hydrocarbons to the matrix; presence of bacterial surfactants etc. Due to these complications the standard methods of analysis, are not exactly comparable from one set of experimental conditions to another. They are better used to see the changes from a 'baseline' in a particular set of experiments. Separation of the *O & G* components into the various standard fractions gives a better profile description of the biotransformation of oil fractions. A 'pattern of oil loss' can be seen from chromatograms of any of the fractions such as saturates or aromatics from day zero of an experiment to its conclusion. Similarly, charting the microbial population growth curves or 'activity' of the population through time should serve as an indicator of the degradation activity.

The parameters measured such as biomass and substrate concentrations are used to obtain growth rates, degradation rates, yield, half velocity coefficients (K_s) etc. The input from these measurements can then be further integrated into models including mass transfers, rate orders and inhibition kinetics that will be discussed in the following sections.

2.4.3 MICROBIAL GROWTH AND SUBSTRATE UTILISATION RATES

All bioreactors, including the slurry reactors proposed here, can be interpreted using the classical model of microbiological growth, given by Monod. Given a complete availability of food, nutrients, oxygen and the absence of inhibitory compounds, bacteria should multiply exponentially. However, in a batch culture, one of the essential requirements (substrate or nutrients) will eventually become depleted and growth would cease. In slurry reactor treatment of wastes, ideally, the limiting element is the waste. The basic assumption of Monod kinetics is that as the substrate decreases, so does the growth rate until a period where the substrate are exhausted (Lawrence and McCarthy 1970). At this point bacteria enter an endogenous phase characterised by high death rate and cell lysis. The specific growth rate can be expressed by the familiar equation of Monod:

$$\mu = \frac{\mu_m \quad S}{K_s + S} \qquad (2\text{-}1)$$

Where;

μ = specific growth rate, time $^{-1}$

μ_m = maximum value of μ, time $^{-1}$

S = residual growth-limiting substrate concentration, mass/unit volume

K_s = half-velocity constant, substrate concentration at one-half maximum growth rate, mass/unit volume.

As various texts (Grady and Lim 1980; Metcalf and Eddy 1991) cover growth kinetics and substrate utilisation in detail, only a brief review of the coupling of the growth rate equation of Monod to substrate utilisation in a batch reactor is covered and it can be found in [1]Appendix D, p. 259.

Many of the assumptions upon which Monod kinetics are based should not be valid in

[1] Equation 2-2 to 2-5 are in Appendix D

its use with the slurry reactor suggested in this thesis, such as; the assumptions that utilisation rate is limited by a single enzymatic process; that there is a constant enzyme concentration; and, that there is no species distribution of the microbial community (Rogers *et al.* 1993). However, approximations to the equations always seem to be applicable and used (Grady and Lim 1980). References show that Monod kinetics are frequently utilised in biodegradation studies involving mixed bacterial cultures as well as isolated strains (Simkins and Alexander 1984; Li *et al.* 1995).

2.4.4 MASS TRANSFER AND REACTION RATE

How fast a microbiological reaction proceeds in the biological degradation of oils is governed by several factors. Some of these are;

I. mass transfer rates of a substrate from the matrix to the microorganism. This rate is influenced by physical-chemical properties such as solubilities, diffusibilities, dissolution, adsorption, desorption, matrix effects, sorbent, mixing, etc.

II. rate of enzyme synthesis for the uptake and metabolism of the substrate with this also being governed by the presence of inducer or inhibitor, metabolic state of the organism, limiting environments, etc.

III. environmental conditions such as pH, temperature, nutrients, redox, electron acceptors etc.

IV. physiological state and growth phase of the microorganisms eg. lag, log, starvation, (Guerin and Boyd 1995).

Clearly there are complex interactions between the microorganism, its environment and the substrate plus matrix which will influence the rate of the reaction. Some of these 'environmental effects' such as temperature, pH, have been comprehensively discussed and reviewed in texts and literature and the commonly used equations and relationships such as Arrhenius (Vail 1991) and Q_{10} rules (Bossert and Bartha 1984) successfully applied. Based on the literature search, most of these environmental parameters have been kept at optimum or maintained within a certain range for the experiments in this research. The choice of parameters controlled and the set points or range will be covered in Chapter 5 on "Experimental Set-Up". The main rate controlling parameter for

this research was thought to be the mass transfer of the contaminant (oily sludge) to its assimilation by the microorganisms and this is reviewed in this section.

Transport limitations for biodegradation have been demonstrated in a study of alkane oxidation by Miller and Bartha (1989). Their experiment involved encapsulation of octadecane in liposomes whereby its uptake was greatly increased. The encapsulation of alkanes into phopholipid vesicles facilitated the delivery of hydrocarbons into bacterial cells, possibly by the fusion of the hydrophobic vesicles with the cell membranes. While unencapsulated octadecane was taken up at only 1.3%, liposome encapsulated octadecane was taken up at 23.5%. This resulted in growth with k_s values of 2453 mg/l in unencapsulated octadecane which decreased to 60 mg/l in liposome encapsulated octadecane. This shows transport limitation of insoluble substrates may be one of the main reasons causing the low degradation rates observed for HOCs.

Oily residues in a reactor may be found bound to organic and inorganic residues suspended in the slurry or existing as oil droplets in the water phase or as a separate oil phase layer and adhering to the walls and stirrers in the reactor. Several rate models have been reviewed which can predict the rate of removal of contaminant organic constituents and some of these models will be used to test the fit of the data generated by this research.

Generally reaction rate is expressed as moles of the components of interest formed or destroyed per unit volume or mass per time. Defining *dNi/dt* as the change in the number of moles of component i, rate of reaction (r) may be addressed as follows based on unit volume (V) of reacting fluid;

$$r_i = \frac{1}{V}\frac{dN_i}{dt} = \frac{\text{moles i formed}}{(\text{volume of fluid})(\text{time})} \qquad (2.6)$$

Based on unit mass (W) of solids in a fluid-solid system (Levenspiel 1972);

$$r_i = \frac{1}{W}\frac{dN_i}{dt} = \frac{\text{moles i formed}}{(\text{mass of solid})(\text{time})} \qquad (2.7)$$

These 2 equations are related by;

$$\text{(volume of fluid) } r_l = \text{(Mass of solids) } r'_l \qquad (2.8)$$

Rate equations are obtained by theoretical mechanistic considerations or by empirical curve fitting. Experimentation is often necessary to determine the constants of the rate equation because of the complexity of the basic mechanistic approach. Biodegradation reactions of interest can be modelled as irreversible unimolecular, first order reactions represented as;

$$A \rightarrow \text{products}$$

In a constant volume batch reactor system the measure of a reaction rate of the disappearance of component A becomes;

$$-r_A = \frac{1}{V}\frac{dN_A}{dt} = \frac{-d(N_A/V)}{dt} = \frac{-dC_A}{dt} = f(k,C_A) \qquad (2.9)$$

Assuming that k is independent of concentration and integrating gives;

$$-\ln C_A / C_{AO} = kt \qquad (2.10)$$

A linear plot of - ln C_A / C_{AO} versus time will confirm the usual classical pattern of exponential kinetics of a first order reaction whilst non linearity would suggest more complex reactions (Levenspiel 1972;Song and Bartha 1990). The uptake of a low solubility substrate is more complex than water soluble substrates as the bacteria are located in the water soluble portion of the matrix and the degradative agents require adaptive mechanisms for uptake of the substrate which is in the non -aqueous portion of the matrix. In soil, rates of oil degradation are rarely always first order but rather is both first order and zero order due to; diffusion and solubility limitations; increasing population growth; different rates of utilisation of the various hydrocarbon groups, as reaction proceeds, recalcitrant ones remain (Higgins and Gilbert 1977). This is also applicable to biological reactor treatment of oily sludges. Mass transfer rates would however, be expected to be higher in a stirred tank reactor based treatment system compared to degradation beds soil treatment systems as under slurry reactor conditions, soil desorption and solubilisation is maximized (Rogers *et al.* 1993).

Various researchers studying the rates of reactions for the degradation of low solubility substrates, have coupled other kinetic expressions into these basic rate models to better simulate some of the rates observed in their laboratory and field trial experiments. Volkering, and his co-workers (1992) took into consideration the rate of mass transfer, in this case from a solid phase to an aqueous phase. They coupled a model based on [2]dissolution kinetics for substrate availability and Monod kinetics for bacterial growth. In studying the [3]dissolution rate effect on growth rate they used solid crystals of PAHs (naphthalene, anthracene and phenanthrene) in shake flask cultures. Each PAH has a different [4]solubility (related to K_{ow} the octanol–water partition coefficient) and dissolution rate and the maximal specific growth rate attained in the shake flask cultures reflected this inherent [5]solubility and dissolution rate. They observed that only at low cell densities did growth show exponential kinetics; where growth was not limited by the dissolution rate of the solid PAH (i.e. there was excess PAH in solution). At high cell densities, the uptake rate of substrate by biomass exceeded the dissolution rate of the substrate and biomass formation rate then became limited. Maximum mass transfer rate of the substrate to the bacteria is therefore limited by the dissolution rate of the hydrocarbon. To confirm their initial findings Volkering *et al.* (1992) included an experiment based on particle size (shown to have an direct relationship with dissolution rate) of the solid PAH and then included a size related factor into the rate model and a relationship with particle size (surface area), dissolution rate and growth rates was established.

$$\frac{dX_t}{dt} = \frac{-Y.(dQ_t)}{V\ (dt)_{max}} = \frac{Y.K_l.A.C_{eq}}{V} \qquad (2.11)$$

where:

X_t = the biomass concentration, kg/m^3

Y = the yield constant, kg biomass formed/kg substrate used

Q_t = the amount of solid or adsorbed substrate

C_{eq} = the equilibrium concentration of substrate in solution, kg/m3

K_l = a mass transfer constant , m/h

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[2] Dissolution is defined as the *changing* of a substance from a solid non soluble or sorbed portion to an aqueous soluble portion.

[3] Dissolution rate is defined as the *rate of the change* ie the rate the substance goes into solution or is desorbed into solution

[4] Solubility is a specific value that is inherent in the substance and determines the limit the substance will go into solution Eg . solubility of phenanthrene is 7.2 x10^{-6} (log Kow =4.57) and naphthalene is 2.5x 10^{-4} (log Kow = 3.36) (Pearlman *et al.* 1984). It determines the *extent* the substance will go into solution.

A = the contact surface, m^2

t = time, h

V = volume, m^3

K_l is the rate constant of mass transfer from the solid phase to the aqueous phase and is described as

$$-\frac{dQ_t}{dt} = K_l \,.\, A.\, (C_{eq} - C_t) \qquad (2.12)$$

where C_t is the concentration of substrate in solution.

Their proposed model in equation 2.11 describes a linear increase with time of the biomass concentrations when the biomass formation rate is limited by the mass transfer rate. Surface and yield are considered constant.

When the mass transfer rate (-dQ t /dt) is equal to the dissolution rate (J), the equilibrium concentration, C_{eq} can be replaced by the saturation concentration C_{max}. The equation for linear growth then becomes:

$$\frac{dX_t}{dt} = \frac{Y \,.\, J_{max}}{V} = Y.\, \frac{K_l \,.\, A \,.\, C_{max}}{V} \qquad (2.13)$$

[6*].

This model has also been proposed by them to be representative of PAH sorbed onto soils and surfaces therefore requiring 'dissolution' (desorption) from their solid attachments before degradation. Desorption limits, similar to solubility limits, determine the extent the sorbed contaminant may enter the aqueous phase and be

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[6*] Volkering and co-workers completed the model describing batch growth on slightly soluble substrate using the Monod equation to yield the relationship of μ_t and C_t:

$$\mu_t = \mu_{max} \cdot \frac{C_t}{C_t + K_s}$$

where;

μ_t is the actual specific growth rate, h

μ_{max} is the maximal growth rate, h

K_s is the saturation constant

C_t is calculated by integration of equation 2.12

microbiologically available for degradation (Rogers *et al.* 1993).

Stucki and Alexander (1987) also found growth rates on the PAH, phenanthrene, to be exponential only up to cell concentrations of about 10^7 cells per ml. Afterward the rate of multiplication declined. They found that the bacteria *Flavobacterium* sp. *and Beijerninckia* sp., growing in 318 μM phenanthrene, displayed linear growth rates between 40 and 80 hours (correlation coefficients of 0.974 and 0.945 for *Flavobacterium* sp. and *Beijerninckia* sp respectively using regressional analysis) when cell counts were higher than 10^7 cells per ml. At lower initial concentrations of substrate, 84 μM phenanthrene, the exponential growth rate declined at densities of 4 X 10^6 cells per ml. Their findings also support the view that growth is limited by the rate of dissolution of the hydrocarbon.

Other rate models, similar to Volkering and co-workers, (1992) address desorption of PAH from the matrix, e.g. soil, sand. Dissolution experiments with solid crystals of PAH as carried out by Volkering *et al.* (1993), carried out to represent desorption of PAH *from solid matrixes, are simplified. This is in order to reduce some of the variables* involved. Desorption experiments using bound contaminants involve other variables, which can be attributed to differences in chemical properties of the sorbate, nature of *the sorbent, the mechanism of sorption,* the time allowed for sorbate-sorbent equilibration (Karickhoff 1980) and the properties of the degradative organism (Guerin and Boyd 1992). Again, some experiments reduce variability by using a pure PAH (sorbate) absorbed to a well characterized soil (sorbent) with identified microorganisms. Abiotic soil desorption tests are performed to compute desorption isotherms (soil/water partition coefficients for particular chemicals of interest). The extent to which chemicals *desorb off soils into an* aqueous phase have been found to be directly correlated with their susceptibility to biodegradation (Rogers *et al.* 1993). This is a widely held observation and the USEPA uses this as criteria to determine if bioremediation is an alternative for contaminated soil treatment (Rogers *et al.* 1993). The USEPA protocol was based on the foregoing premise that the sorbed substrate has to be desorbed and enter the aqueous phase before it can be utilised by the bacteria. However, this is not entirely true in all cases as was demonstrated by Guerin and Boyd (1992) where soil sorbed naphthalene was readily available to a *Pseudomonas putida* ATCC17484. From the results of their experiments, the authors have proposed that strain 17484 directly

mineralised surface-localised labile sorbed naphthalene. While this is an important mechanism for some specifically adapted bacteria, generally, for bacteria to readily pick up substrate it has to be in a soluble form. Mechanisms for the uptake of low solubility substrate by microorganisms is covered in greater detail in the following consequent section. The following reviews the rate model proposed by Guerin and Boyd (1992) which were fitted to experiments where well characterised soil and sorbed naphthalene were used to represent contaminated sediments.

Guerin and Boyd (1992) were developing a model that could determine if desorption was limiting bioavailability of substrate. For the experiments two different bacterial isolates were used and as the results showed, these two organisms demonstrated different dependences on sorption limiting degradation rates. The hypothesis that sorbed naphthalene was unavailable to degradative organisms was tested using a kinetic approach for assessment of whether, and to what extent, sorbed naphthalene was available to the established microbial degraders. Based on experimentally determined partition coefficients of the PAH in sediment slurry and sediment free solutions, the soluble portions of the PAH were quantified against total percent mineralised. Total percent mineralised at higher than that determined by the partition coefficient meant the organisms was enhancing the bioavailability of the sorbed PAH. Rates and extent of mineralization were estimated by non-linear regression analysis of experimentally derived data by using both a simple *[7]first-order model and a new model proposed by them, a three parameter, coupled degradation-desorption model. Guerin and Boyd (1992), in presenting their new model, utilised an alternative first-order model based on one which described the progress of an enzyme-catalyzed reaction over time following addition of a slowly binding inhibitor (Leatherbarrow 1990). The model;

$$p = v_2 t + [(v_1 - v_2)(1 - e^{-kt})] / k \qquad (2.13)$$

where:

p = the enzyme catalysed reaction rate

v_1 = the reaction rate prior to inhibitor binding

[7] According to Guerin and Boyd (1992), the first order equation used to estimate percentage mineralized is as follows

$$P = P_{max} (1 - e^{-kt}) \qquad \textit{(equation 2.1a)}$$

where;
P_{max} is the maximal percentage mineralized
k is the first order rate constant

v_2 = the new inhibited rate

k = the first order rate constant.

This model provided fit to biphasic curves in which an exponential phase is followed by a linear phase of reaction. However, this model based on inhibition kinetics, simulate an increasing inhibition as time progresses, hence the term $v_1 - v_2$. Desorption on the other hand increases the concentration for uptake as time progresses. Guerin and Boyd (1992) then modified the equation by reversing the sign of the rate term and it thus becomes:

$$P = v_2 t + [(v_1 + v_2)(1 - e^{-kt})]/k \qquad (2.14)$$

Here v_1 represents the initial reaction rate (percent minute $^{-1}$) and v_2 represents the mineralization rates resulting from desorption of bound PAH (naphthalene) and is related to the kinetics of naphthalene desorption. The linear-rate term $v_2 t$ describes the mineralization of desorbing naphthalene. The authors presented laboratory experimental data which validated the use of the suggested model in the simulation of the kinetics of sorbed substrate mineralization under [8]equilibrium and [9]non equilibrium conditions. When desorption rates are of the same order as biodegradation rates, nonequilibrium conditions prevail and the use of the classical first order rates result in very poor RSS (Residual sum of squares). The authors found that their simple 3 parameter model provided excellent fits to experimental data in systems not at equilibrium and this model degenerated to the simple first-order model as the systems approximated equilibrium conditions. The RSS in the non-equilibrium conditions dropped from a range of 7.97 - 85.239 using the equation 2.1a (classic first order rate equation) to 1.753 - 10.833 using equation 2.14. Thus experimental data can be fitted to this model or to classical first order models to determine if equilibrium or non equilibrium conditions prevail which would also indicate if the sorbed PAH was biologically available or not.

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t is time .

[8] equilibrium conditions prevail when : i) instantaneous sorption and/or desorption
ii) soil /sorbent free
pseudo-equilibrium conditions are when desorption rates are much slower than degradation rates

[9] non equilibrium conditions prevail when desorption ratese of the same order as biodegradation rates

The fundamental problem of how quickly biodegradable pollutants are metabolized in a solid-water-oil interphase requires that adsorption-desorption, metabolic reaction processes and biofilm interactions etc. are quantitatively assessed. To design and optimize bioreactor treatment systems, verifiable mathematical models are needed to describe the physical and chemical process controlling the availability of a chemical and its resulting biodegradation. There are a lot of other mathematical models presented in literature with different parameterisation and complex equations used to define for example, radial diffusion models in biofilm analysis (Zhang *et al.* 1995). These complex models address in more detail the 'microcosm' process of solid-water-oil-bacteria-surfactant interaction and together with the more simple parameterisation models contribute to greater understanding of the oil degradation process.

Presented here were some simple kinetic models that can be used to fit the experimental data generated by this research and future recommended work and it can be used to show improvements in mass transfer rates from the different reactor design configuration and experimental conditions. While this research dealt with a particularly non homogenous substrate and mixed cultures and are therefore harder to fit into kinetic models, in particular, models that required specific coefficients such as solubility or partition coefficients, simple classical rate order equations are still applicable and information on the reactions that are occurring from within the reactor can be gained from it.

2.4.5 THE UPTAKE OF INSOLUBLE HYDROCARBONS BY MICROORGANISMS

Modelling of growth rates on different substrates and setting different physical, chemical and biological parameters in the biodegradation process (Volkering *et al.* 1993) can help to understand better the microbial rate limiting factors for the biodegradation of hydrocarbons and the conditions that affect it. The uptake of insoluble hydrocarbons by microorganisms have been proposed to occur through several different modes (Higgins and Gilbert 1977; Guerin and Boyd 1992; Bury and Miller 1993). There are two main theories: the first involves the uptake of hydrocarbons that are dissolved in the aqueous phase (free phase hydrocarbon) or "solubilized", "pseudosolubilized" and the second

involves the physical adhesion between the microorganism and oil droplet (oil droplet >> cell size) with the hydrocarbon being taken up directly. Volkering's work supports the requirement of dissolved or solubilized hydrocarbon to be present prior to uptake. However, Guerin and Boyd (1992), found that napthalene had different bioavailabilities to two different microorganisms and that solubilization alone could not account for the higher rates of uptake observed for *Pseudomonas putida* ATCC 17484. Their work suggests that some organisms have direct and immediate access to sorbed PAHs at the sorbent water interface possibly through physical attachment. There are two mechanisms that have been suggested involved in the direct uptake of insoluble substrate (Higgins and Gilbert 1977). One of the mechanisms suggested is the direct attachment of the oil to specific lipophillic regions of bacterial membrane and dissolution into the cytoplasmic membrane. The production of microbial surfactants have also been suggested to enhance the uptake of hydrocarbons. In a *Pseudomonad* sp. a rhamnolipid (microbial surfactant) together with a specific protein, is produced which decreases the hydrocarbon droplet size (Higgins and Gilbert 1977). The hydrocarbon becomes solubilised by a surfactant produced at the interface between the organism and the droplet. Thomas and his co-workers (1986) studied the rates of dissolution and biodegradation of water insoluble compounds. One of the compounds studied was octadecane and they found the mineralization of the compound was about 200 times faster than its normal dissolution rate. This means that the microorganisms were either acting on the insoluble compound or enhancing its dissolution. There are several other references in literature on the enhancement of mineralization rates of PAHs from surfactants, intrinsic surfactants (microbiological surfactants) and synthetic surfactants. The mechanism of surfactant enhancing the rates may possibly be both through a 'facilitated type' i.e. improving physical attachment or increasing the solubilities of the low solubility substrate.

On the account of the emerging importance of surfactants use in bioremediation of low solubility contaminants in soil and ground-water and its application in this research, the subject of biological and nonbiological surfactant mechanism of action and effect on biodegradation will be reviewed further in the next section.

CHAPTER 3
REVIEW OF SURFACTANTS

3.1 SURFACTANTS

Various researchers have shown evidence that surfactants can influence the uptake and consumption of insoluble substrates (Guerin and Jones 1988; Thiem 1994; Volkering *et al.* 1994; Oberbremer *et al.* 1990; Zhang and Miller 1995). Many microorganisms produce extracellular surface-active compounds in response to exposure of an insoluble substrate and these compounds have been suggested to be involved in the mechanism of enhancement of its uptake (Reddy *et al.* 1982; Bosch *et al.* 1988; Parra *et al.* 1989; Mattei and Bertrand 1985). However, the bioavailability of insoluble substrates solubilized by surfactants have remained controversial (Bury and Miller 1993; Laha and Luthy 1991).

Surfactants have also been used in cleaning up oil spills. Their use however have remained controversial due to either the evidence of toxicity of the surfactant or the toxic effect of the solubility enhanced HOCs on the flora and fauna. Furthermore, the surfactant itself may become a pollutant. Recently, surfactants have also been used in washing land contaminated with HOCs. The bioremediation strategy relies on the surfactant properties to desorb the HOC's partitioned in the soil and mobilizing it into the bulk aqueous phase (Scheiborgen 1994) which allows for its recovery and subsequent *in-situ* engineered treatment such as pump and treat systems. The interaction of different surfactants with soil, water, non-aqueous-phase layers (NAPL) and HOCs are complex and this is an important support area of research.

The word *surfactant* comes from *surface active agent* (Swisher 1987). Surface active agents tend to concentrate at surfaces and interfaces of an aqueous solution and alter the surface properties. Surfactants are amphiphilic molecules having two distinct structural moieties, one non-polar (generally one or more hydrophobic groups - alkyl,

aryl hydrocarbon or fluorocarbon, etc.) and the other polar (one or more hydrophilic groups - anionic, cationic or nonionic). The polar group has an affinity for water (hydrophilic) and other polar substances whereas the nonpolar moiety is hydrophobic (Edwards *et al.* 1991). In a polar medium, such as water, the non polar ends of surfactant molecules can associate with one another to form structured aggregates referred to as 'micelles', which will be discussed further in the next section. Surfactants can be man-made or occur naturally. Surfactants are classified according to the charge present or absent in the hydrophilic portion when it is dissolved in water. It can be classified into anionic, cationic, non-ionic and ampholytic types depending upon whether the solution is either acidic, basic or neutral (Zajic 1976).

Surfactants are commercially used in soaps and detergents as well as emulsifiers in products such as creams and lotions. The type of emulsion favoured, i.e. either 'oil in water' (O/W) or 'water in oil' (W/O) is indicated by its relative affinity for oil and water. The parameter used to characterize the emulsification behaviour of surfactants is the hydrophile/lipophile balance (HLB) value. HLB quantifies the relative dominance of the hydrophilic group over the lipophilic portion of a surfactant molecule. A surfactant with strong hydrophilic group relative to the hydrophobic part will have a high HLB value; that is, the HLB value of a surfactant increases with its hydrophilicity. Some studies have indicated the toxicity of surfactants to microorganisms is related to the surfactants lipophilicity i.e. due to cell adsorption (Thiem 1994). Surfactants are used to improve the solubilization or emulsification of hydrophobic substances, such as oil, into a medium, such as water, and solubilizing capacity of surfactants improves with increasing lipophilicity (low HLB value). Thus, with increasing lipophilicity, a surfactant exhibits better solubilizing properties but are also more toxic. Thiem (1994), in his research found that while all the surfactants tested improved the solubilization capacity of the insoluble substrate, the surfactants Marlipal 013/90 and Triton X-102 with HLB values of 13.3 and 14.6 respectively, inhibited the growth of the hydrocarbon degrader *Mycobacterium* sp. and inhibited the degradation of solubilized PAH by mixed cultures. The surfactants, Brij 35, Arkopal N-300 and Sapogenat T-300, all with HLB value of 17 on the other hand enabled the degradation of solubilized PAH to occur. In relation to surfactant's emulsification properties, surfactants with HLB values of 8-18 are classified as O/W emulsifiers and surfactants with lower HLB values are called W/O emulsifiers (Rosen 1989).

Further details and comprehensive descriptions of surfactant structural components, as well as a discussion on natural surfactants are given in :-

(i) Zajic & Panchal's, *Bio-Emulsifiers* (1976)

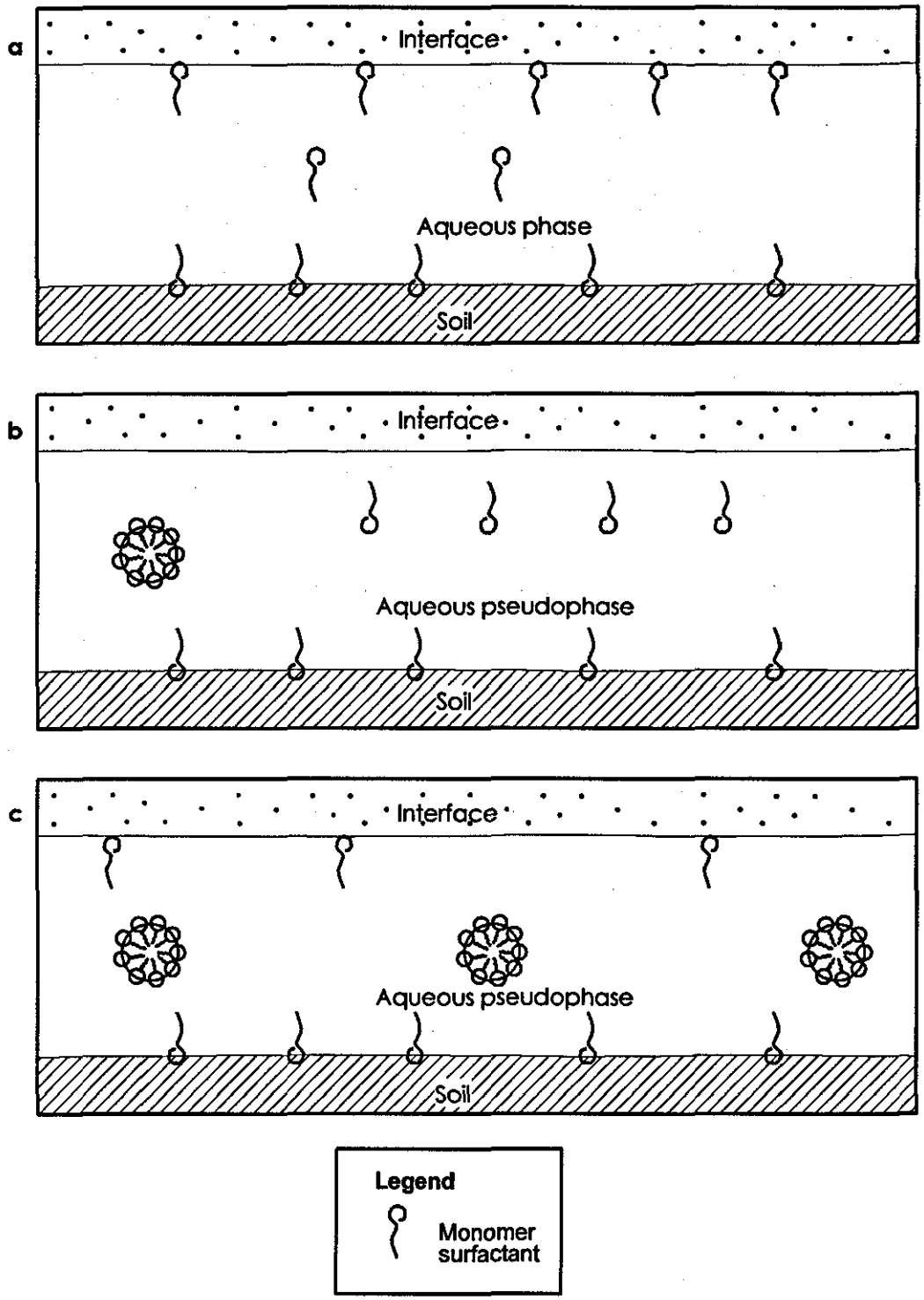
(ii) Swisher's , *Surfactant Biodegradation* (1987)

3.2 SURFACTANT MICELLES AND SOLUBILIZATION OF HOCS

Due to its amphiphilic nature, a surfactant molecule may be dissolved in water as monomers, adsorbed at interfaces of other hydrophobic/hydrophilic layers or exist incorporated with other surfactant molecules as part of a micelle (Figure 3.1). The existence of the surfactant either in monomeric state or micellar form is related to its bulk solution concentration. For each particular surfactant, at a specific bulk concentration greater than a specific threshold value, known as its critical micelle concentration (CMC), the hydrophobic groups of the excess surfactant molecules are attracted to each other in the bulk solution and form micelles. The number of surfactant molecules that form each micelle is referred to as the coordination number (Grimberg *et al.* 1995). The micelles may be spherical, spheroidal or rodlike clusters with hydrophobic interiors with characteristic sizes in the low nanometer range. Hydrophobic compounds, such as PAH, partition into the hydrophobic core of a micelle which can be collectively referred to as micellar pseudophase and the external phase consisting of water and non aggregated (monomer) surfactant at CMC, is the aqueous pseudophase (Edwards *et al.* 1994b). Beyond the point of CMC, the concentration of single molecules in the aqueous pseudophase remains relatively constant. Further increments in surfactant form micelles. The micelles are in equilibrium with the monomers and there is relatively free passage of surfactant molecules back and forth between the two states (Swisher 1987).

Measurement of CMC values for each surfactant is easily taken as at the CMC, an abrupt change in certain solution properties including the lowering of the liquid surface tension is observed. This abrupt change in surface tension is measured to determine the surfactant solution CMC. The conventional way of determining the CMC is through a plot of surface tension versus the logarithm of surfactant concentration. The CMC is found at the concentration where there is a pronounced break in the slope (Thangamani and Shreve 1993).

Figure 3.1 : Surfactant monomeric and micellar state at:
(a) below CMC
(b) at > CMC
(c) at >> CMC



Hydrophobic organic compounds (HOC) are solubilized in aqueous solution to a certain extent by the presence of surfactant micelles. The interior volume space that may be occupied by HOCs depends on the surfactant structure (Zhang and Miller 1995), aggregation number, micelle geometry, ionic strength and chemistry, temperature, solubilizate chemistry and solubilizate size (Edwards *et al.* 1991). Larger size surface groups, such as that from rhamnolipids containing the rhamnose sugar group and two alkyl groups on the rhamnolipid 1 and 3 isomer, form a less dense packing of surfactant monomers. This would provide for a large volume, low density micelle that is able to accommodate a greater amount of HOC (Thangamani and Shreve 1994). At sub-CMC levels there is relatively little HOC solubility enhancement whereas at greater than CMC, comparatively larger amounts of HOC can be solubilized within the micelles, thus enhancing the apparent 'solubility limits' (related to solubility coefficients) of HOC in water. For example the apparent solubility of DDT is increased by a factor of over 200 in the presence of 5×10^{-4} Molar Triton X-100 relative to its solubility limit in water alone (Edwards *et al.* 1994b). It has been found that there is a linear relationship with the HOC solubility and surfactant concentration above the CMC (Thangamani and Shreve, 1994). The slope of this increase defines the solubilising capacity (SC) or also known as molar solubilization ratio (MSR) when expressed in molar units (Edwards *et al.* 1991). The MSR gives a measure of the effectiveness of a particular surfactant in solubilizing a given HOC. An alternative approach in quantifying surfactant solubilization consists of characterizing the partitioning of the organic compound in micellar pseudophase (between micelles) and aqueous pseudophase (monomeric solution) with a mole fraction micelle-phase/aqueous phase partition coefficient. The micelle phase/aqueous phase partition coefficient, K_m , is the ratio of the mole fraction of the compuound in the micellar pseudophase, X_m , to the mole fraction of the compound in the aqueous pseudophase, X_a . Of particular significance is that the parameter of K_m has been found to be related to another hydrophobic-hydrophilic partition coefficient, the octanol-water partition coefficient, K_{ow} which has been shown to be related to the persistance of particular compounds in the environment (Edwards *et al.* 1991). The solubility enhancement effect of surfactants is exploited in the experimental work of Volkering (1995) and Thiem (1994) and others (Churchill *et al.* 1995a; Oberbremer *et al.* 1990; Edwards *et al.* 1991; Grimberg *et al.* 1995) to increase the mass transfer of PAH into aqueous solution.

This research looks at surfactants and whether their use may be beneficial in improving microbial degradation rates of oily sludges in stirred tank reactors. It has been reported by Marks *et al.* (1992), that biodegradation of benzo(a)pyrene (a recalcitrant PAH) can be dramatically enhanced in well mixed aerobic suspended growth systems by the use of surfactants. The surfactant, Triton X, increased the solubility of benzo(a)pyrene thus retaining the compound of interest in the liquid-solid phase (mixed liquor) where it was subjected to microbial degradation. Additionally, the surfactant limited the attachment of the hydrophobic contaminants into surface scum or reactor wall clingage, again maximizing benzo(a)pyrene content in the mixed liquor. Since this research centers on similar treatment in stirred tank reactors, one of the rate improvers tested was surfactants. This review on surfactants will therefore look at;

(i) the existing controversy on surfactants effects on degradation rates

(ii) the mechanism of action of surfactants on bacterial assimilation of HOC

(iii) interaction of surfactant at different concentrations with sorbents and

(iv) how, high and low HOC concentrations in surfactant solution affect bioavailability

3.3 EXISTING CONTROVERSY ON THE EFFECT SURFACTANTS HAVE ON DEGRADATION RATES

While the effects of surfactants on aqueous solutions containing HOCs in solid (Thiem 1994), sorbed (Volkering *et al.* 1992) or non aqueous phase layer (Thangamani and Shreve 1994) can be shown to increase the solubilization or desorption of HOC's; the effect of partitioning of the hydrocarbons into a micellar phase; the effect of surfactants on bacteria toxicity and attachment mechanisms; the partitioning of insoluble compounds into the different compartments of soil/solid, aqueous and micellar at different CMC's; seem to interact to provide contradictory results on overall surfactant effect on biodegradation. Contradictory results can be found in literature as to whether surfactants improve rates or extents of biodegradation (Bury and Miller 1993; Zhang and Miller 1992; Laha and Luthy 1991; Putcha and Domach 1993; Liu *et al.* 1995; Volkering *et al.* 1995; Grimberg *et al.* 1995). A paper by Liu *et al.* (1995) contains a summary of the conflicting results on the use of surfactants being beneficial, detrimental or ineffective to microbial substrate utilization rates and growth yields.

Volkering (1995) tested the influence of nonionic surfactants on bioavailability and biodegradation of PAH. The surfactants tested resulted in increased apparent solubilities as well as increased maximal rates of dissolution of crystalline PAH. This in turn increased linear growth rates of microbial cultures growing in the surfactant and PAH solution. Rates of degradation of naphthalene and phenanthrene in [1]*dissolution limited growth* phase were increased by the addition of surfactants. However, the increase in growth rate of the microbial cultures was less than the increase in maximal dissolution rate of the crystalline PAH. From his experiments, Volkering (1995) concluded that although rates were improved, substrate present in micellar phase is not readily available for degradation by the microorganisms .

Thiem (1994) investigated the use of several different non-ionic surfactants and sodium dodecyl sulfate (SDS) on the solubilization of PAH. His main focus was on improving biodegradation rates by the use of surfactants enhancing the solubility of PAH. At the same time, due to the contradictory results observed by the various researchers, he also looked at the toxicity of the surfactants possibly nulling the effect of the improved solubilities as the microorganisms became susceptible to the surfactants. Thiem also found all the surfactants tested increased the solubilization of PAH. PAH was solubilized by mixing solid PAH, in excess of its aqueous solubility in water, in surfactant containing mineral medium. The 'solubilized' PAH was separated from remaining PAH particles by filteration through 0.2 μm-pore-size polycarbonate membrane filters. Growth of mixed cultures on solubilized phenanthrene and fluoranthene were exponential, indicating the bioavailability of the solubilized PAH. He also found that some surfactants tested were toxic and only non toxic surfactants enhanced the degradation rate of PAH. It is noteworthy that Thiem (1994) found the surfactant Triton X-102, Triton being one of the frequently used surfactant by other researchers, inhibitory to growth of *Mycobacterium* sp. and to several PAH degrading mixed cultures.

Zhang and Miller (1992) tested the biosurfactant, rhamnolipid, on the solubilization of octadecane and its degradation. Dispersion of octadecane in aqueous solution with 300 mg rhamnolipid (7.5 x CMC) was found to be increased by a factor of more than 4 orders of magnitude, from 0.009 to > 250 mg/l. Biodegradation experiments showed

[1] *Dissolution limited growth* occurs when the rate of dissolution/desorption of the substrate is the limiting growth factor .

mineralization was also increased to 20 % in the presence of surfactants compared to 5 % in its absence. However, they also found that the fourfold increase in mineralization rates of octadecane that they observed were not as high as the $>10^4$ -fold increase in aqueous dispersion of octadecane. They, similarly to Volkering (1995), concluded that the octadecane in the micellar pseudophase was not freely available.

Another research that looked at the role of solubilization and biodegradation rates of hydrocarbons were experiments by Bury and Miller (1993). Biodegradation rates of straight chain hydrocarbons solubilized by surfactant showed exponential kinetics when surfactants were added compared to no surfactant added (linear rates). They designed their experiments to exclude degradation occurring by any other method other than micellar solubilized HOC. They needed to exclude degradation occurring at oil water interphase by direct attachment; i.e. on macro-sized oil droplets (at >>cell size) where there could be oil degradation occurring at the oil interphase with water. They therefore, did an experiment to get dissolution quantities for the HOC in surfactant solution and solubilized exact quantities producing only micellar solubilized HOC. Their conclusion was solubilization improved hydrocarbon degradation rates and micellar solubilized HOC was freely available for bacterial metabolism.

While some research have shown the exit rates of micellar solubilized HOC are high enough not to limit mineralization, this finding is not universally agreed upon (Putcha and Domach 1993; Liu *et al.* 1995). Surfactant effect on PAH biodegradation studied from fluorescence monitoring of micellar naphthalene by Putcha and Domach (1993), revealed an increased sequestering of naphthalene in the micellar phase through time which resulted in the inhibition of naphthalene biodegradation. Their finding suggested micelle maturation occurred over the time scale of days, achieving greater order through time, lowered the diffusibility of naphthalene within the micellar phase and thus diminished the rate at which PAH exited the micellar phase. Liu *et al.* (1995) on the other hand, quoted from another research, short residence times of 4 to 13 μs for naphthalene in micelles of sodium dodecyl sulfate with exit rates of 5×10^{-4} s, which suggests that micelle solubilized PAH should be bioavailable to PAH degrading microorganisms.

Grimberg *et al.* (1995) studied the kinetics of phenanthrene dissolution into water in the presence of non-ionic surfactants. They developed a mathematical model to describe the kinetics of the above process by quantifying the apparent aqueous saturation concentration of phenanthrene and the mass transfer coefficient observed in a completely mixed batch reactor as a function of surfactant concentration. The model accounted for the diffusional transport of dissolved phenanthrene and transport of micelle solubilized phenanthrene (exit and entry into the hydrophobic core of the micelles). For the surfactants investigated, there was an increase in phenanthrene dissolution rates in the presence of surfactant micelles, however, the observed mass transfer coefficient of phenanthrene dissolution into water with surfactants actually decreased as compared to water alone.

Laha and Luthy (1991) found the surfactants Brij 30, Tergitol and Triton X-100 at 1% v/v (>1000 x CMC) completely inhibited the mineralization of phenanthrene in soil-water systems. The concentration of surfactants used resulted in the presence of micellar aggregates of surfactant in the presence of soil. Lower concentrations, below micellar surfactant concentrations, of about 0.01 % were found to have either no effect or also inhibitory effects. Although they could not verify the specific inhibition mechanism on the microorganisms, they suggested the inhibition to be a micellar related phenomena.

Another later experiment by Liu *et al.* (1995) showed, mineralization of naphthalene in a mineral salts solution was only slightly inhibited in the presence of 0.15 % Brij (170 x CMC), not affected by 0.20% Triton X-100 (20 x CMC) slightly improved at 0.04% Triton X-100 (4 x CMC). The governing mechanism in the rates and extent of degradation seemed to be the initial concentration of naphthalene and the concentration of surfactant affecting the equilibrium partitionings between the three phases of micellar pseudophase, aqueous pseudophase and sorbed soil phase. From their studies they showed that the naphthalene mineralized from the micellar pseudophase accounted for the majority of the total amount of naphthalene mineralized. They concluded that neither the micelle surfactant nor the micelle-solubilized naphthalene affected naphthalene mineralization. This is in contrast to Laha and Luthy's (1992) finding earlier.

Efroymsom and Alexander (1991) showed Triton X-100 completely prevented the mineralization of hexadecane dissolved in heptamethyl-nonane possibly by preventing bacterial cells adherence to solvent water interface or due to cell membrane effects. Similarly, Churchill *et al.* (1995a) also noted inhibition of phenanthrene mineralization with the use of Triton X-45, Triton X-100 and Triton X-165 at 0.1% v/v with the organism *Pseudomonas putida*. This inhibition was not noted when the organism used was *Pseudomonas saccharophilla*.

The following section reviews possible mechanisms of action by which some of the above contradictions may be explained.

3.4 THE MECHANISM OF ACTION OF SURFACTANTS ON HOC AND BACTERIA

Surfactants effects on the bioavailability of organic compounds was explained by Volkering (1995) to be affected through three main mechanisms : (i) a reduction in the interfacial tension between the aqueous phase and the non-aqueous phase causing a dispersion of non-aqueous phase liquid hydrocarbon, such as in the process of emulsification, where contact area is effectively increased between microorganism and oil droplet (ii) the enhanced solubilization of hydrocarbons into micellar pseudosolubilized state (iii) "facilitated transport" of organic compound from solid or solid sorbed state (desorption), to aqueous non-aqueous interphase, solid and liquid interphase, liquid and non-aqueous phase interphase etc. through the lowering of surface tension of water or water in soil pore (soil columns) or interaction of monomer surfactant at interfaces, or surfactants at diffusion layers on solid hydrocarbons.

All of the above effects of surfactants may result in different adaptations of the microorganism in order to pick up; free phase hydrocarbon; emulsified hydrocarbon (oil droplets); micellized hydrocarbon (micro droplets <<<cell size) and hydrocarbon as a separate phase or dissolved in NAPL. Following is a brief review on the uptake of HOC by bacteria followed with mechanisms by which surfactants;

i. affect bacterial assimilation of HOC.

ii. interact with sorbents at different CMC

iii. affect high and low HOC concentrations.

3.4.1 HOC UPTAKE

There are two main theories involved in the microbial uptake mechanism of hydrocarbons. Without going into specific detail, the first mechanism involve the microorganism taking up free phase hydrocarbons (hydrocarbons dissolved in the aqueous phase) or solubilized hydrocarbons, while the second theory involves physical adhesion of the substrate to the organism. The assimilation of free phase hydrocarbon is easily explained as both the hydrocarbon and bacteria are in the solution in the same phase and diffusion of substrate into the cell can easily occur. Micellar hydrocarbon uptake may occur similarly as free phase as micellar phase hydrocarbon have been shown to display high exit rates to the aqueous pseudophase (Liu *et al.* 1995). The uptake of hydrocarbon by 'attachment', as that would occur when oil is emulsified (oil drops) as well as when it is sorbed in soil or as a solid or a separate oil layer, has also been shown to be one of the possible mechanisms of uptake. Some bacteria have been shown to be chemotactic to hydrocarbons (Guerin and Boyd 1992) and proteinaceous substances associated with the cell wall has also been thought to assist the attachment of possibly free oil or surfactant solubilized oil.

3.4.2 SURFACTANTS AND BACTERIAL ASSIMILATION OF HOC

Biosurfactants may be involved in both 'solubilized' uptake and 'attached mechanism' uptake as biosurfactants can be cell wall associated or secreted into the surrounding media. Solubilized uptake or physical adhesion uptake may involve microbial surfactants assisting solubilization or facilitated transport together with specific lippophilic regions of the bacterial cell (Higgins and Gilbert 1977; Reddy *et al.* 1982). Excreted surfactants act to emulsify water-insoluble substances or re-distribute cells between oil and water phase or mediate transport of hydrophobic substances into the cell.

Cell wall associated biosurfactant have the potential to promote cellular attachment to hydrophobic surfaces. This type of attachment mechanism may make the bacteria using these type of mechanisms more susceptible to surfactant effects. The surfactant may inhibit attachment as shown by Efroymsom and Alexander (1991) where surfactant inhibited the attachment of bacterial cells to the NAPL and aqueous layer interphase or it may affect the cell membrane structure such that the microorganism may no longer be effective in attaching. Degree of affinity or strength of attachment to the oil has been shown to be correlated to the lipophilicity of bacterial cell wall but, this has not been shown to be correlated to mineralization abilities (Baruah 1967). From their investigations, no relationship could be drawn to show affinity or strength of attachment and biodegradation. Zhang and Miller (1994), on the other hand, investigated the rate of biodegradation by cells classified as hydrophobic and hydrophilic cells in the presence of rhamnolipid and found the rates in both hydrophobic cells were inhibited and the rates in hydrophilic cells were enhanced. Zhang and Miller (1995) further investigated the effects of rhamnolipid structure on the biodegradation of hexadecane and octadecane. Their studies showed the importance of surfactant structure in determining hydrocarbon biodegradation rates and possibly one of the reasons for the discrepancies found in a fair amount of the literature since each research used different microorganisms and these organisms may each be producing microbial surfactants with differing physicochemical properties affecting biodegradation rates differently. In this same research, Zhang and Miller (1995) again found inhibition of octadecane degradation when rhamnolipids were added when a strongly hydrophobic *Pseudomonas* sp. was used to effect the biodegradation. This suggests the surfactant attachment to the strongly hydrophobic cell surface removed the cells capability for utilizing the hydrocarbons as substrate.

3.4.3 SURFACTANTS INTERACTION WITH SORBENTS AT DIFFERENT CMC

The following review on partition equilibrium models governing the mass exchange of hydrocarbons between the micellar pseudophase, aqueous pseudophase, sorbed phase covers only a physicochemical processes. The interaction of biological effects are not taken into consideration in these models. Although at the moment, no one author or groups of authors have adequately presented a model that can explain all the

different observations, the models presented by Edwards *et al.* (1994a & b), as well as Grimberg *et al.* 1996 and Volkering *et al.* (1995) gives the following insights that may explain some of the observations.

Edwards *et al.* (1994b) describes the partitioning equilibrium in two types of systems;

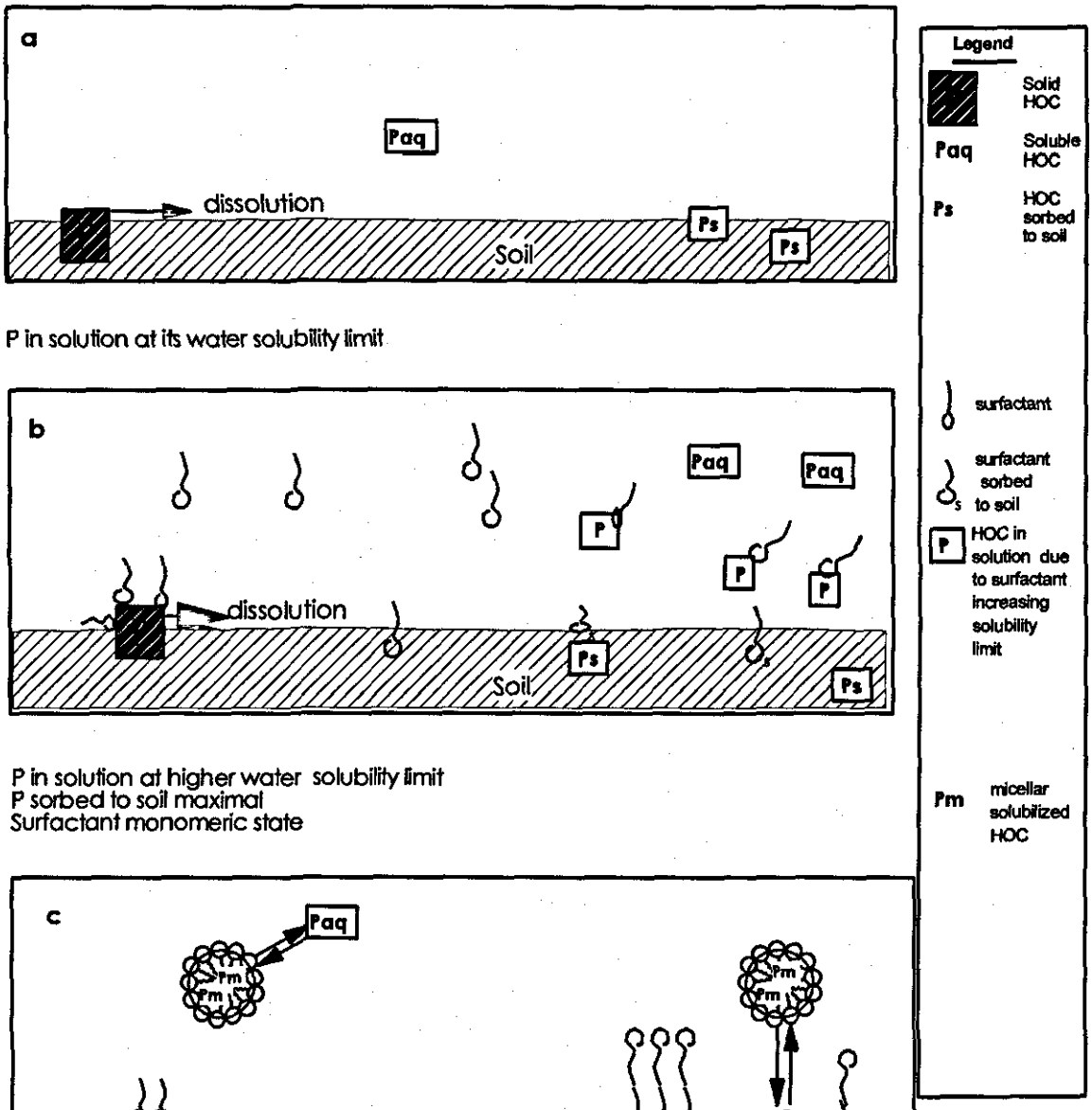
i) in a soil/aqueous system in the absence of surfactant

ii) ii) in a soil/aqueous system in the presence of surfactant.

According to Edwards *et al.* (1994b) in a system where there is only the hydrocarbon and an aqueous phase of soil and water, the HOC is dissolved in solution up to its water solubility limit and this is in equilibrium with the HOC molecules sorbed onto soil (Fig. 3.2a). By their definition; for the attainment of CMC, the surfactant monomer concentration is at a maximum when a surfactant is added just enough for CMC to be attained, beyond this point, the surfactant forms micelles. The presence of soil, alters the amount of surfactant required for the surfactant to form micelles in the aqueous phase as the surfactant initially becomes preferentially sorbed onto soil. In the presence of soil the surfactant becomes sorbed to soil first until it attains complete saturation of the sorbent sites before it reaches maximum monomeric state, then micellar state (Fig. 3.2b). Soil has different maximum sorption capacities for surfactants as this depends on factors such as organic content (humic matter) of the soil.

Bulk solution surfactant concentrations can act to either inhibit or enhance HOC desorption from soil to bulk solution (Edwards *et al.* 1994a). In the cases where there is sorbed HOC and the surfactant present is at much higher than CMC, the HOC may desorb into the micellar pseudophase. Sorption and desorption of HOC depends on a number of factors, such as, surfactant concentration and the nature of the solid sorbent. Edwards and his team (Edwards *et al.* 1994a) found with sandy soils of low organic carbon content, Triton X-100 surfactant concentrations at below CMC or low supra-CMC levels, lowered bulk aqueous concentrations of phenanthrene due to increase sorption of phenanthrene. At what they referred to as intermediate level above CMC, there is an increase in solubilization/desorption of phenanthrene. At much higher surfactant doses the bulk of the phenanthrene is either dissolved or solubilized in solution. A conceptualized model they presented is as follows.

Figure 3.2 : Partition equilibrium of HOC in soil aqueous systems in;
(a) absence of surfactant
(b) presence of surfactant at CMC
(c) presence of surfactant at >> CMC



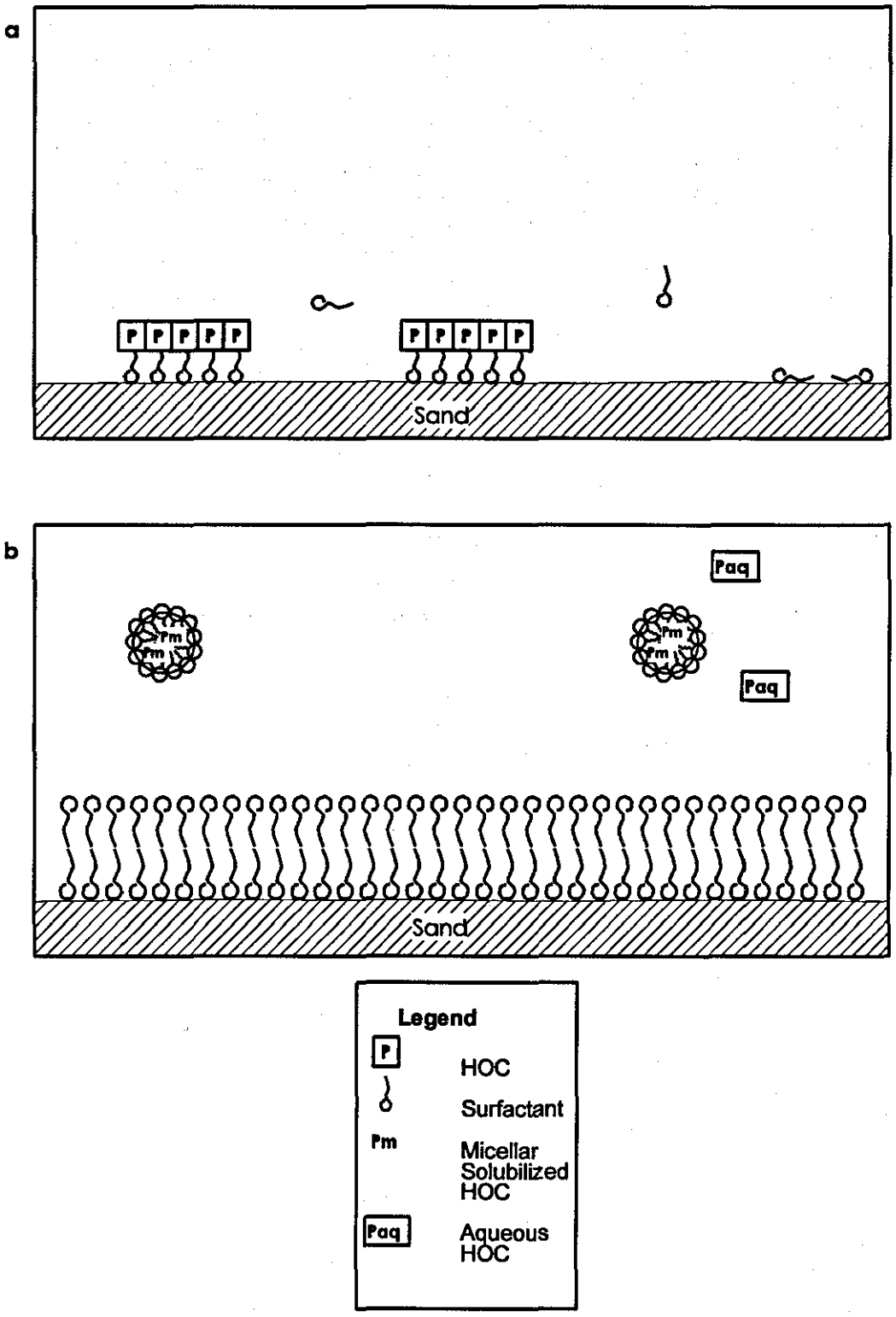
Silica or clean sand are hydrophilic surfaces that attract the hydrophilic groups of the surfactant. At sub CMC levels these surfactant molecules sorb nearly flat on sorbent (sand) surfaces due to the surfactant's different groups being attracted to the different sorbent surface properties. Then at higher bulk solution concentrations, the hydrophobic tails of the surfactant start sticking out into the aqueous pseudophase due to stronger mutual attraction of the adjacent surfactant molecules's hydrophobic groups. These surfactant's hydrophobic tails sticking outwards into the aqueous pseudophase attracts the HOCs and in effect the HOCs become sorbed onto the surface of the sand through the attachment of the surfactant (Figure 3.3a). Addition of surfactants continues this process until there is an almost continuous monolayer of perpendicularly oriented surfactants with their attached HOCs at the sorbent surface. Further additions, at supra CMC levels, the surfactant molecules start forming bilayers due to the mutual attraction of the hydrophobic groups of the surfactant. At this stage the attachment of HOCs (sorbed HOC) are inhibited and they are preferentially 'dissolved' in the micellar pseudophase (bulk solution) (Figure 3.3b). Edwards (1994a) presented a model with calculations for effectiveness at which the surfactant can act as a sorbent and gave a maximum effectiveness for the HOC sorption at the intermediate stage i.e. at CMC and slightly above where the conceptualized model of the surfactant is as a monolayer adsorbed onto the sorbent surface.

3.4.4 SURFACTANT AND LOW AND HIGH HOC CONCENTRATION

With limited amounts of HOC and at surfactant CMC or slightly above CMC surfactants acting as sorbents can result in lower aqueous phase HOC as it is preferentially sorbed. In cases of where there is 'excess' or a reservoir of HOC readily available as that would occur with the use of solid PAH or PAH dissolved in NAPL, or a separate HOC phase, then the aqueous pseudophase concentration of the HOC can remain maximized as even as it becomes depleted by biodegradation, it gets replenished from the excess reservoir of PAH due to the equilibrium partitioning. In the absence of the readily available reservoir of HOC, the aqueous concentration of HOC becomes gradually depleted through biodegradation and biodegradation rate slows down.

Liu *et al.* (1995) however, experimented with low PAH concentration and surfactant concentration of about 170 x CMC which due to the equilibrium process caused most of

Figure 3.3 : Sorption and desorption of HOC:
(a) sorption of HOC onto sand layer
(b) desorption of HOC due to surfactant's preferential bilayer formation



the PAH to occur as micellar solubilized. Aqueous PAH concentration was measured at well below its aqueous solubility limit. However, this did not slow the degradation process down as proposed by Edwards's *et al. (1994b)* model by which Liu *et al.* (1995) concluded micellar PAH was bioavailable to the mixed cultures of bacteria they used. In his experiment Liu *et al.* (1995), as Edwards *et al.* (1994b), determined that in aqueous systems without soil, with PAH concentrations at higher than the pure compound aqueous solubility limit and surfactants (Brij 30 and Triton X-100) at higher than CMC resulted in the micellar solubilization of the PAH. The degradation of aqueous PAH caused the micellar solubilized PAH to be released into the aqueous phase. The equilibrium partition kept shifting due to the degradation of naphthalene in the aqueous pseudophase. The degradation of naphthalene in the aqueous pseudophase caused the release of naphthalene from the micelle i.e. it replenished the aqueous phase naphthalene. However, in a system with no excess solid phase naphthalene, there was a decrease in the aqueous phase naphthalene. At supra CMC surfactant, the aqueous phase naphthalene concentration decreased to lower than pure naphthalene solubility that would have been observed in the absence of surfactant. This, however, did not affect the naphthalene mineralization significantly.

Liu's *et al.* (1995) experiment demonstrated the effect of having excess PAH on the equilibrium partitioning process. Liu *et al.* (1995) also included an experiment to determine if there was direct access to micellar solubilized PAH. From their experiment, by showing there was no inhibition in the degradation rates when most of PAH was micellar solubilized, they concluded that micellar solubilized PAH could be taken up directly by bacteria. What should be noted is that their experiment used mixed cultures and does not discount a surfactant-mediated-attachment type mechanism for biodegradation and therefore is not purely a physicochemical explanation for enhancement or inhibition of PAH degradation but includes biological influences of surfactant on biodegradation. The model proposed by Edwards *et al.* (1994b) is a pure physicochemical process of partitioning based on concentrations of PAH and surfactants.

Controversy seems to center upon the accessibility of the HOC when it is micellar solubilised. Further work by Volkering *et al.* (1995) and Grimberg *et al.* (1996) provide a little bit more insight, although their reports seem to contradict. Grimberg *et al.* (1996) in quantifying the biodegradation of phenanthrene in the presence of surfactants used a

specific bacterial species that was previously confirmed as to only utilize soluble (aqueous) phenanthrene. His model is similar to that as proposed by Edwards *et al.* (1994a) in that when there is no excess or pool of PAH available and surfactants are present in micellar form results in low aqueous phase PAH and this causes a decrease in the biodegradation rates as micellar PAH was biounavailable. However, rates of biodegradation are limited by the dissolution rate of PAH into solution in the absence surfactant. Grimberg *et al.* (1996) proposes that surfactants not only increases aqueous solubility limits of PAH in water, it also increases the rate of dissolution when there is an excess of PAH, either solid PAH, sorbed or micellar. This can be similarly related to the 'desorbing' that was noted by Edwards *et al.* (1994b) at >> higher surfactant concentration and the replenishing process observed by Liu *et al.* (1995) at >> surfactant concentration and excess PAH. Volkering *et al.* (1995) attributed the rates of degradation he observed, which were higher than that would be occurring if only increased solubilities were the only effect of surfactant addition, to an increase in maximal rates of dissolution of solid PAH. This he termed as facilitated transport of the PAH. Volkering *et al.* (1994b) also concluded that micellar PAH served as a protected reservoir that replenishes the aqueous phase PAH when it was depleted by biodegradation. Grimberg *et al.* (1996) support the finding of Volkering *et al.* (1994b) that micellar solubilized PAH was not available for biodegradation and that in the absence of surfactant, the mass transfer rate of the PAH may not be high enough to replenish the degraded PAH from the aqueous phase (Grimberg *et al.* 1996) thus rates become dissolution limited. They both place more emphasis on increase in dissolution rates to be an important mechanism that increases the biodegradation rates of PAH. This dissolution as proposed by Volkering *et al.* (1994b) is a facilitated transport (mediated by surfactants) process whereas Grimberg *et al.* (1996) proposes a model based on equilibrium partitionings between the aqueous phase and micelles .

This recent work by Luthy's group (Edwards *et al.* 1994a; Edwards *et al.* 1994b; Liu *et al.* 1995) with the conceptualized model explains some of their earlier observations (Laha and Luthy 1991; Laha and Luthy 1992) of inhibition of mineralization rates with the use of surfactants. While the model described by Edwards (1994a & b) and further research by Volkering *et al.* (1995) and Grimberg *et al.* (1996) provide some explanation accounting for the inhibition versus enhancement effect of surfactants based on variabilities in hydrocarbon concentration, surfactant concentrations and sorbent effects, this still does not explain the other observations such as; different toxicity of the same

surfactant; different bioavailabilities of HOCs; as outlined earlier in section 3.3. These effects can be caused by the different mechanisms of uptake of hydrocarbons by the microorganism.

Researchers have used synthetic surfactants as well as microbially derived preparations to gain further insight into the mechanisms of microbial degradation of hydrocarbons in the presence of surfactants. What is becoming clearer at this stage is that this is a complex process and generalizations about it are inappropriate. Properties of the surfactant (CMC, molar solubilization ratio, HLB value) (Thiem 1994), microbial or synthetic (Thangamani and Shreve 1994), its quantities used at sub CMC, CMC and supra CMC (Laha and Luthy 1991; Liu *et al.* 1995); micellar structure and its differences under different conditions such as pH (Zhang and Miller 1992; Zhang and Miller 1994), the type of HOC (Zhang and Miller 1995), the bacteria and its properties such as degree of hydrophobicity (Zhang and Miller 1995), presence of specific proteins on cell surface as well as other influences such as the presence of solids and type of solids such as quantities of organic fractions present (Edwards *et al.* 1994a), all affect the uptake process of HOC by microorganisms. The overall interaction of these various mirobial, surfactant, substrate and sorbent effects will determine the biodegradation, rate and extent, of HOCs. As much as a lot of the results on the overall effect of surfactants on biodegradation rates seem to differ in their findings all the experiments have contributed towards progress in terms of understanding the microbial process for the uptake of HOCs as well as surfactant interaction with HOCs, microorganisms and sorbent. However, research in this area should continue vigorously as it will be needed in order to try and exploit the potential of surfactants in assisting the biodegradation of insoluble and recalcitrant substrates frequently found polluting the environment either in soil or subsurfaces and aquifers as well as improving waste treatment processes for recalcitrants.

CHAPTER 4
RESEARCH OBJECTIVES AND SCOPE OF WORK

4.1 AIM OF RESEARCH

The aim of this research was to determine if oily sludges from refineries could be efficiently biodegraded in specialized aerobic reactors and to determine the design limits achievable.

A reactor based treatment was chosen for several reasons;

- it has been determined from the literature that it was likely to be a technically feasible process
- it was also determined as 'environmentally safe' as it can be a closed treatment unit with all the emissions of VOC and leachates controlled
- enclosed reactor treatment means better process control
- the limitations of mass transfer would be reduced as compared to other biological methods such as landfarming and composting

More specific objectives were the treatment limits of the 16 Polynuclear Aromatic Hydrocarbons (PAH) to EPA limits specified by the method of incineration, currently determined as the Best Demonstrated Available Technology (BDAT) for oily sludge in the USA.

Changes in reactor design and the possible benefits of surfactant addition were also studied to try and improve reaction rates and degradation extent for the sludge treatment. Very little information was available in the literature on the influence of reactor design on rates of degradation/transformation therefore, a novel airlift reactor design was compared to a conventional continuously stirred batch operated reactor. The fundamental objective was to investigate the possibility of improving the mass transfer rates through reactor design.

Experiments with surfactants added to desorb oil off from particulate matter has had

contradictory results (see Chapter 3). While some research have shown that surfactants can improve the dissolution of PAH into the aqueous phase and therefore make it 'available' for bacteria to degrade, other research have shown that 'micellar solubilized' PAH is not available when it is sequestered within the micelle structure. Some research has also shown, surfactants prevented attachment of the bacteria to the hydrocarbon and other research has shown the reverse i.e. helping in the attachment. Further work might help to resolve this controversy.

4.2 SCOPE OF WORK

At the start of this research, methods for analyzing the parameters of interest were investigated. Available standard methods would be the first choice; however, these methods had to be applicable and practical to the research requirements. After surveying the literature on the current laboratory practices for similar research, the standard methods were tested then developed to improve the specificity of the standard technique to the particular academic objectives of this research. Following, are some of the analytical methods developed.

Experiment A
Oil and Grease determination for mixed liquor samples and sludge samples
Part of Experiment I
Oil and grease determination in the presence of surfactant
Experiment B
Oil degrader plate count
Experiment C
HPLC determinations for the 16 PAH

Experiment A was undertaken due to solvent extractability differences and standard methods surveyed specified different solvents with different lengths of extraction, Experiment I further adapted O&G determination because surfactants interfered with the method. Experiment B was developed as research progressed as there was dissatisfaction with the methods found in literature. The final method is an adaptation from several different research literatures. The HPLC method, Experiment C had to be developed owing to the differences in column matrix used for separation, sample types and sample matrix interference's. The published methods would need to be investigated

and adapted to improve the separation of the sample PAHs for the best chromatograms.

Prior to the bench scale reactor runs, a series of experiments were planned to determine the critical experimental conditions to be used during the bench scale reactor studies. These experiments were carried out under idealized conditions in a well-controlled environment and were used to determine:

Experiment D

A source for the microbial inocula to be used and the acclimatization conditions necessary to promote initially high biomass concentrations

Experiment E

Significance of primed and unprimed microbial cultures and their growth rates on hydrocarbon

Experiment F

Additional nutrient requirements of the microbial cultures

Experiment G

Rates of degradation in relation to sludge loading

Experiment H

Abiotic control for oil loss at the selected sludge loading

Experiment I

Selection of surfactant

In order to determine the mixing conditions in the bench scale reactors, tracer studies were also carried out using lithium bromide for several different sets of stirrer mixing speed, air flow rates and solids loadings. Several test runs of the novel airlift reactor design were also carried out using oily sludge to determine true mixing conditions. The airlift prototype was slightly modified after each test run to improve the design.

After all the determinations of the above, the bench scale continuously stirred batch reactors were run for approximately sixty days. The Basic Batch Stirred Tank Reactor (Basic BSTR) was run till degradation of oil was no longer apparent which was at about sixty days and this was used as the end point for the other reactors to compare extent of degradation for the same time period. Bench scale reactor experiments consisted of three runs;

Experiment J

Basic 'Batch Stirred Tank Reactor' (Basic BSTR)

Experiment K

Airlift 'Batch Stirred Tank Reactor' (Airlift BSTR)

Experiment L

Basic 'Batch Stirred Tank Reactor' with surfactant (surfactant amended BSTR)

The results were then used to compare reactor design and surfactant additions as a means to improve reaction rates and extent of degradation for the treatment of oily sludges.

CHAPTER 5
EXPERIMENTAL SET- UP AND ANALYTICAL METHODS

5.1 INTRODUCTION

In this following section, the analytical details on the scope of work performed in this project are presented. The section is divided into the three subsections; analytical techniques, preliminary shake flask experiments and reactor runs detailed in Chapter 4.

The literature review in Chapter 2 indicated some of the literature which were reviewed to determine the operating environmental conditions. The operating conditions selected for this research and their supporting literature are set forth in this chapter.

Unless otherwise stated, all broth media used in the experiments consisted of a minimal basal salts media supplemented with 0.05 percent (wt./vol.) yeast extract for essential vitamins and [1]topped crude oil at one percent (vol./vol.) for a source of carbon. Plate count methods used either solubilized PAH crystals or crude oil as a sole source of carbon, utilised the same minimal basal salt media with fifteen percent purified agar but without the yeast extract. In order to characterise 'oil' only degraders, the omission of yeast and the use of purified agar were necessary as the contaminants on non-purified agar and the contents in the yeast extract allowed growth. This was tested in the laboratory and has also been referred to by Eismann *et al.* (1991) in that non-purified agar gave false positives. The minimal basal salt solution used throughout the studies was formulated by Bushnell and Haas (1941), hereafter referred to as BBH media (recipe in *Appendix E*, p. 261), and this media, with various sources of carbon supplements, is widely used by researchers for cultivation of oil degrading cultures (Deziel *et al.* 1996; Huesseman *et al.* 1993; Berwick and Stafford 1988; Prince and Sambasivam 1993; Sexstone and Atlas 1977; Atlas and Bartha 1972; Cooper and Hedrick 1976).

[1] topped crude is crude oil with the lighter fractions allowed to evaporate. In all the experiments where crude oil is used as a source of carbon, the crude oil was first autoclaved in a loose cover bottle and the lighter fractions were allowed to evaporate. The crude oil used was from the North Sea.

After sterilisation by autoclaving, the BBH media was adjusted to pH 7, using a Mettler Delta 340 pH meter, with the use of either sodium hydroxide or sulphuric acid. Supplements of nitrogen and phosphorus, from ammonium nitrate and phosphoric acid respectively, were added at the start of all the biodegradation experiments at C:N of 150:1 and C:P of 750 :1 by weight, where the carbon loading from the sludge is taken as C. Previous research on landfarming had already determined that the large amount of hydrocarbon substrate present at the start created a nutritional imbalance which retarded rates of degradation if left uncorrected (CONCAWE 1980; API 1980). Thus, N and P additions to landfarming operations were considered a necessity. There was some variation in the C:N and C:P ratios found in literature for landfarm studies. The American Petroleum Institute (1980) for example, recommends N-fertiliser ratios of 160:1 for C:N. Bartha and Bossert (1984), in their review of previous experiments on landfarming have listed ratios ranging from a C:N of 15:1, 200:1 and also 300:1 as being used by other researchers. In a previous research, Dibble and Bartha (1979) recommended the ratios of C:N of 60:1 and C:P of 800:1. Rogers *et al.* (1993) recommend as a rule of thumb, a ratio of C:N:P of 120:10:1 on a weight basis, based on the oxidation - reduction reactions method of Mc Carthy (1972), which result in larger amounts of N and P additions. The different ratios recommended for C:N additions in landfarming activities are probably the result of the complexity and variability present in soil (Leahy and Colwell 1990).

In reactor studies, where minimal basal salt media has been used to resuspend sludge, N and P are also an important supplement. N and P are usually part of the formulation of the minimal basal salt media in the form of ammonium nitrate and di- or mono-potassium phosphate respectively. However, it has been determined that at high sludge contents, nitrates and phosphates became quickly depleted from the standard BBH media (Prince and Sambasivam 1993). Another research by Stroo *et al.* (1989) determined a C:N ratio between 120 and 240:1. They used calculation based on respiration monitoring in a liquid-solid contact reactor using varying solids loadings. Using the lower value would result in cost savings on the reactor operation. For this research, the ratio C:N of 150:1, which is approximately similar to the range recommended by API on landfarming and within the range recommended by Stroo *et al.* (1989) was used for the addition of N. P was added at ratio 750:1 which is C:P close to the API recommendation. These nutrient supplements were added on top of the N and P supplied by the BBH media (0.35 g N/l BBH and 0.41g P/l BBH). For all the

experiments in this study, the carbon content from sludge is calculated as being eighty-ninety percent of the oil and grease (O&G) content from the sludge (Bailey *et al.* 1973; Bartha and Bossert, 1984). An example of the calculation for the nitrate and phosphate supplementation based on sludge loading is covered in section 5.1.4.

Inoculum source

Oily sludge used for all the experiments came from a sludge pit from an oil refinery refining crude oil from the North Sea. At the time of collection, the sludge was very wet due to a recent rainfall. When it was transported back to the laboratory, water was allowed to separate and this was decanted. The sludge was then allowed to air dry for a few weeks. The consistency of the sludge at the end of this period can be described as a viscous black semi-solid material. There were chunks of grease, also intermixed with the sludge which probably came from machine maintenance operations. To eliminate sludge characteristics as a variable, the sludge was thoroughly mixed by hand and passed through a 425μ standard sieve to eliminate larger particles. This was stored in a sealed container in the cold room at $4\ ^{0}\ C \pm 1\ ^{0}$ until used. The sieved oily sludge was initially characterised according to its physical and chemical properties using standard laboratory methods for wastewater and sludges (SW 846). Sieved sludge characteristics are detailed in *Appendix* F, p. 262. Characterisation tests by standard methods using the methods detailed below, included dry solids fraction (dsf), O&G content, total solids content, asphaltene content, heavy metals and the PAHs by HPLC.

5.2 ANALYTICAL TECHNIQUES

As mentioned in the literature review, standard methods are largely available for most of the analysis carried out. As such, these methods are only briefly discussed with the changes made to the standard procedures pointed out. The Table 5.1 lists the analysis and the standard methods employed or the literature used for reference for the analytical method.

Table 5.1: Standard Methods employed in this research

Sect.	Analytical techniques for mixed liquor and sludge	
5.2.1	TS, TVS,TFS	APHA 2540 B, E
	TSS, TVSS, TFSS,	APHA 2540 ,D, E
5.2.2	pH	Black C.A. Methods of Soil Analysis 61-3 Alexander, M. Introduction to Soil Microbiology
5.2.3	O&G (mixed liquor)	Developed from APHA 5520 B
	O&G (Sludge)	Developed from APHA 5520 E. SW 846 Method 3540
5.2.4	Heterotrophic Plate count	APHA 907A (pour plate with Plate Count Agar)
5.2.5	Oil Degrader Plate Count	Sexstone and Atlas, 1977, Bartha and Atlas 1972
5.2.6	Naphthalene Plate Count	Developed from Ogunseitan *et al.* 1991, Bogardt and Hemminsen, 1992
5.2.7	MPN	Huesseman *et al.* 1993
5.2.8	Dissolved Oxygen Uptake Rate	Using YSI 5739 dissolved oxygen probe
5.2.9	Nitrate	Hach Method 358
5.2.10	Orthophosphate	Hach Method 492
5.2.11	Total Organic Carbon	Using a Rosemount Dohrman DC/90 Carbon analyser
5.2.12	PAH	SW 846 Method 8100, Smith *et al.* ;1996

5.2.1 SOLIDS ANALYSIS

The APHA 2540 method was used for all the determinations using five or ten ml of sample. TS was determined by drying a well mixed sample in a weighed dish and drying it, first, on a water bath, then to constant weight in an oven at 103 to 105 ^{o}C. The residue, cooled in a dessicator, was the TS. TFS was determined by igniting the TS residue to constant weight at 550 ± 50 ^{o}C. The temperature of the furnace was raised slowly to ensure no combustion losses by explosion. The remaining residue was the fixed solids while the weight loss on ignition was the TVS. TSS was determined by filtering a well mixed sample through a glass-fibre filter disk and drying the filter in an

oven at 103 to 105 °C to constant weight. The increase in filter weight was the TSS. The TFSS was determined by igniting the same filter to 550 ± 50 °C. The remaining residue was the TFSS while the weight loss on ignition was the TVSS. Soluble solids were determined by subtracting the TSS from TS. Almost all solids analysis were carried out in triplicates. The standard deviation for the triplicates (samples TS of range 0.08 – 0.7 g) were in the range of 0.0001 g to 0.009 g for the 10 ml samples (see *Appendix J*, p. 285, 288, 290, 292; *Appendix H*, p. 294; *Appendix L*, p. 304; *Appendix M*, p. 308; *Appendix N*, p. 312).

5.2.2 pH

pH for sludge samples were determined by mixing approximately ten gm of sample with twenty-five ml of distilled water and allowing the mixture to stand for thirty min. This was then stirred and the pH measured with a glass electrode meter. The Mettler Delta 340 meter was calibrated prior to the measurement using the pH 4.0 and pH 7.0 buffer. Mixed liquor samples were measured directly with the glass electrode meter. For monitoring pH changes daily, pH paper (pH range 0 to 7 and 7 to 14) was used as a rough estimate.

5.2.3 O&G

Presented below is the final procedure adopted for O&G analysis for mixed liquor and sludge. These methods were tested in the laboratory for recovery using crude oil or extracted O&G from sludge as the spike. The results of these tests, as well as other tests carried out in the process of developing the method below, are presented in Chapter 6.

O&G had to be determined for two different types of samples i.e. liquid-solid slurry from mixed liquor and a semi solid sludge from the original sludge or from the treated sludge. Mixed liquor O&G determinations were calculated on a per volume basis while for sludge, O&G was calculated on a dry weight basis of solids. The methods were developed with references to *APHA method 5520 B Partition - Gravimetric* for the mixed liquor samples and *APHA method 5520 E Extraction Method for Sludge Samples* and *SW 846 method 3540 for Soxhlet Extraction* for the sludge samples. In neither case

were the samples acidified in the process as the O&G samples were later used for PAH analysis.

5.2.3.1 MIXED LIQUOR O&G

Ten ml of sample were collected by either pouring out a well mixed sample into a volumetric cylinder or by using a volumetric syringe with a large bore needle to pull out ten ml from the container. The cylinder or syringe was then rinsed several times with distilled water and the rinse and sample were collected in a 100-ml Duran bottle. Twenty to twenty-five ml of dichloromethane (DCM) were then added to the bottle and this was extracted in the shaking incubator for twelve hours. This was then transferred to a separatory funnel and the Duran bottle was rinsed with fifteen ml of DCM and added to the separatory funnel. The layers were allowed to separate. If an emulsion formed, it was stirred gently with a glass rod to improve the separation. The solvent layer was then drained through a glass funnel containing phase separation filter paper. The collected solvent was distilled off in a rotary evaporator up to about five ml which was then pipetted into a tared dish. The distilling flask was rinsed a few times with small volumes of DCM that were added to the tared dish. The tared dish was then placed in a 40 ^{0}C oven in a fume cupboard to evaporate the last traces of solvent. The dish was cooled in a dessicator before weighing. The gain in weight of the tared dish is the O&G which is reported as g O&G per litre of mixed liquor. When this method was tested on crude oil from the North Sea, recovery of O&G from the crude oil was 66.71 % (δ = 0.03 %) of its weight and when [2]*pre-extracted* O&G from sludge was used, the recovery was 85.55 % (δ = 1.92 %) of its weight. DCM was chosen as the solvent as earlier tests for extraction of O&G from sludge using DCM and Trichlorotrifluoroethane (TCTFE) showed higher recoveries, DCM at 33.88 % (δ = 1.5 %) and TCTFE at 29.86 % (δ = 1.24 %) O&G per dry solids. DCM was also chosen as in the next step, the method for PAH determination from the O&G fraction uses DCM as the solvent and DCM also has lower boiling points and will allow for recovery of low boiling point volatiles. DCM however, will extract degradation intermediates and as such O & G determinations will include degradation products and therefore, degradation rates are likely to be under estimated and will show the most minimum possible.

[2] *Pre-extracted O&G* from sludge was obtained by solvent (DCM) extraction of the sludge sample

For samples of mixed liquor, the standard deviation using this method was mostly less than 10 %. Higher than 10 % (up to a maximum of 55%) standard deviations were apparent in the reactor runs, at the start when the slurry was less homogenous or when events such as clingage addition occurred (see *Appendix* L, p. 304; *Appendix* M, p. 308 and *Appendix* N, p. 312).

5.2.3.2 SLUDGE O&G

Five to ten gm of sludge samples were weighed into a small beaker for extraction. At the same time, as O&G for sludge is determined on a dry solids basis, the dry solids fraction (dsf) of the sample was determined as shown below.

Dry solids fraction determination

Three clean glass-evaporating crucibles were prepared by drying overnight in an oven at 103 -105 ^{0} C. The crucibles were then cooled in a dessicator till used. Well mixed sludge samples were then weighed, five to ten gm, in the crucibles and the weight noted to four decimal points. This is the wet weight (WW). These samples were then dried in the oven at 103 - 105 ^{0} C overnight and then cooled in the dessicator. The weight was then measured using an analytical balance accurate up to ±0.0005 gm. The drying and cooling were repeated till a constant weight was noted. This is the dry weight (DW). To get the dsf;

dsf = DW (g)/WW(g)

The wet weight of the samples taken for O&G analysis is multiplied by the dsf to report O&G per dry weight.

For the O&G analysis, the sludge sample in the beaker was blended together with fifteen gm of anhydrous sodium sulphate. The dried sample was then placed in an extraction thimble and plugged with glass wool. The extraction thimble was placed in a Soxhlet extractor. A 300 ml volume of DCM was then measured into a 500 ml round bottom flask together with one or two solvent rinsed boiling chips. The flask was then attached to the extractor and allowed to extract for eight hours. An experiment was carried out to determine the length of extraction and it was determined that there was no appreciable difference between extracting for four hours as recommended in APHA 5520 E or sixteen hours as in SW 846 method 3540. The extract was allowed to cool

before it was removed from the assembly and the solvent was evaporated in a rotary evaporator until about five ml. The O&G extract was then pipetted out into a tared crucible and the distilling flask was rinsed with small volumes of DCM which was then added into the tared crucible. The balance of solvent in the crucible was evaporated in a warm oven (40 ^{0} C) under a fume cupboard. The crucible was cooled in a dessicator before the gain in weight was determined for O&G. The results are reported as wt of O&G per wt dry solids. Soil samples spiked with crude oil yielded recoveries of an average of 65.03 % O&G from four samples (δ = 1.09 %) which were approximately equal to the recovery of a liquid-liquid extraction of water spiked with crude oil. Standard deviation for this method on the sludge from the reactor runs averaged 2.7 %. (triplicates of 4 sets).

For both these analyses, it was felt that it was not relevant to proceed with TPH or TOE determination (passing the extract through a silica column to remove fatty acids) after O&G determinations as the oils were from a known hydrocarbon source. Therefore it is assumed that all the O&G recovered is TPH.

5.2.4 HETEROTROPHIC PLATE COUNT

APHA method 907 A Pour Plate Method was used for this procedure.

Five or ten ml of mixed liquor sample were measured and mixed into, forty-five or ninety ml respectively, sterile phosphate buffered saline solution in a Duran bottle. The 10^{-1} dilution was then placed in a shaking incubator for five minutes. The dilution was allowed to settle for two minutes and one ml from the upper layer removed for the 10^{-2} dilution. Decimal dilutions up to 10^{-9} were then prepared by serially diluting one ml of the previous dilution in phosphate buffered saline to the next 10^{-1} dilution using sterile pippetes. After each dilution the sample was shaken by hand for one minute. One ml of the required dilutions were then pippeted into labelled sterile disposable petri plates before cooled molten plate count agar was dispensed into the plates. Triplicate analysis for each dilution was carried out together with control plates from the phosphate buffered saline dilution water that was used. The plates were incubated at an elevated temperature of 37 $^{\circ}$ C using a rich media to enable the plates to be read earlier. While ideally, incubation should be done at the same temperature as the preliminary experiments, results shown in Chapter 6 do indicate good correlation of bacterial counts

to oil degradation. The plates were read with a Gallenkamp colony counter after forty-eight hours using the protocols in the APHA method.

5.2.5 OIL DEGRADER PLATE COUNT

The oil degrader plate count method was developed as research progressed. The initially adopted methods from literature had drawbacks, therefore after a few adaptations, a naphthalene degrader plate count procedure was established. The progress was as follows.

The literature reference by Atlas and Bartha (1972), and Sexstone and Atlas (1977) were used in determining the first method. Oil plates were prepared by adding one percent crude oil as the test compound to heat sterilised BBH agar. BBH without added oil served as controls. Counts of oil utilizers were corrected by subtracting the control plate counts from the oil agar counts to give a final count for oil degraders.

Several trials were carried out to determine the best method to incorporate oil into the media and plating technique. Initially, oil agar plates were prepared by adding sterile topped crude oil to hot molten agar that was then vortexed and plated. The prepared plates were sealed in plastic bags and kept at 5 ^{0}C till used. Prior to use, the plates were brought to room temperature. The mixed liquor sample dilutions were then spread plated onto the agar and incubated at 37 ^{0}C for 2 weeks. This method was found to be prone to spreaders. The method was then adapted by using a pour plate technique, whereby the molten agar with the added oil was cooled first before pouring it into the plates with the pipetted dilutions. The medium and dilutions were mixed by rotating the dish first in one direction then in the opposite direction. All dilutions and plating were carried out under the laminar air flow cupboard, model Gelmann Science BH 48. After the medium solidified the plates were stored in plastic bags, inverted and incubated at 37 ^{0}C for two weeks. Although this method resulted in less spreaders, colonies were hard to count as the colonies were quite small and it had to be read against a dark background. Later, the use of purified agar in the method, deleted the need to prepare control plates as the counts on purified agar was zero. There was no longer a need to subtract the counts from the oil-free agar plates from the oil agar plates to determine total hydrocarbon-utilising bacterial counts. Instead, a control was prepared from the dilution water and oil agar plate to check for dilution water contamination. As in the

heterotrophic plate counts, triplicates of each dilution were plated and controls were plated using the dilution water for each set of results.

5.2.6 NAPHTHALENE DEGRADER PLATE COUNT

Based on a few literature references including, Shiaris and Cooney (1983), Ogunseitan *et al.* (1991), Bogardt and Hemmingsen (1992), Grifolli *et al.* (1995), another method of estimating oil degraders was determined with the use of specific hydrocarbon compounds. Since the use of crude oil resulted in the background being dark which made the plate hard to read, another method was sought to estimate oil degraders. The use of pure compounds of PAH as substrate was considered as an acceptable alternative. Initially, naphthalene vapour was introduced into the plates by immersing a sterile filter paper in a solution of ethanol and naphthalene and placing it into the cover of the petri dish and closing the dish then inverting it for incubation. The plates were inoculated through the pour plate method. The plates were also stored in plastic bags to reduce dehydration as it was incubated for two weeks before being read. This method yielded easy to count colonies but was not reproducible. Occasionally there was not enough naphthalene vapour to support growth. The method of Bogardt and Hemmingsen (1992), reviewed earlier, was then adapted for use.

Naphthalene crystals (at > than soluble limits in water) were placed in sterile distilled water and placed on a hot plate with stirring. The solution was then heated till right before it boils (small bubbles appear). One ml of this was then quickly pippeted into portions (9-12 ml) of warm molten BBH agar. Sample dilutions at one ml were then added to the cooled molten agar and plated. Similar protocols to the other methods for replicates, controls, incubation and reading were carried out. All oil degrader plates were also incubated at 37 ^{0} C to speed the bacterial growth. The plates would otherwise take over a month to develop and would cause the agar to dry out. The colonies on naphthalene plates were small but distinctive. The plates could be read after seven days but were easier to read a few days later as the colonies became more distinguishable from dispersed naphthalene solids.

For all the bacterial counts, sample dilutions were prepared as in the heterotrophic plate count method.

5.2.7 MOST PROBABLE NUMBER

Another method that was used in this research was the MPN for total heterotrophic bacterial counts. The procedure of Huesseman *et al.* (1993) was adopted. Ten ml of sample were transferred to a 250-ml sterile Duran bottle and mixed with ninety ml sterile BBH medium. Slurries were shaken in a shaking incubator for sixty minutes and serially diluted (1:10) up to 10^{-13} in nine ml sterile trypticase soy broth which served as both mineral and energy source for bacterial growth. Cultures were incubated for seven days at 37^{0} C and the highest dilution showing turbid growth were selected to compute the approximate number of bacteria/ml slurry of reactor e.g. if the 10^{-5} dilution tube were the highest dilution showing turbid growth, the number of bacteria present was about 10^{5}/ml slurry. Three tubes were inoculated per dilution with control tubes made with the dilution water used to check for contamination.

5.2.8 DISSOLVED OXYGEN UPTAKE RATE

This was determined by measuring the dissolved oxygen consumed during a fixed time interval in a fixed volume and calculating the uptake rate.

Dissolved oxygen (DO) readings were taken with a YSI 5739 Oxygen probe fitted tightly into a BOD bottle in which the contents were gently mixed by a magnetic stirrer . The membrane in the probe had to be replaced after each set of readings due to the adhering oil. The probe was calibrated before use in water saturated air. (Probe held over twenty ml of water, not touching, in a BOD bottle). Dissolved oxygen uptake rate (DOUR) analysis was not carried out very often due to the difficulty of obtaining meaningful results. The readings had to be repeated several times for one data set as the adhering oil interfered with the free passage of oxygen into the membrane for detection.

5.2.9 NITRATE

Nitrate was determined by HACH method 358 from filtered samples. A HACH kit was used with premeasured vacuum ampoules. Nitrate is determined by reducing the nitrate to nitrite with cadmium metal. The nitrate is converted to an intermediate diazonium salt with sulfanilic acid. The diazonium salt couples to gentisic acid to form

an amber coloured product. The intensity of the blue and amber colours determines the concentration.

5.2.10 ORTHOPHOSPHATE

Orthophosphate was determined using HACH method 492. Orthophosphate reacts with molybdate in an acid medium to produce a phosphomolybdate complex. Ascorbic acid then reduces the complex, giving an intense blue colour which is then measured to get the concentration of orthophosphate.

5.2.11 TOTAL ORGANIC CARBON

Total organic carbon (TOC) was determined on two types of sample, the liquid-solid slurry from the mixed liquor samples and soluble TOC from filtered mixed liquor samples. Filtered samples were injected into the ports of a TOC machine, model Rosemount Dohrman DC/90 with a Sartec Analytical System, and readings were taken for total carbon (TC) and total inorganic carbon (IC). TOC was then determined from the difference. Sludge slurries were placed in the boat and TC was measured from its combustion. For sludge slurries, only TC was measured.

5.2.12 POLYNUCLEAR AROMATIC HYDROCARBONS

HPLC was chosen as the method of choice to separate and quantitate PAH for several reasons. Although GC's are highly efficient at separation, GCs will not determine > C35 which are more recalcitrant (Bossert and Compeau 1995). HPLC also offers a variety of stationary phases capable of providing unique selectivity for the separation of PAH isomers that are often difficult to separate by GC. The selectivity in the HPLC is achieved because of interaction of the solute with both stationary phase and the mobile phase rather than only the stationary phase as in the GC. Detection by UV absorption and fluorescence spectroscopy also provide extremely sensitive and selective detection for PAH (Bartle *et al.* 1981).

SW 846 method 8100 was used as a reference as well as other literature for modifications to the method. The method had to be developed in order to resolve the peaks from the chromatogram of the 'unknown' samples as well as to maximise the

peak height for detection. The matrix from which the PAH was extracted from O&G for HPLC determinations were very tarry and complex and this resulted in an extract that was also very complex. The O&G extract, either from sludge, treated sludge, mixed liquor slurry, foam, organic trap, had to be cleaned and concentrated with the use of solid phase extraction and the PAHs were identified and quantified by means of a reverse-phased HPLC using programmed UV-visible and fluorescence detectors in series. The total PAH was calculated as the sum of 15 individual fluorescent PAH and acenapthylene, which is not fluorescent. The limit of quantification is 1 ppb and typical concentrations of the samples ranged from 0.001 ppm to 100 ppm. The linearity is 500 to 2000 μg/l in the extraction solution for each of the 16 PAH using UV absorption and 500 to 1000 μg/l using fluorescence. The HPLC system consisted of a Hewlett Packard 1050 Model with components as listed below. The Liquid Chromatograph was fitted with a Rheodyne injection valve and a 100 μl sample injection loop.

Instrumentation

Pump and Controller	Hewlett Packard 1050
Interface	Hewlett Packard 35900
UV visible detector	Hewlett Packard
Fluorescence detector	Hewlett Packard 1046A
Software	Chemstation 3365 series II
Computer	Hewlett Packard Vectra 486/33N
Printer	Hewlett Packard Laserjet III P
Analytical Column	Phenomenex (125 x 4.60 mm) C18 monomeric , 5 μm particle size Lichrosphere 100 RP-18 (244 x 4.00 mm) C18 monomeric, 5 μm particle size Vydac 201 TP (150 x 4.6) C18 polymeric, 5 μm particle size
Guard Column	C18 guard column

It is noted here that several chromatographic columns were used throughout the study period and each time the method had to be adapted to accommodate for the difference in column separation efficiency, selectivity and retention characteristics of PAH. Columns were not equal in their capacity to resolve the 16 PAH, with the Vydac column found to be the most efficient. Calibrations were carried out per column.

All samples of O&G, prior to cleanup, were kept in foil covered glass bottles at 4 ^{0} C. For the clean up, approximately 0.05 gm of O&G sample was weighed to four decimal places and was redissolved in a calculated amount of ethyl acetate and an aliquot,

equivalent to approximately 0.005 gm, of this was taken up in a precision syringe. To this, 2 ml of the internal; spike, decafluorobiphenyl at 80 mg/l was added. This was followed by a solid phase extraction procedure, detailed below, for sample concentration and clean up. This step was necessary as the samples contained a lot of polar and other particulate substances that compromised the integrity of the chromatographic column as well as interfered with the chromatogram. Without this step, the chromatographic column became blocked very fast and the PAH compounds were retained longer and elution times varied. Another clean up method, from SW 846 method 3630 silica gel clean up, was tested but it was found that the recoveries were even more variable than the solid phase extraction. Moreover, the chromatograms had peak tailings with consequent less sharp fractionation and poor reproducibility. This is probably due to the high absorptivity of silica as well as adsorption of trace amounts of water. The clean up procedure from the solid phase extraction did result in lower recoveries of the PAH as determined from recovery of the spike.

5.2.12.1 SOLID PHASE EXTRACTION CLEAN UP OF PAH SAMPLES

The following procedure was carried out:

An 3M Empore extraction disk (8 μm bonded silica based) was first prepared. An all glass assembly for membrane filtration was set-up and a 3M Empore extraction filter placed on the 47mm diameter stage. Five ml of ethyl acetate and five ml of DCM were pipetted onto the disk and a brief vacuum was applied to draw a portion of the solvent through the disk. This was left to soak for three minutes and then vacuum was further applied. The process was repeated using methanol. Without allowing the methanol to dry out, the sample prepared earlier in ethyl acetate was placed in the sample reservoir and the sample filtered through the disk. At this stage, the PAH and polar compounds are held within the silica pores. Full vacuum was then applied to dry the extraction disk. A solvent rinsed boiling tube was placed in the Buchner flask of the filtration assembly to receive the filtrate. Five ml of ethyl acetate was then used to rinse the sample container and the rinse was poured over the extraction disk. A brief vacuum was applied to pull some ethyl acetate through. The disk was soaked in ethyl acetate for three minutes. Then all of the ethyl acetate was pulled through and collected in the boiling tube. Five ml of DCM was then poured over the disk and allowed to soak for five minutes. Then this was pulled through in the boiling tube and the process was repeated with a further five ml of DCM. The DCM eluted the PAH into the filtrate collection

chamber, while the polar and more complex (large molecules) compounds remain trapped within the silica pores. Omitting this cleaning step, invariably means, these polar and complex compounds would otherwise block the front ends of the silica based chromatographic columns in the HPLC.

Anhydrous sodium sulphate was added to the sample collected in the boiling tube which was then decanted into a clean boiling tube. The old boiling tube was rinsed with DCM and the washing was combined in the clean boiling tube. The sample was then dried by passing a slow stream of dry nitrogen over it. As soon as it was dry, the sample was redissolved in 2 ml of filtered (0.45 μm pore size) acetonitrile (ACN) and shaken gently to dissolve components. This was then passed through a 0.45 μm teflon syringe filter.

Samples cleaned by this method were analysed immediately by HPLC and the balance of the samples were kept in teflon sealed screw capped vials wrapped in aluminium foil and kept refrigerated at 4 ^{0} C for back up. Samples were diluted accordingly if the concentration range did not fit the range selected (between 10 – 25 units on y axis of the chromatogram chart).

5.2.12.2 HPLC PROGRAMME

Preparation of PAH standards for calibration

Before samples were run, PAH standards were prepared for calibration. A PAH mixture containing the 16 PAH, each at 0.1mg/ml in methanol was purchased from Supelco. From this stock standard, dilutions were prepared as below for 500, 1000 and 2000 mg/l PAH concentrations.

Calibration standards

The stock solution of the 16 PAH was at 0.1 mg/ml for each PAH and it was dissolved in methanol. A 10 μg/ml dilution of PAH was prepared by taking 1 ml from the stock, using a precision syringe, into 9 ml of filtered ACN.

- For 2000 μg/l
- Using a precision syringe, 2 ml of the PAH 10 μg/ml dilution was taken and placed into 8 ml of filtered ACN.

- For 1000 μg/l

Using a precision syringe 1 ml of the PAH 10 μg/ml dilution was taken and placed into 9 ml of filtered ACN.

- For 500 μg/l

Using a precision syringe 0.5 ml of the PAH 10 μg/ml dilution was taken and placed into 9.5 ml of filtered ACN

A few single solutions of PAH such as acenapthene, chrysene etc. were also purchased to place peak elution times of individual PAH as well as to determine minimum detection limits. All dilutions were kept in teflon lined screw capped vials, wrapped in aluminium and kept at 4 ^{0} C. Subsequent dilutions were prepared using volumetric flasks with a solution of 50:50 ACN:deionised water. All solvent used were HPLC grade and were filtered with a 0.45 μm nylon filter prior to use.

The standards prepared for calibration were used to prepare calibration curves and tables as well as checking the integrity of the column and elution time drifts during analysis from between sets (time frame). Calibration tables were prepared using the Chemstation software with manual integration. Most peaks had to be integrated manually due to the peaks eluting very close together such that any small drift in the elution time affected the automated calibration which was based on elution time. Therefore, chromatogram pattern was used to identify peaks more often than elution times. The internal standard decafluorobiphenyl helped to place the degree of drift in the unknown samples. Unknown sample concentrations were estimated based on the calibration standards with peaks being integrated manually. Using the stock solution dilution of the 16 PAH as well as a few single standards, a three point calibration using the UV detector gave R^2 values between 0.9039 to 1 (calibration charts in *Appendix* J, p. 269) for the linear regression curve.

The chromatography was established to resolve each component of a mixture of the 16 PAH standards at ppm and ppb concentration levels. The detection of the 16 PAH was aided by a UV-visible detector coupled to a fluorescence detector. The procedure for the chromatography employed an ACN and water (18 MΩ de-ionised) gradient which is detailed below together in the Table 5.2 with the temperature of the column and the flow rates. A timetable was also set for the fluorescence detector, also detailed below in Table 5.3. The variable wavelength detector was set at 254 nm.

Table 5.2: HPLC Pump timetable

Column Temp = 38 °C Flow rate = 1 ml/min		
Time (min)	% pure water	% Acetonitrile
0.00	50	50
35.00	0	100
45.00	0	100
55.00	50	50

Table 5.3: Fluorescence detector time table

time (min)	Exitation (nm)	Emission (nm)	PMT gain
0.00			7
0.01	220	322	7
13.00	210	312	7
16.50	247	375	7
19.00	230	420	7
22.00	230	380	7
25.00	230	453	11

This set of programme was used for the Lichrosphere column. Everytime the column was changed, a new timetable had to be set as elution times and separations vary according to the column matrix and packing. For the Lichrosphere column, a few of the peaks, peaks number 3 and 4 and peaks number 9 and 10 could not be separated although a number of different gradient programmes as well as detector time table changes were made. Some of the chromatograms that were produced as a result of determining the best separation gradient and best detection time table are in *Appendix* O. A fair amount of time was spent in developing the method to determine column temperatures, solvent gradients, absorption wavelengths and fluorescence timetable for detection of the 16 PAH. Each peak has a different optimum value of λ_{ex} and λ_{em} in its fluorescence spectrum and it was necessary to set these parameters for each peak in the flourimeter using a timetable. However, switching wavelengths takes real time and some peaks elute so close together that it is not possible to achieve ideal λ for each peak. Switching has to occur between peaks and small drifts in retention times result in some chromatograms 'jumping' the baseline when the switch occurs during a peak elution. Therefore, time was taken to devise a timetable that would maximise the use of

optimum λ_{ex} and λ_{em} for the 15 PAH. Even then, small drifts in the retention times (RT), i.e. RT fractionally gets longer each time due to gradual increasing blockage of filters, columns etc., will require the time table to be updated to compensate for the drift. Optimum values for λ_{ex} and λ_{em} are found in *Appendix* G (p.264). UV absorption, although could have been set to a timetable, was finally maintained at 254 nm throughout after several trials.

Prior to starting the HPLC, solvents were degassed and the solvent lines were purged. The HPLC was always started on 100 % ACN before gradually putting in a 50:50 ACN:water baseline. Injections of 25µl samples were preceded by filling the loop with filtered ACN. Standard PAH runs were conducted daily during the use of the HPLC to check the integrity of the column and the drift in retention times. Chromatographic areas of each identified peak from the samples were manually integrated using the Chemstation software and calculated based on the equivalent areas of the peak from the standards.

5.3 PRELIMINARY EXPERIMENTS

As mentioned in Chapter 4, a series of preliminary experiments were planned first in order to determine the experimental conditions to be used for the biological treatment of oily sludge in a reactor. Following is the sequence of experiments that were carried out and the analytical methods employed in each one. All the experiments were carried out as shake flask culture experiments in a light obscured Gallenkamp Orbital Incubator at 37 ^{0} C and shaking speed, 160 rpm.

At the start of this research it was decided that cultures, already acclimated and at high density, would be introduced into the reactors in order to ensure a shorter lag time period as well as to overcome the possibility of low biomass restrictions on the biodegradation rates. It had already been determined from the literature reviewed that seeding reactors may prove beneficial as system parameters could be optimised and competition between autochthonous bacteria microflora reduced as compared to its use in open systems such as in landfarming (Leahy and Colwell 1990). Previous research, based on landfarming found mixed results from inoculation studies which may be due to the difficulty of the seed organism having to compete with the already adapted cultures present in soil as well its restricted movement in the soil. The mass transfer problem is

easily overcome in a reactor system and the use of high numbers of preadapted cultures may overcome factors such as predation, competition etc. Corseuil and Weber (1994) and Wiggins *et al.* (1987) have cited critical population development period, related to the time taken for the adapted population to build sufficient biomass to yield demonstrable contaminant degradation, as being a possible limiting factor on rates. Thus, the use of initially high concentration of bacterial culture should overcome this.

The selection of micro-organisms able to grow on xenobiotic compounds was addressed first together with determining a method to enhance population size before acclimating the cultures for all the other experiments. The first experiment was set-up to determine a source for the microbial inoccula to be used throughout all the experiments. Through the literature reviewed, it was decided that a mixed microbial culture would be preferred to single pure cultures. Several authors have pointed out that mixed cultures are important when it comes to the complete mineralization of complex and toxic organics for several reasons:

- Single organisms may not have the ability to mineralise the xenobiotic completely due to; the accumulation of toxic intermediates and the susceptibility of single cultures (Buitron and Gonzales 1996).
- no single organism may have all the required characteristics e.g. enzymes, to degrade the complex compound and its intermediates (Ascon-Cabrera and Lebeault 1993).

Based on this, it was decided not to use isolated strains or mixtures of isolated strains but to determine a source of microbial consortia that was already equilibrated and that had potential to degrade oily sludge.

5.3.1 EXPERIMENT D. MICROBIAL INOCULA SOURCE:

Three sources for the microbial inoculum were chosen to test its potential to degrade oily sludge;

- an active landfarm treating oily sludges
- activated sludge from a nearby sewage treatment plant
- oily sludge from a sludge pit

The experiment to determine the culture source was carried out in conjunction with determining a method that could be used to enhance population size of the cultures. Cultures were grown in either nutrient media or BBH with one percent crude oil (BBHO). Nutrient media, being rich, was used to boost the cell numbers. However, from the literature reviewed, cultures previously adapted to degrading oil could loose this 'adaptation' if grown in oil-less media for prolonged periods of time (Guerin and Boyd 1995; Goldstein *et al.* 1985). Therefore, experiments were carried out with cultures being grown in both nutrient and BBHO. A small amount of landfarm soil (one to two gm), sludge (one to two gm) and activated sludge (ten ml) were each placed in fifty ml of nutrient or BBHO in 250 ml Erlenmeyer flasks which were then stoppered with a cotton bung and incubated in a gyratory shaking incubator for five days for enrichment. Then the flasks were decanted without the solids and resuspended in 500 ml of the respective media in 2-l Erlenmeyer flasks, which were again incubated and shaken for five days. The cells were harvested by centrifugation of the flask contents at 4000 rpm for fifteen minutes. The recovered cell pellet was then washed three times with BBH to remove the media and adhering oil. The cell pellet was then resuspended in BBH and incubated in the shaking incubator for a short time period to allow the pellet to completely and homogeneously resuspend in the media.

Table 5.4 : Experiment D - Microbial inoccula source

CULTURE SOURCE	MEDIA	SAMPLE	INFERENCE
Oily sludge	Nutrient	OSN	Nutrient media to boost numbers, cultures present likely to be acclimatised, to check against LFBBH culture
Landfarm	Nutrient	LFN	Nutrient media to boost numbers, cultures present likely to be acclimatised, to check against LFBBH culture for possibility of losing adaptation by prolonged growth in oil-less media
Landfarm	BBHO	LFBBH	acts as baseline, sets lowest limit
Activated sludge	Nutrient	ASN	Nutrient media to boost numbers ,hardy culture, possibly not acclimated, to check against LFN and OSN

Biodegradation potentials of the cultures were then tested. Topped crude oil at one percent was added into each flask and the flasks incubated with shaking at the

previously set conditions. Subsequently, twenty ml were taken out for each sample of TSS and O&G analysis and each analysis were carried out in triplicates for five consecutive days. The Table 5.4 shows the different sets of culture and media used for this experiment and the objective of each combination.

The results of this experiment were used in the next experiment.

5.3.2 EXPERIMENT E. PRIMED VS UNPRIMED CULTURES

After the determination of oil degradation potential of the cultures, the consortia chosen was cultured in the same manner and resuspended in BBH or nutrient media. This experiment's objective was to note the growth rates of cultures that have been primed on oil as a substrate and those that were not to determine the significance of having adapted cultures at the start of the experiment. Growth rate was observed through VSS and DOUR. The microbial consortium that was chosen was transferred repeatedly from the original population to fresh BBHO for acclimation. Initially, topped crude oil was added at one percent vol./vol. At the end of the first week the cells were harvested and resuspended in fresh BBH with one percent crude oil. Subsequent harvesting and resuspension utilised higher amounts of crude oil, i.e. increasing amounts up to five percent vol./vol. The same procedure of harvesting and resuspension was done for the 'unacclimated' culture but using nutrient media only. After six weeks the cells were harvested, washed and resuspended in BBH and crude oil was added to both sets of acclimated and unacclimated cultures. The growth rates of the two were then compared.

After determining the benefit of primed cultures, this set of results were then used to set-up the continuous cultivation conditions for the culture as detailed in *Appendix* H (p. 265). The principals of a 'chemostat' (Tempest 1970) was initially practised in a 750 ml Erlenmeyer flasks in the shaking incubator with removal of a fixed volume of spent media at fixed time intervals and its simultaneous replenishment. A 'chemostat' housed in a 4-l Erlenmeyer flask with aeration was placed in the warm room ($37C^0$ ±) in the laboratory and was used for all subsequent inoccula source. Everytime an inoculum was required, fifty ml of the chemostat contents were taken out and used to seed 450 ml nutrient media which was incubated overnight in the shaking incubator. The cells were harvested as before and resuspended in the required amount of fresh BBH media.

5.3.3 EXPERIMENT F. MINERAL SALTS REQUIREMENT

Another experiment was also carried out to determine if the minimal basal salts used met the requirements of the mixed cultures. Two concentrations were chosen, one similar to that used by Bushnell and Haas (1940) and the other one at twice the concentration. Topped crude oil was added at one percent vol./vol. The VSS and DOUR measured was used as the basis for measurement for the growth rate of the culture. The set-up was as Table 5.5 below.

Table 5.5 : Experiment F - Mineral salts requirements

Media	*Analysis*
25 ml @1X concentration in 250 ml Erlenmeyer Flasks	TSS, TVSS in triplicates daily for 5 days
50 ml @1X concentration in 250 ml Erlenmeyer Flasks	DOUR daily
25 ml @2X concentration in 250 ml Erlenmeyer Flasks	TSS, TVSS, triplicate daily for 5 days
50 ml @2X concentration in 250 ml Erlenmeyer Flasks	DOUR daily

5.3.4 EXPERIMENT G. SLUDGE LOADING RATES

Before the reactor runs could be set-up, the amount of sludge or solids to be treated in the bioreactors needed to be determined. The amount of solids in the slurry of a reactor is important for several reasons. For one, due to the toxicity of the oily sludge there could be an inverse relationship between sludge load and degradation rate of the consortia. High rates of degradation could require low solids load. In order to be cost effective, sludge loads need to be as high as possible. High solids content would require more power input for stirring to get a uniform mix and therefore will cost more. All these factors, have to be taken into consideration when deciding the solids content of the reactor.

In order to determine the optimum loading for the bioreactors based on these factors, a series of shake flask experiments with 5, 10, 15 and 20 % dry solids load (wt/vol) from the oily sludge were carried out as follows:

Following the complete characterisation of the oily sludge, as in *Appendix* F (p. 262), the sludge load to get the correct percentage of solids was determined. The experiment was set-up to run for 21 days with sampling on day 0, 3, 6, 9, 12, 15, 18, 21 for each sludge load with triplicate analysis for each data point. Prior to the start of the experiment, the dry solids content of the sludge, was determined. This was then used to calculate a wet sludge loading that would give the required dry solids load of 5, 10, 15 and 20 % (wt/vol) in the 25 ml BBH media or 50 ml BBH media e.g.

10 % (wt/vol) of 25 ml is 2.5 gm dry wt
Wet weight of sludge required to give 2.5 gm dry wt?
(1÷ dsf) x 2.5 = amount required

The calculated wet weight of the sludge was then weighed at ± 0.03 g of the calculated amount into a 250 ml Erlenmeyer flask and labelled accordingly. The flasks with the sludge was then stoppered with a cotton bung with the tops wrapped in aluminium foil which was then autoclaved together with BBH media separately for dispensing later. After autoclaving, the sterile BBH media was cooled to room temperature before harvested cells from the chemostat were resuspended in it. Following this, 24 ml or 48 ml of the inoculated BBH media was dispensed into the 250 ml Erlenmeyer flask. The balance of 1 ml/2 ml that was added after this to make up to 25 ml and 50 ml, respectively, was made up of a nutrient solution. The nutrient requirements were determined as follows:

DATA USED IN CALCULATIONS

The dsf of the sludge that was used = 0.714
The O&G content of the sludge used = 33.11 % of dry weight
C is calculated as being 90 % of the O&G
N is 35 % of NH_4NO_3
P is 31 % of H_3PO_4 , 1 ml H_3PO_4 weighs 1.75 g

Based on C:N of 150 :1

For a 15 % (wt/vol) dry solids sludge content in a 25 ml solution

- 0.15 X 25 = 3.75 g dry wt of sludge
- based on 33. 11 % O&G content per dry weight = 1.242 g O&G
- based on 90 % of O&G is C , C = 1.1178 g
- to get C:N of 150:1 = 1.1178 ÷ 150 = 0.007452 g N
- to get amount of NH_4NO_3 needed = (100 ÷ 35) x 0.007452 = 0.0211 g NH_4NO_3

Based on C:P of 750 :1

For a 15 % (Wt/Vol) dry solids sludge content in a 25 ml solution

- 0.15 X 25 = 3.75 g dry wt of sludge
- based on 33. 11 % O&G content per dry weight = 1.242 g O&G
- based on 90 % of O&G is C , C = 1.1178 g
- to get C:P of 750:1 = 1.1178 ÷ 750 = 0.0015g P
- to get amount of H_3PO_4 needed = (100 ÷ 31) x 0.0015 = 0.0048 g H_3PO_4
- ml of H_3PO_4 weighs 1.75 g, ∴ 1 ml ÷ 1.75g = 0.57 ml /g
- to get 0.0048 g = 0.0048 x 0 .57 = 0.0027mls = 2.7 µl

The nutrient solution was then made up as the total amount on NH_4NO_3 and H_3PO_4 needed for all the flask for the run e.g.

For a total of 48 flasks with 25 ml. BBH, add (0.0211 x 48) NH_4NO_3 and (2.7 µl x 48) H_3PO_4 in 48 ml of BBH. 1 ml of this solution was then added to each flask.

The flasks were incubated at 37 ^{0}C at 160 rpm. Each 25 ml media flask were analysed for, O&G and total solids content. For each flask, five ml was taken up by a volumetric large bore syringe for total solids analysis and the balance of twenty ml was used for O&G analysis. Thus, triplicates were sampled for these two analyses, per sampling point per sludge loading. It was also planned that on each sampling day, another 250 ml Erlenmeyer flask with fifty ml of media would also be analysed for MPN and nitrate and phosphate to determine bacterial numbers and to ensure nutrient requirements were adequate. From the fifty ml sample, twenty ml each were taken for nitrate and phosphate analysis and the balance of ten ml were used for seeding dilution samples to carry out MPN. Due to lack of space in the incubator some analysis were done less frequently. A summary of the experimental set-up is as in Table 5.6.

The results of this experiment were used in setting up the bioreactor test runs as well as determining kinetics of growth and degradation.

Table 5.6 : Experiment G - Sludge loading rates

% solids load	no of flasks	analysis	ml
5 %	25 ml media flask X 8 sampling points	O & G	20
	X triplicate analyses	total solids	5
	= 24 flasks		
	50 ml media flask X 8 sampling points	Nitrates	20
	= 8 flasks	Phosphates	20
		MPN	10
10 %	25 ml media flask X 8 sampling points	O & G	20
	X triplicate analyses	total solids	5
	= 24 flasks		
	50 ml media flask X 8 sampling points	Nitrates	20
	= 8 flasks	Phosphates	20
		MPN	10
15 %	25 ml media flask X 8 sampling points	O & G	20
	X triplicate analyses	total solids	5
	= 24 flasks		
	50 ml media flask X 8 sampling points	Nitrates	20
	= 8 flasks	Phosphates	20
		MPN	10
20 %	25 ml media flask X 8 sampling points	O & G	20
	X triplicate analyses	total solids	5
	= 24 flasks		
	50 ml media flask X 8 sampling points	Nitrates	20
	= 8 flasks	Phosphates	20
		MPN	10

5.3.5 EXPERIMENT H. ABIOTIC OIL LOSS – CONTROL EXPERIMENT

After selecting a sludge load at ten percent solids loading, another experiment was set-up as the abiotic control to measure the losses from other than biodegradation processes e.g. volatilisation, photodegradation. The control experiment consisted of a similar set-up as the ten percent solids loading set of flask experiment in 5.1.4 except

that the BBH used was uninoculated and all other solutions were kept sterile after autoclaving.

5.3.6 EXPERIMENT I. SURFACTANT CHOICE

The last shake flask experiment was actually carried out after the two reactor runs i.e. the Basic BSTR and Airlift BSTR. Both the runs highlighted the problem of the sludge agglomerating at the bottom of the reactors. Stirring seemed to promote this process. Surfactant was selected as an amendment to be added to the Basic BSTR to try and address this problem. It was felt that surfactant could reduce the viscosity of the sludge and that would reduce the agglomeration. Agglomeration reduced the amount of sludge entering the mixed liquor phase where most of the biodegradation was taking place. Surfactant was also selected as an amendment to increase rates of degradation through the processes outlined in the literature review section.

Several experiments were carried out prior to setting up the third surfactant amended BSTR run and all are presented in this section. One of the experiments presented here is a determination of an analytical method, which were covered in the previous section. This analytical test is covered here as it was developed only for this experiment. The method used in all the other experiments could not be used due to the interference from the surfactant. Therefore, this set of experimental results, for that one particular analysis, is not comparable with the rest of the project data as the method utilised was different.

The experiments carried out as part of choosing the surfactant for the reactor run are as follows;

- 5.3.6.1 Extraction and measurement of O&G in the presence of surfactant
- 5.3.6.2 CMC of the 3 chosen surfactants
- 5.3.6.3 Testing for the presence of microbially produced surfactant of the culture used against a *Pseudomonas* produced surfactant
- 5.3.6.4 Selection of 'highest degradation rate enhancer' surfactant
- 5.3.6.5 Testing the addition of surfactant to sludge or mixed liquor

Efficiency of surfactant in releasing oil into the mixed liquor was deemed as the main essential effect of surfactant addition as the objective was to increase the amount of oil in the mixed liquor phase. Reference for methods to determine abiotic desorption tests are available in literature (Roger *et al.* 1993). However the best 'solubility enhancer' surfactant is not necessary the best degradation rate improver as has been shown from the research by Harmsen (1991). There are other factors determining the overall rate of biodegradation such as toxicity, competitive inhibition etc. which may reduce the rate of an otherwise efficient surfactant. Thus, it was decided to use 'best degradation rate enhancer' rather than most efficient surfactant in releasing oil into the water phase as the selection criteria. A number of options were available to check for degradation rate, as has been reviewed earlier i.e. DOUR, bacterial counts, VSS and O&G measurements. While the former three methods were indirect and biodegradation is inferred from the increase in DOUR, bacterial counts and VSS respectively, only O&G measurements provide the most direct evidence of degradation occurring. Indirect methods, such as determining DOUR, may be overestimated if the microorganisms are respiring the surfactant or contaminant. These disadvantages have been reviewed earlier. From the literature reviewed, there were no mention of any O&G determination for measuring rates in the presence of surfactant. Most research used pure radioactive isotopes (Zhang and Miller 1995; Churchill *et al.*1995a; Stucki and Alexander 1987; Foght *et al.* 1989; Guerin and Jones 1988, Laha and Luthy 1991) or measured using HPLC (Grimberg *et al.* 1996; Volkering *et al.* 1993), GC (Oberbremer *et al.* 1990; Bury and Miller 1993) and one reference used a fluorospectrophotometer determination of pure PAH concentration (Putcha and Domach 1993).

5.3.6.1 EXTRACTION AND MEASUREMENT OF O&G IN THE PRESENCE OF SURFACTANT

In order to determine if the method used in all the O&G analysis in this research was still applicable, it was carried out in the presence of surfactant. From the results it was obvious that gravimetric determination of O&G in the presence of surfactant did not work because all the solvents tested either extracted the surfactant as well which contributed to the weight or caused too much emulsification which hampered recovery. The method had to be adapted if determination of O&G needed to be done. Determination of O&G using infrared adsorption, is a standard method (APHA & HMSO). However, this option was not considered earlier as gravimetric determinations

were more commonly used. In order to run the set of experiments to select the surfactant based on the best 'degradation rate enhancer', the analytical determination of O&G had to be developed first. Following are the tests that were carried out:

1. extraction and gravimetric determination of O&G in the presence of 3 types of surfactant using (DCM) as solvent
2. extraction and gravimetric determination of O&G in the presence of 3 types of surfactant using cyclohexane and n-octane as solvent
3. extraction and FTIR determination of O&G in the presence of 3 selected surfactants using TCTFE as solvent

Experiment 1

In order to determine if gravimetric determinations of O&G were possible, duplicate samples of sludge in ten ml BBH solution or samples of pre-extracted O&G from sludge in ten ml BBH solution were mixed with each of the three surfactants and extracted as in the method delineated in section 5.2.3. and determined gravimetrically. Experimental set-up as in Table 5.7.

Table 5.7 : Experiment I - Gravimetric O&G determination

no	sample	wt (gm)	surfactant	wt(gm)
1	sludge x 2	1.2 -1.4	Brij 35 at 80 CMC	0.096
2	sludge x 2	1.2 -1.4	Sapogenat at 80 CMC	0.448
3	*extracted O & G x 2	0.29-0.35	Brij 35 at 80 CMC	0.096
4	extracted O & G x 2	0.29 -0.35	Sapogenat at 80 CMC	0.448
5**	sludge	1.36	Inipol at 10 X recommended dose	300 µl

*pre-extracted O&G from sludge, recovery using the same solvent should be close to 100 % i.e. eliminating solvent selectivity and matrix effects of extraction

** Inipol EAP 22 was carried out separately at a later date as it had not arrived yet at the time this experiment was carried out

After determining the solvent used, DCM, extracted surfactants together with the O&G it was decided to test other solvents mentioned in the literature.

Experiment 2

Cyclohexane (Oberbremer *et al.* 1990) and n-octane (Thiem 1994) were tested for extracting O&G from sludge. The experimental set-up is as in Table 5.8.

The sludge, BBH and surfactant amounts were the same as the previous experiment with DCM. The volume of solvent used for extraction were the same as the literature reference. The extraction was carried out in a 100 ml closed Duran bottle in the orbital shaker overnight. The contents of the bottle were then centrifuged and the solvent layer recovered. When there was a strong emulsion the samples were frozen, thawed and recentrifuged to improve separation. The solvent was evaporated at lower than boiling point temperature of the solvents in an oven placed in a fume cupboard. O&G were determined by gravimetry.

This experiment was carried out only as a screen to determine the possibility of using gravimetric determination of O&G, hence, only one of each combination was tested. Again, all results showed gravimetric determinations would have problems. Another method had to be determined.

Table 5.8 : Experiment I Testing of solvents for gravimetric determination of oil and grease

Sample no	Sludge wt (gm)	Surfactant	Solvent (ml)
1 , 2, & 3	1.3 - 1.35	Brij 35/ Sapogenat/Inipol EAP 22	n- octane 5 ml
4, 5 & 6	1.3 - 1.35	Brij 35/ Sapogenat/ Inipol EAP 22	Cycylohexane 10 ml

Experiment 3

The method used by Oberbremer *et al.* (1990) was adapted. O&G was pre-extracted from sludge samples and used in these experiments. Approximately 0.7 gm of the O&G in twenty-five ml BBH was amended with surfactant and extracted with thirty ml TCTFE by mixing or agitation and then separated by centrifugation at 4000 g x 20 min. The upper five ml of solvent layer was taken up in a volumetric syringe and dried with anhydrous magnesium sulphate. The solvent was collected by decanting and the remaining magnesium sulphate was rinsed with a few ml TCTFE and the two portions of solvent were added and made up to ten ml. This was then measured in the Fourier Transformed Infrared Spectrophotometer (FTIR), model Perkin Elmer 1600 series. The sample was held in a very thin paper film in between sodium chloride plates. The FTIR was set to scan the infrared absorbance of the extract between 2400cm^{-1} to 3400 cm^{-1}. A compensating cell containing pure solvent was placed in the reference beam and the spectrum was stored in the FTIR data bank. After the sample was scanned, the solvent's spectrum is compensated for in the sample spectrum and the peaks at 2957

(2960) cm^{-1}, 2927 (2930) cm^{-1} and 2857 (2860) cm^{-1} were added up and compared to a calibration chart to get O&G content. The samples for the calibration chart was made up of dilutions of pre-extracted O&G from the sludge itself to get accurate results. This method yielded satisfactory results and was used for the selection of surfactant experiment. The set-up for the series of flasks were as follows;

Table 5.9 : Experiment I - Infrared spectrometry determination of O&G

No	*Extracted O & G (gm) in 25 ml BBH	surfactant
1a	0.7184	0.24 gm Brij 35
1b	0.7318	0.24 gm Brij 35
2a	0.7039	1.12 gm Sapogenat
2b	0.7159	1.12 gm Sapogenat
3a	0.7050	100 µl Inipol EAP 22
3b	0.7320	100 µl Inipol EAP 22

* pre-extracted from sludge

5.3.6.2 CMC OF THE THREE CHOSEN SURFACTANT

Prior to the selection of surfactant experiment, surface tension measurement of different molar concentrations of the surfactants were carried out to confirm the CMC values reported in literature. For this a White Digital Balance, model DR2 ks, with a du Nouy interfacial tensiometer was used to measure triplicates of surface tension in dynes/cm.

5.3.6.3 TESTING FOR THE PRESENCE OF MICROBIALLY PRODUCED SURFACTANT OF THE CULTURE USED AGAINST A *PSEUDOMONAS* PRODUCED SURFACTANT

It was also initially planned to test biosurfactants against the three synthetic surfactants that were pre-selected. For this, first, an experiment was set-up to determine if the microbial cultures used in the reactors were surfactant producers. At the same time a known surfactant producer was also cultivated in shake flasks to provide a comparison of the liquors from a known surfactant producer and the cultures used. Surface tension and emulsification behaviour of the *Pseudomonas* culture broth and the Airlift BSTR culture broth were compared.

This set-up would also enable a selection for the source of biosurfactant if it were found that the cultures used were surfactant producers. The experimental set-up to determine if the cultures used were surfactant producers was as follows:

Cultures from the Airlift BSTR (ten ml) were pippeted from the foam layer into 250 ml of nutrient media in 750 ml Erlenmeyer flasks. This was incubated overnight in the shaking incubator at 180 rpm and at 30 ^{0} C. The cells were harvested the next day by centrifuging at 4000 rpm for twenty minutes and washed two times in BBH and finally resuspended in 300 ml BBH. Twelve 250 ml flasks were then prepared by measuring out approximately two gm pre-extracted O&G for each of the flasks which was stoppered with cotton and the tops wrapped in aluminium foil and autoclaved. The sterile flasks were then filled with twenty ml of the washed and resuspended cultures from the Airlift BSTR. All the flasks were incubated at 180 rpm at 25 ^{0} C. One flask was taken out at times 0, 5, 15, 29, 48 and 74 hours to measure surface tension. All surface tension measurements were carried out on the same White Digital Balance mentioned earlier. The platinum ring was kept clean by dipping in alcohol and flaming it. In the case where there was adhering oil, the ring was cleaned in chromic acid briefly first.

Pseudomonas aeruginosa (NCIMB 8295) maintained on caso broth was used as a known surfactant producer. Two loops from the broth was inoculated into sterile thirty-five ml of Kays Minimal media (Zhang and Miller 1992) in 250 ml Erlenmeyer flask which was incubated at 200 rpm and 37 ^{0} C for 24 hours. Two ml of this was then inoculated into sterile 200 ml PPGAS (pH 7.2) media (Zhang and Miller 1992) for a total of 60 hours. This culture was periodically removed at times 6, 32, 48 and 60 hours to measure surface tension. The results for the two broths were then compared.

Emulsification ability of the mixed microbial cultures and *Pseudomonas aeruginosa* was also investigated. Reduction in surface tension and emulsification ability are two characteristics of surfactants with the surfactant showing greater reduction in surface tension and greater durability of emulsification denoting a more efficient surfactant (Bosch *et al.* 1988). For the mixed microbial cultures from the Airlift BSTR, emulsification tests were carried out per flask at the same time surface tension measurements were carried out. For the *Pseudomonas aeruginosa*, emulsification tests were carried out at the end of sixty hours as according to previous research (Zhang and Miller 1992) the surfactants were harvested at this time.

Emulsification tests as referenced in Churchill. *et al.* (1995b) and Bosch *et al.* (1988) were carried out by mixing equal volumes of aqueous culture with equal amounts of oil and the volume occupied by the emulsion measured after an equilibration period. For this research, the mixed microbial cultures at twenty-five ml per flask were removed at the stipulated times and was vortexed with twenty-five ml of vegetable cooking oil. The volume occupied by the emulsion was compared at two hours then at twenty-four hours. The same was carried out for the *Pseudomonas* culture but only at the end of sixty hours.

At this point, an attempt was made to isolate the biological surfactant so that it could be used in the 'selection of surfactants experiment'. The method used by by Zhang and Miller (1992), with the slight adaptation of centrifuge speed, was employed. The culture of *Pseudomonas aeruginosa* was cultivated as previously mentioned. Then at sixty hours the whole culture was centrifuged at 6,800 x g for twenty minutes and biosurfactant was recovered from the culture supernatant. The biosurfactant was then precipitated out of solution by acidification to pH 2.0 then centrifuged at 10,000 x g for twenty minutes. The precipitate, which was an oily brown liquid, was dissolved in 0.05M bicarbonate, reacidified and recentrifugeed at 10,000 x g for twenty minutes. Following centrifugation, the precipitate was extracted in chloroform:ethanol (2:1) three times. The organic solvent was evaporated on a rotary evaporator and the recovered biosurfactant, which was barely discernible, was redissolved in 0.05 M bicarbonate. The low yields may be due to the method employed yielded very low recoveries or this could also be due to the surfactant being non extractable as it could be cell or membrane bound. Since the method employed by Zhang and Miller actually centrifuged at 12,100 g x 20 minutes, a second attempt was made to recover more biosurfactant using the higher speeds to isolate the extract. However, at the second attempt, the centrifuge broke. The other methods for recovery used freeze dry techniques, which could not be attempted due to lack of facilities. At this stage in the research it was too late to wait for a centrifuge replacement and thus the research was limited to synthetic surfactants although it is strongly felt that biological surfactants may offer distinct advantages. For one, it would be more environmentally friendly (biodegradable) and less likely to cause ecological imbalance. There is also some evidence as reviewed earlier, that they are more specific in action than synthetic surfactants, (Guerin and Boyd 1992; Zhang and Miller 1995) and can therefore be applied for a more specific use, such as to aid attachment to oil, rather than used as an emulsifier only.

5.3.6.4 SELECTION OF 'HIGHEST DEGRADATION RATE' ENHANCER SURFACTANT

As the biological surfactant could not be isolated due to the above reasons, the selection of surfactants experiment investigated three synthetic surfactants against one control experiment without surfactants.

For the selection experiment, three surfactants were pre-selected for testing based on the literature reviewed i.e. Sapogenat, Brij 35 and Inipol EAP 22. Earlier work with the surfactant Sapogenat (Tributyl phenol polyglycol) by Thiem (1994) showed it was a good rate enhancer. Sapogenat was then acquired through the kind donation from Hoechst UK limited. Brij 35 was selected as it had also been used a number of times with good results in the literature reviewed for similar work (Volkering *et al.* 1995; Thiem 1994; Liu *et al.* 1995). Both these surfactants have a HLB of seventeen which means it is less likely to be toxic as other surfactants such as Triton X-102 with a lower HLB of 14.6 and these two surfactants also have high molar solubilization ratios (MSR), 0.152 for Brij 35 and 0.150 for Sapogenat compared to 0.112 for Triton X-102. Both Sapogenat and Brij 35 were used at eighty CMC. As previously reviewed, there is a proportional increase in dissolution of insoluble compounds in water with increasing amounts of surfactant above CMC levels.

The other surfactant that was chosen was an oleophilic fertiliser, Inipol EAP 22 (referred subsequently as Inipol) developed by Elf Acquitane of France for use in the biodegradation of oil spills. Inipol was developed following the grounding of the Amoco Cadiz in 1979. Although it has been widely marketed as a fertiliser for oil, it also has lauryl phosphate and oleic acid, which are surfactants, in its formula (Lindstrom *et al.* 1991). Chemical composition and other physicochemical details are in *Appendix* I (p.266). There have been very encouraging reports from the use of Inipol in treating oil spill on land (Churchill *et al.* 1995b;Glaser 1991;Chianelli *et al.*1991;Bragg *et al.* 1990). The research by Glaser, Chianelli and Bragg investigated the use of Inipol in actual field applications as a nutrient enhancer to treat the Exxon Valdez oil spill. They determined that Inipol substantially contributed to the cleaning up of seventy miles of oiled beach at Prince William Sound and Gulf of Alaska. However, there was little reference to its surfactant effects. Churchill *et al.* (1995b) measured several physiochemical properties of the Inipol formulation such as its surface tension effect on water, its emulsification

ability of a mixture of oil and water and its effect in increasing the aqueous solubility of methyl-naphthalene. Inipol, as compared to rhamnolipid, a natural surfactant produced by microorganisms, was almost equally efficient. In their research, Churchill et al. (1995b) concluded that the use of Inipol increased the degradation rate of hydrocarbons and that was strongly related to its surfactant effects.

Elf Acquitane of France kindly donated 100 ml of Inipol EAP 22 for the experiments.

The experiment, set-up in a series of 250 ml Erlenmeyer flasks, were run in duplicates with sampling for O&G and total solids carried out on days 0, 2, 5, 8 and 12. Experimental set-up for the series of flasks are as in Table 5.10.

Table 5.10: Experiment I : Selection of surfactant

No of flask	Surfactant	Sampling day	Analysis per sampling day
20 flasks	Brij 35 at 80 CMC	0, 2, 5, 8, 12	2 flask for O&G 2 flask for TS, TVS, TFS
20 flasks	Sapogenat at 80 CMC	0, 2, 5, 8, 12	2 flask for O&G 2 flask for TS, TVS, TFS
20 flasks	*Inipol EAP 22 at 100µl	0, 2, 5, 8, 12	2 flask for O&G 2 flask for TS, TVS, TFS
20 flasks	control	0, 2, 5, 8, 12	2 flask for O&G 2 flask for TS, TVS, TFS

* at slightly higher than recommended dose of ten percent of O&G content

Culture inoculums were prepared in the usual manner by inoculating 250 ml of nutrient media with fifty ml cultures from the chemostat, growing overnight, harvesting and resuspending the cells in sterile two litres of BBH. Twenty-four ml of this culture solution was then pippeted in 250 ml Erlenmeyer flasks. Prior to that, sludge was weighed and placed per flask at 3 ± 0.05 gm wet sludge. The nutrients N and P were added by dissolving 1.12 gm NH_4NO_3 and four ml H_3PO_4 in 80 ml BBH and pipetting one ml per flask. This gives the ratio of 150 :1 for C:N and 750:1 for C:P. Brij 35, Sapogenat and Inipol were then added to twenty flasks each. Brij 35 and Sapogenat were both added at eighty x CMC (wt/v) 0.24 gm and 1.12 gm respectively and Inipol was used at slightly higher than the recommended dosage of ten percent of the weight of oil. The calculations for all were as follows;

Brij 35

CMC - 0.12g/l

80 x CMC in 25 ml = (0.12 /1000) x 80 x 25 = 0.24 gm

Sapogenat

CMC - 0.56 gm/l

80 x CMC in 25 ml = (0.56 / 1000) x 80 x 25 = 1.12 gm

Inipol EAP 22

Dry solids fraction of sludge = 0.7194

3 gm wet weight = 2.1582 gm dry weight

oil at 33 % O&G per dry weight of sludge gives 0.73 gm O&G in 2.1582 gms dry sludge.

10 % of 0.73 gm = 0.073 gm = 73 µl Inipol EAP 22

100 µl of Inipol EAP 22 was pippeted into twenty flasks. Twenty flasks were also run as control with no surfactants added. Four flasks per set of surfactant or control were taken out of the shaking incubator on each sampling day and two flask were analysed for O&G by FTIR and the other two flask for TS, TVS, TFS.

Based on the overall results plus visual observation, as will be discussed further in the next chapter, Inipol was chosen as the surfactant to be tested in the reactor run.

5.3.6.5 TESTING THE ADDITION OF SURFACTANT TO SLUDGE OR MIXED LIQUOR

The final test carried out prior to the addition of surfactant to the reactors was to determine if Inipol should be added to the sludge first prior to placement in the reactor or whether it should be added to the reactor slurry once the reactor was started up. Since one of the reasons surfactant was selected as an amendment was to increase the oil fraction in the mixed liquor, this was selected as the criteria for determining where the surfactant was to be added. For this, two flasks in duplicates were set-up. The first flask had 3 ± 0.05 gm sludge that was mixed thoroughly with 100 ul Inipol, before twenty-five ml BBH was added to it. The second flask had sludge and BBH mixed first then Inipol was added to the slurry. Both flasks were placed on to magnetic stirrers and samples were taken out after one hour and checked for O&G using the FTIR method.

From the results it was quite clear that addition of Inipol directly to the sludge released more oil into the mixed liquor and this procedure was adopted in the surfactant amended BSTR run.

5.4 REACTOR RUNS

As stated in Chapter 4, three sets of reactor runs were planned. A Basic BSTR was to set a baseline for the lowest treatment limits based on a conventional design and without other additives other than basal salts, inoculum, nitrogen and phosphorus. These additives were predetermined based on the literature reviewed and shake flask experiments that were carried out. The Basic BSTR was to be compared to an Airlift BSTR that was designed with an improved solids mixing in mind. The third reactor run was to investigate the addition of surfactants selected to reduce agglomeration as well as increase the oil fraction in the mixed liquor fraction of the reactor.

Prior to the runs, tracer tests were carried out on the Basic BSTR and Airlift BSTR to determine different mixing conditions. From the shaker flask experiments previously, ten percent solids was already selected as optimum. However, with the airlift design, one of the prime concerns was the lift of solids. To investigate the degree of mixing affected by the percentage of solids, tracer tests were conducted at zero, five and ten percent solids to determine the difference. The air flow rate was determined at where ten percent solids could be lifted. The stirrer speed was fixed. For the Basic BSTR, ten percent solids was a fixed parameter and the variables were stirrer speed and air flow rates. For both reactors the air feed was not just to provide oxygen but also functioned as a mixing device. Table 5.11 shows the mixing conditions that were tested for both reactors.

Table 5.11 Mixing conditions tested with tracer

AIRLIFT	***% Solids***
Air only	0, 5, 10
Stir only	0, 5, 10
Both	0, 5, 10
Basic STR	***Air feed (l/min)***
stirrer speed 150 rpm	2, 4, 6
stirrer speed 250 rpm	2, 4, 6

For these tests, a known concentration of tracer (C t) was added to the reactor mixed liquor and samples were taken out at fixed time intervals and the concentration was measured (Cm). (Cm)/(C t) will give an indication of the efficiency of mixing. A value of one will show 100 percent mix with no dead spaces. Less than or more than one will show dead spaces in the reactor. Tracers were carried out using lithium chloride. Lithium was measured in the flame photometer, model Corning 410.

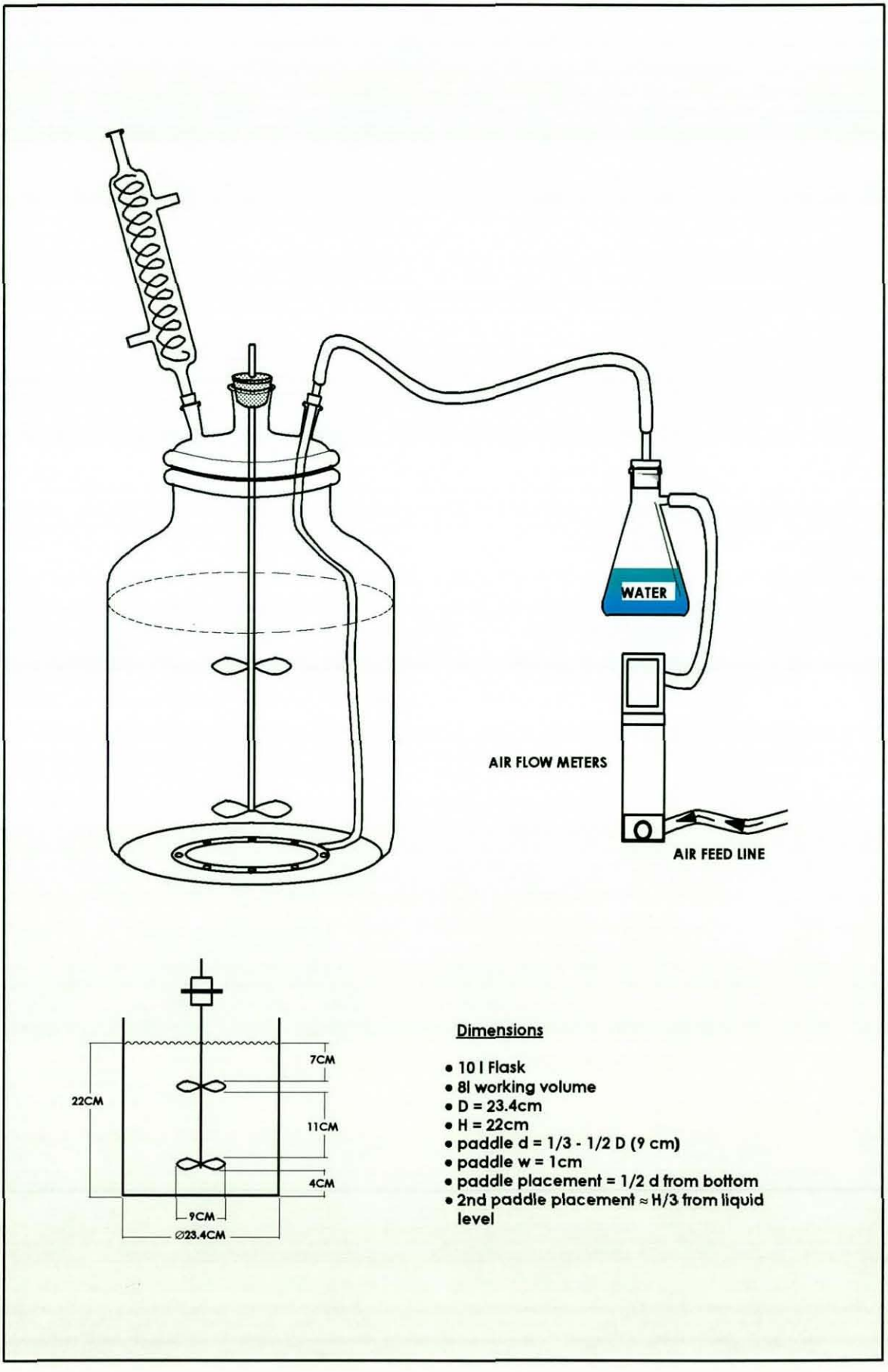
5.4.1 BASIC BSTR

The Basic BSTR was set-up using prefabricated glassware. The run was planned to be stopped when oil degradation was no longer apparent.

5.4.1.1 REACTOR CONFIGURATION

Treatment was carried out in the apparatus shown in Figure 5.1. Humidified air was sparged into the bottom of the ten l vessel (working volume eight l) at 4l /min. The reactor was stirred continuously at 250 rpm with a stainless steel paddle stirrer with a diameter (d) at 1/3 diameter of flask (D) and placed at 0.5 d from the bottom i.e. 1/2 diameter of paddle distance from the bottom. At this height, according to Talaga, (1994) energy consumption for mixing a three phase system is minimised. With this design, the stirrer being directly over the air feed line, the bubbles are dispersed fairly rapidly. However, the stirrer speed must be high enough i.e. beyond the flood point, for the bubbles to disperse. For the solids to be well mixed the speed has to be increased even further. A second paddle was placed a few cm below the surface of the liquid (11cm above the first paddle) to improve mixing in the upper layers. The paddle was placed perpendicular to the one at the bottom on the same shaft. Exit gases were condensed from the top with a cold water condenser. The condenser was also fitted with an activated carbon trap for the VOC that did not condense. The activated cabon was later extracted for O&G.

Figure 5.1 Basic BSTR



Dimensions

- 10 l Flask
- 8l working volume
- D = 23.4cm
- H = 22cm
- paddle d = 1/3 - 1/2 D (9 cm)
- paddle w = 1cm
- paddle placement = 1/2 d from bottom
- 2nd paddle placement ≈ H/3 from liquid level

5.4.1.2 BASIC BSTR RUN

O&G content and the dsf of sludge were first determined from the sieved sludge. A ten percent dry solids for the 8l working volume was then determined. N and P was then calculated based on the O&G content. Calculations are as follows;

Table 5. 12 Basic BSTR Set-up

Working volume	**8 l**
10 % solids	800 g dry solids
dsf of sludge Amount of wet sludge needed to get 800 g dry solids = (1 ÷ 0.734) x 800 ≈	0.734 1090 g wet sludge
O&G content in sludge	33% per dry weight of solids
O&G content in reactor	33 % of 800 g = 264 g O&G
Carbon (C) of reactor (90% of O&G is C)	90 % of 264 g = 237.6 g C
N at C:N 150:1	1.58 g N as NH_4NO_3 = 4.51 g
P at C:P 750:1	588 µl H_3PO_4

From the calculations, 1,090 g of sludge was weighed and placed in the reactor before 8 l of inoculated BBH media together with the added nutrients N and P were added. The BBH media was inoculated in a similar manner as that for the shake flask cultures. i.e. fifty ml sample from the chemostat grown overnight in 450 ml nutrient media then harvested and resuspended in BBH. The reactor was then started and a sample taken out for day zero after one hour of mixing, with the use of a long large bore needle syringe. Each time when samples were withdrawn, the new liquid level was marked to indicate the new operating volume and prior to each sampling this level was brought to mark by adding BBH media. Sampling was carried out at approximately five-day intervals. Parameters that were measured are as in Table 5.13.

Table 5.13 Parameters measured in the Basic BSTR

Analysis		
Mixed liquor	O&G	10 ml
	TS, TVS, TFS	10 ml
	TSS, TVSS, TFSS	5 ml
	Bacterial counts - PCA plates	
	- naphthalene plates	
	pH	
Filtered Mixed liquor	TC (boat)	
	TOC & IC (injection)	

pH was monitored and maintained at 7. On day fifty-one of the run the reactor was opened and clingage was put back in and treatment proceeded. The reactor was stopped on day sixty-five of the run when the mixed liquor no longer showed substantial oil losses. The reactor was turned off and allowed to settle before decanting the mixed liquor portion. The bottom solids were then sampled as were the clingage. Mass balance for O&G and total solids were then calculated.

5.4.2 AIRLIFT BSTR

The Airlift BSTR was fabricated in the laboratory and was made out of perspex. It was set to also run for approximately the same time as the Basic BSTR. An airlift design was selected as airlifts typically consume less energy i.e. 25-50 % of that needed in a conventional stirred tank design using turbine or surface aerators (Brox and Hanify 1989). Furthermore, airlifts can handle higher solids content. The draft tube together with the axial impellers were used to direct suction and discharge. This improved the top to bottom circulation, which is of particular value for suspension of heavy solids and for dispersion of gases (Walas 1988). There are less shearing forces in an airlift compared to a strongly stirred tank reactor which promotes the development of attached growth, such as in biofilm formation, which increases the active biomass in the reactor (Heijnen *et al.* 1992). Therefore it was hypothesised that, compared to the Basic BSTR, the Airlift BSTR would result in better mass transfer of the solids to the

degrading organisms through both better solids mixing and formation of a higher active biomass from biofilm formations.

5.4.2.1 REACTOR CONFIGURATION

The Airlift BSTR configuration is as in Figure 5.2 Internal volume capacity was approximately 4 l and the working volume was 3.7 l. Humidified air was fed from the top of the reactor, through a perspex tube with a stone diffuser at the end for better bubble distribution, at 4 l/min. from the central airlift and at 3 l/min at the other two side aerators. A hollow draft tube of 6-cm diameter, assisted the lift of the solids. The solids were lifted through the draft tube and were recirculated at the top of the reactor through holes in the draft tube. Initially, the draft tube was designed as a completely hollow cylinder and the solids were lifted over the top of the hollow cylinder. This design caused a lot of spilling and solids crept up the stirrer shaft. The draft tube was redesigned after two trial runs and the top of the draft tube was closed off and holes were instead made along the sides of the draft tube near the top for the solids to recirculate. This stopped most of the spillage and the creeping up of the solids onto the stirrer shaft. However, the airlift reactor still spilled slightly but this was more due to foaming caused by the bacterial cultures.

A condenser was also fitted for the exit gases similar to the Basic BSTR. The stainless steel paddle stirrer, diameter 9 cm was placed approximately 3 cm from the bottom. The stirrer was required to shift the solids off from the bottom of the reactor where it could then be lifted and recirculated.

5.4.2.2 AIRLIFT BSTR RUN

The Airlift BSTR was set-up in a similar manner to the Basic BSTR at 10 % solids. The contents at the start are as in Table 5.14.

The sludge was weighed and placed in the reactor through the open top. Inoculated and N and P amended BBH was then added. The top of the reactor was then screwed on tightly and the reactor started. Sampling for day zero was done an hour later. Sampling was done with the use of a long large bore needle at a fixed depth. Sampling

Figure 5.2 - Airlift BSTR

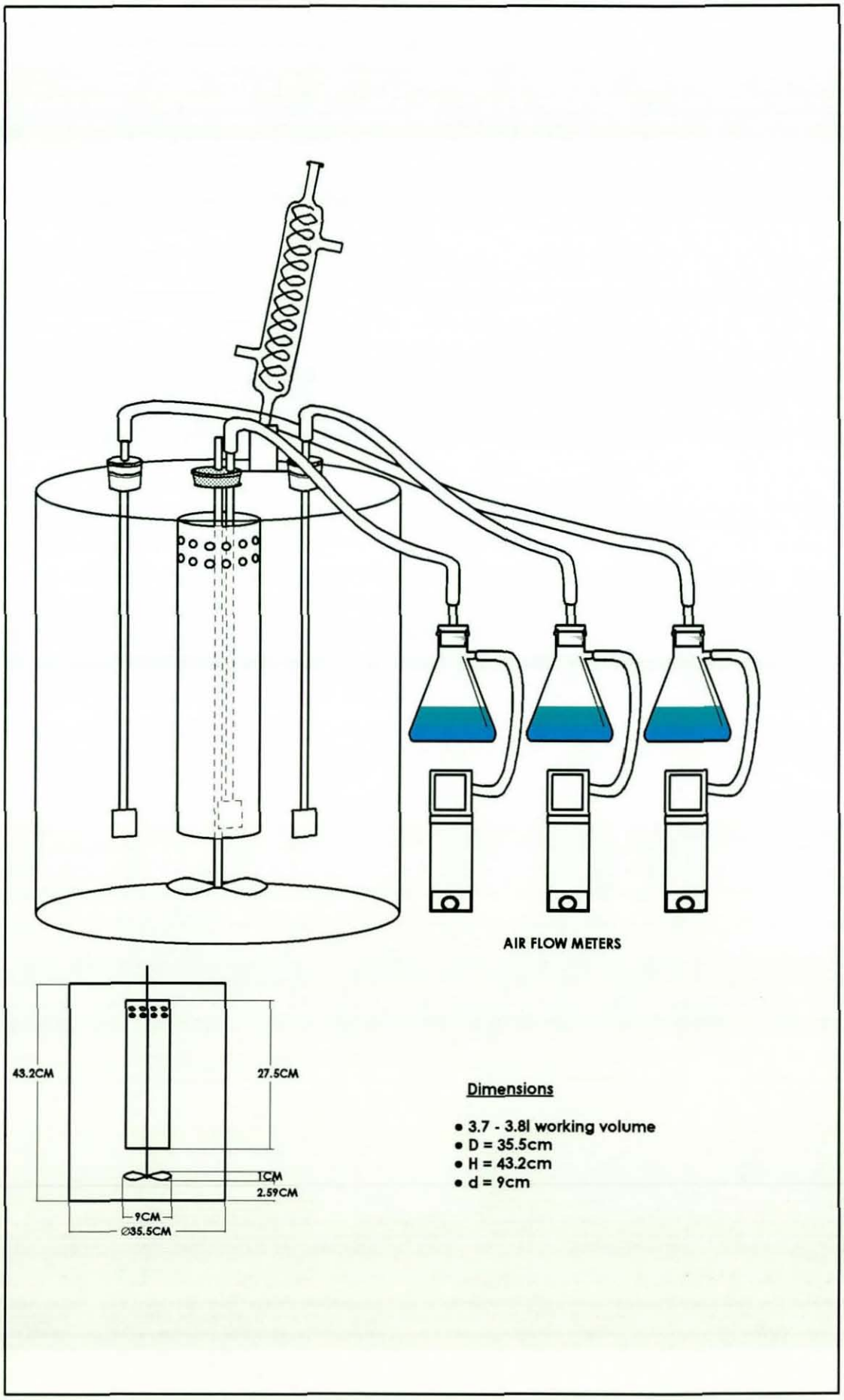


Table 5.14: Airlift BSTR set-up

Working volume	**3.7 l**
10 % solids	370 g dry solids
dsf of sludge Amount of wet sludge needed to get 370 g dry solids = (1 ÷ 0.7509) x 370 ≈	0.7509 493 g wet sludge
O&G content in sludge	33.73% per dry weight of solids
O&G content in reactor	33 % of 493 g = 124.36 g O&G
Carbon (C) of reactor (90% of O&G is C)	90 % of 124 ≈ 112 g C
N at C:N 150:1	0.7461.58 g N as NH_4NO_3 = 2.13 g
P at C:P 750:1	270 µl H_3PO_4

was always carried out at the fixed depth in order to reduce the difference between samples that could possibly come from stratification of layers in the Airlift BSTR. Sampling parameters were the same as the Basic BSTR but with the volumes reduced on a few parameters as the total working volume in this reactor was smaller. Sampling was carried out at approximately five day intervals. Similar to the Basic BSTR everytime a sample was taken out, the reactor volume was marked and before each sampling the reactor volume was made up to the mark with BBH. The air feed lines were passed through a moisture filled chamber prior to entering the airlift, but some mixed liquor still vaporised. The Airlift BSTR was run for sixty-eight days. Due to one spill on the sixteenth day the reactor was stopped and restarted on day nineteenth with more sludge and BBH added. For this, the bottom solids as well as the mixed liquor were taken out and analysed for a second baseline data set, before further additions. On day forty-three, the reactor was opened and clingage was put back into the mixed liquor phase. At the end of the run, the reactor was stopped, allowed to settle and the clingage and mixed liquor were sampled. The reactor was the decanted and the bottom solids sampled. A mass balance for O&G and total solids were then calculated.

5.4.3 SURFACTANT AMENDED BASIC BSTR

The surfactant amended BSTR was set exactly as in the first reactor run with the addition of ten percent (based on O&G weight) Inipol. Surfactants were thought to be

able to improve rates of oil degradation through several means: increase solubility, increase dissolution rate, assist attachment of bacteria to oil (Refer to Chapter 3). Slurry treatments reduce intraparticle mass transfer limitations but with insoluble substrates the mass transfer from the solid or sorbed phase to the aqueous phase might be rate limiting (Volkering 1992;Zhang *et al.* 1995;Irvine and Earley 1993). Based on an earlier test the surfactant was added to the sludge prior to the sludge being placed in the reactor. Midway through the run, on day twenty-four, Inipol was again added. Sampling procedures and protocols were the same as in the Basic BSTR. Clingage was put back on day forty-eight. The reactor was stopped on day sixty-two, and the contents were settled then sampled. A mass balance for O&G and total solids were then calculated.

CHAPTER 6
RESULTS AND DISCUSSION

6.1 INTRODUCTION

The results of the experiments carried out as delineated in Chapter 5 are presented in the order of reference in Chapter 4, except for 'O&G determination in the presence surfactant' which is presented together with the 'Selection of surfactant experiment'.

6.2 ANALYTICAL METHODS

Only the results for the methods developed during the course of this research for: O&G determination; oil degrader plate counts ; and, PAH by HPLC are presented and discussed.

6.2.1 EXPERIMENT A - O&G DETERMINATION

After reviewing the standard methods and other research literature associated with O&G and PAH analysisit was concluded that the choice of solvent was important as it determined the extractable amount as well as class of substrate. The choices were narrowed down to two solvents, DCM and TCTFE. Carbon tetrachloride, which was a regularly used solvent for these determinations, was deemed as too hazardous and was a controlled item. With the chosen solvents it was assumed that apart from undegraded O&G, partially broken down metabolites such as fatty acids would also be extracted, thereby the oil loss is underestimated. However since PAH analysis was to follow, it was felt that the two solvents could exhaustively extract these compounds into solution. The first experiment was then to check, which of the two would extract the higher amount of undegraded, and partially degraded substrate.

6.2.1.1 SLUDGE SAMPLES

Method SW 846–3540 which is similar to APHA Method 5520E, Soxhlet extraction for

sludge samples, were employed for this test. The samples were extracted for four hours at six cycles/hr (300-ml extractor). Sludge samples weighed between fifteen and seventeen gm wet weight. The dsf was determined at 0.7546. Percentage O&G was determined per dry solids. Calculations was as follows for percentage O&G;

[Recovered O&G (g) ÷ (Wet weight sludge (g) x dsf)] x 100 = % O&G per dry solids

The results are tabulated in Table 6.1

Table 6.1 Percentage O&G extracted by solvent

No	solvent	% O&G (per dry solids)	Average	± diff.
1	TCTFE	31.66		1.8
2	TCTFE	29.73		0.13
3	TCTFE	29.05		0.81
4	TCTFE	29.00	*29.86	0.86
5	DCM	33.43		0.45
6	DCM	35.30		1.42
7	DCM	34.90		1.02
8	DCM	31.87	**33.88	2.01

* δ = 1.24 %

**δ = 1.56 %

Based on the average values, DCM gave higher recoveries and the extracts were darker in color. The fact that DCM has a lower boiling point than TCTFE was also considered in selecting DCM as the solvent for O&G determinations.

Comparing methods *SW-846-3540 to APHA Method 5520E*, the length of extraction varied between four hours and sixteen hours. To determine if there were appreciable differences between using a shorter extraction time and 16 hours, the Soxhlet extraction was carried out for both lengths of time. O&G analysis was to serve as the main parameter determining biodegradation and as such it would be much used throughout the research and therefore has to be a practicable option. Results were as follows. The set up was the same as previously but this time the soil was spiked with crude oil at between 6 -11 % dry weight of soils and recovery was determined based upon recovery of the spike as a percentage of initial spike weight.

Table 6.2 Percentage recovery of spiked crude oil at four hours and sixteen hours' Soxhlet extraction with DCM

NO.	HRS OF EXTRACTION	% RECOVERED	AVERAGE	DIFF. ±
1	4	66.66	65.66	1
2	4	64.66		1
3	16	64.4	64.4	0
4	16	64.4		0

Although there was a slight difference, the difference cannot be due to length of extraction as the four-hour set up extracted marginally higher amounts. Therefore it is assumed that there is no difference between four hours and sixteen hours of extraction and that an average of 65.03 % recovery (average recovery of the 4 samples) can be expected from using crude oil as spike from the above standard method (APHA Method 5520E with DCM as solvent instead of TCTFE). For all the sludge analysis that followed, Soxhlet extraction was carried out for eight hours.

It is also noted that Soxhlet extraction for soil samples spiked with crude oil gave similar recoveries as liquid-liquid extraction of water samples spiked with crude oil i.e 65.03 % for soil and 66.71 % for water. As mentioned in the literature review, oil extraction can be compromised by the complexity of the matrix. However, the above results in Table 6.2 and Table 6.3 show that recoveries were similar, which would indicate that the method used for extraction might be more important than the matrix.

6.2.1.2 MIXED LIQUOR

Mixed liquor samples were extracted by shaking the mixed liquor with DCM in a 100 ml Duran bottle in an orbital shaker. As previously mentioned, O&G analysis would be used extensively throughout this research and the practical application of a chosen method was very important to facilitate progress. The overall analytical requirements from the research was very intense and in order to carry out sufficient tests, further simplification of the method was required. For this, the standard method in APHA Method 5520 B, the 'three times extraction in a separatory funnel' was substituted with shaking overnight in an orbital shaker.

0.2 ml topped crude oil was spiked into twenty ml of water and extracted for twelve

hours in the orbital shaker with twenty-five ml of DCM. Recoveries of spiked crude oil samples were equivalent as in Soxhlet extraction as shown below in Table 6.3. Recovery of solvent was maximally about 80 %, accounting for the solubility of DCM in water. When DCM extracted O&G was used as spike material in mixed liquor, recovery was 85.55 % (Table 6.4). For this 0.3 - 0.4 g of DCM extracted O&G from the original sludge was spiked into twenty ml of water and extracted for twelve hours in the orbital shaker with twenty-five ml of DCM.

Table 6.3 Recovery of spiked crude oil

WT OF 0.2 ML CRUDE OIL (G)	RECOVERY	% RECOVERED
0.1748	0.1166	66.7
0.1787	0.1185	66.31
0.1770	0.1188	67.11
average		66.71
δ		0.4

Table 6.4 Recovery of DCM extracted O&G

SPIKE	RECOVERED	% RECOVERED
0.3604	0.3061	85
0.3522	0.2977	84.55
0.3048	0.2654	87.1
	average	85.55
	δ	1.3

All raw data to the above derivations are in *Appendix J,* p 267.

6.2.2 EXPERIMENT B - OIL DEGRADER PLATE COUNT METHOD

Methods for counting microbial oil degraders were selected from literature and applied. However the results were unsatisfactory. Outlined in Chapter 5 and the literature review in Chapter 2 were the references for the applied methods. Following were some of the results and observations made that led to further adaptations in the oil degrader plate count method.

Spread plate oil agar plates – results from the experiment 15 % and 20 % solids loadings using this method were irregular and could not be used to be representative of

hydrocarbon degraders. The counts varied between 68 and 122 for 1 dilution and on some days there were no growth recorded. The control plates used at this stage was non purified agar and gave rise to between 1 – 5 colonies on the BBH agar without oil. Spreaders were also common, possibly due to the oil sitting on top of the agar.

Pour plate oil agar - This method was not found from the literature. It was an adaptation of the above method in response to the spreaders as well as the 'no growth'. It was thought that the oil sheen could be doing both, promoting the swarmers when it was at the right concentration and not toxic and preventing growth when it was too high. Using this method produced more reproducibility in the counts as the dilution showed a proportionality increase/decrease in the counts according to dilution. However, there were still spreaders, possibly from the colonies that were growing on top of the agar. Crude oil as a substrate had a lot of drawbacks. Besides the above problems, crude oil made the background very dark and the colonies in the pour plate hard to read.

Naphthalene vapour pour plate method - From a few of the literature reviews, naphthalene crystals were commonly used in the cover of inverted petri dishes. This method was adapted by dissolving naphthalene in some solvent (ethanol) and emersing filter paper to soak the naphthalene solution which was then lifted and air dried for a few brief moment before placing it in the cover of the pour plate. This method yielded satisfactory results. However on one occasion there was no growth possibly due to not enough naphthalene in the soaking solution. The same solution was used to soak a number of filters. This method could be developed further with more controls put into the 'soaking' stage and 'placing in the covers' stage.

Naphthalene pour plate method – After reviewing a paper by Bogardt and Hemmingsen, (1992) another technique was tried. Bogardt and Hemmingsen used a more complicated procedure whereby, they first pre-prepared a vitamin enriched mineral salts agar underlayer then the agar with the dilution and substrate was plated over. In an effort to simplify, BBH media, (as detailed in section 5.1) was used to which the dissolved naphthalene was first added, cooled then the dilution added which was then poured onto the plate. It was found, when the method was first tested as laid out by Bogardt and Hemmingsen, due to the small amount of agar to be poured into the half filled plate, the agar comes close to solidifying. Although it is realized that could be overcome with the use of water baths, the whole process of plating becomes cumbersome as it was carried out on a laminar air flow cupboard which had space restrictions. The adapted method yielded results that were useful in determining

hydrocarbon degraders. The colonies were small but distinct. After one week experienced personnel could already read the plates. However, the plates were easily read at the end of two weeks as the naphthalene crystals were appreciably smaller and the colonies larger.

This method was used for all the reactor runs. Also as pointed out in Chapter 5, the use of purified agar discarded the need for controls grown on BBH without oil as the controls were always zero counts. Finally, controls were run on naphthalene plates with the dilution water to check for contamination.

The raw data showing each count of the triplicates that were done per dilution can be seen in *Appendix M (p. 307) and N* (p. 311).

6.2.3 EXPERIMENTS C - HPLC DETERMINATIONS FOR THE 16 PAH

A more definitive means for determining biodegradation was the monitoring of PAH compounds and HPLC was chosen as the analytical method for reasons mentioned in the literature review section. In this following discussion, referred chromatograms are either in the *Appendix* J (p. 271, 272, 273, 277, 278, 279 & 280) or *Appendix* O of this thesis. Due to updating the HP Chemstation software, some of the earlier chromatograms could not be reproduced from the saved diskette files as the program could no longer read the earlier chromatograms. As such, the hard copies initially produced during method development, have been photocopied and compiled in *Appendix* O.

Standard methods from SW 846 and APHA as well as British Gas in-house methods were used as starting points in developing the separation and identification method. Initially, only UV–visible absorption spectrometry was available for identifying PAH. The elution gradient and solvents as recommended by the above standard methods were applied with relatively good results for separation of a mixed PAH standard detected by UV-visible absorption. Adjustments were made to optimize the efficiency of the system by determining effects of flow rate changes and column temperature. The determination of an optimum flow rate as well as column temperature was effective at eluting peaks earlier as well as improving the resolution of the peaks. The first column that was used during these determinations was the Vydac column which had good selectivity such that all the sixteen peaks were well resolved. The temperature selected was 35 ^{0} C and flow rate was at 0.8 l/m. With the other columns the temperature was adjusted to 38 ^{0} C and

flow varied from 1 -2 l/m depending on column.

With the Vydac column, runs using Standard PAH solutions were well separated and could be integrated easily from the baseline (Chromatogram 0024 & 0013 in *Appendix* O). However, late eluting peaks had some interference from ACN and pure water (blank run) which were being absorbed at the same elution times (Chromatogram 0016 in *Appendix* O).

In an effort to improve the sensitivity of the detector, the variable wavelength detector was programmed to monitor different wavelengths at different times during the chromatographic process to ensure that the peaks were monitored at close to their maximum absorbance wavelengths. Some of the chromatograms produced during the wavelength changes (e.g. Chromatogram 0327 & 0316) and its effects on standard PAH peaks as well as 'sample 5' are in *Appendix* O. In the standard PAH mix, there were a limited number of components to resolve and the higher wavelengths enhanced peak detection, however in the samples where a large number of peaks had to be resolved, the late eluting peaks became very reduced in height when wavelengths were switched. Switching the wavelengths affected the sensitivity possibly due to more light being scattered and lost. When a fixed wavelength was used the peaks were higher, i.e. there was more sensitivity in the detection. The baseline was still drifting upwards due to the solvent gradient but the peaks were coming off the humped baseline and could possibly be integrated manually using the Chemstation software. With some low PAH samples, peak attenuation had to be increased to resolve the peaks and this caused the baseline to become even more 'humped' and thus, integration was a more difficult task. Hard copies of some of the chromatograms produced in determining the application of the UV-Visible detector time table are in *Appendix* O.

Fluorescence spectroscopy is 1000 to 10,000 times more sensitive than UV-visible spectroscopy (Phar *et al.* 1992) and since there was difficulty in identifying PAH due to the low PAH quantities, a fluorescence detector was purchased to try and improve the identification of the PAH peaks. For this, a new time table for fluorescence detection had to be developed. Based on earlier UV visible absorption chromatograms and identified PAH peaks, the λem and λex was programmed for optimal peak resolution (sharp and differentiated peaks). Several runs had to be carried out in order to determine switching wavelenghts did not occur during a peak elution time which would result in the baseline 'jumping upwards'. The chromatograms produced during this selection process are in *Appendix* O.

In testing if the detector's timetable and gradient elution program was good enough for identifying PAH peaks from a real sample, a sample that was spiked 50:50 with a standard PAH mixture of 16 compounds was used (the sample was named *sample 5* in the chromatograms). The chromatogram of sample 5 was compared to Standard PAH chromatogram as well as the unadulterated sample (Chromatograms 0330, 0062, 0063, 0066, 0067, 0068, 0069 in *Appendix* O). This enabled the identification of the PAHs from the sample as well as determined other interfering PAH. Due to having the fluorescence detector, connected in series to the UV-Visible detector, the UV-Visible detector was monitored at a fixed wavelength at 254nm.

Other steps taken to check the method during development were;
determining the repeatability of a sample recovery through Empore extraction discs by doing a triplicate from a single sample point through the Empore discs;
determining the recovery of different samples through filter syringe with the use of a spike;
checking the linearity of UV-visible absorption spectrometry by injecting the standard PAH mixture at 500, 1000, and 2000 µg/l;
checking the reproducibility of RT and peak areas between injections by using duplicates;
determining the lower limits of detection of PAH through Empore disks with fluorescence detection.

Triplicate analysis of a day forty-three Airlift BSTR sample to check the repeatability of the recovery of a sample through the Empore extraction disk revealed that RT was hardly variable at differences of 0.03 or less as seen from RT of spike decafluorobiphenyl (Refer to spreadsheet together with Chromatograms 200, 201 and 202 in *Appendix* O). The integrated areas under the peak, representing quantities of PAH, on the other hand were more variable depending on the peak. It would seem, by looking at the integrated results, some peaks were more reproducible than others. However, if the chromatograms were compared, there is very little variability. The variability is due to the difficulty of integrating the peak areas to an irregular baseline. The baseline is not flat and some peaks come off at a tangent as well. The program in the Chemstation software has several parameters that can be manipulated to allow for integrating each chromatogram differently. However, there were still limitations as to how it could be manipulated to integrate all peaks from the start of the peak base to the bottom at where it ends. Furthermore, the true baseline may actually be curved rather than a straight line from valley to valley when peaks are eluted off a sloping baseline

(Weston and Brown 1997) which cannot be drawn by the Chemstation software.

From the triplicate analysis of the day forty-three sample, the results showed that the spike was recovered in similar quantities. Approximately 19.64 % of the spike was recovered which was initially thought to be representative of the recovery of the samples. However, when a sample of 16 standard PAH compounds at 12.5 and 25 ug/l and spike at 40,000 ug/l was processed through the Empore extraction disks to determine minimum limits detected by HPLC using Empore for sample clean-up, what became clear was the spike was preferentially retained in the Empore disks. The other PAHs were also differentially retained however not to the extent of the spike as can be seen in the summary spreadsheet for Minimum Detection Levels in *Appendix O.* Unfortunately, at this stage all the samples were already processed. In order to change the spike for use as an internal calibration standard, more investigations using the Empore disks, which were expensive, would be required. Although preliminary investigations of decafluorobiphenyl indicated it would be a good spike choice as it was well differentiated from the other peaks, (chromatograms 0088 and 0089) and it was eluted in a similar manner to the other PAHs of interests, once it was processed through the C-18 bonded silica, the spike became preferentially retained and its use as an internal standard was no longer applicable. However, since the RT was reproducible, the spike was used to measure the degree of drift in the elution times of early eluting peaks.

A fluorescence detector was used for detecting the much lower PAH quantities found in the samples from the reactor runs. As seen from the calibration spreadsheet in *Appendix J* (p. 274) there was less difference in areas recorded for PAH at 500 μg/l quantities than at 1000 μg/l quantities indicating reproducibility is better at the lower PAH quantities. For each column, the standard PAH mixture solution was used to identify RT of the individual peaks and a few single PAHs were used when there was uncertainty in the elution order when the column was changed. Standard PAH mixture at 500 mg/l and 1000 mg/l were used for calibration for fluorescence detection. The spreadsheet are in *Appendix* J (p. 274- 280) together with sample chromatograms. Other chromatograms used in PAH calibration are in *Appendix* O.

Reproducibilty of an injection was also determined as can be seen from the duplicates for the standards. A duplicate of injection for sample processing through the injection filter can also be seen in chromatograms 187 and 190 in *Appendix* O.

For regular maintenance, the analytical equipment were checked for system integrity by doing duplicate injections of the standard PAH mixture to determine peak areas, RT and chromatogram pattern. Single PAH compounds were also regularly used to place RT drifts. Some of the problems that were detected included injection port leaks detected through the loss of peak areas, column blockage with peaks eluting later, dirty frits with irregular chromatograms etc. The HPLC manual contained a guide that was used in troubleshooting.

6.3 PRELIMINARY EXPERIMENTS

Following are the results to the shake flask culture experiments determining the conditions for the reactor runs. Unless otherwise stated, the gyratory orbital shaker was run at 160 rpm and 37 ^{0}C.

6.3.1 EXPERIMENT D - MICROBIAL INOCULA SOURCE

Experiment D, which was carried out to determine a source for the microbial inocula yielded some very interesting results. The cultures were sampled from; activated sludge from a treatment plant, designated AS; oily sludge, designated OS; and from a landfarm treating oily sludges, designated LF. With reference to the Table 5.4 for the set up combinations for the growth media, Figure 6.1 shows the TVSS content, taken to represent bacterial mass, of the four cultures at the start for up to about five days. As can be immediately seen, there is a boost in the numbers for the cultures grown in nutrient broth. Turbidity readings of the 10^{-1} dilutions and MPN tubes for dilutions 1×10^{-8} to 1×10^{-13} carried out for the day zero cultures also showed a difference as can be seen in Table 6.5. LFBBHO which served as a control for cultures not exposed to a rich media, yielded the lowest numbers of microorganisms from the start to finish. LFBBHO cultures also served as a control to the LFN cultures to determine if prolonged growth in an oil-less media (LFN) would affect their capacity to degrade hydrocarbons. This result is discussed later.

Table 6.5: Bacterial mass at start of experiment

CULTURES	10^{-1} DILUTION MPN	TURBIDITY
LFBBHO	1×10^{9}	65 NTU
LFN	1×10^{10}	69.5 NTU
ASN	1×10^{11}	87 NTU
OSN	1×10^{11}	90 NTU

Figure 6.1 : Growth pattern of LFBBHO,LFN,ASN,OSN cultures

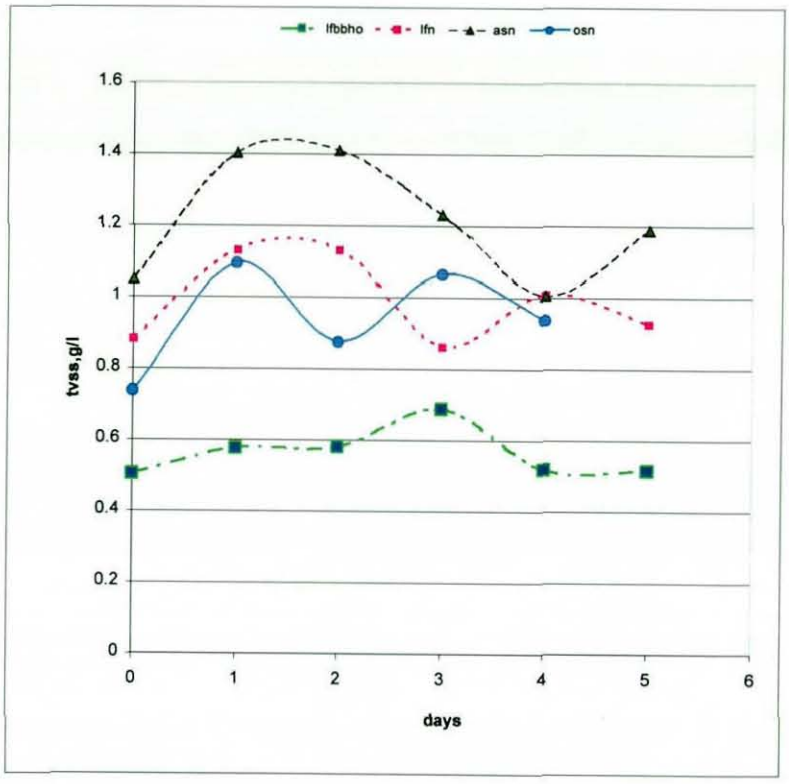
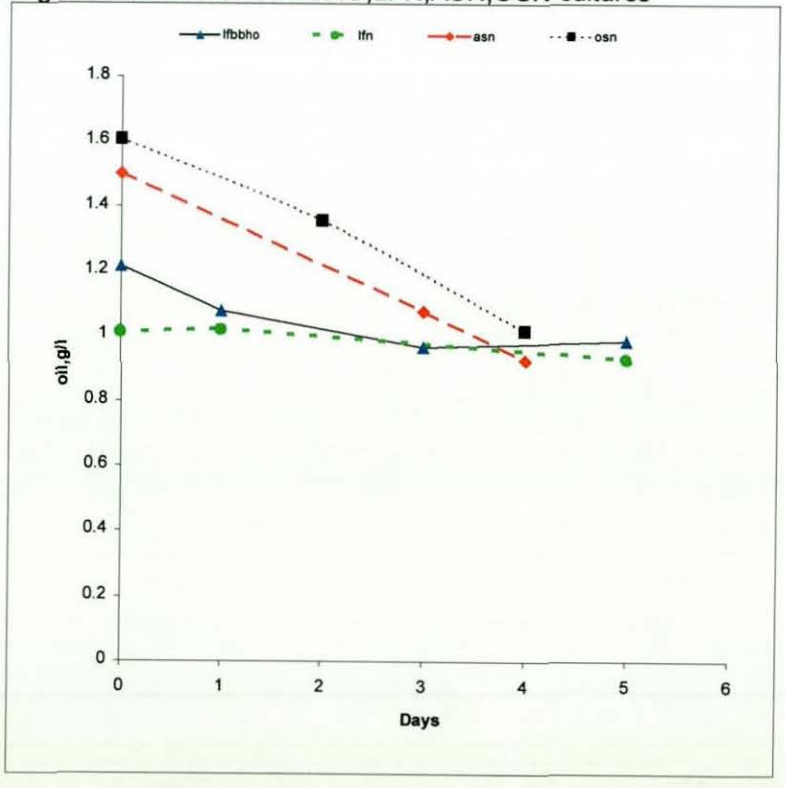


Fig 6.2 : Oil loss for LFBBHO,LFN,ASN,OSN cultures



Another observation is the pattern of growth for the cultures. They all exhibit, to different degrees, a diauxie (biphasic) growth pattern with first a sharp increase then followed with a drop in growth rate followed by another growth spurt. The first spurt of growth could be due to growth on easily metabolizable substrates present at the start. When these substrate were no longer available, growth declined. At the same time, some cells could be producing extracellular products to enable the utilization of the remaining substrates and thus another spurt of growth occurs when these compounds were formed. An example of this would be the production of emulsifying compounds which increase the fraction of hydrocarbons in the aqueous phase, or the induction of specific enzymes as required to further breakdown the hydrocarbon chain. Another explanation could be that the death of some of the susceptible organisms provided more easily metabolizable products, which resulted in the second spurt in growth. This diauxie growth pattern will be discussed further in section 6.2.4. Another observation from Figure 6.1 is that the growth curves do not show a lag period at the start.

Figure 6.2 shows the rate of oil loss for the four sets, LFBBHO, LFN, ASN, and OSN cultures. There is a difference between the rates shown by the OS and AS cultures compared to the LF cultures as seen from the slope of the lines. This possibly indicate that the source of culture (being induced) is a far more important criteria for degradation rates than the higher numbers at the start. The rate, as seen from the slope, is steepest for OSN with ASN next . The other two cultures have much lower rates. From Figure 6.2, it is obvious that the LF cultures do not provide a good culture source for the degradation of oil. In LFN cultures, which had higher numbers throughout, there were lower degradation rates. LFN cultures 'lost' some of their potential capacity to degrade hydrocarbons when grown for prolonged periods in an oil-less media. This may mean that 'source of cultures' (microbial culture mix) is a far more important criteria for oil degradation rates than cell numbers at the start. Guerin and Boyd (1995) found that adaptation period for the degradation of naphthalene differed significantly for a *Pseudomonad* sp and an *Alcaligenes* sp when grown on non-selective media (nutrient) first. The induction of naphthalene degrading activity was rapidly induced to high levels in the *Pseudomonad* culture (15 min) while *Alcaligenes sp* had long lag (adaptation) period of several hours. The maintenance of naphthalene utilization activity in the *Alcaligenes* sp. depended on being induced by the presence of the substrate or its metabolites. While their research cannot be directly correlated to the results of Experiment D it does show that the organisms involved in the degradative process have properties that affect induction and maintenance of substrate utilization. This could explain why the LFBBHO cultures exhibited slightly higher oil degradation rates

compared to LFN as it could be that the cultures require constant exposure to the substrate in order to remain induced toward its utilization. In this experiment, 'source' is essentially the microbial consortia that was used whereby there could be a dominating species directly involved in the degradation process and therefore the organism would be 'dominating' the culture's properties.

The source of culture, or microbial consortia, is an important factor as this determines how well adapted the cultures are towards degrading hydrocarbon compounds. An environment that has a long history of hydrocarbon contamination provides a selective advantage to hydrocarbon-utilizing bacteria and these organisms become 'selectively enriched'. This is based on the concept of "microbial infallibility" first introduced by Alexander (1965). However, all the growth curves as seen in Figure 6.1 did not show a lag phase that would be indicative of 'adaptation'. Again this could be due to the utilization of soluble hydrocarbon compounds at the start for which induction was rapid, however, upon depletion of this ready assimilable substrate, the organisms had to 'adapt' to utilize the remaining fractions resulting in the observed diauxie or biphasic curve. It is concluded that all the cultures were adapted (possibly to various degrees) but they had intrinsic properties that were related to the culture source that affected the rate of degradation. Conjugal transfers of plasmids with xenobiotic degradative genes among a microbial population has been suggested as one method by which populations become adapted and thus the expression of a dominating characteristic from a microbial population is a possibility (Leahy and Colwell 1990; Shailubhai 1986).

From Figure 6.1, ASN cultures gave the highest bacterial mass while in Figure 6.2 OSN gave the best oil degradation rates with ASN cultures giving slightly lower rates but of the same magnitude. The population size may not have made a big impact in this experiment as all the cultures were relatively dense (10^9 and 10^{11}) at the start and may already meet the critical population size, first referred to by Corseuil and Weber (1994) and Weber and Corseuil (1994), required to achieve good rates and therefore the boost in numbers did not become a critical factor in achieving high degradation rates.

From the experimental results, it would seem that ASN or OSN cultures would be a good choice for the microbial inocula as they gave high oil degradation rates as well as bacterial growth. OSN cultures have been residing in the oily sludge and are probably well adapted to utilize hydrocarbons as substrate. ASN cultures also gave good rates which may be due to the cultures constant exposure to oil in wastewater as well as being hardy; possibly fairly used to shock loads of toxic compounds present in

wastewater. LF cultures grown in nutrient broth or BBHO showed lower rates. Although LF cultures would have been expected to have high rates as they have been previously exposed to hydrocarbons, not enough information on the length of exposure and the conditions of the landfarm were available for evaluation. Possibly, the land has been allowed to be fallow for quite sometime as is commonly practiced in landfarming (Bartha and Bossert 1984) and therefore the hydrocarbons concentrations may have been too low to keep the cultures induced.

Visual observations of the four cultures in the BBHO media during the experimental run as well as observations made during analysis gave further insight which were used in the interpretation of results. As can be seen from Figure 6.3: photoplate of the three cultures (AS, LF and OS in BBHO), taken during the experimental run, the oil layer in AS remains as a floating oil layer whereas oil in the other two have 'dissolved' to various degrees into solution as seen from less oil on the surface and the darker water column. This could be the result of biosurfactant production in OS and LF cultures.

During O&G extraction on day zero of the experiment, it was also observed that the AS cultures were very emulsified which hampered the solvent recovery process. It was observed that it was difficult to separate the solvent and oil from the water fraction and some moisture was breaking through the phase separator paper. This was probably due to either the presence of surfactant affecting the property of the phase separator paper or the longer residence time of the emulsion in the filter paper caused the water to break through. The O&G content may then be overestimated at the start resulting in what is seen as high rates of degradation due to the bigger difference in weight. It is interesting to note that the extraction step in the AS cultures produced a strong colloidal layer on day zero. This property may be indicative of the presence of surfactants but the oil layer, as seen in photo plate 6.3.1, did not disperse into the water column as compared to OS and LF cultures. The possibility of both cultures producing surfactants but of different natures cannot be ruled out.

In order to ensure the use of a consortium of bacteria that could achieve high rates as well as possibly reducing the partitioning effect of oil, it was decided that cultures from OS and AS would be mixed and cultured in a chemostat-like set-up primed with topped crude oil. The cultures in AS provided hardy and adapted cultures and the OS cultures were already primed towards utilizing sludge and possibly, both cultures were producing surfactant compounds, which would be investigated at a later stage. For each

Figure 6.3 Photoplate 1 - From right to left, cultures of AS, AS, LF and OS and
Photoplate 2 - From right to left, cultures of AS, LF and OS

consequent experiment, 50 ml of the chemostat culture were added to 450 ml nutrient media overnight that was then centrifuged and resuspended in BBH. The cultures were not grown for 10 (5+5) consecutive days in nutrient media as was carried out for the LFN cultures in Experiment D, but nutrient media was used to boost the numbers overnight only. The adoption of this procedure for all other experiments was verified by the next experiment which determined priming requirements for the microbial consortia. The raw data collected from the Experiment D, which were used to generate the graphs in this section, are in *Appendix J* (p. 281-284).

6.3.2 EXPERIMENT E - PRIMED VS UNPRIMED CULTURES

As outlined in Chapter 5, after determining a source for the inoculum, another experiment was set up to determine the effect of 'priming' the chosen microbiological consortia on crude oil. As seen in the results of Experiment D, there is inference that cultures can loose their adaptation to utilize a particular substrate if not constantly exposed to it in the environment. This was variable depending on the culture source. While the OS and AS cultures grew well on the oil even when it was grown on non selective media first, LF did not. In order to confirm the mixed cultures response to the procedure of boosting cell numbers with the use of a rich media over a several day period, Experiment E, as detailed in Chapter 5, was set up.

In order to see the effects of 'priming' and its role in adaptation and the subsequent effect on substrate uptake, the mixed cultures from Experiment D were grown successively on different media for six weeks then isolated and washed and put through another oil biodegradation experiment. The culture that was repeatedly transferred to nutrient media was named culture A while the one successively transferred to BBHO was named culture B. At the end of six weeks the cultures were transferred to fresh BBHO and the growth of the two cultures were compared as well as their activity as measured by DO. The results are as in Figure 6.4 and 6.5. Culture B showed higher mass when grown in minimal media with oil as the only substrate while culture A showed lower mass throughout and a lag period at the start. Mass of the cultures is taken to be directly correlated with substrate utilization, as only oil was present as substrate. The MPN at the end of five days were for culture A 10^{9} and culture B 10^{10}. Activity was measured by the DOUR, which was taken on day two and is shown in Figure 6.5. Graphs of the activity show less of a difference but culture B still show higher rate as seen in the higher gradient. Since increase in cell numbers is due to

Figure 6.4 : Growth of culture A and culture B

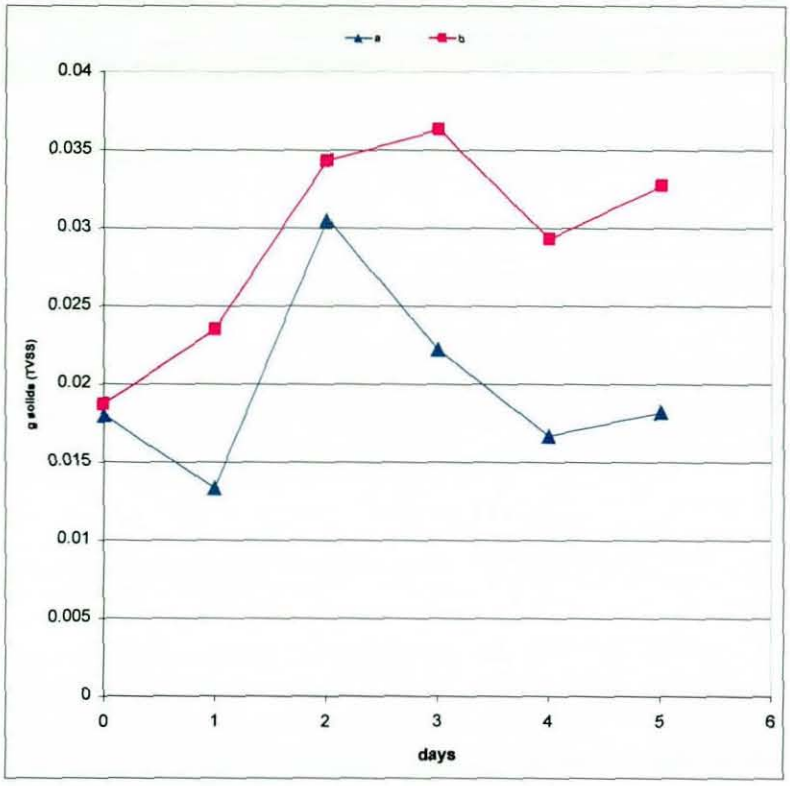
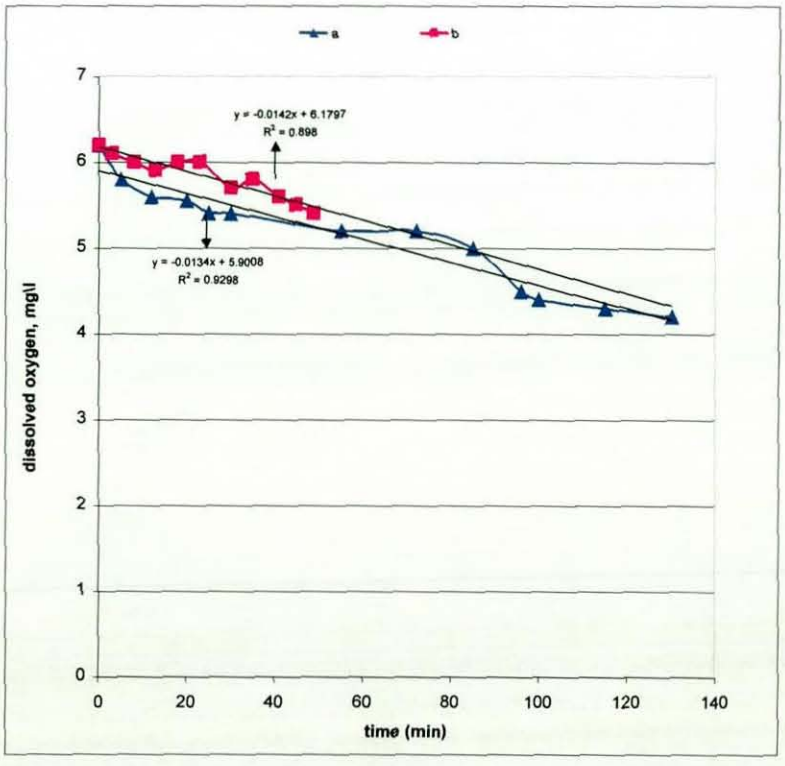


Figure 6.5 : DO consumption of culture A and culture B



better substrate utilization rates[1], priming cultures on crude oil can be seen to have a substantial effect on growth. While not disregarding the fact that the use of a rich media to boost bacterial numbers at start will have a substantial effect on substrate utilization rate, the procedure was not adopted as it can be seen from the results of this experiment that the numbers were higher from cultures primed with oil. However, nutrient media was used for a short period of time before the cultures were resuspended into BBH. Twelve to fifteen hours were felt to be adequate for using the rich media for increasing cell mass without compromising the cultures adaptation.

The results from culture B were then used to calculate the dilution rate for the chemostat as shown in *Appendix H* (p. 265). As mentioned in Chapter 5 the microbial consortia from Experiment D were initially kept in the orbital shaker. When the cultures were transferred to the chemostat, it was allowed to equilibrate for a week then fifty ml of chemostat content was taken out to confirm the cultures growth was proceeding as it should be. The sample was taken and processed through the same procedure carried out at the start of all experiments i.e. added to 450 ml nutrient media, grown overnight, harvested and washed and resuspended into fresh BBH. For this experiment twenty ml of the cultures were resuspended into thirty-six 250 ml Erlenmeyer flasks to which topped crude oil was added at one percent volume/volume. This is for a whole flask analysis (whereby the contents of the whole flask is used for one data point) for TSS and O&G in triplicates for five days, including day zero. Another series of flasks were also set up for the measurement of DOUR for the five day run. This experiment was run in the orbital shaker and the result is as shown in the growth curve and oil loss in Figure 6.6 and the DOUR in Figure 6.7. As can be seen, the growth was rapid initially, with no lag period then there was a reduction in biomass momentarily then another increase with a smaller gradient. The kinetic coefficient for oil loss is 0.158 day^{-1} based on a linear regression of the oil remaining. The results are fairly similar as that obtained for ASN and OSN cultures previously. The DOUR is maximum on day 1. The results were not analyzed at this stage for first order reaction rates since these preliminary runs were short with minimal analysis. The raw data used in generating the graphs are in *Appendix J* (p 285).

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[1] $Y = dx/ds$

where; Y is the growth yield , x and s are biomass and substrate concentration

Figure 6.6: Growth of mixed culture and oil degradation

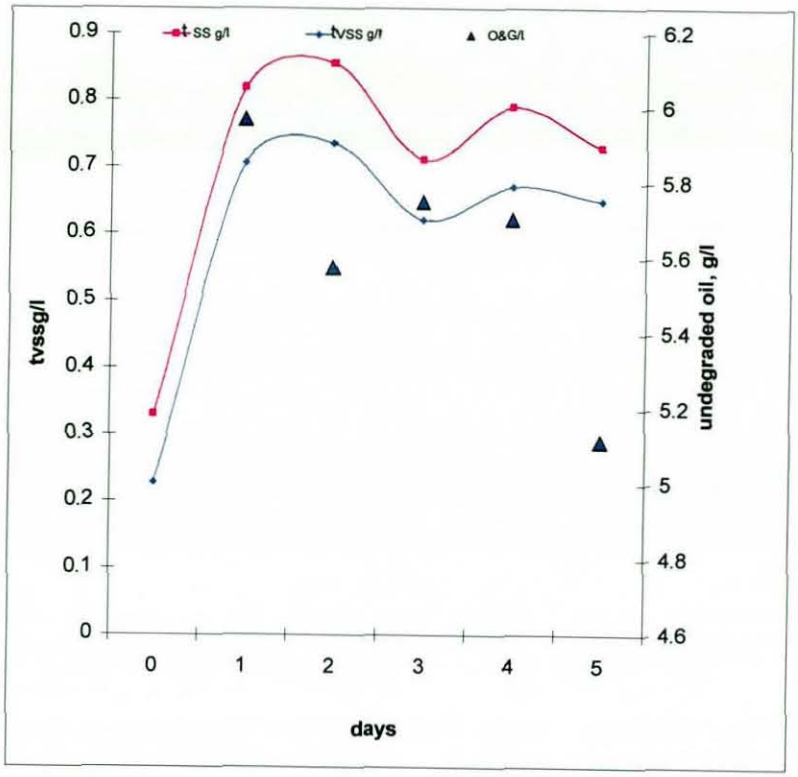


Figure 6.7 DOUR for the mixed cultures

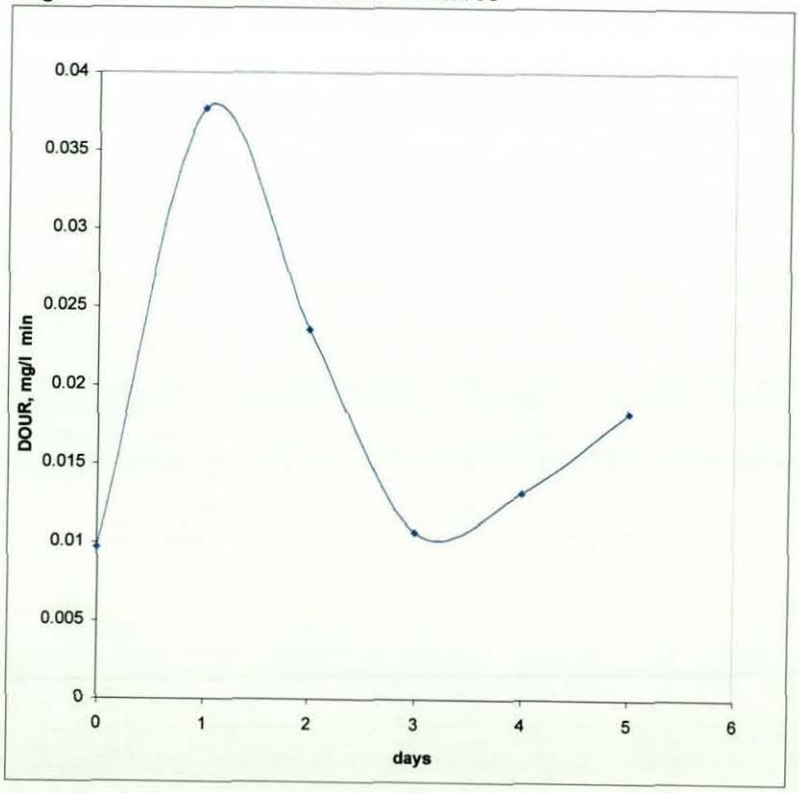


Figure 6.8: Growth of mixed cultures in 1X and 2X Basal Salts Solution

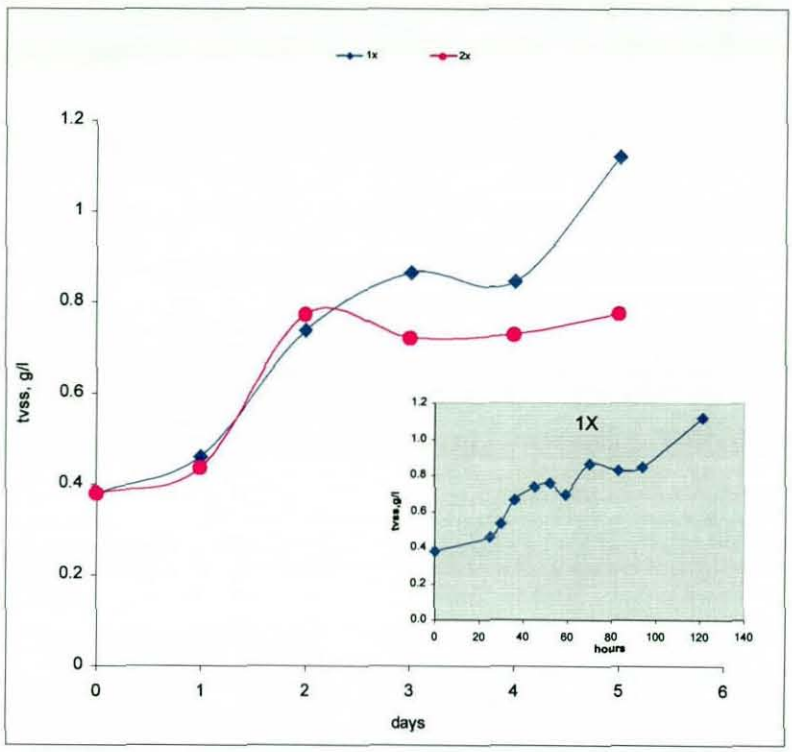
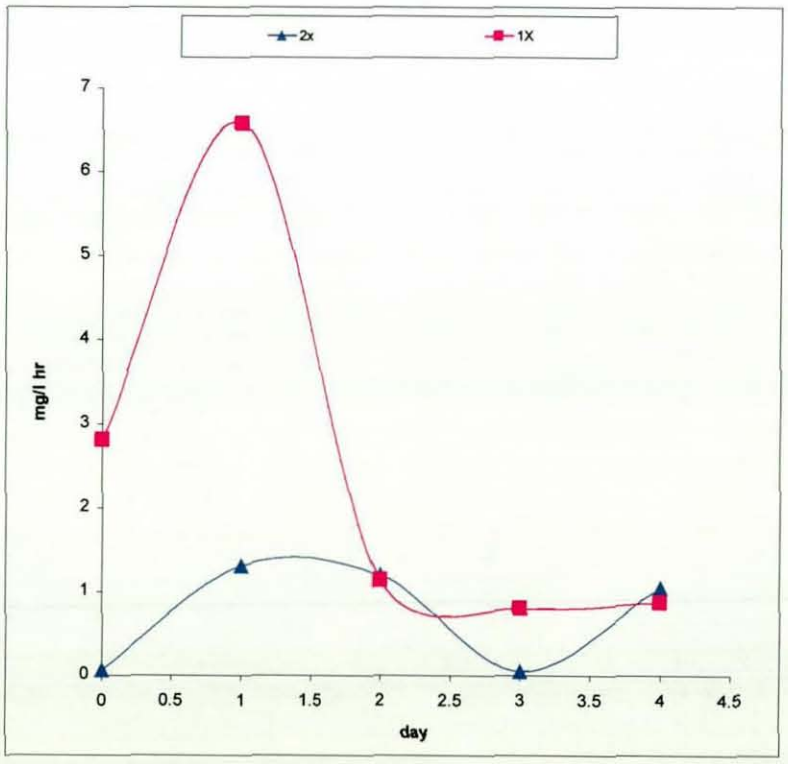


Figure 6.9 DOUR for 1X and 2X



6.3.3 EXPERIMENT F - MINERAL SALTS REQUIREMENT

To determine if the micro and macro-nutritional requirements were being met with the basal salt solution to be used, 2 concentrations of the minimal basal salt solution were tested. The results are as in Figure 6. 8 and 6.9 for growth (TVSS) and activity (DOUR). Spreadsheets from the experiment are in *Appendix J* (p.286). From the graphs it can be seen the 1X concentration was adequate and this was used throughout the experiments. The numbers at the end of the experiment was high, MPN of 10^{10} for 1X and 10^{9} for 2X and therefore would be reflective of the requirements from high cell numbers.

As a manner of comparison, the graphs for the growth of the microbial consortia from the chemostat and the microbial consortia grown in 1X and 2X basal salt media and DOUR are graphed in Figure 6.10 and Figure 6.11. The oil content used for these experiments was at one percent vol./vol. The graphs are similar and the maximum mass attained from the first spurt of growth by day two was almost equal. The second growth phase is seen earlier in the experiment for the 1 X concentration. The dissimilarities probably arise due to the fact it is a mixed culture and not single cultures. The chemostat was used to maintain some degree of control in the use of mixed cultures for all the other experiments. The cultures were kept maintained at late log phase by the dilution rate. However once the cultures were harvested from the chemostat and resuspended for the shake flask experiments and lab scale runs, there would be differing environmental pressures being exerted due to the different microcosm set up for the experiments such as in the next experiment where the substrate (oil load) concentration is varied.

6.3.4 EXPERIMENT G - SLUDGE LOADING RATES

The results of this experiment were used to determine some of the kinetic parameters of growth from which the sludge loading for the bench scale reactors were selected. The results were evaluated using Monod's growth equation (2-1) and first order rate removals for O&G, equation (2-10), for the selection of best degradation rates. Earlier experiments were preliminary in nature, were of short duration with less data points, and were of limited value to determine kinetic parameters. Furthermore, they were carried out using crude oil as substrate.

To enable the solids load calculations, the sludge's properties *(Appendix F,* p. 262*)* were

Figure 6.10 Comparison of growth of mixed cultures (MC) from chemostat, growth on 1X and 2X

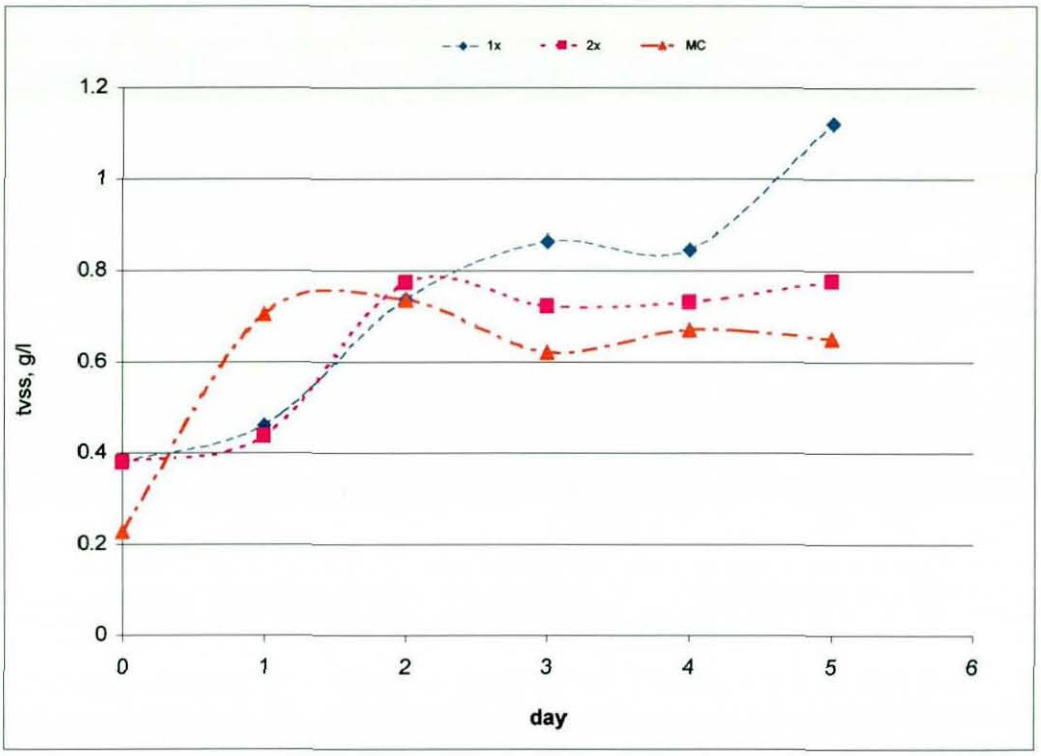


Figure 6.11 Dissolved oxygen uptake for mixed cultures and in 1X and 2X basal salt solution

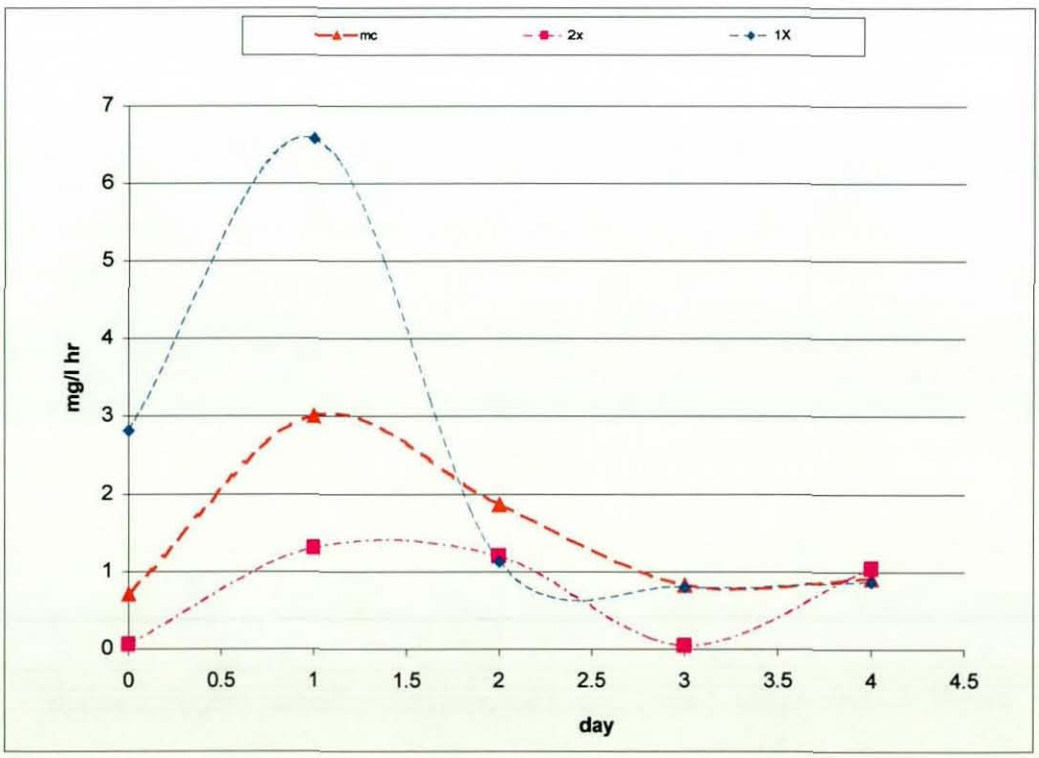


Figure 6.12 : Growth curves of 5, 10, 15, 20 % solids load of sludge in shake flask cultures

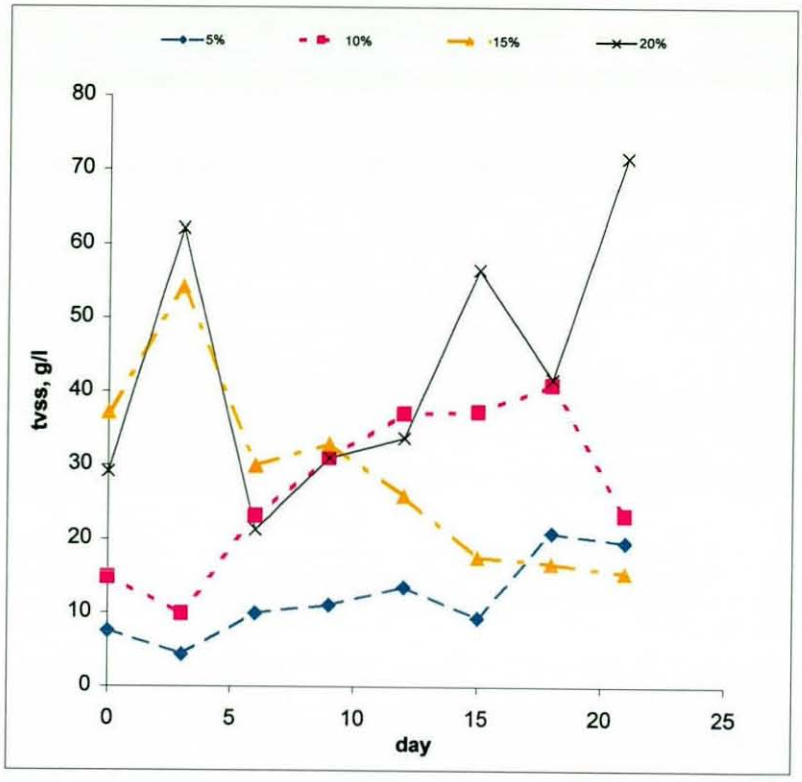
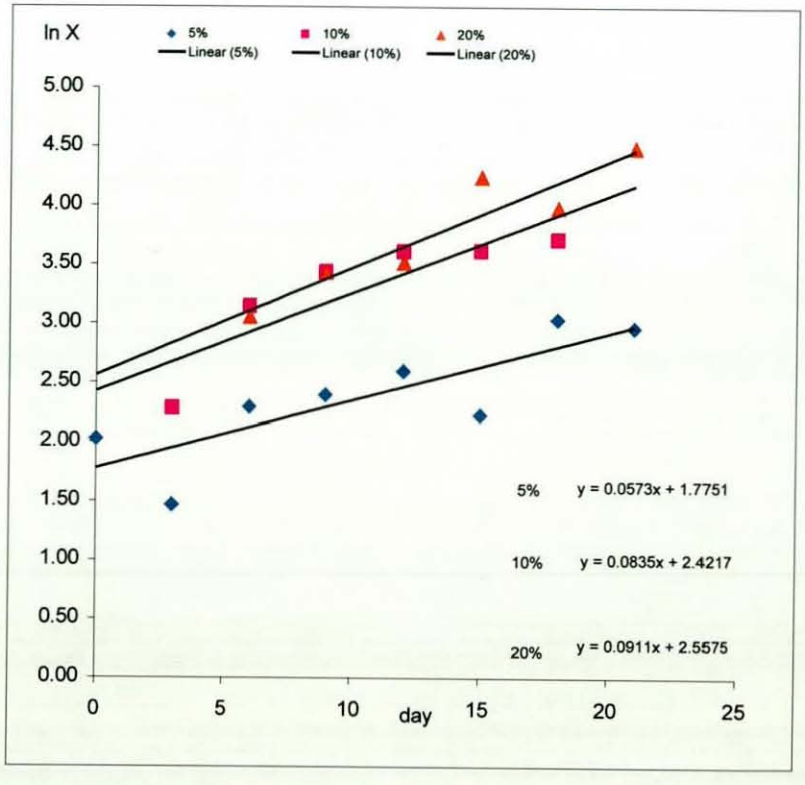


Figure 6. 13 : Growth rate of 5, 10, 20 % solids load in shake flask cultures



first determined. The O&G determination was based on an average value of six replicates. The method used for oil extraction was also tested for recovery. This was presented in section 6.1.

Figure 6.12 shows the trend in biomass growth for the four concentrations of solids selected for testing. Generally, the shapes for the two higher solids load (15% and 20%) were similar at the start and the two lower concentrations (5% and 10%) share a similar pattern. Each data point was from the average of triplicates, with one flask being used per data point. In essence each flask is a separate microcosm environment for which some control on growth per flask (to maintain identical environments) was imposed by using one source of broth for culture and media which was then measured out into the flasks. The sludge was premixed before being weighed per flask to ± 0.03 g of the required weight. However, even with these precautions, growth may still be variable within each flask, possibly due; to flask position resulting in slight temperature differences in the temperature controlled orbital shaker; or to sludge inconsistencies; or to different species dominating etc. The raw data used to generate all the results for this section are in *Appendix J* (p. 287-292).

From the Figure 6.12, it can be seen that the 20 % solids content show a very clear diauxie growth pattern while the 15 % solids shows a less obvious diauxie growth. The 15 % growth curve show a sharp increase initially which then declines and rises again very slightly before drastically reducing till it reached low steady numbers. The 15 % growth curves showed that the maximum rate of growth was reached by the third day (second data point) and the rates declined thereof. Due to this, this set of data points were not used in generating the Monod coefficients.

Guerin and Jones (1988) also showed biphasic growth patterns in their study of phenanthrene mineralization which they have construed to be due to a redirection of the organisms cellular metabolism to the utilization of phase 1 growth products. They showed that at high phenanthrene concentrations, there was a build up of phenanthrene metabolites. Phenanthrene degraders (as opposed to mineralizers) dominated the cultures and phenanthrene degradation was the preferred pathway as more energy per substrate was gained in oxidizing phenanthrene rather than its metabolites. Phenanthrene metabolites were treated as a second substrate, similar to competitive inhibition of substrate, and its uptake only occurred when the 'first' substrate became depleted. Hence, the growth slow down before a second growth phase would be due to the cultures 'adapting' towards the second substrate. Morphological changes, detected

in the ribosomal proteins of crude cell lysates, have been associated with the shift in metabolism (Guerin and Boyd 1995). They also coincided this activity with the shutting down of the cell's degradation activity momentarily, therefore retarding degradation rates.

Guerin and Boyd (1995) and Guerin and Jones (1988) experimented with a single PAH substrate while Experiments G was carried out with a very complex substrate consisting of a mixture of hydrocarbon compounds, each group displaying different susceptibility to biodegradation : short straight chain alkanes > branched chain > 2 – 3 ring benzene compounds > 4-5 ring benzene compounds etc. with short straight chains being the most susceptible and decreasing in order. 'Susceptibility to biodegradation and the relationship to compound structure' literature is exhaustive (Higgins and Gilbert 1977; Bartha and Bossert 1984;Shailubhai 1986; Morgan and Watkinson 1994; Sutherland *et al.* 1995). Similarly, the susceptibility to biodegradation by virtue of compound structure and the concept proposed by Guerin and Boyd (1995), that the preferred substrate is utilized to depletion before a less efficient substrate is used for energy and growth, can be applied here. The diauxie growth pattern could be due to the cultures producing extracellular biosurfactants in response to the diminished soluble compounds. At the start of the experiment, there would be soluble substrate present, subject to solubility limits, which is depleted very fast due to high bacterial numbers at the start as well high growth rates. The substrate being mostly insoluble is only very slowly desorbed from the 'solid support' they are attached too. Degree of binding to the solids is very dependent on the nature of the support (Means *et al.* 1980; Rutherford *et al.* 1992). Sorption capacity of the sorbent and hence the partition coefficient and desorption isotherms of the solute, is a subject of active research as has been discussed previously in surfactant's literature review. In the case of petroleum refinery sludges, the sorbent is made up of fines and rust residues as well as produced sand. Either the rate of desorption or the solubility of the substrate limits the reaction, whereby the cultures have to 'adapt' to reduce this mass transfer limitation. This could account for the step response in the growth curve seen clearly in the 20 % solids load. This pattern was repeated in other experiments as will be shown later.

As previously mentioned, the two higher concentrations show a sharp increase in microbial biomass from day zero to approximately day three while the two lower concentrations show a short lag phase followed with a more gradual increase in biomass with both reaching maximum biomass on day eighteen. The dissimilar pattern for the high substrate and low substrate could possibly be related to the proportionally higher

amount of easily metabolized substrate at the start for the higher concentrations. For the low substrate concentrations, the concentration of soluble substrate was possibly limiting growth hence it required the synthesis of enzymes to break down the oil or produced surfactants to enhance solubilization and therefore this resulted in a lag phase as well as a hardly discernible diauxie growth pattern. A similar observation at high and low substrate concentration was noted by Guerin and Jones, (1988) as previously reviewed. However, they postulated two possible reasons for this; at low phenanthrene concentrations, concurrent utilization of phenanthrene and its metabolite would allow the degraders to mineralize the substrate more efficiently. Hence they postulate that due to their inherent efficiency of substrate utilization phenanthrene mineralizers would be more competitive at low substrate concentrations and would therefore dominate, hence less diauxie.

While the rate of growth, do not follow that of typically soluble substrates, other than the 15 % solids load, bacterial mass generally increased over time. The 15 % solids load also showed a mass increase but it was for a period for which only two data point were available. As such, little information could be inferred from the data. All the graphs show after an initial increase, either a decline in mass or a plateau followed by an increase. However, in the determination of growth rate, this biphasic pattern was ignored and the mass increase for the time period of the experiment was evaluated for the growth rate. Some data points (mostly first and last data point) were dropped to evaluate growth at increasing biomass only.

Based on the growth curves as shown in Figure 6.13, ln X of the biomass (TVS) was plotted against time to determine the growth rate, μ, which was then fitted to Monods growth curve to determine *Ks* and μ_{max}. (Pirt 1976). The graphs are as in Figure 6.14, and the derived biokinetic constants are as in Table 6.6 Yield, Y, was also determined from a plot of substrate and maximum mass attained, Figure 6.15 (Pirt 1976).

Table 6. 6 Summary of Biokinetic Constants

PARAMETER	VARIABLE	VALUE	Truax *et al.* (1995)
Half saturation constant	K_s	292 mg O&G / l	977 mg O&G/kg
Maximum Yield coefficient	Y	1.061 g TVS/g O&G	0.98 g TVS/g TPH
Maximum specific growth rate	μ_m	0.0901 day^{-1}	0.11 day^{-1}
Maximum substrate utilization rate	k at 5% load	0.0302 day^{-1}	
	k at 10% load	0.043 day^{-1}	
	k at 20% load	0.0327 day^{-1}	

Figure 6.14 MONOD growth curve and double reciprocal plot

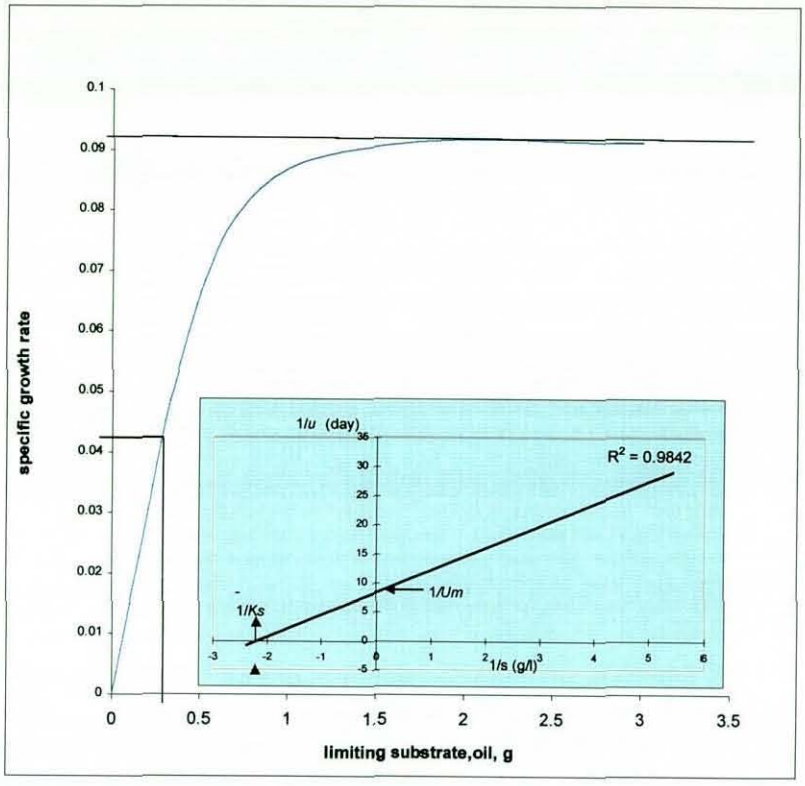
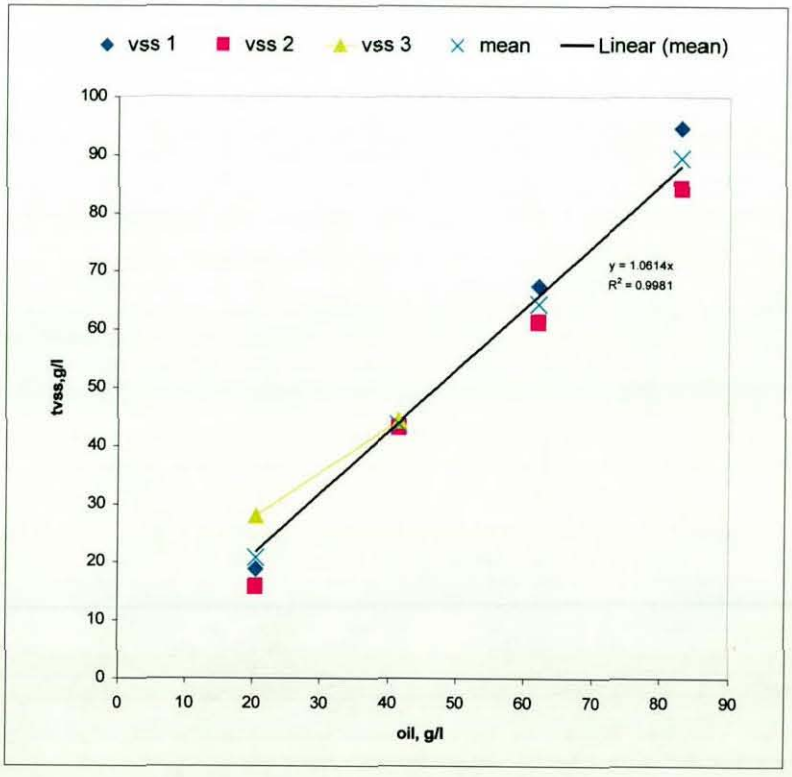


Figure 6.15 : Growth yield



The coefficients may be compared to those derived by Truax *et al.* (1995) in his investigation of bench scale reactor treatment of fuel contaminated soils. From his investigations, he derived a μ_{max} of 0.11 day $^{-1}$, *Ks* of 977 mg TPH /kg sand and yield coefficient of 0.98 mg TVS /mg TPH. While kinetic coefficients would be sensitive to the organisms, substrate and environmental conditions and are therefore very specific, a comparison of the magnitude of the derived constants serves as a useful reference. In general, growth on hydrocarbons, as can be seen from the growth rate, are slower than those on more soluble compounds such as those in municipal wastewater. An observation by Truax *et al.* (1995), in his review of other similarly derived constants from wastewater is that, low μ_{max} and high *Ks* values are typical of wastes that are degraded slowly and growth substrate plots based on classical Monod or Michaelis-Menten show flatter rectangular hyperbolas. Yields on the other hand are higher, possibly due to long chain hydrocarbons being in a more reduced state and therefore requiring less energy for synthesizing new cells. This observation is similarly supported by the results of this experiment.

Figure 6.16 show the pattern of oil loss in the shake flask cultures. A plot of the remaining oil versus time show different gradients for the concentrations tested. The 15% solids load data did not follow a similar pattern to the other solids load. The observed growth curves, Figure 6.12, may explain the shape of oil loss pattern. As seen in the growth curve, growth progressed very fast initially and then declined substantially compared to the other loads tested. There was no significant second phase of growth as in the others. The oil loss showed a fast drop in concentration (Figure 6.16) which corresponds to the high bacterial mass initially (Figure 6.12). Oil was then seen to increase from day six to day twelve and then stabilize. This may be attributed to the organisms dying and releasing stored oil. As the organisms continue to die, the oil recovered increases until it shows a constant amount which correlates to the time which the TVSS curves show a constant biomass being recovered. Bacteria storing oil in storage vesicles (intracytoplasmic membrane complex) upon uptake is a phenomena that is much speculated about based on freeze fracture electron microscopy of oil degrading bacteria (Marie-Jose de Smet *et al.* 1983).

Due to the rate removals being anomalous for the 15 % load data, the following first order rate removals were only carried out for the other three solids loading. A plot of '–ln Ca/Cao versus time' gave reaction coefficients of 0.030 day^{-1} for 5%, 0.043 day^{-1} for 10 % and 0.033 day^{-1} for 20 % solids load (Figure 6.17). Regressional analysis for

Figure 6.16 : Pattern of oil loss for 5, 10, 15, 20 % solids load in shake flask cultures

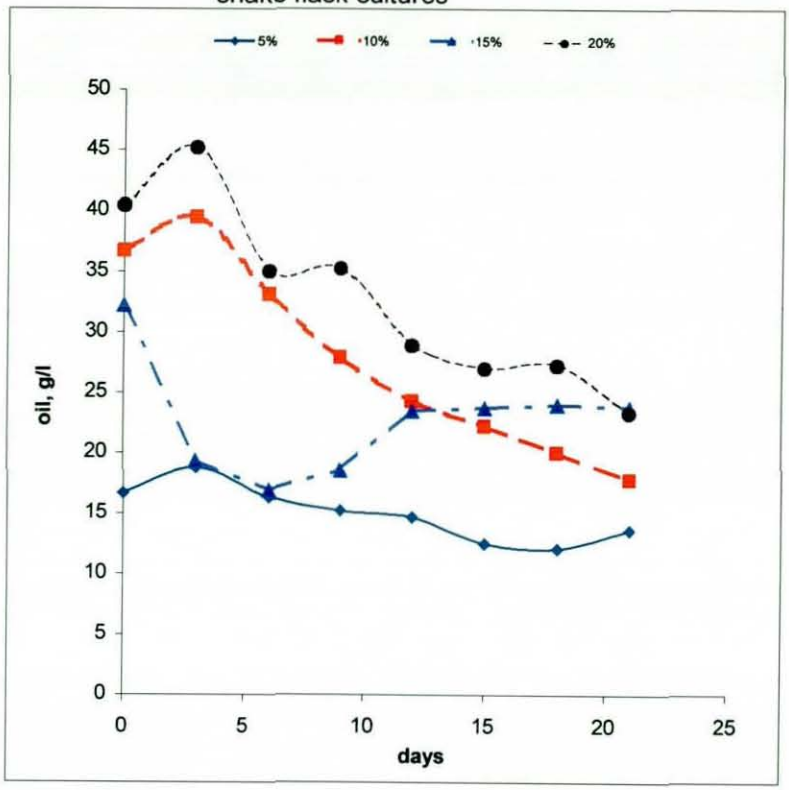
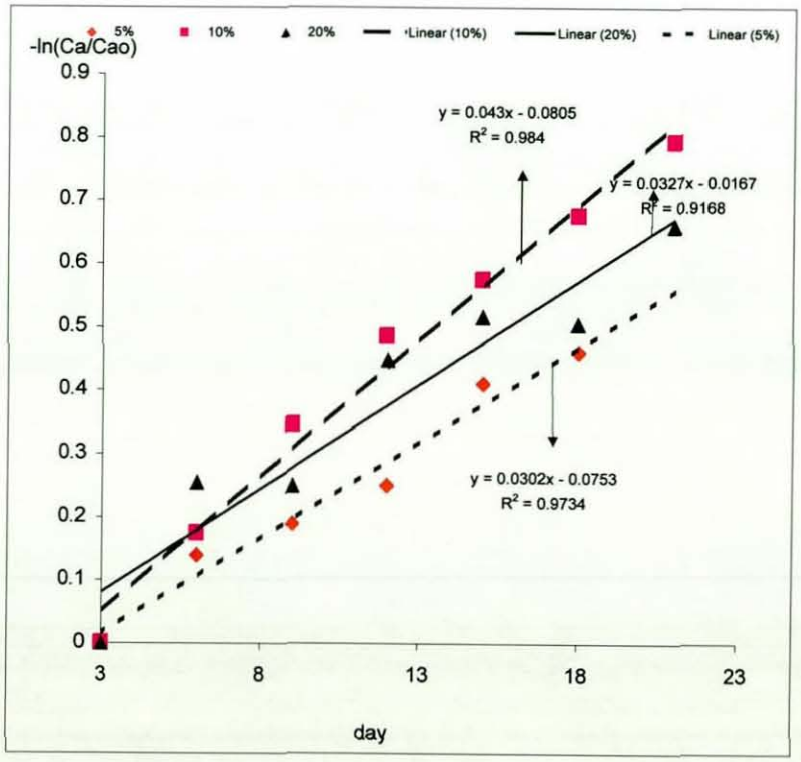


Figure 6.17: First order rate removals for oil and grease for %, load in shake flask culture experiment.



the fit to the line drawn gave R^2 values of 0.91 to 0.97. This means that the rate of removal was the best for the 10 % solids load, this is discounting the 15 % solids data. From the rate coefficients, it could possibly be that the best rates fall between 10 % and 20 %. At 20 % there is a drop in the removal rate possibly due to inhibition from the high load of toxic compounds. As already observed by others, Bartha and Bossert (1984), there is a concentration dependence for rates. Bartha and Bossert (1984) observed rates in landfarming to increase as the oil load concentration increases from 1.25% to 5% thereafter, remaining the same up to 10% then to decline slightly at 15 % oil load (per dry solids). Deul *et al.* (1978) observed activity increases linearly from 0.1-5 % and declines at more than 10 % oil load.

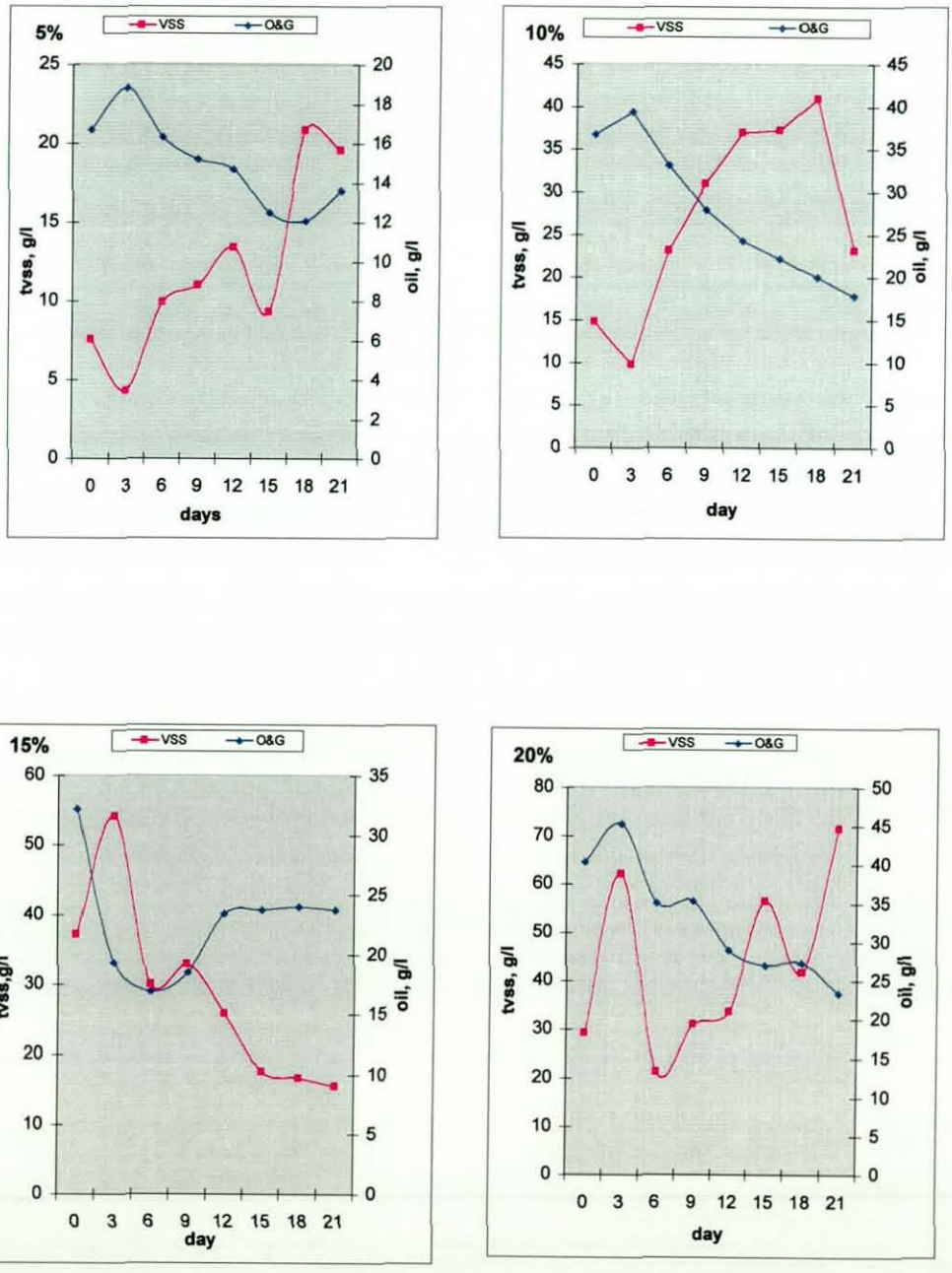
Figure 6.18 show the pattern for oil loss and biomass growth for each of the solids concentration tested. The analytical method for determining the remaining O&G was standard throughout the research. As an observation, second data point O&G recovery was almost always higher than day zero throughout this research. There is no 'increase' in oil between day zero and day three, rather due to the changes in the mixed liquor, recovery is improved. This difference in recovery accorded to differences in sample or 'matrix' has been discussed earlier and other analysts have noted this difference which is unavoidable (Lauch *et al.* 1992). The standard method for PAH analysis in SW 846, has multiplication factors for the difference in recovery for water versus sludge samples however this will not take account of differences within one type of matrix. This is a limitation, which is unavoidable. For this reason, most of day zero data point for O&G is dropped when calculations are involved.

From the results of this experiment, 10 % solids were selected for the bench scale reactors based on the best rate coefficients for oil degradation and relatively good growth rates. The loading at 10 % was also felt low enough not to compromise mixing in the stirred tank reactor or the 'lift' in the Air lift reactor. However, that was to be confirmed from tracer studies. The tracer studies testing 0, 5, 10 % solids load was carried out after this to determine the mixing conditions were adequate before confirming the solids load in the reactor. The results of this are discussed in section 6.4.

6.3.5 EXPERIMENT H – ABIOTIC OIL LOSS (CONTROL EXPERIMENT)

After confirming, the solids load to be at 10%, an abiotic control was set up to determine how much oil loss was through volatilization, photodegradation etc. The results are as

Figure 6.18 : Oil loss vs mass increase for 5, 10, 15 and 20 % solids load shake flask culture experiment



in Figure 6.19. It can be seen that the fluctuations in the oil content are more likely to be variations in extractability. However, there is some oil loss that can be attributed to volatilization, absorption to the vessel i.e. abiotic loss. Calculated as a percentage of initial oil content it is 12.5 % loss for a 10 % solids load. The TVS content do not show any significant changes. Raw data are in *Appendix J* (p. 293-294).

6.3.6 EXPERIMENT I - SURFACTANT CHOICE

Experiment I is a series of short experiments that was required prior to starting the surfactant amended reactor run. Results are as follows.

6.3.6.1 EXTRACTION AND MEASUREMENT OF O&G IN THE PRESENCE OF SURFACTANT

The experiment as laid out in Section 5.3.6.1, determined that gravimetric determinations for O&G in the presence of surfactants was not viable. The results for the extractions are as in Table 6. 7 and pictured in Figure 6.20. The control sample's (pre-extracted O&G from sludge) extraction efficiency was comparable as in the analytical method determination experiments (results in Table 6.4).

Following this, the new method with FTIR determinations of O&G was developed. So far there have been no literature reference to any O&G determination in the presence of surfactant. This method is fast and gives satisfactory results to allow for comparing surfactant effects on oil degradation per say rather than increases in solubility of insoluble compounds as the criteria for surfactant selection. The results are as in Table 6.8. As the method subsampled five ml from thirty ml of solvent (with the extracted oil), the oil recovered was compared to the aliquot of oil expected in five ml assuming an even distribution of oil in solvent. In calculating the correction factor, an average recovery percentage was used. The correction factor is multiplied with the O&G content to compensate for the differences. The differences may be due to differences in recovery or minor interference with the absorption spectra. The differences were slight but the correction factor might be required in instances where there is a fine margin of difference between surfactant choices. This control on variable recovery would need to be determined per surfactant. The percentage recovery was determined from extrapolating absorption from the surfactant and oil samples to a standard graph also prepared using extracted O&G from sludge. The sample chromatograms can be viewed

Figure 6.19 Abiotic Control experiment showing extracted oil and TVS content for a 10% solids load

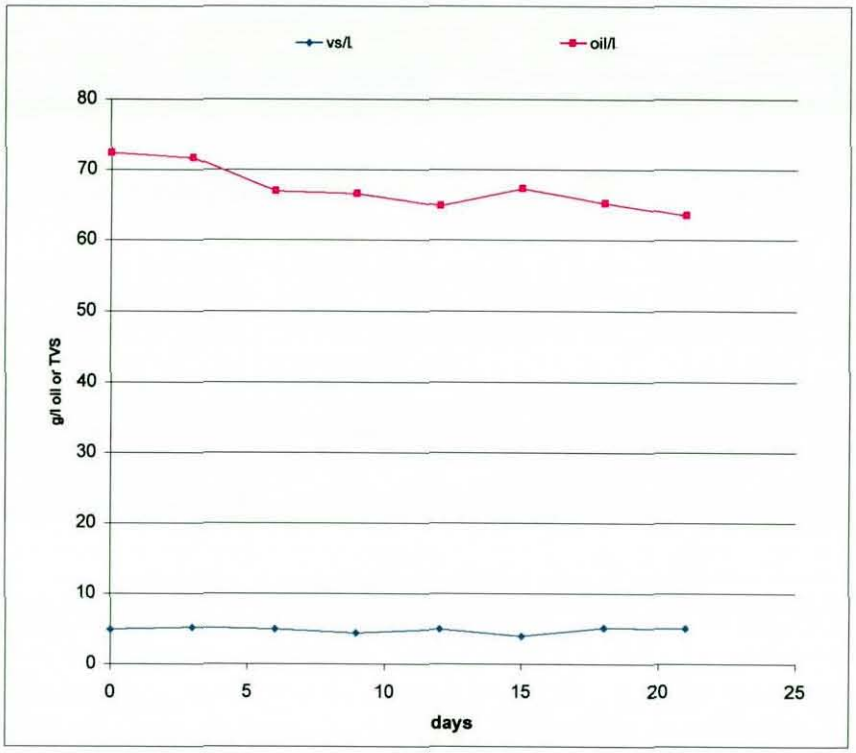


Figure 6.20 Gravimetric determination of O&G in the presence of surfactants using various solvents

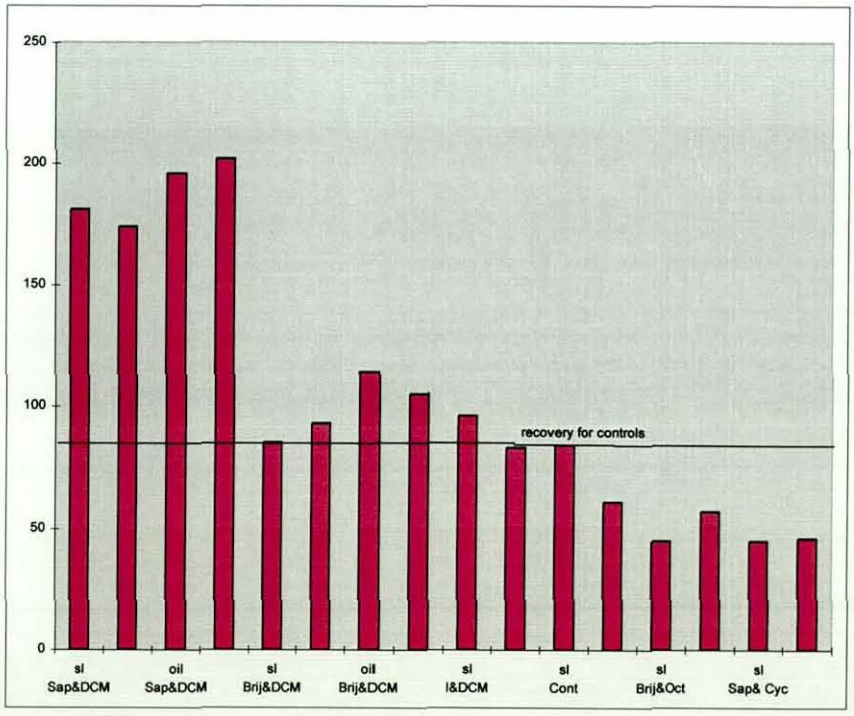


Table 6. 7 : Gravimetric determination of O&G in the presence of surfactants

Surfactant	Solvent	Sample	Sample wt	Recovered	% recovery
Sapogenat	DCM	Sludge	1.31 g	0.6 g	181
Sapogenat	DCM	Sludge	1.36 g	0.6 g	174
Sapogenat	DCM	ext. oil	0.31 g	0.61 g	196
Sapogenat	DCM	ext. oil	0.31 g	0.63 g	202
Brij 35	DCM	Sludge	1.26 g	0.27 g	85
Brij 35	DCM	Sludge	1.34 g	0.31 g	93
Brij 35	DCM	ext oil	0.29 g	0.33 g	114
Brij 35	DCM	ext. oil	0.34 g	0.36 g	105
Inipol	DCM	Sludge	1.36 g	0.33 g	96
Control	DCM	Sludge	1.34 g	0.28 g	83
Control	DCM	Sludge	1.34 g	0.28 g	84
Sapogenat	n-octane	Sludge	1.34 g	0.21 g	61
Brij 35	n-octane	Sludge	1.32 g	0.15 g	45
Inipol	n-octane	Sludge	1.35 g	0.19 g	57
Sapogenat	cyclohexane	Sludge	1.32 g	0.15 g	45
Brij 35	cyclohexane	Sludge	1.33 g	0.15 g	46
Inipol	cyclohexane	Sludge	1.33 g	NR*	-

*NR - not recoverable

Table 6. 8: FTIR determination of O&G in the presence of surfactant

Surfactant	Sample	Wt (g)	In 5 ml (g)	Recovered	% recovery	Cor. Fact.
Brij 35	Ext. oil	0.7184	0.1197	0.112	93.57	1.06
Brij 35	*Ext. oil	0.7318	0.1220	0.116	95.08	
Sapogenat	Ext. oil	0.7039.	0.1173	0.118	100.6	1.02
Sapogenat	Ext. oil	0.7159	0.1193	0.114	95.56	
Inipol	Ext. oil	0.7050	0.1175	0.120	102.13	0.98
Inipol	Ext. oil	0.7320	0.122	0.124	101.64	

*Ext. oil is O&G pre-extracted from sludge

in *Appendix* O. The standard chart, determinations of absorption for each peak, its summation and extrapolation from O&G for the standard charts are in spreadsheet format in *Appendix J* (p.295-296).

From a survey of the literature, there has always been more concern on the increase in solubility as a factor for choosing surfactants to aid biodegradation, but there has also been instances where the best solubility enhancing surfactant is not the most efficient at increasing degradation rates. This has been highlighted in the literature review section. This method can provide a quick screen using degradation as a criteria rather than solubility enhancement for surfactant selection. To determine oil degradation other research have relied on more sophisticated and time consuming analyses using chromatographic methods or radioisotope assays. FTIR provides a fast and sensitive determination for O&G.

6.3.6.2 CMC OF 3 CHOSEN SURFACTANTS

Following this, the CMC of the surfactants were tested to confirm the values found in literature. The graphs for determining the CMC are as in Figure 6.21, 6.22 and 6.23 for Brij 35, Sapogenat and Inipol EAP 22 respectively. They correspond well to the values found in literature as shown in Table 6. 9.

Table 6.9 : CMC of selected surfactants

Surfactant	CMC	Lit. ref.	Conversion	Exp. Found CMC
Brij 35	0.10mM	Thiem, 1994	123 mg/l	120-130 mg/l
Sapogenat	0.35mM	Thiem, 1994	569 mg/ l	400 –500 mg/l
Inipol EAP 22	0.009 % V/V	Churchill, S. *et al.* 1995	900 ul/l	700-800 ul/l

6.3.6.3 TESTING FOR THE PRESENCE OF MICROBIALLY PRODUCED SURFACTANT OF THE CULTURES AGAINST A PSEUDOMONAS PRODUCED SURFACTANT

The cultures from the Airflift BSTR were grown and tested for the presence of surfactants by testing the broth for emulsification ability and a reduction in surface tension. The production medium was extracted O&G from sludge in BBH. As can be seen in Table 6.10, there is decreasing surface tension through time with the lowest reading at 30.63 dynes/cm. Broth CMC was probably reached at approximately 29 hours as seen from Figure 6.24. This can be correlated to the time the second phase of growth starts, after maximum mass (plateau) of phase one is reached, as can be seen in

Fig. 6.21 CMC of Brij 35

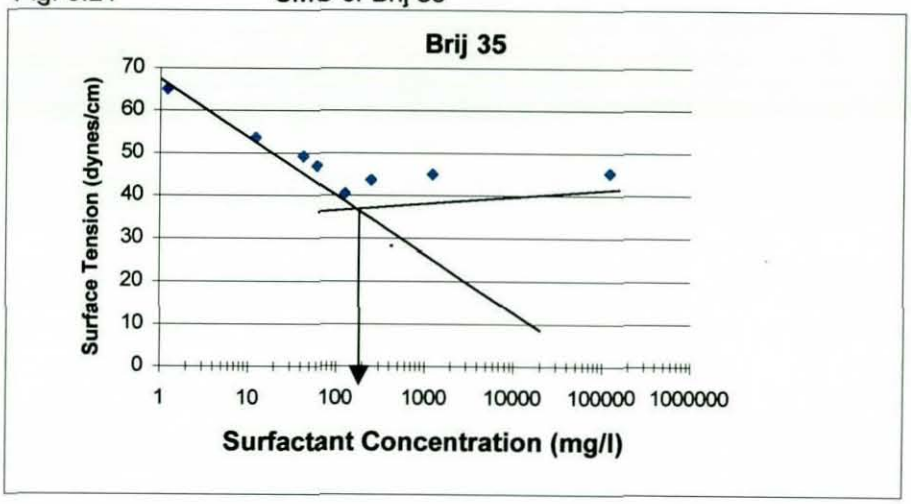


Fig. 6.22 CMC of Sapogenat

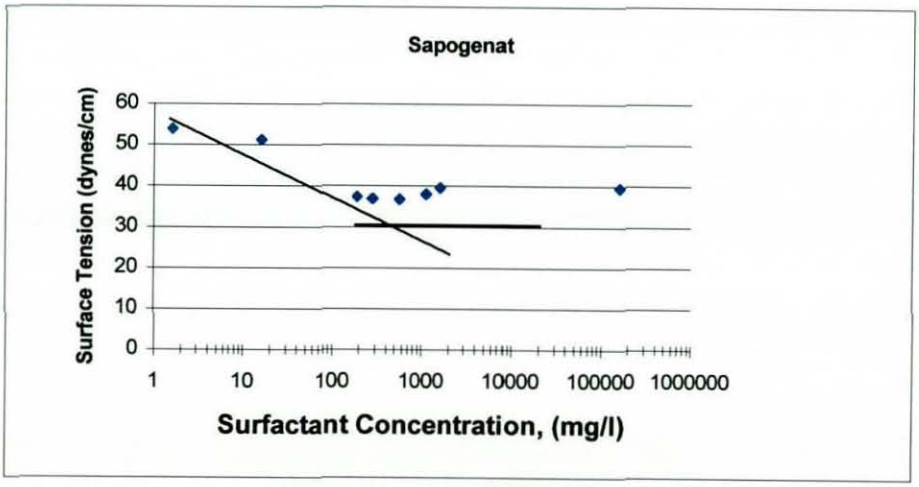


Fig. 6.23 CMC of Inipol EAP 22

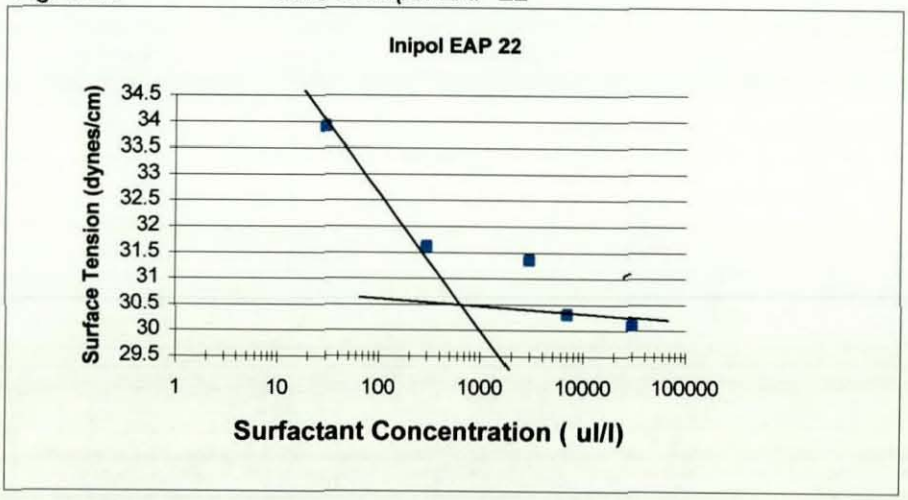


Figure 6.24 Airlift culture surfactant production

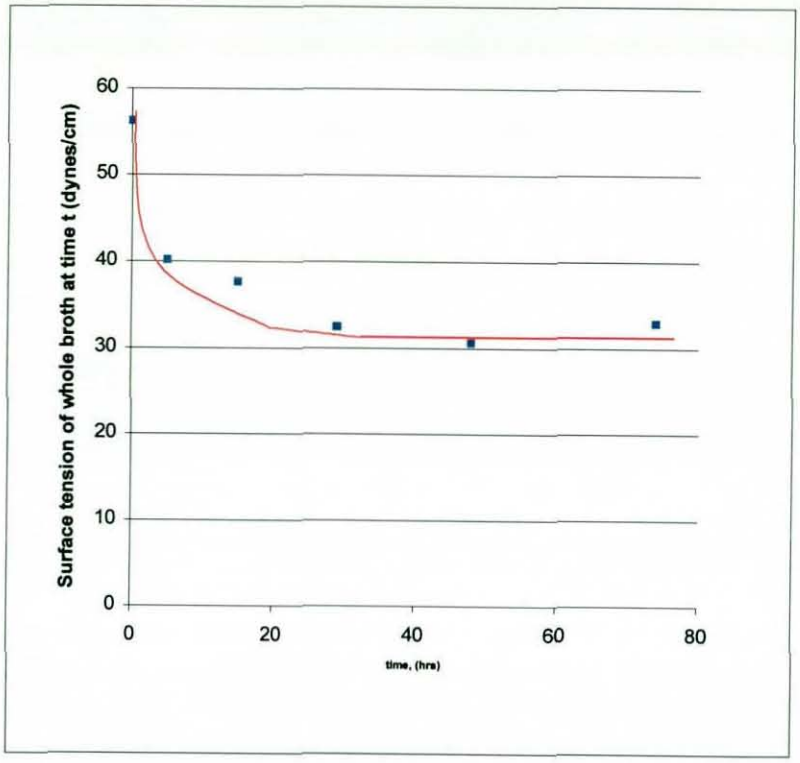


Figure 6.25 *Pseudomonas* sp culture surfactant production

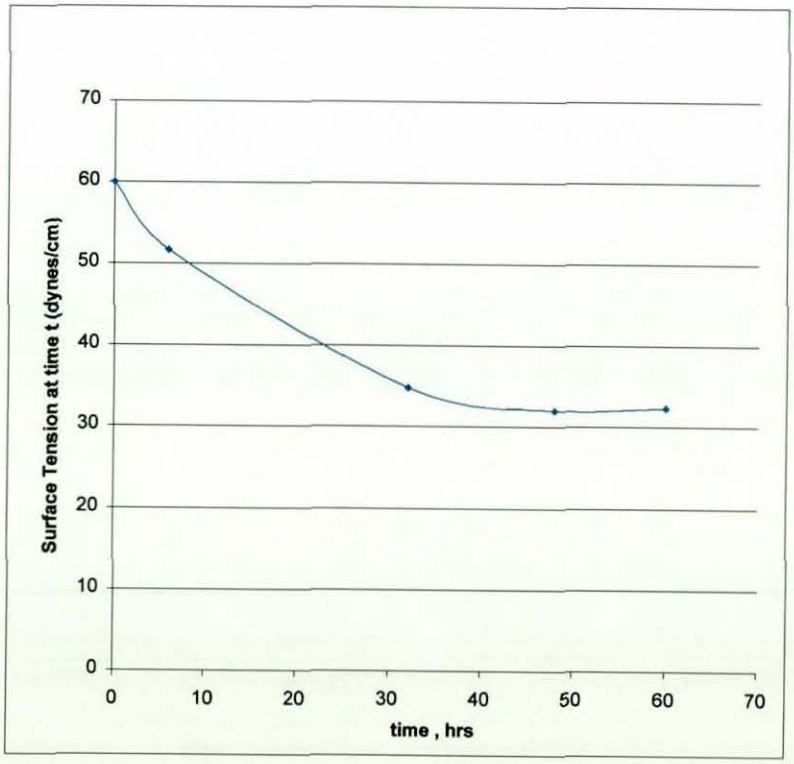


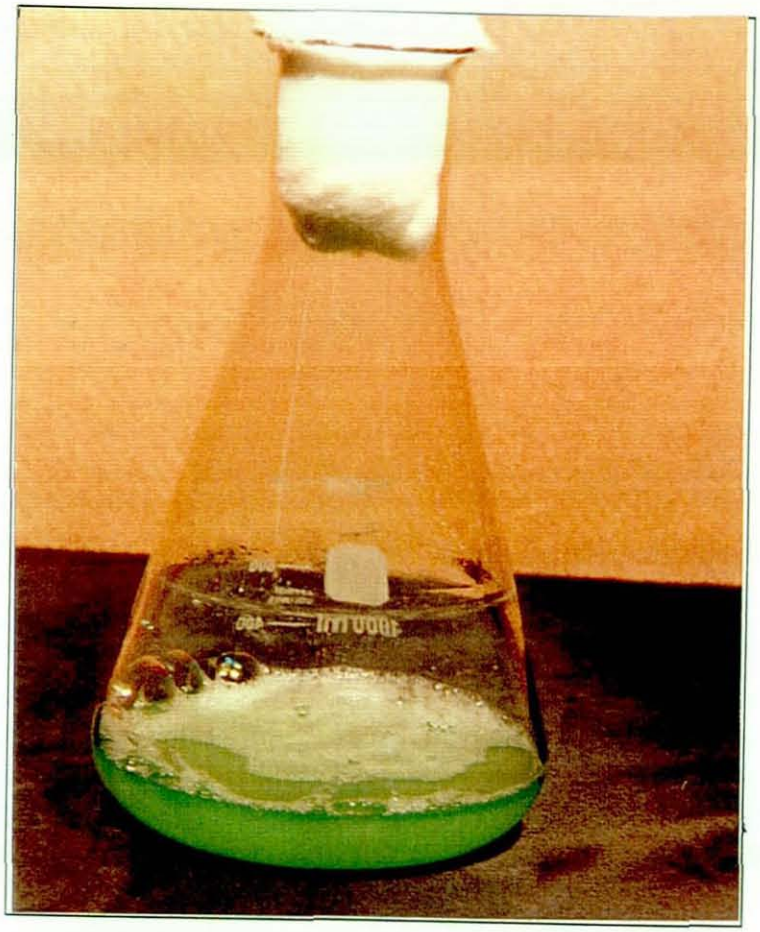
Figure 6.6. Research by Bosch (1988), on surface-active compounds from microbial cultures, show that surface activity can be detected during early logarithmic phase of growth. This may show that cultures are induced to produce surfactants upon immediate exposure to oil and that the first phase of growth are by bacteria that can either utilize insoluble compounds by direct contact or are utilizing soluble substrate. Subsequently, the production of biosurfactants enhance solubilization or facilitated transport of oil into bacteria resulting in a second growth spurt arising out of this adaptation.

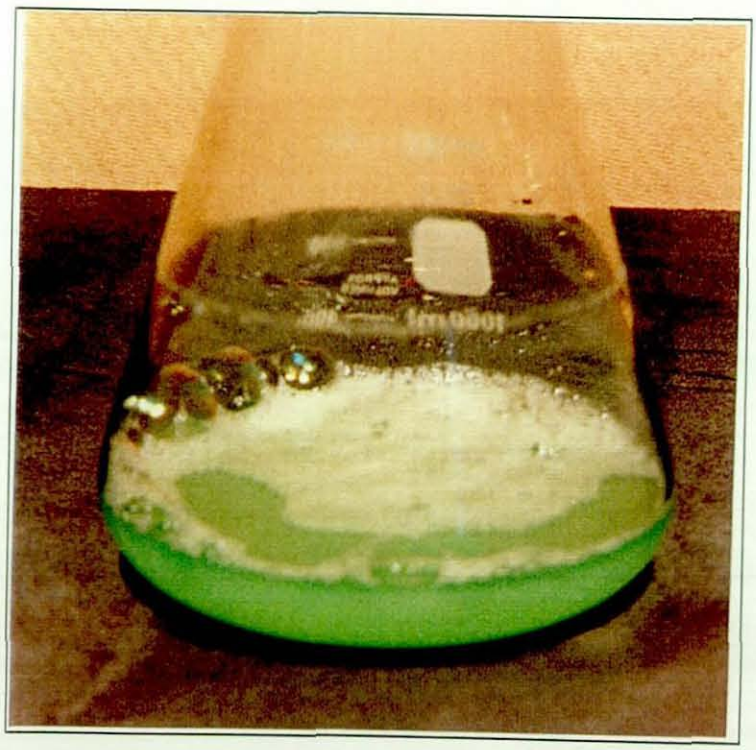
Table 6.10: Surface tension readings through time for *Pseudomonas* sp. and airlift microbial consortia

Culture							
Pseudomonas	Time (hr)	0	6	32	48	60	
ST		60	51.7	34.8	32	32.3	
Airlift	time	0	5	15	29	48	74
ST		56.3	40.2	37.7	32.5	30.6	32.9

Bosch (1988) screened a total of 1025 microorganisms for the ability to produce surfactants and selected forty that were potentially useful as surfactant producers based on their ability to reduce surface tension significantly and maintain high emulsions for longer periods. Their best strains reduced surface tension to 29 to 33 dynes/cm in a basal salt and kerosene medium. The airlift cultures reduced surface tension to similarly low levels, 30.6 dynes/cm. In comparison, the *Pseudomonas* strain grown in Kays minimal media (Recipe in *Appendix* E, p.261) and surfactant production induced in PPGAS media (Recipe in *Appendix* E), reduced surface tension to 32 dynes per cm. Figure 6.25 demonstrate the drop in surface tension through time. Surface tension reduction with the *Pseudomanad* sp was concomitant with the production of a bluish green pigment as seen from Figure 6.26, Photoplate of *Pseudomonas aeruginosa* broth at 29 hours. Surface tension readings did not show much difference for the Airlift BSTR cultures and the known surfactant producer used but the emulsification test results were very different as shown in Table 6.11. The *Pseudomonas* strain while showing some emulsions at ten minutes (8-ml emulsion out of 50 ml vegetable oil and broth), at the end of twenty-four hours showed no emulsion at all. The Airlift BSTR cultures showed good emulsification at the end of twenty-four hours. Although there was variation in the amount of emulsification, the best figure indicates approximately 67% remained emulsified after twenty-four hours. The variability possibly arises as each flask is a

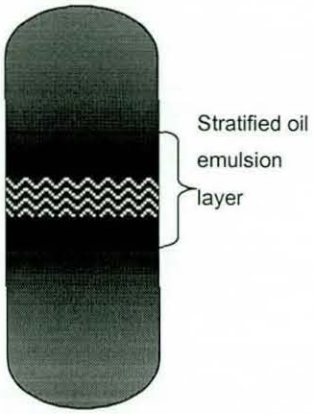
Figure 6.26 : Photoplate 1 of Pseudomonas aeruginosa grown for surfactant production in PPGAS media





microcosm environment by itself. The phases were stratified but there was barely any free oil phase floating on top, with the next layer of emulsified oil also stratified into two layers as shown in the following illustration. The bottom layer was composed of water. The layering was distinctive enough for demarcation. Bosch *et al.* (1988) noted that surface tension and emulsification ability were not well correlated.

Table 6.11 Emulsification ability of airlift cultures using culture broths at time (t) of growth



Time(hr)	Total vol	At 2 hrs	At 24 hrs	% emulsion
Airlift cultures				
0	46	0	0	0
5	48	27	18	66.6
15	44	24	10	41.66
29	44	26	9	34.6
48	42	30	10	33.33
74	46	36	14	38.8
Pseudomonas sp	50	0	0	0

It was not part of the scope of this research to go deeper into surfactants other than testing their use as an amendment to reduce mass transfer limitations. However, further work to investigate the relationship between emulsification ability of microbially produced surfactants and their effect on degradation rates of oil may prove to be useful. There is not enough information available on biological surfactants for its potential use in remediation of oily waste or soils. Thus far work on surfactants have been mostly on synthetic surfactants and have been controversial possibly due to the different microorganisms utilized in the experiments. Work by Zhang and Miller (1995) have shown that bacteria have different mechanisms for picking up insoluble substrates. Due to this difference, some of the synthetic surfactants have inhibited uptake and some have enhanced uptake. Since biological surfactants have intrinsic factors associated with these mechanisms, their study would give insight into the use of surfactants, both synthetic and microbial, to enhance insoluble substrate uptake. The fact that microbial surfactants differ widely in their emulsification ability while reducing surface tension almost equally may provide more clues as to how surfactants work to inhibit or enhance insoluble substrate uptake. Further work along this line should be able to give more insight into how organisms pick up insoluble compound for growth and energy and thus allow for exploitation of this mechanism.

Further research planned for this study using isolated biological surfactants from *Pseudomonas* or the airlift cultures could not proceed beyond confirming that the cultures from the airlift were producing biological surfactants. The method for isolating surfactants taken from Zhang and Miller (1992) yielded very low quantities of surfactants and a fair amount of time would be required to develop better conditions for harvesting and isolating techniques. These are recommended for further work.

At this point in the research, the Airlift BSTR and the Basic BSTR runs were already finished. The results of the above tests confirmed the presence of the microbial surfactants, which were produced from the microbial consortia grown in the semi chemostat. Observations of microbial surfactant effects in the reactors will be discussed later in section 6.4 under the reactor experiments. The observation that the sludge was agglomerating at the bottom of the Basic BSTR prompted the decision of further adding chemical surfactants in order to break the clump. The next experiment determined the choice of surfactant

6.3.6.4 SELECTION OF 'HIGHEST DEGRADATION RATE ENHANCER' SURFACTANT

The results of this experiment, set up as detailed in section 5.3.6.4, are graphically displayed in Figure 6.27 and 6.28. The experiment was run for twelve days. Initially it was decided that the parameter to measure bacterial growth was TVSS. This was decided based on the fact that the surfactants were soluble and would filter through therefore allowing the TVSS to be representative of bacterial mass. However, after the first day of which TVSS was measured, the samples could not filter through. The samples were slimy and blocked the glass fiber filter. As an alternative to TVSS, TVS was then substituted. As can be seen in Figure 6.27, the TVS were measuring both the surfactants as well as bacterial mass, leading to the Sapogenat amended flask having constantly highest TVS. Deleting the weights of the surfactant did not result in any meaningful results, possibly the surfactants were also being used as substrate. This was realized before the run was finished, therefore on the twelve day, bacterial mass was measured by centrifugation. The cell pellet was dried then weighed. The highest mass was found in the control experiment without surfactant with the other three at almost equal weights. Fixed solids was highest in the Inipol amended flask. Inorganic carbon, as measured by the Total Carbon Analyser, was highest also for Inipol amended flask while TOC was highest for the Sapogenat amended flask. These results can be

Figure 6.27 TVS of surfactant selection experiment

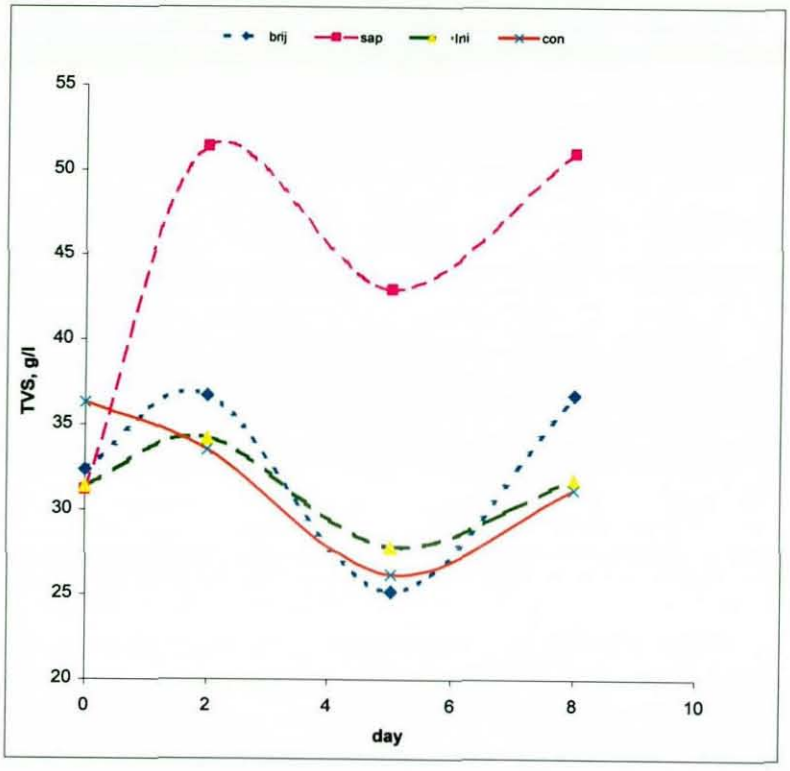
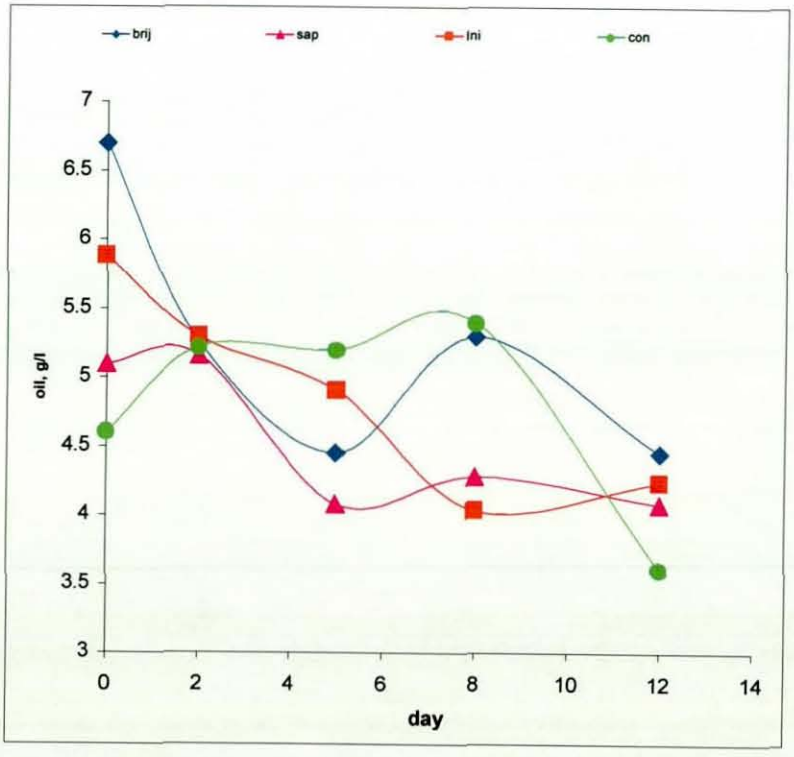


Figure 6.28 Rate of oil loss for selection of surfactant experiment



viewed in the raw data spreadsheet in *Appendix J (p. 297-300).* These results were not very conclusive. However, based on the linear rates of oil degradation, Figure 6.28, Inipol amended surfactants yielded the best rates. Visual observations also noted that Inipol amended flasks were darker with the solids well dispersed in solution. Hence Inipol was selected as the surfactant for the Basic BSTR. The O&G determinations were based on extrapolating absorbance values of the TCTFE extract (by FTIR) onto Standard O&G Chart 2 which is also in *Appendix J* (p. 300). A second standard chart was necessary as between the time the first standard chart that was determined for the experiment 5.3.6.1 and this experiment, the FTIR was serviced and the lamp was realigned. While the proportionalities were still good as seen from the fit of the curve in Standard O&G chart 1 at $R^2 = 0.995$, the absolute values for absorbance from the two charts are different. All the absorbance chromatograms are compiled in *Appendix* O. Peak wavelengths and corresponding heights and determination of O&G from conversion from the Standard Chart and Correction Factor are also available in *Appendix J* (p. 298-300).

6.3.6.5 TESTING THE ADDITION OF SURFACTANT TO SLUDGE OR MIXED LIQUOR

The results of this experiment are seen in Table 6.12. Flasks 1A and 1B had Inipol added directly to sludge while 2A and 2B, Inipol was added to the mixed liquor. The O&G content in the ML in flask 1A and 1B shows much higher levels indicating better desorption of oil from the sludge when Inipol was added to the sludge prior to slurrying. The absorbance chromatograms are in *Appendix* O and the peak wavelengths and corresponding heights and determination of O&G from conversion from the Standard Chart are also available in *Appendix J* (p. 301).

Table 6.12: Addition of surfactant experiment

Sample	Total absorbance (cm-1)	Extrapolated O&G (g)	Average O&G (g)
1A	1.6801	0.083	0.0755
1B	1.4033	0.068	
2A	0.423	0.28	0.0385
2B	0.9774	0.49	

6.4 REACTOR RUNS

Prior to setting up the Airlift BSTR and Basic BSTR, extensive tracer tests were carried out to determine mixing characteristics in the reactors. The tests were carried out with lithium chloride and are presented here. There were problems measuring lithium chloride, associated with using 'solids' (which were silica), as the samples measured in the flame photometer for lithium chloride detection was aspirating silica and blocking the tubes. Each tracer had to have its own standard carried out immediately after the (approximately) 15 samples due to the drift in readings. An experiment was also carried out to determine what would be significant results. An 800-ml beaker was filled with 500 ml water and stirred with a magnetic stirrer and a measured amount of dissolved lithium chloride was injected in. The syringe was immediately rinsed by syringing up water and dispensing back into solution. This was also carried out in the tracer experiments in the reactors. Equilibrated readings were taken 15 minutes later. The results determined that Cm/Ct was not significantly different between 0.97 and 0.99. Cm /Ct results for the Airlift BSTR and Basic BSTR are in Table 6.13 and 6.14. The readings were taken from time 0 up to 15 minutes.

Table 6.13: Tracer studies for the Airlift BSTR

% solids	Tracer	Std. Chart (Listan)	Mixing	Cm/Ct	Range	*Equil. Time	Range
0	20	5	Air	0.92	0.92–1.02	1m20s	1m20s-2m
	24	9	Air	1.02		1m20s	
	27	12	Air	0.92		2m	
	18	4	Stir	1.05	1.02-1.05	3m20s	3m20s-3m50s
	25	9	Stir	1.02		3m20s	
	28	12	Stir	1.02		3m50s	
	16	3	Both	1.04	0.94-1.04	1m40s	1m40s-2m
	29	13	Both	0.94		2m	
5	32	16	Air	0.995	1-1.03	1m40s	1m20s
	34	16	Air	1.027		1m40s	
	31	12	Stir	1.176	1.12-1.18	5m	5m
	35	15	Stir	1.121		5m	
	15	2	Both	0.943	0.94-1.08	40s	40s-1m20s
	33	16	Both	1.075		40s	
	36	16	Both	1.042		1m20s	
10	41	19	Air	1.094	1.08-1.09	4m	3m20s-4m
	42	19	Air	1.082		3m20s	
	39	18a	Stir	1.138	1.14	3m50s	3m50s-5m
	40	18b	Stir	1.148		5m	
	37	17a	Both	0.926	0.93-1.08	1m20s	1m20s
	38	17b	Both	1.08		1m20 s	

*m – minutes s - seconds

After reviewing the values of Cm/Ct for the Airlift BSTR, what was apparent was that, stirring on its own resulted in the highest Cm/Ct values (range 1.02 – 1.14) and would correspond to the highest amount of dead space. Having both air feed and stirrer resulted in similar Cm/Ct values for with or without solids; range 0.94 – 1.08 for 5% solid and 0.93 –1.08 for 10% solids. With just air feed, more solids resulted in less thorough mixing; range 1-1.03 for 5 % solids and 1.08–1.09 for 10 % solids. Therefore, the tracer studies showed that both stirrer and air feed were required in order to reduce the amount of dead spaces in the Airlift BSTR when solids were present. The Cm/Ct value at 10 % solids and with both air feed and stirrer range 0.93 –1.08 and straddled the values found for full mix at 0.97 and 0.99. While it cannot be discounted that there are dead spaces using these mixing conditions for the Airlift BSTR, the amount is low and the length of time the reactor is run, two months, makes it insignificant.

The time for equilibrium to be reached were also similar to the Cm/Ct results. It was seen that stirring on its own resulted in the slowest equilibration. Mixing from the air feed resulted in a faster equilibration. The amount of solids do not seem to affect the time it takes to reach equilibrium when both air feed and stirrer were used, however with just air feed, the higher solids load lengthened the time it takes to equilibrate. However, overall, equilibrium was reached in five minutes or less and would be insignificant as the reactor was run for sixty days. The only significance for this study was for sampling protocols. Whenever the stirrer and air feed in the reactor was temporarily shut off, sampling was only carried out after five minutes upon being turned back on.

Tracer tests carried out for the Basic BSTR are summarized in Table 6.14. The range for Cm/Ct is 0.97-1.16 which indicated dead space was still small and equilibrium was reached within 60 seconds even at the slowest stirrer speed of 36 rpm (flood point at air = 4l/m). A middle range was then selected for running the Basic BSTR at 250 rpm and 4l/m air. The spreadsheets for the tracer studies are in *Appendix* K (p. 302). The tracer and standard lithium chloride charts are in *Appendix* O.

The Basic BSTR, described in section 5.4.1 was used in part to represent a simple or basic design for comparison with the airlift design on biodegradation rates and extent of degradation. The reactor was also to be used to investigate the effects of surfactants. The biodegradation rates in the Basic BSTR are expected to be the minimum rates achievable. It was hypothesized that in the Airlift BSTR, the solids mixing would be improved due to the draft tube and airlift and this would effect higher degradation rates through better mass transfer rates of HOC to bacteria. It was also assumed that the gas

Table 6. 14 Basic BSTR tracer studies

STIRRER SPEED AIR FLOW RATE	150 RPM	250 RPM	FLOOD POINT
2 l/min			
Cm/Ct	0.99	1.13	
Equil. time	20 sec	30 sec	
4l/min			
Cm/Ct	1.06	1.03, 0.95	1.158
Equil time	20 sec	30 sec, 40 sec	50 sec
6l/min			
Cm/Ct	0.93	1	
Equil. time	50 sec	20 sec	

bubbles would act as hydrophobic liquid membranes and cause stripping of the O&G. The addition of surfactant is hypothesized likewise to improve mass transfer but not in a similar manner. Surfactant was added in a separate experiment to mediate desorption of the oil from the solids. It was observed that in the first reactor run of the basic stirred tank design, the stirring tended to agglomerate the sludge into a sticky mass, therefore preventing the solids entering the mixed liquor fraction. It was felt that surfactant could overcome this limitation.

6.4.1 OVERALL PERFORMANCE OF THE THREE REACTORS

The overall performance of the three reactors are reviewed first before the detailed results of each reactor in the following sections.

6.4.1.1 OIL AND SOLIDS

The determinants for efficiency of degradation were primarily oil loss. To determine oil loss, at the end of the treatment period, the oil remaining in the mixed liquor and bottom solids were deducted from the initial oil and expressed as a percentage for comparison. The initial and end values for dsf, total dry solids and total O&G are shown for all the reactors in the following Tables: Table 6.15 and Figure 6.29 for the Basic BSTR; Table 6.16 and Figure 6.30 for the Airlift BSTR and; Table 6.17 and Figure 6.31 for the Inipol added BSTR.

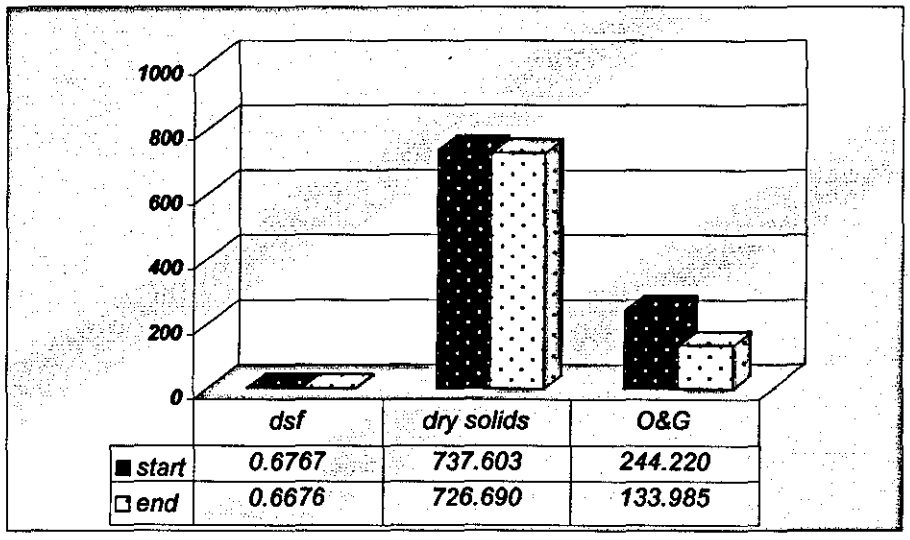
BASIC BSTR

Table 6.15: Mass balance of solids and O&G in the Basic BSTR at start and end of reactor treatment

At start (day 0)					
Fresh sludge	**Wet weight of sludge (g)**	**Dsf**	**Dry sludge (g)**	**% O&G**	**Total O&G (g)**
	1,090.0	0.6767	737.603	33.11	244.2204
At end (day 65)					
Treated sludge and clingage	1006.3	0.6676	671.8059	19.41	130.4
Mixed Liquor	**Vol (l)**	**TS, g/l**	**TS for Mixed Liquor (g)**	**O&G, g/l**	**Total O&G**
	6.895	7.96	54.8842	0.52 g/l	3.5854
		TOTAL	726.6901	Oil remaining	133.9854
				Oil loss	**– 110.235**
				% oil loss	**– 45.14 %**

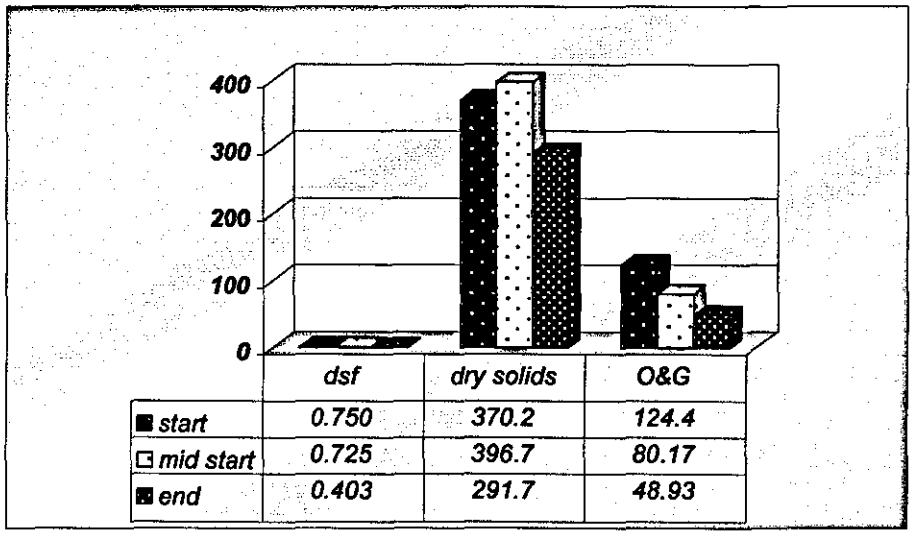
As seen from Table 6.15, the initial dry solids content remained relatively unchanged (738 g at start and 727g at end). The dry solids content of the settled solids were added to the total solids content of the mixed liquor to get the total dry solids content. The Basic BSTR removed 45.14 % of the oil load as measured by adding the O&G content from settled solids and mixed liquor. Most of the O&G (97.3%) came from the settled solids fraction. The mixed liquor, after the solids were settled, contained 0.52 g/l O&G. The dsf of the sludge (calculations shown in section 5.2.3.2) gives an indication the degree of change in the sludge characteristics and in this case showed very little difference from initial (0.6767) and end (0.6676) although the treated sludge, was visibly more viscous and elastic at the end of treatment. Rates of oil degradation normalized to total dry solids content in the reactor gives the rate of oil loss to be 2,360 mg oil lost per kg dry solids per day. Irvine and Earley (1993) showed rates of 1,080 mg/kg/day for total petroleum hydrocarbons (TPH) lost in a nutrient amended, soil slurry sequencing batch reactor with an initial solids content of 10 % (by dry weight). Irvine and Ealey's research determined nutrient requirements to ensure against nutrients limiting degradation rates. Based on the similarities of the reactor conditions from their research and this study and the rates achieved in their reactor and the Basic BSTR, gives support that the Basic BSTR was not nutrient limited. Calculations for N and P requirements based on the carbon content in the sludge are in section 5.3.4. Calculations based on the requirements of a 10^{11} bacterial count per ml was also determined not to be limiting (assumptions made in calculations: C:N:P at 10:5:1, cell is 50 % C, 1cell = 2×10^{-13} kg (Volkering *et al.* 1993).

Figure 6.29. Mass balance at start and end for Basic BSTR



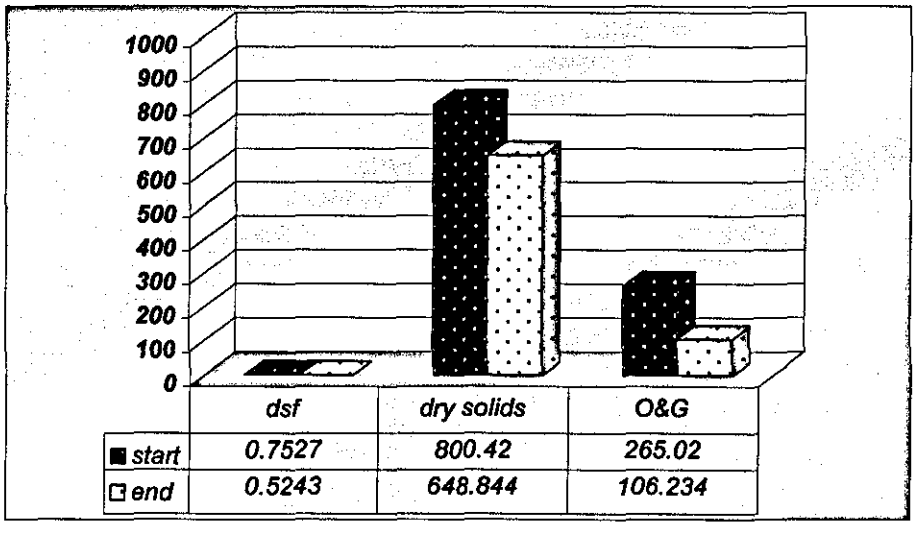
	dsf	dry solids	O&G
start	0.6767	737.603	244.220
end	0.6676	726.690	133.985

Figure 6.30 Mass balance at start and end for Airlift BSTR



	dsf	dry solids	O&G
start	0.750	370.2	124.4
mid start	0.725	396.7	80.17
end	0.403	291.7	48.93

Figure 6.31 Mass balance at start and end of Inipol added BSTR



	dsf	dry solids	O&G
start	0.7527	800.42	265.02
end	0.5243	648.844	106.234

AIRLIFT BSTR

The results from the Airlift BSTR, as seen in Table 6.16, show an improvement in the extent of degradation. At the end of the treatment period, 60.68 % of the oil load was degraded. There was a reduction in the dry solids fraction from 397 g (from the second start date) to 292 g (26% reduction). The dsf showed that the solids at the end of the treatment contained more water or was less 'dense' than the original sludge. The settled solids contained fairly low amounts of oil, 10.52 % of its dry weight compared to the Basic BSTR at 19.41 % by dry weight.

Table 6.16 : Mass balance of solids and O&G in the Airlift BSTR at start and end of reactor treatment

At start (day 0)					
Fresh sludge	**Wet weight of sludge (g)**	**dsf**	**Dry sludge (g)**	**% O&G**	**Total O&G (g)**
	493.03	0.7509	370.2162	33.61	124.4297
Restart due to spill (day 21)					
Reactor sludge	160	0.7255	116	23.84	27.6735
Fresh sludge top up	200	0.7509	150	33.73	50.6557
Reactor mixed liquor added back	Vol (l)	TS (g/l)		(g/l)	
	2.8	46.7	130.76	0.66	1.848
		TOTAL	396.76		80.1772
At end (day 68)					
Bottom solids	566.75	0.4037	228.797	10.52	24.0694
Clingage	84.9	0.5193	44.0886	50.54	22.2823
Mixed liquor	Vol (l) 2.25	TS, g/l 8.36	18.8174	1.1467	2.58
		TOTAL	291.703	Oil remaining	48.9317
				Oil loss –	75.498
				% oil loss –	60.68 %

A large fraction of the remaining oil in the Airlift BSTR came from the clingage, 45.5 % of total remaining oil. The Basic BSTR had minimal clingage, most of which came from the vessel surface (glass) at the mixed liquor level as well as on the stirrer and the clingage, due to the small amounts, was mixed with the settled solids before analysis. In the Airlift BSTR, there was a lot of oily scum on the vessel wall (Perspex) as well as on the draft tube and stirrer shaft. The airlift was designed to lift solids and when the solids were lifted up the draft tube, the solids splashed onto the walls. The Airlift BSTR also experienced foaming which brought the solids and oil into the foam layer and when it receded, the oily scum stuck to the vessel wall. Before the end of the treatment period,

the Airlift BSTR was opened and the clingage was collected and put back inside (this was done for all reactors) but due to the design of the reactor, which lifted solids and oil, the oil was re-deposited on the vessel walls. As seen from the percentage of oil in the clingage (50.54 %), the oil became concentrated compared to the original solids (33.61%). Due to the oil from the clingage which is actually a portion of the waste that did not actively receive treatment, the total oil content in the treated sludge was higher by almost 50 %. Comparing the oil in the mixed liquor fraction, the settled mixed liquor had 1.1467 g/l O&G, which was higher than the Basic BSTR but this, is probably a reflection of the higher solids in the mixed liquor fraction. The rates of oil degradation, normalized to total dry solids content from settled solids and mixed liquor, is 2470mg/kg/day. For comparison, if the clingage were not taken into account, the oil content per solids at the end of the treatment was 108 g/ kg dry solids compared to 168 g/kg dry solids with the clingage.

INIPOL BASIC BSTR

From Table 6.17 it is seen that the extent of oil degradation in the Basic BSTR amended with Inipol was higher than the Basic BSTR and almost the same as the Airlift BSTR at 59.92 %. However, the oil in the treated settled solids and mixed liquor in the Inipol amended BSTR was higher at 16.93 % dry solids and 1.6 g/l respectively compared to the Airlift BSTR. Compared to the Basic BSTR results, the use of Inipol resulted in an improved extent of oil degradation in the settled solids possibly by desorbtion of the oil from the solids into the mixed liquor fraction as evidenced by much higher oil content in the mixed liquor. However, when the amount of oil in the mixed liquor was accounted to the solids in the mixed liquor, as shown in Table 6.18, the mixed liquor solids in the Inipol amended reactor had less oil per solids than the Airlift BSTR. The rate of oil loss per dry solids in the Inipol amended BSTR was 2700 mg/kg/day.

Table 6.18 and Figure 6.32 shows a profile of all three reactor, contents, rates and quantities of oil lost in the two fractions of settled solids and mixed liquor. For comparison with other literature values, the oil loss and oil degradation rates have been accounted to the dry solids content.

Table 6.17 : Mass balance of solids and O&G in the Inipol added BSTR at start end of reactor treatment

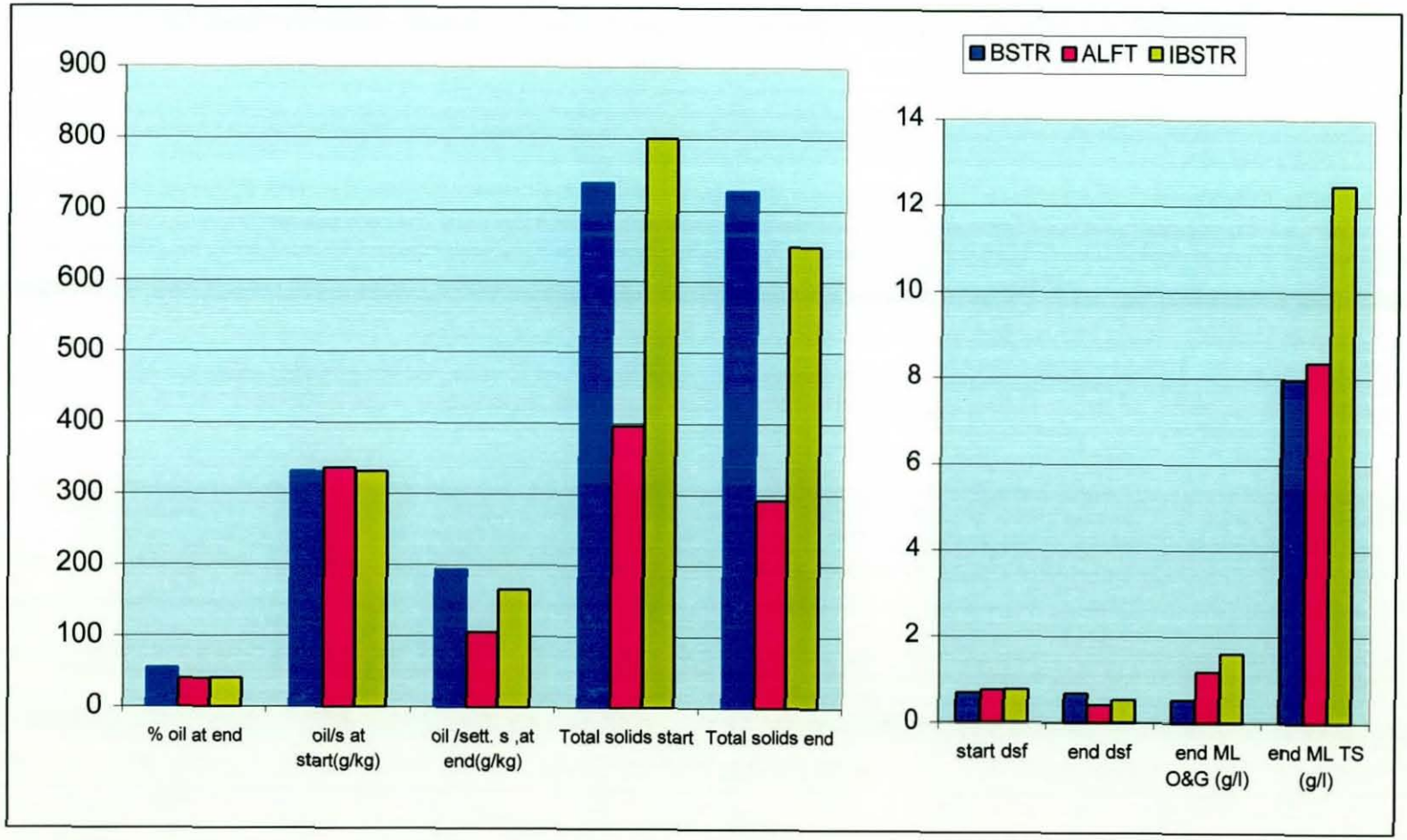
At start (day 0)					
Fresh sludge	**Wet sludge(g)**	**dsf**	**Dry sludge(g)**	**% O&G**	**Total (O&G)**
	1,063.4	0.7527	800.42	33.11	265.02
At end (day 62)					
Treated sludge & clingage	1109.37	0.5243	581.6427	16.63	96.7272
Mixed liquor	**Vol (l)**	**TS, g/l**			
	5.93	12.47	73.95	1.6	9.488
		TOTAL	648.844	Oil remaining	106.2152
					Oil loss – 158.8048
					% oil loss – 59.92 %

Table 6.18 : Reactor profiles

	Basic BSTR	**Airlift BSTR**	**Inipol added BSTR**
Oil per dry solids at start	331 g/kg	336 g/kg	331g/kg
Total percentage oil degraded	45.14 %	60.65 %	59.92%
Percentage oil in settled solids	19.41 %	10.52 %	16.63 %
Oil content in settled mixed liquor	0.52 g/l	1.1467 g/l	1.6 g/l
Solids content in mixed liquor	7.96 g/l	8.36	12.47 g/l
Oil per dry solids in mixed liquor	0.065 g oil/g solid	0.137 g oil/ g solids	0.128 g oil/ g solids
Rate of oil degradation based on total dry solids	2360 mg/kg/day	2470 mg/kg/day	2700 mg/kg/day

From the foregoing results, both the Airlift BSTR and the Inipol amended BSTR achieved a greater extent and slightly higher rates of degradation (based on total solids in the reactor) than the Basic BSTR in the treatment period. The Airlift BSTR produced the best reduction in oil content in both the two fractions (settled solids and mixed liquor) of waste. In comparing the Airlift BSTR and the Inipol amended BSTR the Inipol amended reactor produced slightly cleaner solids in the mixed liquor fraction. This could be a result of the surfactant desorbing the oil off from the particulates in the mixed liquor.

Figure 6.32 Reactors Profile



The settled solids in the Basic BSTR had the highest O&G as was expected. In the Basic BSTR, the mixed liquor fraction had low O&G content because; firstly the sludge solids were agglomerating in the bottom of the vessel, preventing the solids (with attached oil) from going into the mixed liquor and secondly the smaller amounts of oil in the mixed liquor were degraded up to the maximum possible. The O&G content in the mixed liquor of the Basic BSTR had plateaud indicating the maximum extent attainable. The O&G content in the mixed liquor of the other two reactors had not reached a plateau when they were stopped as they were set to run for a similar length of time as the Basic BSTR for comparison.

The Airlift BSTR, had high amounts of untreated residue, as evidenced from high clingage quantities, which upsetted the overall performance results of the run. Both runs using the Basic BSTR design resulted in very little clingage. In the surfactant amended BSTR, there was almost no clingage and the walls were relatively scum free. There was some foaming in the surfactant amended BSTR but the foam was light and carried no solids and disappeared when the stirrer was turned off.

For the following discussion of all the reactor results, reference is made to three different phases as determined by its physical location in the reactor; bottom layer, mixed liquor and foam layer. While there were no stratification of the layers in the mixed liquor visually, the amount of foaming in the Airlift BSTR was significant enough to consider it as a separate but discontinuous (not always there) phase. For all the reactors, reference to the bottom layer of the reactor as the replenishing source of solids and oil being dispersed into the mixed liquor was also significant enough to designate it as a different phase.

6.4.1.2 PATTERN OF OIL DEGRADATION AND BACTERIAL GROWTH

Crucial determinants for the oil degradation process are the causative vectors for degradation. Plate counts for heterotrophic bacterial numbers as well as oil degrader counts were carried out throughout the study with significant findings. While total volatile solids measurements as well as those for total volatile suspended solids were also carried out to represent the organic or bacterial fractions, these could be misleading due to the residual oil, hence the use of plate counts to represent the population growth was required. Literature reviews on results obtained by the use of plate count methods were not very encouraging (Castaldi and Ford 1992) but from the results of this study, plate counts provided an excellent tool to monitor the degradation process. However,

incubation periods required to develop growth on naphthalene plates (oil degrader plates) takes two weeks and were therefore slow to produce results and precautions had to be taken to stop the plates from drying out. From the following results it will be shown that heterotrophic counts using plate count agar provided a quicker method for monitoring and are representative of the oil degradation process. However, oil degrader plate counts provided greater insight into the details of the oil degradation process.

Figure 6.33 shows the oil content of the mixed liquor fraction through time and Figure 6.34 shows the oil content per solids in the mixed liquor fraction through time. The Figure 6.33 shows the pattern of oil [2]dispersed or desorbed from the solids into the mixed liquor fraction without taking into account whether it is solids associated or not. Figure 6.34 indicates the subtle changes occurring in the mixed liquor fraction. From Figure 6.33, the Basic BSTR showed a slow desorption of oil into the mixed liquor fraction, maximum on day seventeen then reducing and plateaud approximately after day forty. The Inipol amended BSTR showed maximum oil in the mixed liquor occurred earlier than in the Basic BSTR which is likely due to the surfactants effecting faster desorption. The Airlift BSTR showed that oil was desorbed in two cycles with oil in the mixed liquor peaking twice on day twenty-six and day forty-six. Figure 6.34 is more interesting as details of the differences between the reactor's behavior become apparent. What can be interpreted from the pattern for the Basic BSTR from Figure 6.33 and the inset of TS is that initially (day 0 –7) the solids were slowly dispersed from the sludge mass to go into the mixed liquor. This resulted in a drop in the oil per solids ratio, however following day seven, TS were reducing in the mixed liquor up to day seventeen whereas the oil was increasing (Figure 6.33) resulting in an increasingly higher oil per solids ratio which peaked on day seventeen. This pattern possibly indicates oil desorption from the bottom layer of the reactor into mixed liquor occurred as the oil and solids pattern were contrasting. The appearance of the mixed liquor on day seventeen was noticeably different. There were bits of floating oil at the liquid level and results from oil degrader plate counts (discussed later with Figure 6.35) showed the highest numbers. From the results it is surmised the bacteria were producing surfactants that were desorbing the oil from the solids .

From the pattern shown in both Figure 6.33 and 6.34, it can be seen that the oil being released (Figure 6.33) in the Inipol amended BSTR is possibly through the steady

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[2] disperse in the context used here refers to the loosening and release of particles from the sludge mass at the bottom of the reactors that were being vigorously stirred.

Figure 6.33 Oil and grease in mixed liquor in the three reactors

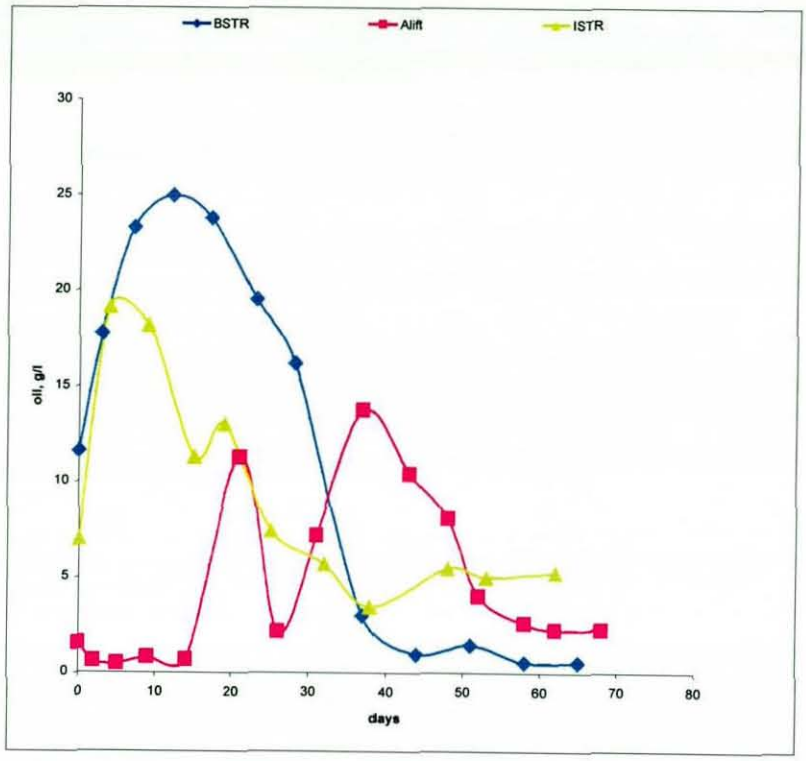
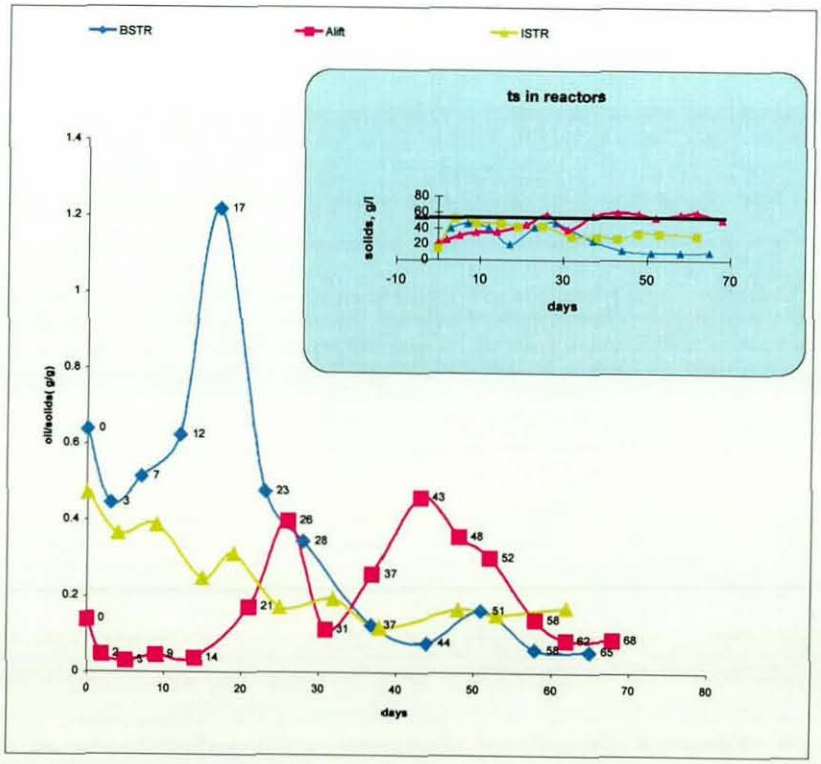


Figure 6.34 Oil and grease per dry solids in mixed liquor in the three reactors



dispersion of oil associated solids from the sludge mass into the mixed liquor and the subsequent reduction of oil has a close relationship with the solids in the mixed liquor. Figure 6.33 shows that oil in the mixed liquor increased at the start then reduced or degraded while Figure 6.34 show that oil was clearly associated with the solids as the decline in oil per solids is fairly constant i.e. there was no increase at the start. The Inipol added to the sludge before reactor start up caused a 'loosening' of the sludge mass making it easier to disperse the solids with the attached oil. From the Table 6.18, it was shown that the solids content in the mixed liquor of the surfactant-amended reactor was high.

Figure 6.33 and Figure 6.34 for the Airlift BSTR show a similar pattern indicating there is no difference in, 'oil in mixed liquor' and 'oil associated with solids' in the mixed liquor . Initially (day 2 - 14), there is very little oil in the mixed liquor in spite of the airlift and stirrer effecting good mixing of the solids. This was actually due to the reactor foaming from a very early stage in the process as foaming caused the solids and oil to be trapped in the foam layers leaving less oil associated solids in the mixed liquor. Analysis of the foam layer oil and solid contents confirmed this visual observation (results and further discussion in section 6.4.3.1). Following a spill incident on day sixteen, due to foaming, the reactor was reloaded with fresh sludge on day nineteen, which resulted in the first oil peak on day twenty-six. Possibly through the early production of biosurfactants there is a slow increase of oil in the mixed liquor up to day twenty-six then the oil and solids sharply decreased as shown by the dip on day thirty-one. On day twenty-four, a change occurred to the foam layer, which was noticeably more thick and non-compressible. Switching off the stirrer did not cause the foam to disappear. However, the foam slowly changed and on day thirty-one it was noted that the sides of the reactor was cleaner and the foam was less thick. After this day the oil and solids content started to increase again in the mixed liquor, possibly as a result of the production of biosurfactants, peaking on day forty-three, then reducing again through degradation.

Figure 6.35 show the heterotrophic counts for all the reactors and there are close similarities in pattern and peak times with oil per solids as shown in Figure 6.33 (inset). Only the Airlift BSTR showed slight differences in that there was a pronounced peak in heterotrophic counts at the start of the reactor run. However, as seen from Figure 6.33, there was initially high oil per solids in the Airlift BSTR that could correspond to the first peak in counts in Figure 6.35. The high numbers of bacteria at the start, possibly caused the foaming. The similarities show that there is a close relationship of bacterial

counts with the oil associated with the solids in the mixed liquor; either attached growth occurred or oil desorption was closely linked with solids dispersion. Only the Basic BSTR showed there might be dissolved or emulsified oil as discussed previously. From the similarity of the oil degradation pattern per solids content and the heterotrophic counts, it is surmised that heterotrophic counts can be used to monitor degradation.

Oil degraders are essentially a subset of the heterotrophic counts, hence their counts are always lower. However the difference in pattern between the two is noted to gauge the reactions that are occurring within the reactor. Oil degraders have specific adaptations that allow the uptake of insoluble substrates, hence they may be selectively advantaged over heterotrophs in media with insoluble substrate. From Figure 6.36, the oil degrader counts from the Basic BSTR have an early peak around the seventh day where the heterotroph numbers are declining (Figure 6.35). It is postulated that these oil degraders were producing surfactants that solubilised substrate which then became available to the heterotrophs resulting in the increase in numbers. Subsequently the pattern for both heterotrophs and oil degraders are the same. Heterotrophs and oil degraders in the Airlift BSTR essentially had the same pattern, possibly due to the very early production of surfactants as evidenced from the early foaming and high oil degrader bacterial counts by the second day of the reactor run. The Inipol amended BSTR show that the oil degraders were inhibited early in the run while the heterotrophic counts were steady throughout the run then towards the end, the number of oil degraders started to rise. One possible explanation for the results could be that the oil degraders were no longer competitively advantaged at the start as the added surfactant made the substrate soluble, hence oil degraders were inhibited but once all the substrate that could be solubilised by the added Inipol was utilized, the oil degraders became competitively advantaged again leading to a rise in their numbers. Another observation is that while the Basic BSTR and the Airlift BSTR exhibited the diauxie growth pattern, observed earlier in the shake flask cultures, the Inipol amended BSTR did not. Since the stepped pattern has been associated with adaptation period or bacterial succession (Guerin and Jones 1988), it is surmised that the Inipol caused the cultures not having to adapt to utilization of substrate except for towards the end of the run.

Further detailed results of the analysis carried out on the three reactors provide information for characterizing the oil degradation reaction in each of the reactors. In particular; adaptation to oil degradation, order of degradation and extents of degradation in the different phases.

Figure 6.35 Heterotrophic counts in the three reactors

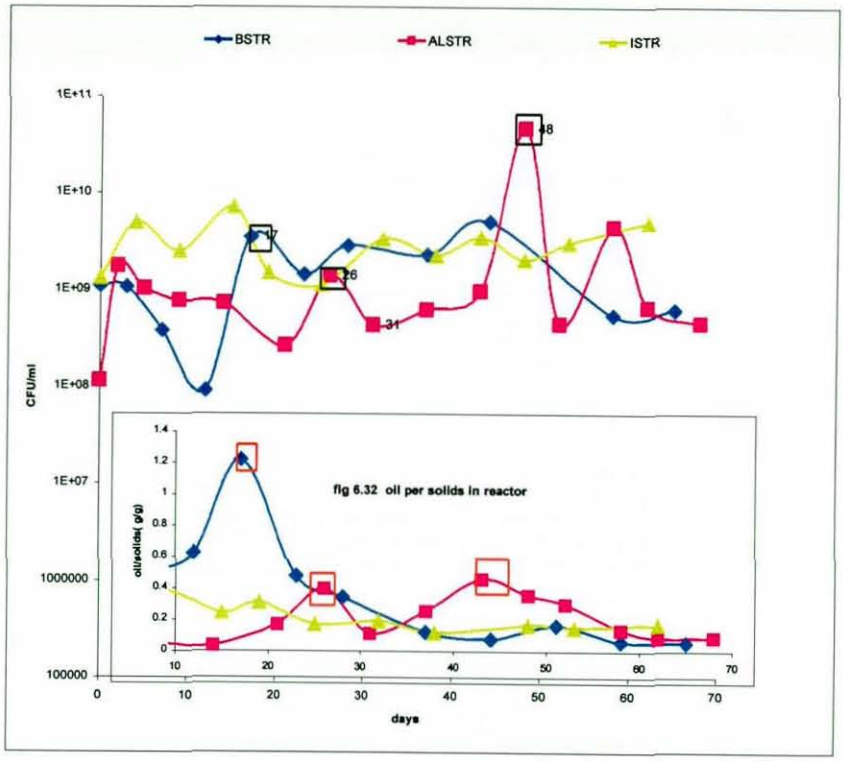
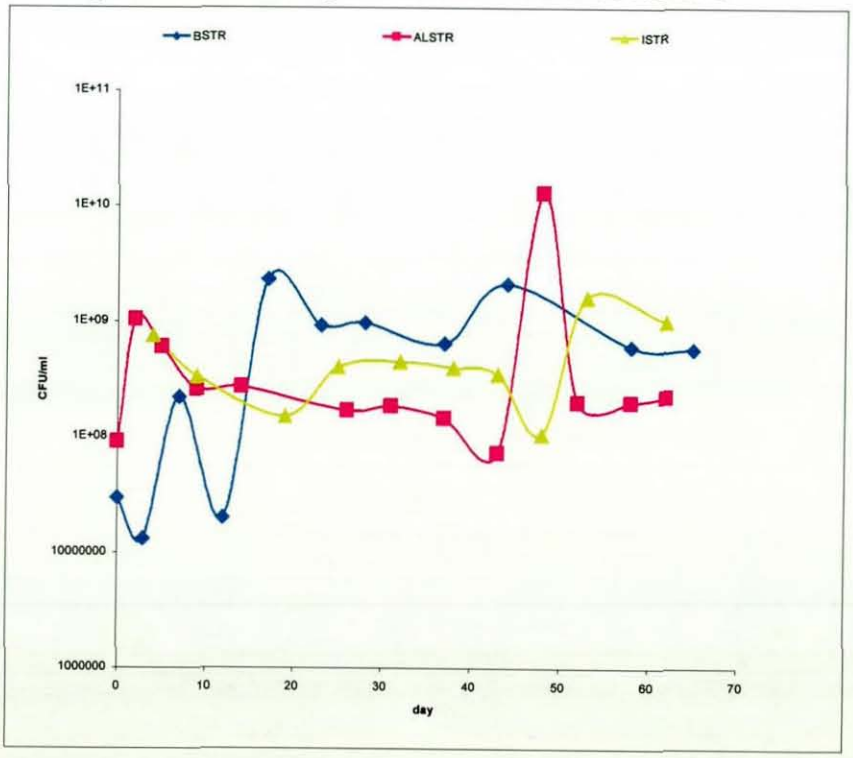


Figure 6.36 Oil degrader counts in the three reactors



6.4.2 BASIC BSTR

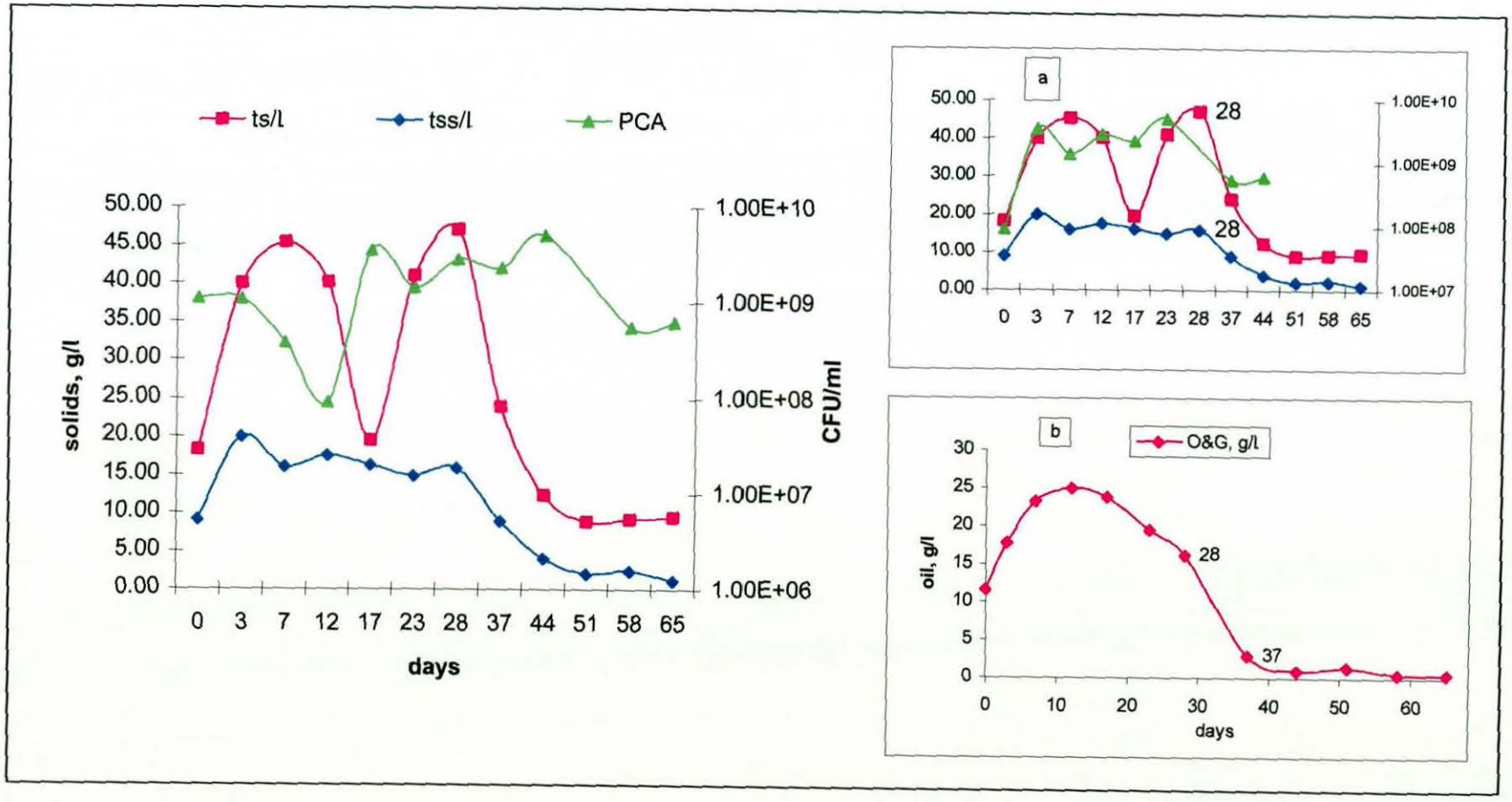
The parameters that were monitored are as listed in Table 5.13. The full results are laid out in spreadsheets in *Appendix* L (p. 303-306).

6.4.2.1 RELATIONSHIP OF TOTAL AND SUSPENDED SOLIDS WITH BACTERIAL GROWTH AND SUBSTRATE

When total and suspended solids were plotted against bacterial growth through time as shown in Figure 6.37, it was observed, that the peaks do not coincide although the general shape of curve for the solids and bacterial growth were similar. This result indicated that the TS/TVS and TSS/TSVS were not completely representative of bacterial mass but there was an indirect relationship between the parameters. When the bacterial growth curve was set back by four sampling points to determine if the patterns were similar (Inset graph a) it can be seen that for the TS, except for one data point i.e. day seventeen, the pattern closely matched the other two curves of TSS and bacterial growth curve. When remaining oil in the Basic BSTR was plotted, Figure 6.37 inset graph b, it became clearer that the solids were much affected by adhering oil or substrate. When oil reduced sharply between day twenty-eight and thirty-seven, the solids showed a similar steep reduction then a plateau, while bacterial counts remained high on day twenty-eight and only latterly dropped, though actual counts were still high. Other research have observed a similar relationship; Vail, (1991) noted in his research study for treating an oily surge water basin, that most of the O&G in the mixed liquor was associated with solids. Considering that oil was the substrate for the bacteria explains the growth curve following slightly behind, but in tandem with the solids content, which were most likely having bound oil.

As reviewed in Chapter 2, increases in TVS and TVSS have been used in reactor studies to represent bacterial growth. However, it has been shown in these reactor studies, these parameters were not fully representative of bacterial growth and suspended and total solids were used to represent the degree of dispersion of solids from the sludge mass and the suspension of solids in the mixed liquor as well as it was used to determine dissolved solids content in the mixed liquor. In the earlier shake flask experiments such as Experiment G, TVS was used to represent bacterial mass and it was representative as can be seen in the increases recorded (Figure 6.12). This was probably due to solids not being a variable (solids was a fixed parameter) as the whole

Figure 6.37 Correlation of solids and bacterial growth



flask was used per TVS analysis. TS and TVS and TSS and TVSS from Figure 6.38 and 6.39 respectively, share similar curves and the patterns are representative of one another. The graphs plotted on the insets show triplicate values of TS and TSS.

Analysis of day seventeen total solids (Figure 6.38) and total suspended solids (Figure 6.39) revealed that the large difference in the two on day seventeen was due to the reduction in the dissolved solids in the total solids fraction; Figure 6.40. Dissolved solids contributed to a large portion of the solids in TS and when there was a decrease in dissolved solids on day seventeen, there was a corresponding drop in TS, Figure 6.40 inset. The dissolved solids fraction could well be related to dissolved oil or soluble substrate.

Observations on the reactor contents and during sample analysis were taken into account when trying to interpret the data. The extracted oil from the ML showed that it was 'increasing' between day zero and reached a maximum on day twelve (Figure 6.32). Since no new oil was added then this is most likely due to the oil being desorbed from the heavier portions of the sludge in the bottom layer which were not being mixed.

Tracer studies were used to identify the best operating conditions for mixing. Silica was used to model the solids. However, in practice, the sludge itself had a pasty consistency and the particles resisted separation. The heavier oil laden particles settled on the bottom of the vessel and visual observations noted that the solids were being shifted round but that the reactor bottom was not fully mixed. The mixed liquor was well slurried but there was stratification in the lowest part of the reactor due to solids partially settling, rising for a short distance then resettling again. The sampling in all the reactors was carried out at a fixed middle depth to avoid any inconsistencies due to stratification of either solids or oil.

During sample analysis of the extracted oils, it became obvious that there were reduced viscosities of the extract from day twelve onwards. Strings of oil were also apparent, floating towards the surface of the reactor on day twelve. Day seventeen, which is the following sampling point and when the day dissolved solids dropped to the lowest point, had a less oily appearance in the settled ML samples collected for analysis. Day seventeen also recorded the highest oil degrader counts (Figure. 6.36).

Figure 6.38 Total solids and total volatile solids in the Basic BSTR

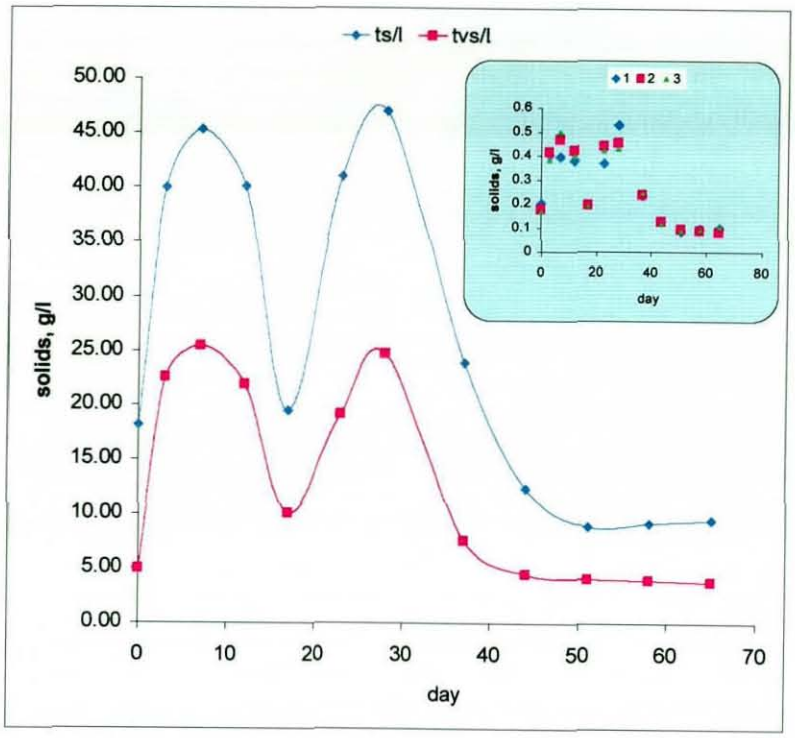


Figure 6.39 Total suspended solids and total suspended volatile solids in the Basic BSTR

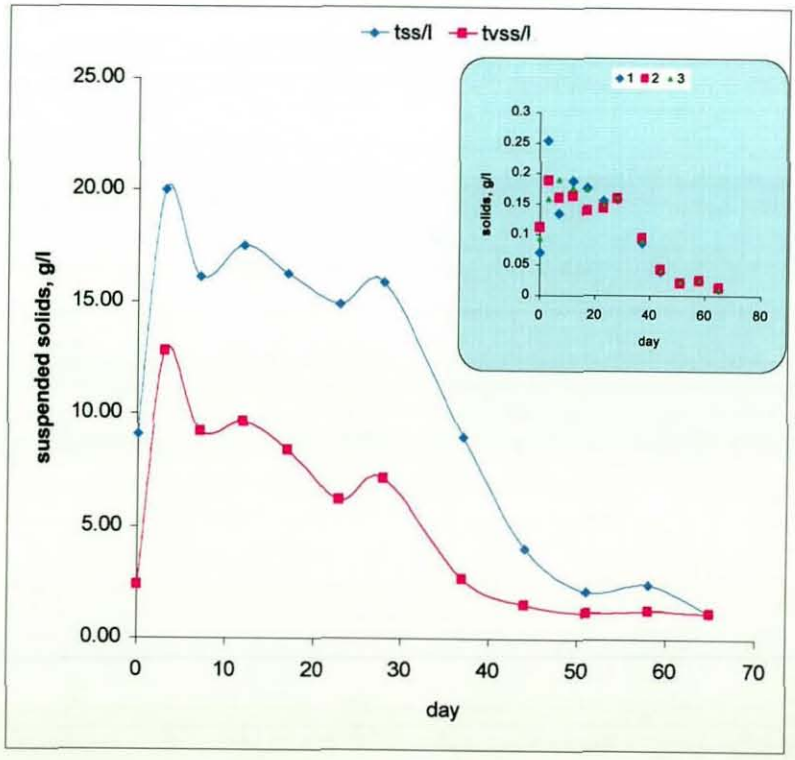


Figure 6. 40 Dissolved solids in the Basic BSTR

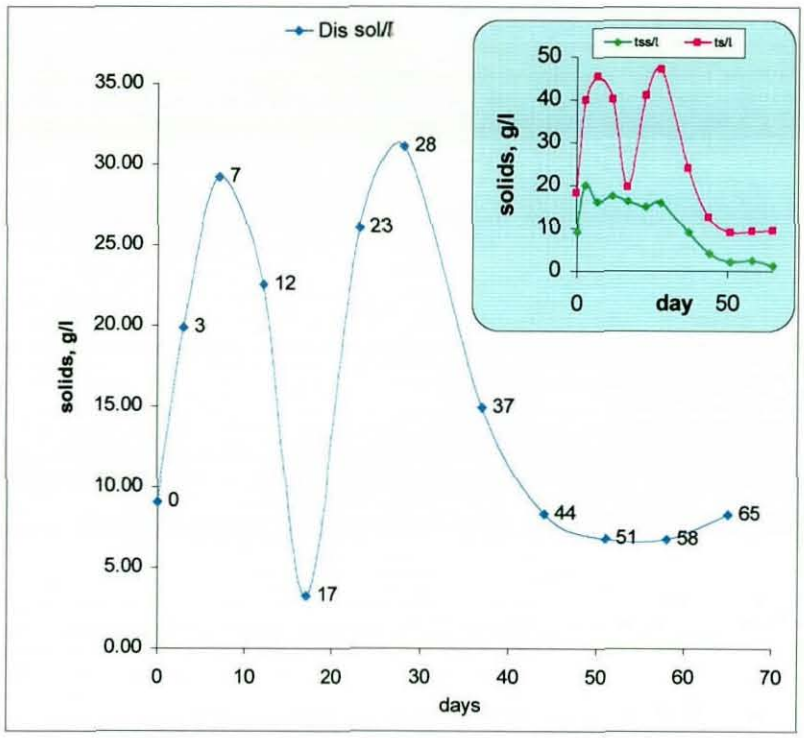
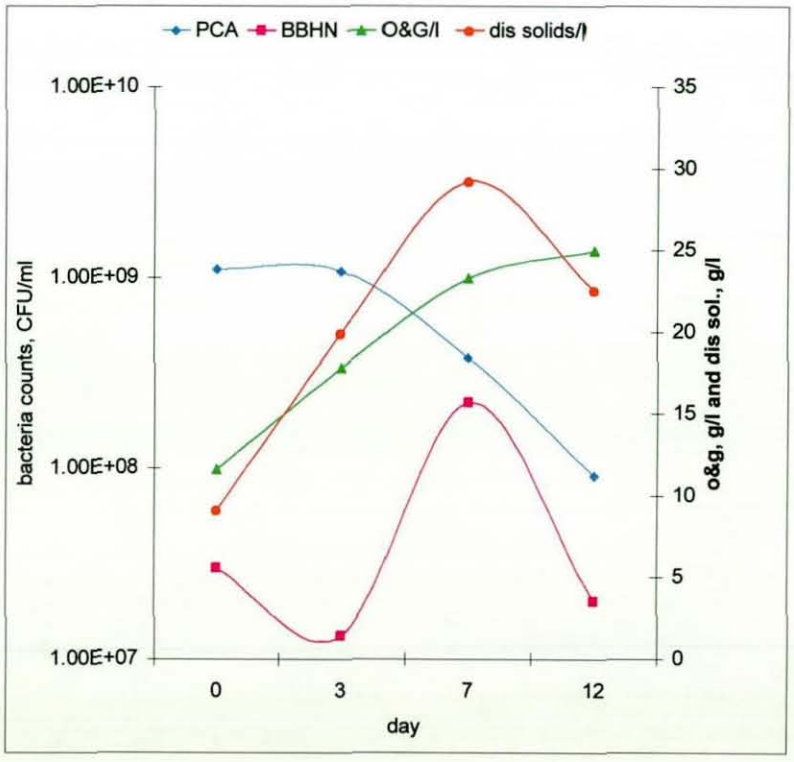


Figure 6.41 First 12 days in the Basic BSTR - Surfactant production



From these results it is surmised that the cultures were actively producing biosurfactants during in the first twelve days of growth and the biosurfactants were; desorbing the oil from the sludge resulting in the 'increase' in measured oil from day zero to day twelve; reducing the viscosity of the extracted oils; and effecting the release of oil (oil strings) observed on day twelve. Thus, the increasingly high dissolved solids fraction from day zero to day seven, Figure 6.41, are possibly due to the result of surfactant solubilising the oily substrate. Figure 6.41 demonstrates the relationships. As is shown, the oil degraders, the most likely group of bacteria that would be producing surfactants was increasing and therefore responsible for the increasing dissolved solids and oil desorption. Heterotrophic counts are slightly inhibited at this stage, probably because the dissolved or soluble fractions of the substrate are still limiting growth. Once the soluble substrate was above a critical point, the heterotrophs benefited from the solubilised substrate and growth was accelerated. Once the bacterial populations started increasing, the dissolved solids fraction dropped according to its uptake and bacteria numbers declined. Subsequently, bacterial numbers rose again when dissolved solids or soluble substrate increased and a sinusoidal offset pattern established between bacterial populations and dissolved substrate

6.4.2.2 TOC

When the oil degrader bacterial counts were plotted against soluble TOC a correlation was apparent. This is shown in Figure 6.42. Counts of oil degraders followed a similar pattern as soluble TOC. When the experiment was planned, taking the TOC reading of the ML filtrate was to measure the amount of readily available substrate for the degrading cultures. Research by Vail, (1991) determined soluble oil contents from filtered portions of mixed liquor and this research substituted O&G determinations by solvent extraction with TOC determinations.

The TOC of filtered ML or soluble TOC, show a similar pattern to dissolved solids (Figure 6.43). However, the dissolved solids reduced when the amount of oil in the mixed liquor (Figure 6.32) dropped steeply following day thirty-seven. The highest oil loss was observed during the period day twenty-eight to thirty-seven. This resulted in much lower O&G for the period following day thirty-seven which corresponds to the sharp decrease in dissolved solids content in the same period. Possibly there was less soluble oil due to the fact that there was less total oil. Following this, the pattern of dissolved solids and soluble TOC became less apparent. In the later period of the reactor run, after day thirty-seven, filtered TOC provided better correlation with oil

Figure 6.42 Correlation of soluble TOC with growth of oil degraders

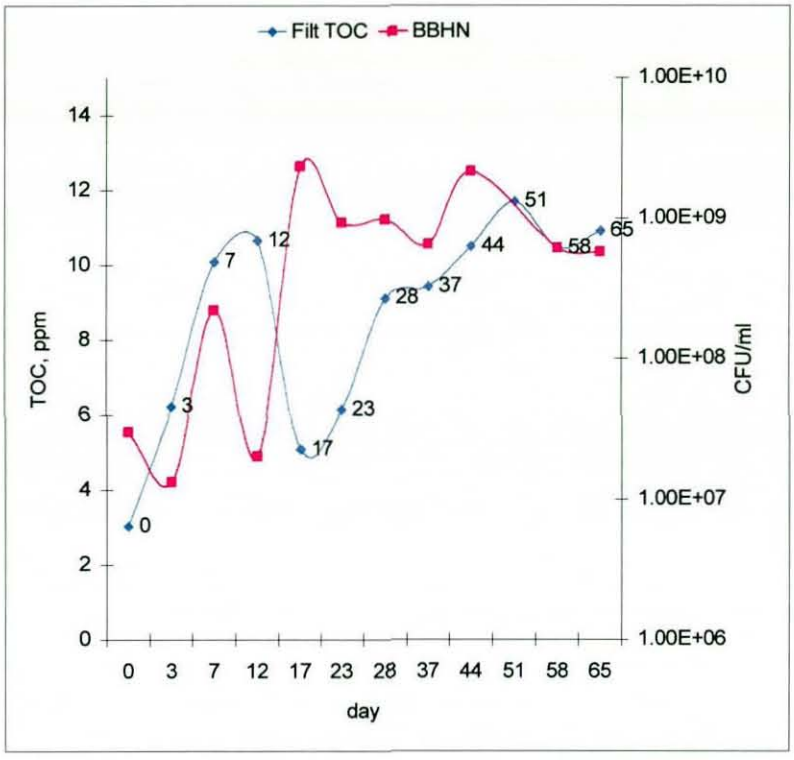
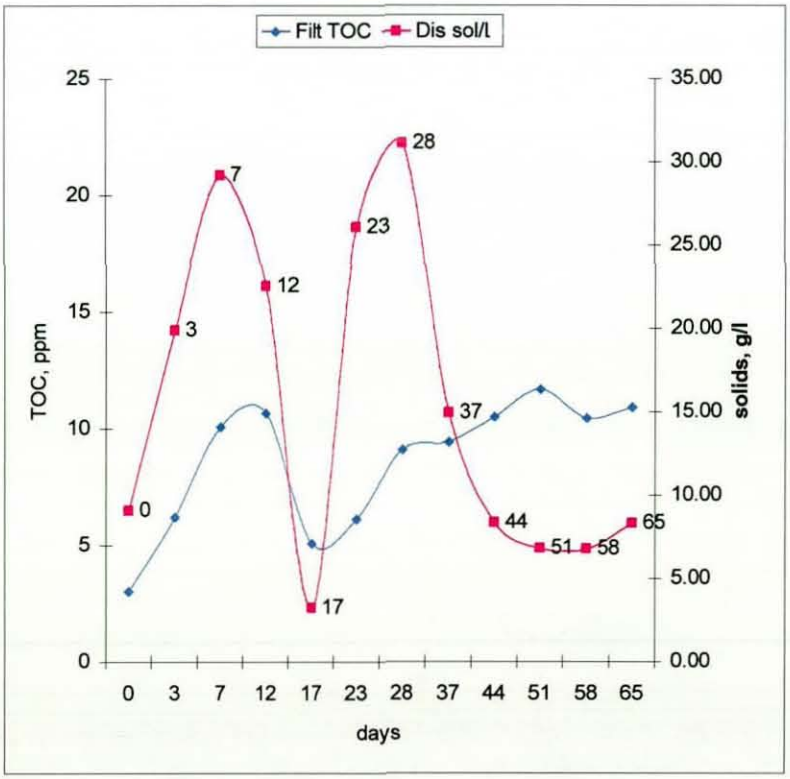


Figure 6.43 Soluble TOC and dissolved solids as possible soluble substrate



degrader bacterial counts.

The TC of mixed liquor was also recorded throughout the three reactors and in the Basic BSTR can be seen to be representative of the substrate , shown in Figure 6.44, and can be a useful parameter to monitor substrate loss due to its relative ease in analyzing this parameter .

Figure 6.45 shows the inorganic carbon compounds relative to the soluble TOC. The initial increase is possibly related to the general increase in solids in the mixed liquor. From day 28, where degradation rates are high, the inorganic carbon can be seen to be increasing and is probably related to the degradation process converting organic carbon to inorganic carbon.

6.4.2.3 GROWTH PATTERN

A pattern of growth for the bacterial cultures observed earlier in the shake flask experiments are repeated in the Basic BSTR. Both the heterotrophic counts, as cultured on PCA, and the oil degrader plate counts, on BBHO, showed two growth phases as seen in Figure 6.46. The difference in pattern for the heterotrophic counts and the oil degrader counts were only apparent in the beginning. While both showed a short lag period of ten to twelve days, the heterotrophic counts showed a decrease while oil degrader counts were maintained. The lag period in the case of the oil degraders is probably more due to the limiting soluble oil content. Although the cultures were primed on the substrate, the use of washed cells required an adaptation period before reaching a maximal rate of surfactant secretion. Diezel et al. (1996) also noted this where the used of washed cells compared to whole culture broths affected the kinetics of biosurfactant production. As seen in Figure 6.39, dissolved solids peaked on day seven, approximately the same duration for the lag period, ten to twelve days. Heterotrophic counts were always a magnitude higher than oil degrader counts and this is to be expected as the oil degraders are a subset of the heterotrophic counts. i.e. while oil degraders are more than likely able to grow on PCA, not all heterotrophs would be adapted to grow on an oil substrate. As also reviewed earlier on surfactants, the surfactant producing bacteria may be producing a solubility enhancing substance that would in general increase the amount of soluble substrate available to all bacteria. Research by Thangamani and Shreve (1994) indicated that hexadecane, initially sorbed on soil, increased quantities in the aqueous soluble phase, when the biosurfactant rhamnolipid reached the CMC . This explanation could also be used to explain the short

Figure 6.44 TC of ML representative of substrate in Basic BSTR

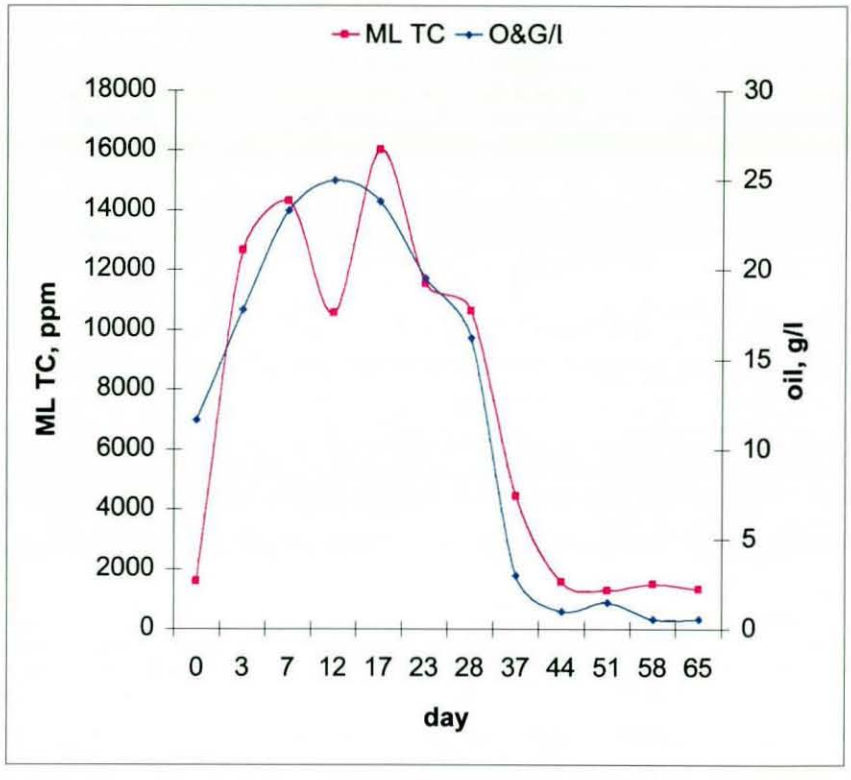


Figure 6.45 TOC and IC of filtered ML from Basic BSTR

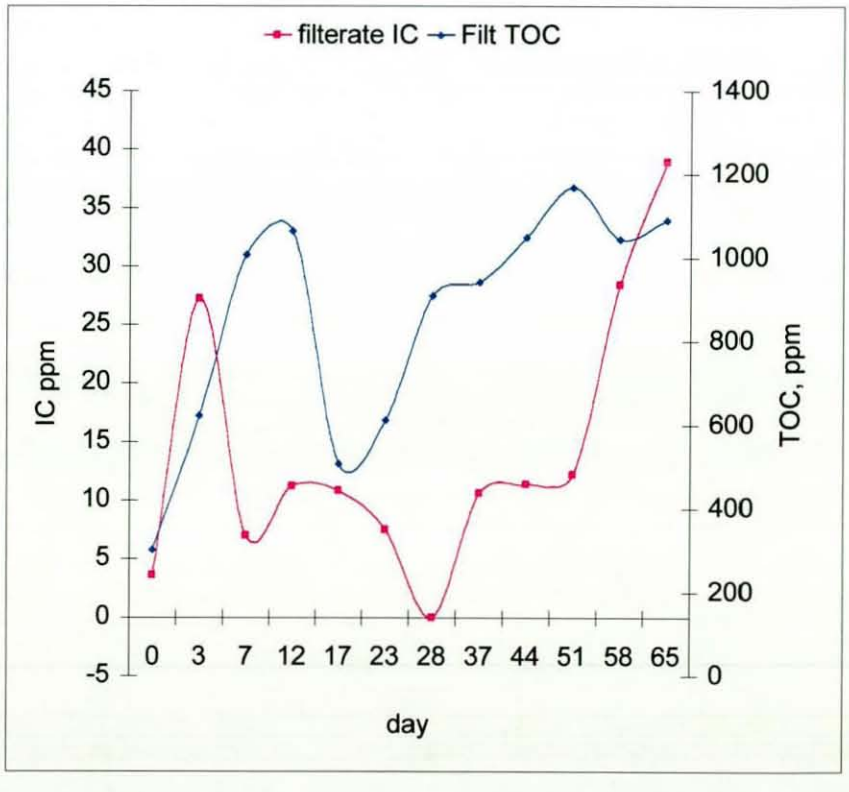


Figure 6.46 Heterotrophic and oil degrader growth curves in the Basic BSTR

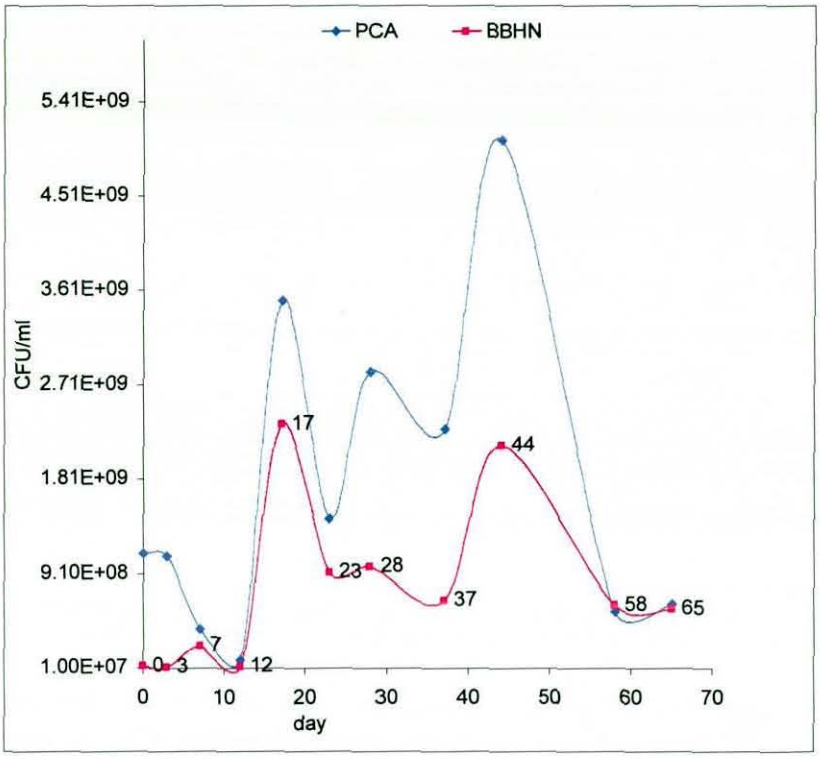
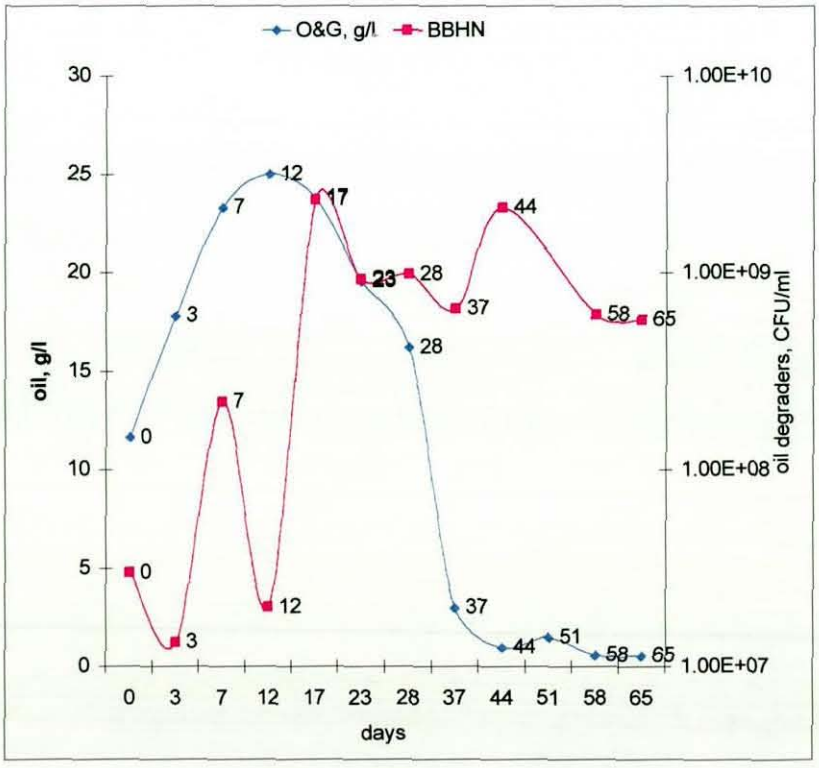


Figure 6.47 Remaining oil and grease and oil degrader bacterial counts in the Basic BSTR



lag phase observed for up to ten days whereby the soluble TOC were slowly increasing, then on the twelfth day, biomass increased exponentially when a large fraction of the substrate became available to the bacteria i.e. soluble.

Figure 6.47 is a plot showing the remaining O&G and the oil degrader plate count results. From the profile of oil remaining it is apparent that the rate of oil loss was highest between days seventeen and thirty-seven where the oil degrader counts grew exponentially to reach densities of 2.33×10^{9} CFU/ml on the seventeenth day, then it was stationary at between $6.5 - 9.7 \times 10^{8}$ CFU/ml from day twenty-three up to day thirty-seven. Stucki and Alexander (1987) have also noted similar detectable mineralization of hydrocarbon occurred when cells entered the stationary phase after initial exponential growth. Between days thirty-seven to forty-four there was another growth phase, with linear growth rates, and a maximum on day forty-four at 2.13×10^{9} CFU/ml of oil degraders, but the remaining oil did not show any further big reductions. From day thirty-seven till the end of the reactor run, the remaining oil was at a low of 3 to 0.52 g/l. The sampling right before day thirty-seven showed oil to be substantially higher at 16.2 g/l. The cell densities reached by exponential growth as well as linear growth are most likely the result of oil consumption as shown from the Figure 6.48 and inset b,c,d and e. The rate of consumption of substrate is reflected in the rate of growth of the bacterial populations.

Research by Guerin and Jones (1988) showed a two-stage mineralization pattern of phenanthrene and a diauxie growth pattern. The researchers showed that at high concentrations of phenanthrene, there was concomitant biomass increase and a build up phenanthrene metabolite intermediates. The subsequent consumption of the metabolite intermediate resulted in the second growth phase. This pattern is similarly recorded in the above results for bacterial counts seen against filtered or soluble TOC. Figure 6.42 show what is possibly a representation of available substrate, i.e. soluble TOC against microbial growth, and a relationship is apparent. While the substrate cannot be as well defined as Guerin and Jones's (1988) primary substrate and substrate metabolites, 'primary' and 'substrate metabolite' could be represented by oil and soluble TOC. While total oil gives a fair indication of substrate loss, total oil is not fully representative of substrate as it is not soluble and from Figure 6.42, while the total oil quantities have dropped following day thirty-seven, soluble TOC is increasing and oil degrader counts experienced a second growth phase. Coincidentally when oil quantities dropped, dissolved solids concomitantly dropped, though at this stage it is unclear if dissolved solids could be representative of the primary substrate.

The inference from the work of Guerin and Jones (1988) and the results of this study is that the diauxie growth pattern may arise from either a change in the original substrate, which would cause the degrading cultures to exhibit a different growth 'curve' based on the different substrate or the same substrate became limiting resulting in a 'succession' of primary degradative organisms to ones that were better adapted at utilizing low quantities of substrate. From Figure 6.48, the two growth peaks correspond to the two degradation phases. With reference to the earlier discussion on the growth pattern exhibited; cultures become substrate limited when the cell counts reached maximum on day seventeen, leading to lowered growth rates as seen in the growth plateau from day twenty-three to thirty-seven. Cultures are maximally producing surfactants at this substrate limited phase leading to an increase in dissolution of substrate (seen as increase in soluble TOC) which supports the second growth phase. Similar to what was observed by Guerin and Jones, (1988) the second growth phase is possibly bacteria that are competitively advantaged to utilize oil at low concentrations, possibly by having the capacity to utilize non solubilised oil from 'attached' surfactant mediated uptake or increasing soluble substrate concentrations. Observations by Stucki and Alexander, (1987), and Volkering *et al.* (1992) noted that at high cell counts, growth became limited due to the rate of dissolution limiting growth. Stucki and Alexander determined that dissolved biphenyl would only become limiting when cell densities reached 2.5×10^8 cells per ml. From the Basic BSTR, results, dissolution limited growth was possibly reached at 2.33×10^9 oil degrader cells per ml on day seventeen in phase one, where soluble TOC was also at the lowest point. At this point exponential growth ceased and the rate of uptake of substrate could not exceed the desorption rate and as a consequence the biomass formation rate is limited to the mass transfer rate.

6.4.2.4 RATE OF REACTION

As the following discussion of results have shown there are two phases of growth and these two phases resulted in two different rates of oil degradation as seen from the biphasic degradation curves.

Oil degradation was split to the two phases as determined by the results as discussed in the previous section. The first phase was determined to be from start untill day thirty-seven and the second phase from day thirty-seven untill the end of the reactor run. While the best fit was deemed to be an important determining factor in deciding rate orders, other results were also taken into consideration which is why day thirty-seven was taken as being the end of phase one rather than day twenty-eight which would have

given better R^2 values for both phases. Figures 6.48 illustrate the facts that were taken into consideration in determining the onset of phase two. Figure 6.48 a shows the relationship of substrate consumption and bacterial growth and it can be seen that the rate change in substrate loss correspond to the start of the second growth peak on day thirty-seven.

The points in both phases were fitted to zero and first order plots to determine the best fit and the reaction rate coefficient. Table 6.19 compares the zero and first order degradation rates. As mentioned in literature review, the first few data points, where oil was increasing in mixed liquor due to desorption, were not used in these graphical determinations of rate orders and reaction rate coefficient.

Table 6.19 : Rate of reaction summary

Rate order	First phase rate coeff.	R^2	Second phase rate coeff.	R^2
First order	0.1043 day $^{-}$	0.8514	0.0577 day $^{-}$	0.7765
Zero order	0.8747 day $^{-}$	0.9236	0.0759 day $^{-}$	0.6881

From the R^2 values , degradation occurred by linear rates in the first phase followed by a much lower rate in the second phase. However, the second phase did not fit well with linear or first order.

From the points plotted in the first phase first order plot, Figure 6.49 b, what is seen is the rates were changing between day twenty-eight and day thirty-seven resulting in the poorer fit. During this time, the counts plateaud and uptake rates were maximal. As discussed previously during comparison of the three different reactors, one of the obvious influencing parameters for oil degradation was the solids content in the mixed liquor. This is not surprising as the oily sludge was strongly sorbed to the solid particulates made of soil and iron fines. One of the objectives of the research was to investigate if this is limiting mass transfer of oil to the bacteria as it could require the oil to be first desorbed off from the particulates into the aqueous phase. Guerin and Jones (1992) as discussed earlier in Chapter 2, showed from their three parameter model how first order plots fitted poorly when the oil degradation was limited by sorption and varying desorption of oil associated with the solids. As previously determined by Guerin and Boyd (1995), when oil is sorbed onto particulates, the rate constant keeps changing due to the surfactants improving desorption and fits to classical first order plots are poorer. The dependence of whether, sorption limited degradation rates, were influenced by

Figure 6.48 Determination of phase I& 2

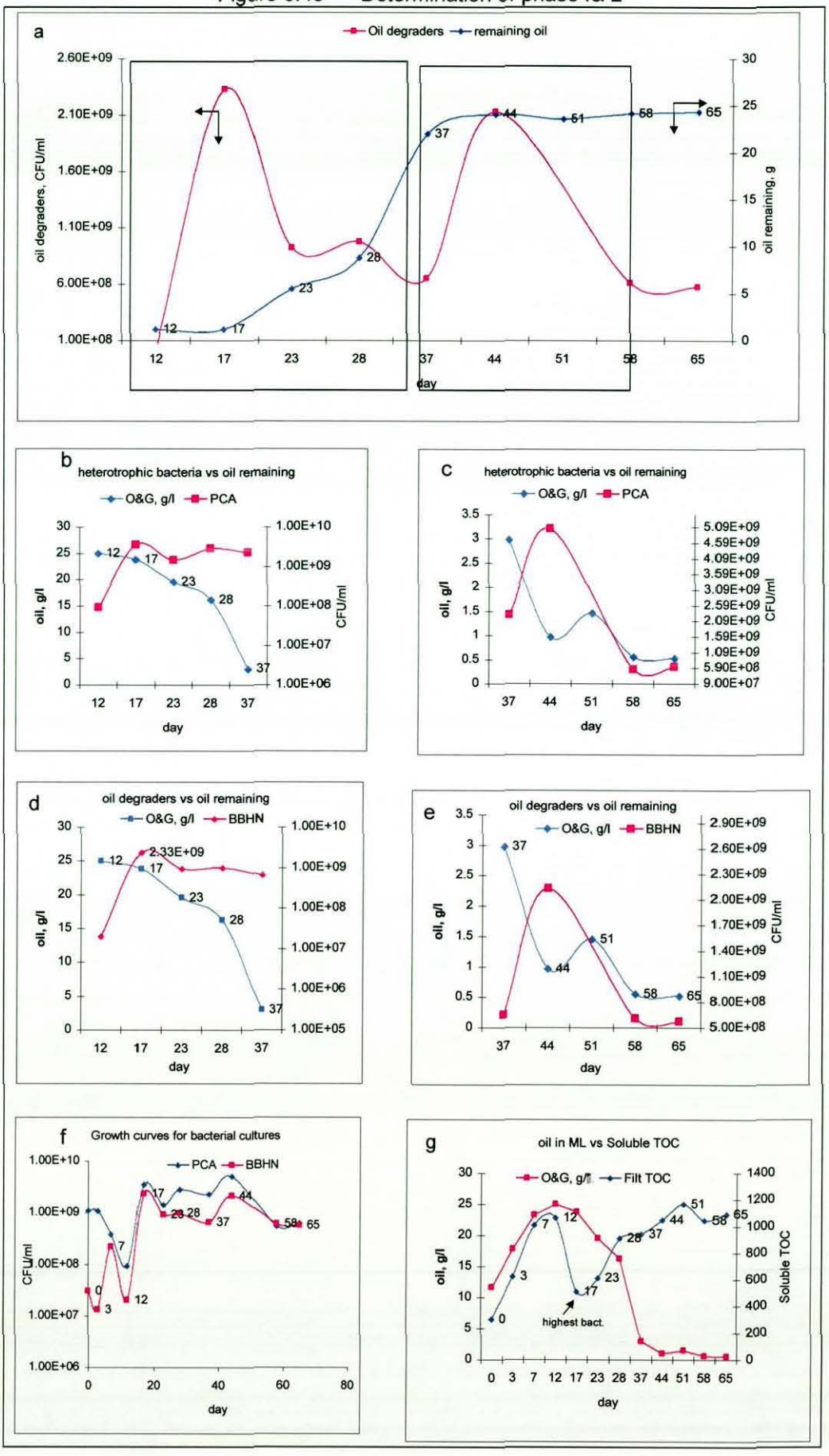
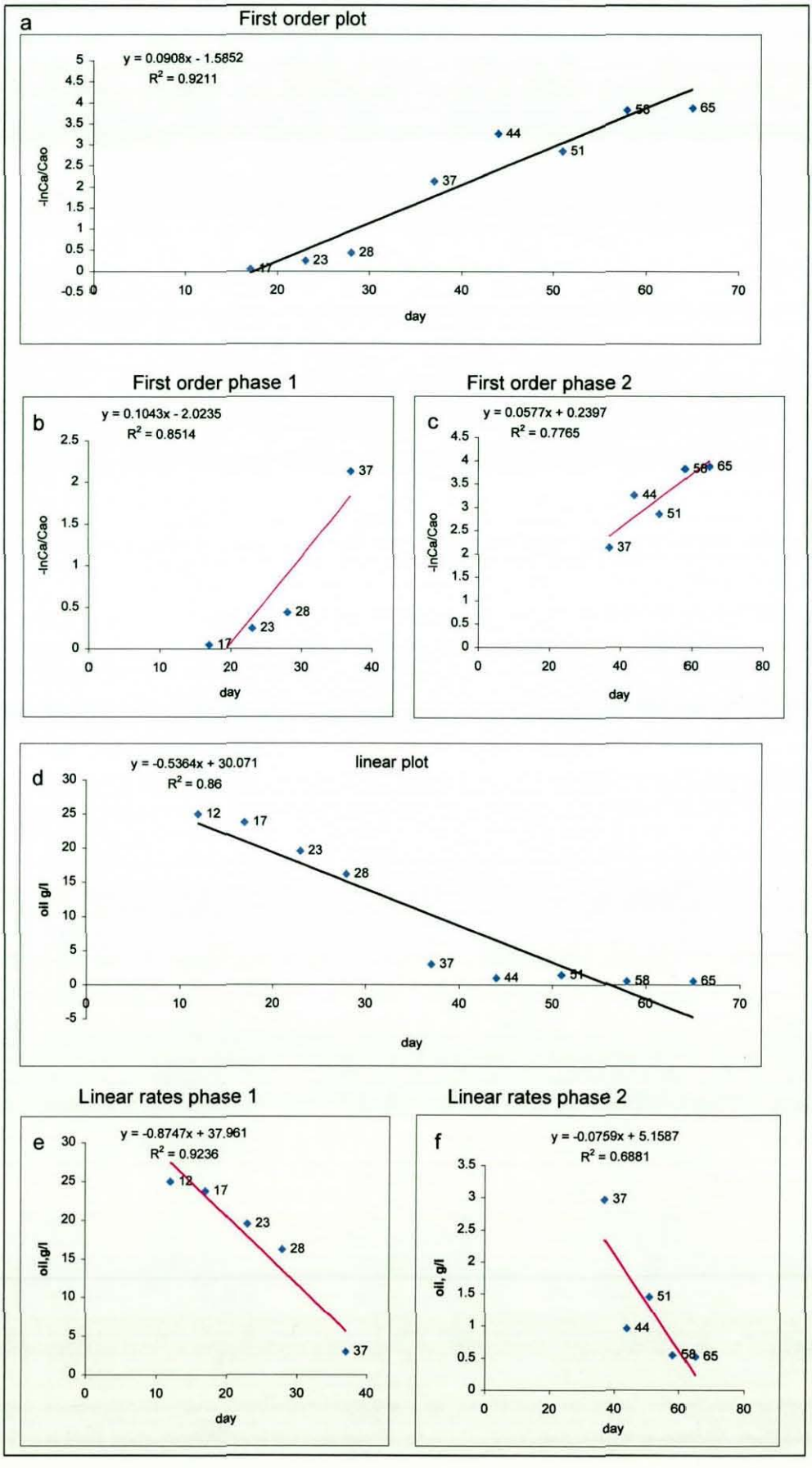


Figure 6.49 Comparison of linear and first order degradation reaction rates



other factors such as the organism effecting the degradation. From analyzing the loss of oil associated with solids in the mixed liquor fraction from the three reactors as discussed in section 6.4.1.1, it was seen that degradation of the oil was mostly from oil associated with the solids, therefore oil in the mixed liquor was normalized to total solids in mixed liquor and plotted to first and zero order plots as seen in Figure 6.50 and tabulated in Table 6.20. Fewer points were available for plotting as the first point where oil per solids was highest was on day twenty-three. However, with oil degradation normalized to the solids content in the mixed liquor, there was higher R^2 values with first order plots with rates of reaction at 0.0981 day $^{-}$ for phase one and better R^2 values for the second phase with reaction rate coefficient 0.027 day $^{-}$.

Table 6. 20 Degradation rate coefficients per dry solids in the Basic BSTR

Rate order	First phase rate coeff.	R^2	Second phase rate coeff.	R^2
First order	0.0981 day $^{-}$	0.984	0.027 day $^{-}$	0.8854
Zero order	0.0507 day $^{-}$	0.8163	0.0022 day $^{-}$	0.8152

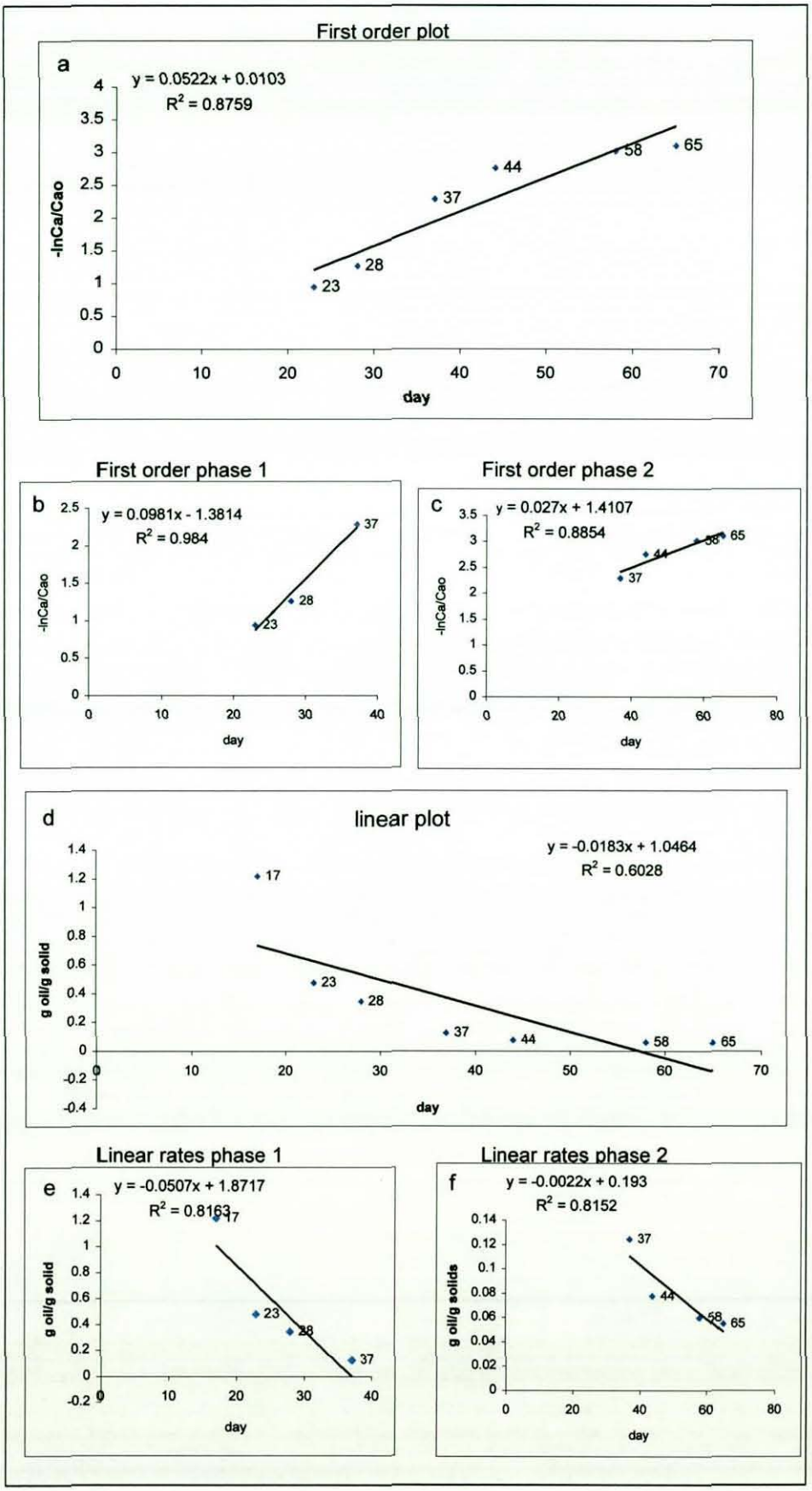
6.4.3 AIRLIFT BSTR

The parameters that were monitored are as listed in Table 5.13 and full results of the sixty-two day run are as in spreadsheets *Appendix* M (p. 307-310). For the Airlift BSTR, the ambient temperature range during operation was between 18 – 22 C ^{0} and the lowest pH recorded was 6.9 whereby sodium hydroxide was added to bring it back to approximately 7.2. As also mentioned previously in Chapter 5, the Airlift BSTR experienced excess foaming at the start which resulted in an overspill on day fourteen when it was topped up and restarted on day nineteen.

6.4.3.1 SUBSTRATE, SOLIDS AND GROWTH

In the Airlift BSTR, the oil was closely associated with the solids as seen from Figure 6.51 where the oil per mixed liquor and oil per solids were superimposable. Clearly this means in order for oil to enter the mixed liquor, the solids have to be in the mixed liquor phase of the reactor. Any portion of the solids not entering the mixed liquor would impair the mass transfer of oil to the actively treated portion of the reactor. As already discussed in section 6.4.1, this was a set back in the Airlift BSTR as the high amount of clingage resulted in reduced overall extent of oil degradation while the treated portions

Figure 6.50 First order and zero order degradation rates normalized to total solids in mixed liquor for the Basic BSTR



showed extensive oil loss. Chapter 8 will discuss further modifications in design and operation of the reactor. The following discussion will focus on the degradation of the oil in the mixed liquor. Besides mixed liquor, since volatile hydrocarbons could be stripped in the Airlift BSTR, the activated carbon trap was analyzed for O&G and the amount of O&G was found to be minimal at a total of 0.7028 g.

As in the Basic BSTR, the bacterial growth was closely related to the solids dispersion into mixed liquor, i.e. there was a rise in bacterial counts as total solids in mixed liquor increased as shown in Figure 6.52. Figure 6.53 shows heterotrophic and oil degrader counts plotted against oil per solids in mixed liquor. In the Airlift BSTR, bacterial counts increased almost immediately and foaming also commenced early. As seen from Figure 6.53, the oil per solids decreased at the start and remained low until the reactor was stopped on day sixteen. As previously mentioned, foaming trapped the solids with the associated oil, and this was recorded as low oil per solids in the reactor mixed liquor at the start. However, from Figure 6.51, TS and TSS in the mixed liquor increased from day zero until day fourteen but this increase was thought to be representative of the increase in bacterial mass, rather than the increase in the dispersion of solids into the mixed liquor. An increase in the dispersion of solids off from the bottom of the reactor was likely but it was not well represented in the mixed liquor phase as the solids and oil were trapped in the layer of foam when the airlift returned the mixed liquor at the top. Solids and O&G analysis of the foam layer confirm the much higher amount of solids and oil in the foam compared to mixed liquor during these times (Table 6.21).

During the initial trial run, thick foam crept up the stirrer shaft and spilled over, which lead to redesigning the sludge return outlet. However, even after the redesign, the sludge still caused one overspill. This time it spilled into a tube that was placed for catching spills. Oil and solids analysis of the foam layer on day five and day twenty-four, which was characteristically similar to the foaming at the start, is tabulated in Table 6.21 together with the solids and oil partitioning in a mixed liquor sample taken on day twenty-one as well as another foam analysis on day thirty-one when the foam layer structure was shown to start collapsing into the mixed liquor. Each foaming incident was followed with an increase in the oil in the mixed liquor and subsequent foaming incident had less oil in the foam which is probably a reflection of oil degradation. The data for day twenty-four and day twenty-one were from an average of triplicate five ml samples of the foam/mixed liquor and day five and day thirty was from a single five ml sample as there was not enough foam.

Figure 6. 51 Oil in mixed liquor associated with solids in the Airlift BSTR

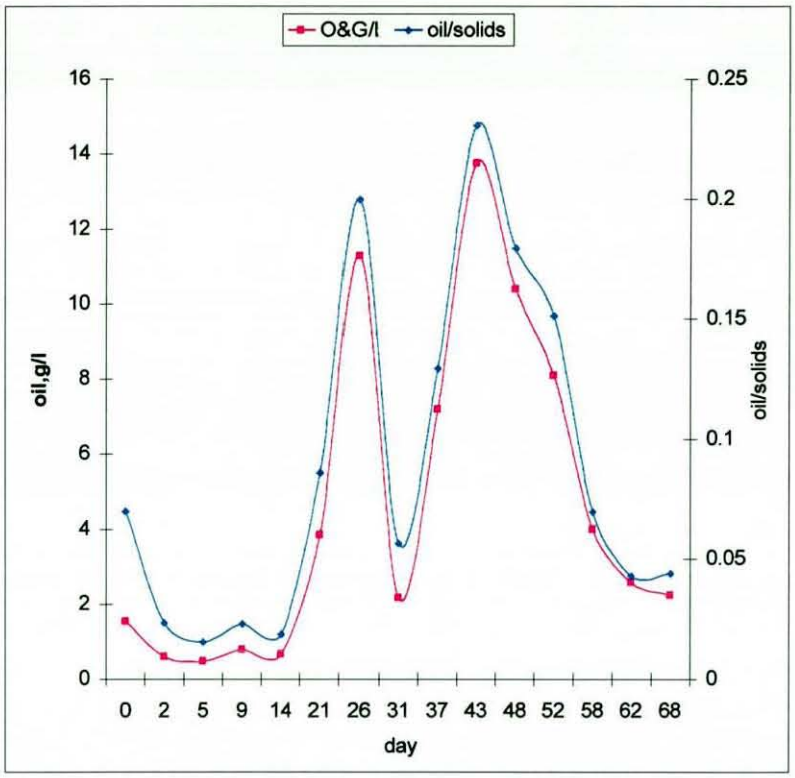


Figure 6. 52 Dispersion of solids into mixed liquor and bacterial growth in the Airlift BSTR

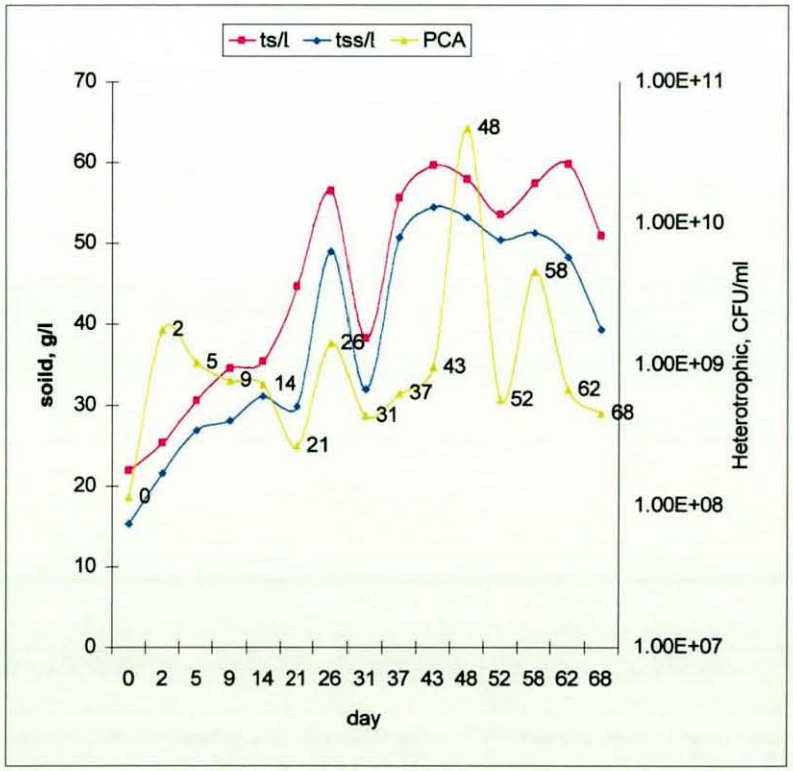


Figure 6. 53 Relationship of bacterial growth with oil associated solids and foaming incidences

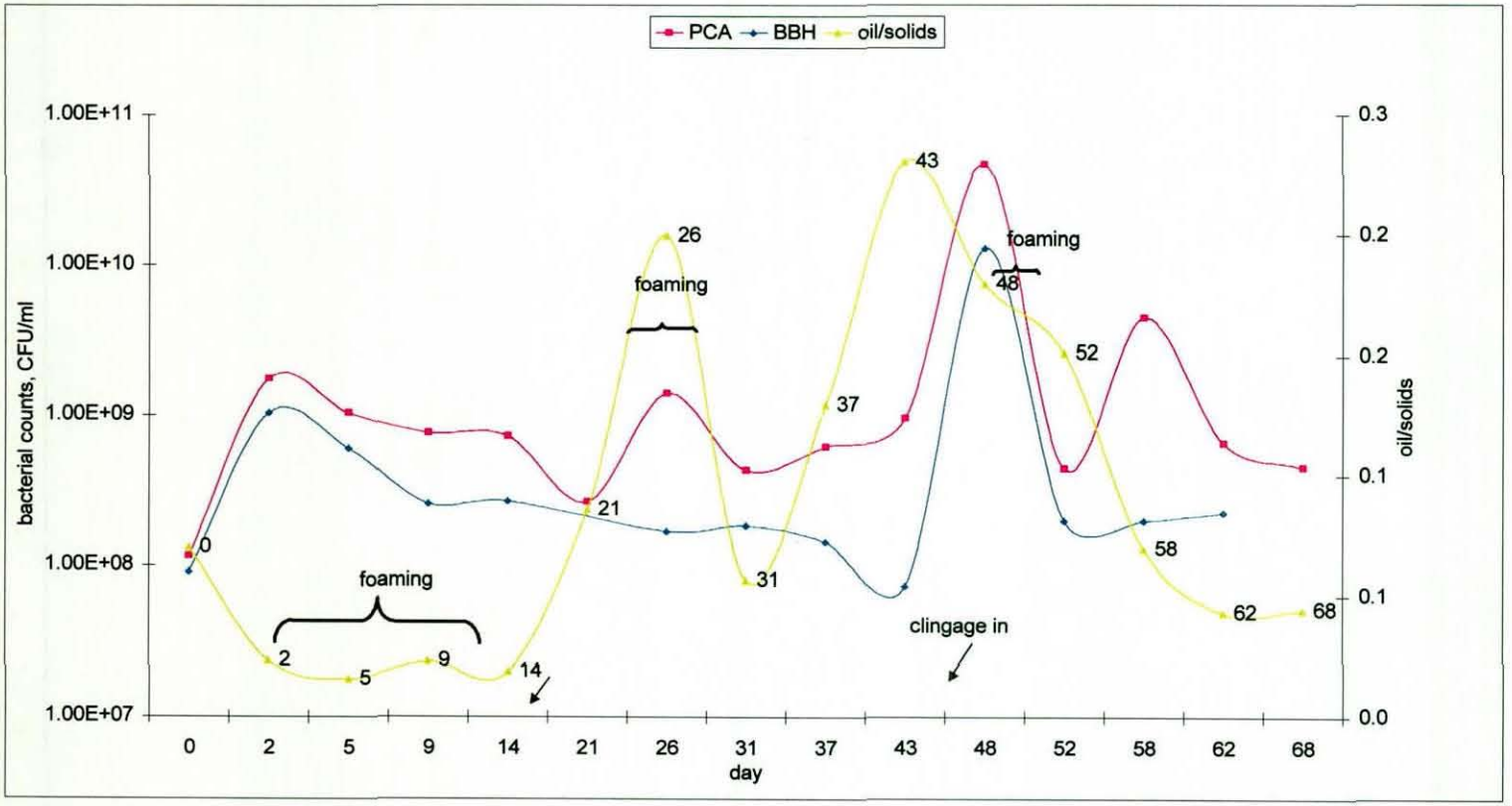


Table 6.21 Solid and O & G partitioning in foam and mixed liquor

	Day 5 Foam	Day 24 Foam (g)	Day 31 Foam (g)	Day 21 Mixed liquor (g)
TS	0.85	0.75	0.09	0.22
TVS	0.71	0.56	0.07	0.05
TFS	0.14	0.19	0.02	0.18
O & G	-	0.49	0.07	0.04
Oil/solids	-	0.65	0.82	0.17

From Table 6. 21, the solids in the day five and twenty-four foam are 3.4 - 3.8 times higher than the solids in the mixed liquor from day twenty-five confirming the observation of solids being trapped by the foam layer. The solids in mixed liquor averaged at 0.15 g between day zero and day fourteen and 0.26 g between day nineteen to end. The fluctuations in oil per solids in the mixed liquor shown in Figure 6.53 and consequently bacterial counts, as that was dependent on the substrate in the mixed liquor, were related to the incidences of foaming in the reactor. The Airlift BSTR had foaming at the start straight up to day sixteen when it spilled. When it was topped up, the foaming continued and on both occasions, the solids in mixed liquor dropped due to the foam trapping the solids. Since the oil was solids associated this also resulted in elevated oil levels in the foam layer, and oil reduction in mixed liquor. While the low oil recovery in the mixed liquor may have been due to its degradation it is hard to determine how much of the reduction was due to degradation or this partitioning into the foam layer. Also evident, after a period of time, which seemed to get shorter each time, the foam layer seemed to disintegrate which was then followed by an increase in mixed liquor oil and solids. The first foaming incident lasted approximately fourteen days but it ended with an overspill. While the reactor was topped up with fresh sludge, the remaining mixed liquor and solids were left in place together with whatever biosurfactants that were produced. When the reactor was started there was a quick dispersion of the solids into the mixed liquor as shown by the steep incline in TS and TSS from day fourteen to day twenty-six in Figure 6. 52. This was not seen in the first sludge addition and is thought to be effected by biosurfactants already in the mixed liquor. Following this, foaming occurred but of a much shorter duration and the reduction in oil per solids in the mixed liquor spanned between only one sampling period. The next foaming incident was after the addition of clingage. On day forty-eight, foaming occurred again possibly as a result of the added substrate in the form of the clingage, but this time there was no discernable

dip in oil per solids in the mixed liquor as the general trend at this stage in the treatment was a steady reduction. From the foregoing it is assumed that biosurfactants were being produced in discontinuous batches and each time it took a shorter time and the overall effect was to increase the dispersion of solids from the bottom layer into the mixed liquor phase.

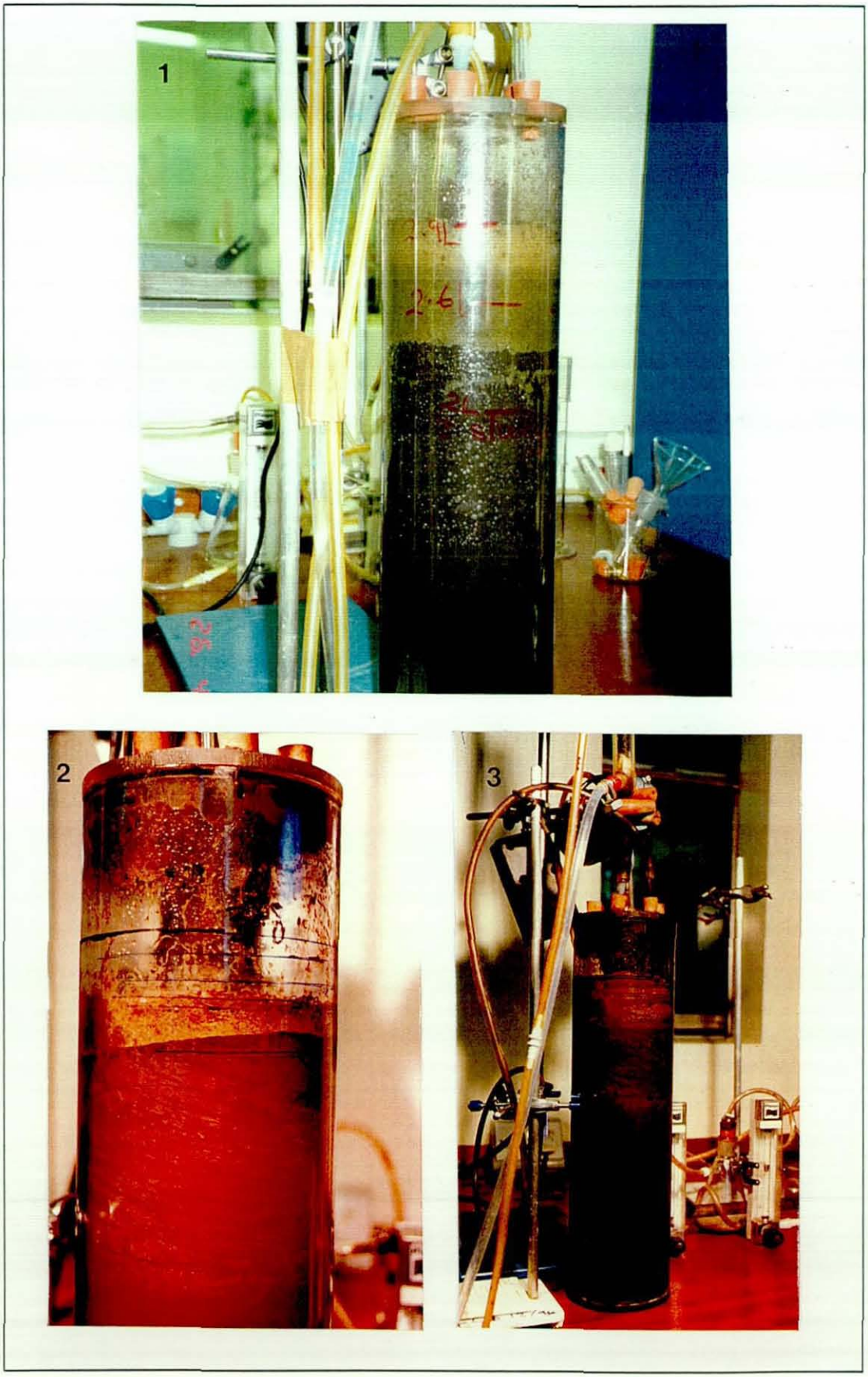
The foaming was due to bacterial surfactant production. Filtered mixed liquor samples were taken on days twenty-one, twenty-six and thirty-seven and the surface tensions were compared to the surface tension of demineralised water and sterile BBH media . As shown in Table 6. 22 there was a substantial drop in the surface tension of the mixed liquor throughout those days. Furthermore, samples were taken from the foam layer of the Airlift BSTR on day twenty-six and were cultured and confirmed for surfactant production as discussed in section 6.3.6.3. It was shown that the broth caused lasting emulsification of oil in water which was related to the drop in surface tension. Furthermore, during the initial trial runs of the Airlift BSTR, it was placed in a temperature controlled room at 37 ^{0}C and the foaming was very severe most likely due to the much higher bacterial growth rate at the higher temperature, resulting in more biosurfactants being produced.

Table 6.22 : Surface tension measurement of mixed liquor in the Airlift BSTR

Sample	Surface tension dynes/cm
Day 21 ML	42.8
Day 26 ML	44.5
Day 37 ML	46
BBH	65.9
Demin. water	70.2

Figure 6.54 show Photoplate 1 which was taken at the start of the reactor run whereby the sides of the reactor were still clean and the foam layer occupied approximately a fourth of the mixed liquor volume. The picture was taken with the airlift and stirrer switched on. Photoplate 2 and 3 of Figure 6.54, was taken at the second foaming incident but with the airlift and stirrer turned off. The foam layer, while reduced in height, was persistent and the wall of the reactor near the top show the heavy clingage due to the sludge return splashing. The sides of the reactor near the liquid level, i.e. where the foam touched, was clean, possibly due to the surfactant reducing surface tension

Figure 6.54 : Photoplate 1 - Airlift BSTR foaming at start
Photoplate 2 and 3 Airlift and Stirrer turned off, second foaming incident



resulting in less creeping of the liquid on to the walls.

Closer observation of the foam layer, showed that the foam layer had small (possibly 1-2 mm range) elongated creamy white beaded substance floating in the liquid. Jose de Smet *et al.* (1983) also observed a whitish substance, being produced by *Pseudomonas oleoverans* when grown in hydrocarbons. The authors have inferred this white substance to be a biosurfactant and they have characterized it as a lipopolysaccharide with high fatty acids, which acts as an emulsifier. Margaritis *et al.* (1979), in their research on the production and properties of biosurfactants, also found that as biosurfactant was produced it had a tendency to accumulate and float on the surface of . the broth together with emulsified oil. They found that the bulk aqueous phase of the broth had very small amounts of biosurfactant. This would account why the biodegradation of substrate in the Airlift BSTR occurred in a sinusoidal manner as it was related to the incidences of foaming (biosurfactant production) and the collapse of the foam into the bulk aqueous phase (mixed liquor) which increased the oil in the mixed liquor thereby its subsequent degradation. The sampling protocol could only measure degradation that was occurring in the bulk aqueous phase (mixed liquor). It was very likely, oil degradation also occurred in the foam layer which could not be measured. A study by Stangle and Mahalingam (1990) on the use of foam bed contactors determined that mass transfer in foams is dramatically enhanced through the much larger gas-liquid interfacial area and long contact times. They also noted, the occurrence of small solid particles in the foam phase, led to foam stability enhancement thereby prolonging this foam phase. From the observation of these researchers, Margaritis *et al.* (1979), Jose de Smet *el al.* (1983) and Stangle and Mahalingam (1990) and the results of the Airlift BSTR, it is concluded that foaming, which is strongly correlated with biosurfactant production, was a very significant factor in the degradation of the substrate in the Airlift BSTR .

6.4.3.2 TOC, IC & TC OF MIXED LIQUOR

As in the Basic BSTR, TC of mixed liquor in the Airlift BSTR mimicked O&G analysis quite closely, Figure 6.55. However, the Airlift BSTR behaved fairly different to the Basic BSTR. In the Basic BSTR, there was only one foaming incident and the foam was very much less however it did effect some oil desorption as well as solids dispersion. The soluble TOC was seen to increase as time progressed in the Basic BSTR and this was thought to be due to surfactant effect, similarly in the Airlift BSTR, soluble TOC

peaks at end of the run (Figure 6.56).

Mineralization was also evident, shown from the increase in inorganic carbon from day forty-three, Figure 6.56. The sharp rise from day forty-three was due to the addition of the clingage. Clingage was removed from the side walls, stirrer shaft, stirrer blade, top cover and sludge return cover. Some of the sludge clingage from the covers was thick and extended a few mm over the surface. The inside layers of the sludge that was thicker, received minimal treatment as noted from the oily and very dark colour of the clingage. Possibly, exposure of the inner layers to bacteria, nutrients and oxygen was diffusion limited. The outer surface of the clingage were likely to have been treated to some extent as they were continuously 'washed' by the mixed liquor and was exposed to air and hence not oxygen limited. This portion of clingage probably contributed to the increase in fixed carbon. Another indication that the clingage was degraded to some extent was the increase in soluble TOC and the reduction in pH (pH dropped to 6.9 possibly due to the influence of oil degradation products such as carboxylic acids) when it was added to mixed liquor.

6.4.3.3 OIL DEGRADATION AND RATES OF REACTION

The degradation of oil in the mixed liquor of the Airlift BSTR was more difficult to monitor due to the occurrence of foaming. How much oil was lost during foaming was unclear as during this time the foam was almost a separate phase in the reactor. However, since the rates in the mixed liquor was not high, and the total amount of oil degraded was high it is inferred that substantial oil was lost from the foam phase. Oil degradation in the Airlift BSTR occurred in cycles with oil associated solids being desorbed from the bottom layers into the bulk aqueous phase where it was degraded. Observations on the physical appearance of the reactor showed the bottom solids were being well lifted off the bottom, but the sticky sludge was still agglomerating with the stirring although this was reduced compared to the Basic BSTR. This bottom layer was slowly releasing more solids into the mixed liquor fraction and oil and solids were seen to increase up to day forty-three, although on two occasions there were dips due to foaming trapping the solids. Oil loss then could only be measured from day forty-three up to day sixty-eight although oil loss may have also occurred throughout the treatment.

Figure 6. 55 TC of mixed liquor pattern similar to remaining O&G

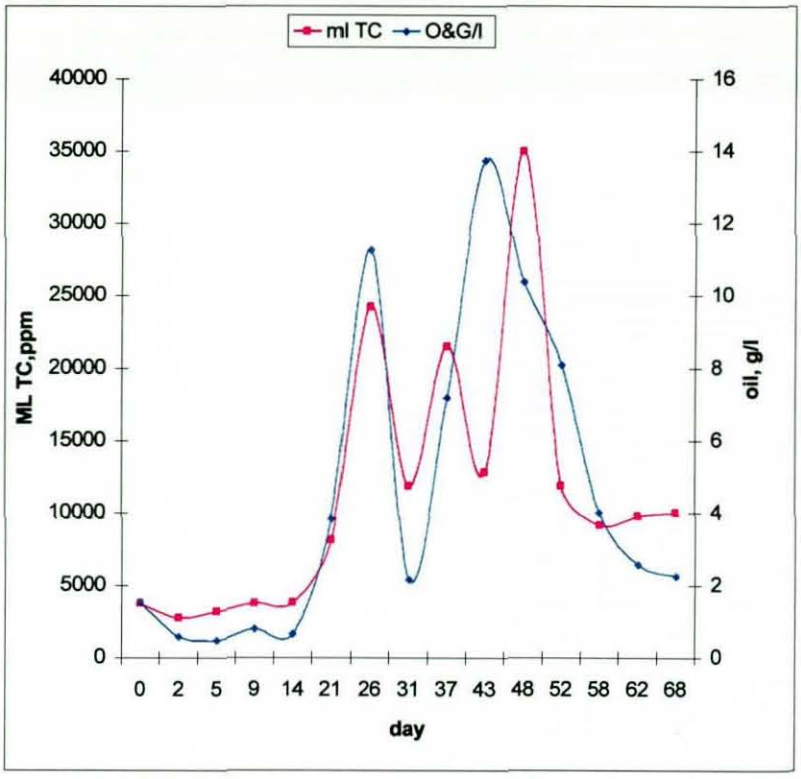
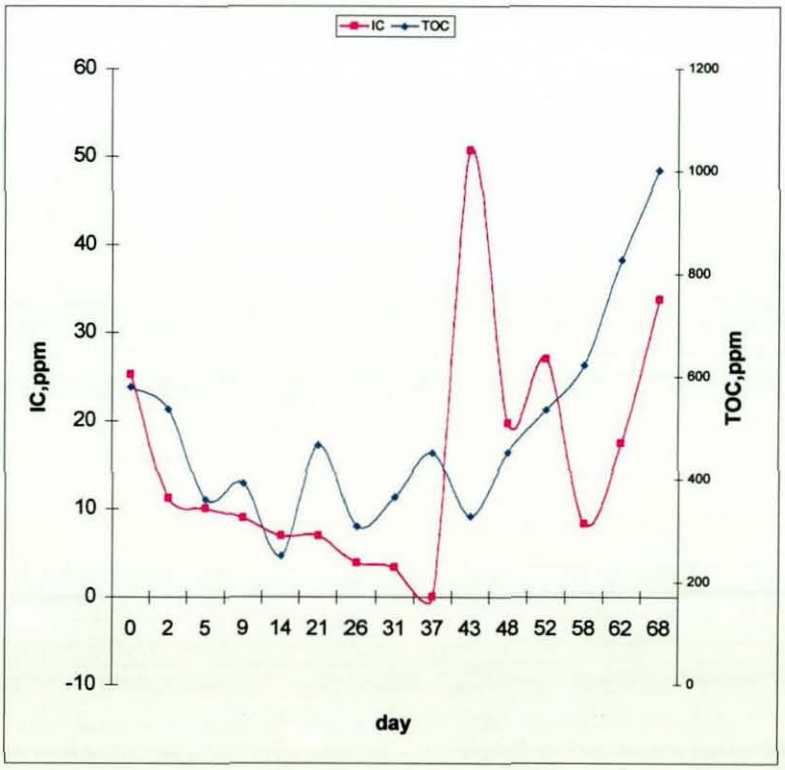


Figure 6.56 Increase in mineralised carbon (IC) after clingage addition



For example, from the graph of Figure 6.57, oil desorption and degradation seemed to occur in cycles related to both bacterial counts and foaming incidences, the first cycle was from day zero to day five, second from day twenty-six to thirty-one and the last and most extended cycle day forty-three to day sixty-eight. The first two earlier cycles were not taken into account when plotting rates as the data points were insufficient. When oil degradation was plotted to zero and first order plots, either normalized per liter mixed liquor or total solids in mixed liquor, rates were seen to fit a linear plot better. The following Table 6. 23 summarizes, R^2 values denoting the fits and reaction rate coefficients of the plots. Figures 6.57 to Figure 6.60 show the plots.

Table 6.23 Reaction rate coefficients

Rate order	Normalized	Time period	Reaction rate coeff.	R^2
Zero	l, Mixed liquor	43-62	0.6003	0.9934
Zero	Solids in mixed liquor	43-62	0.0102	0.9904
First	l, mixed liquor	48-62	0.1025	0.9888
First	Solids in mixed liquor	48-62	0.1063	0.9739

Linear rate coefficients at 0.6 is lower than the Basic BSTR at 0.9 and this is possibly due to inhibition (due to increase in toxicity) by the higher oil content in the mixed liquor. However, even with lowered rates, the Airlift BSTR achieved a greater extent of oil degradation as the degradation phase was extended and oil desorption from the bottom was not limiting. In the Airlift BSTR , substantial amounts of oil was lost from the solids (Table 6.18) and this has been attributed to the improved solids dispersion into the mixed liquor effected both by the airlift, stirring and biosurfactant production. The production of biological surfactants possibly aided this process of oil desorption as well as solids dispersion. This was seen in the increase in oil and solids in the bulk aqueous phase after each foaming incident. The airlift design promoted the production of biosurfactants, seen from the extensive and persistent foaming, compared to the Basic BSTR and this is possibly significant to the success of the Airlift BSTR in treating oily sludges.

Figure 6. 57 Linear rates for oil degradation from day 43 to day 62 in the Airlift BSTR

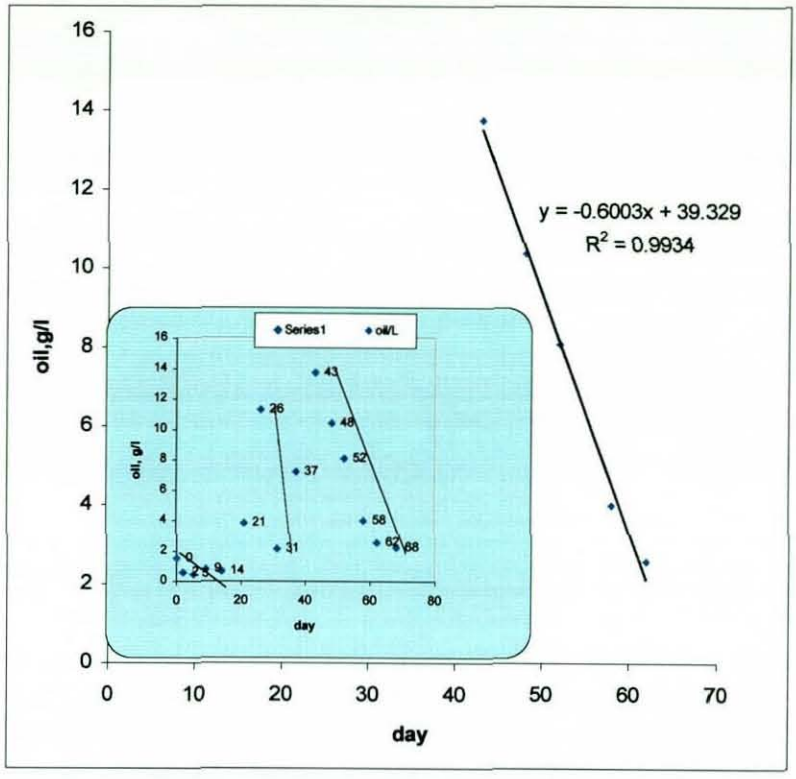


Figure 6. 58 Linear rates for oil degradation, normalised to total solids in mixed liquor, for day 43 to 62 in the Airlift BSTR

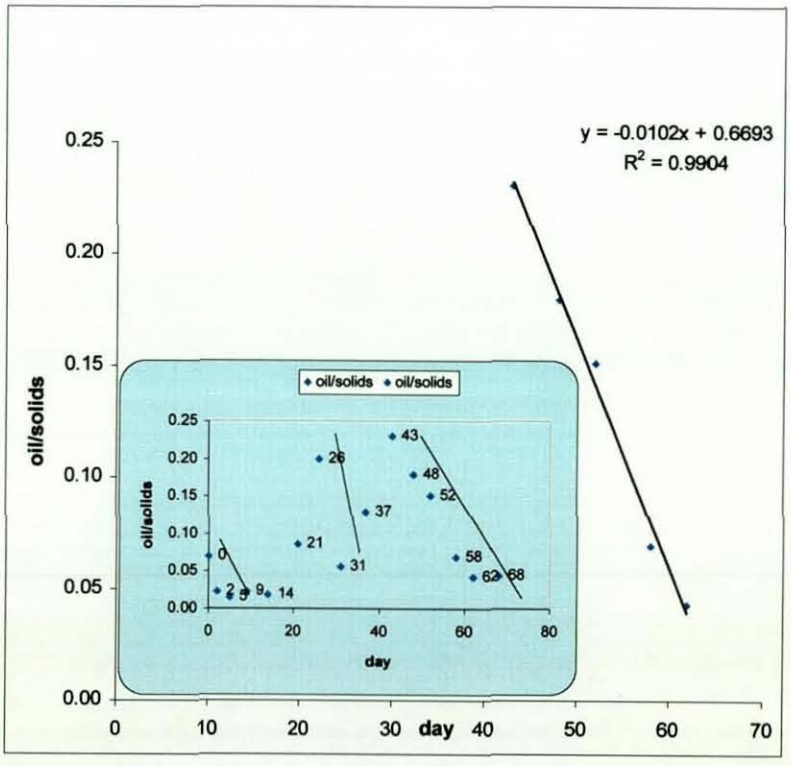


Figure 6.59 First order oil degradation rates from day 48 to 62 for the Airlift BSTR

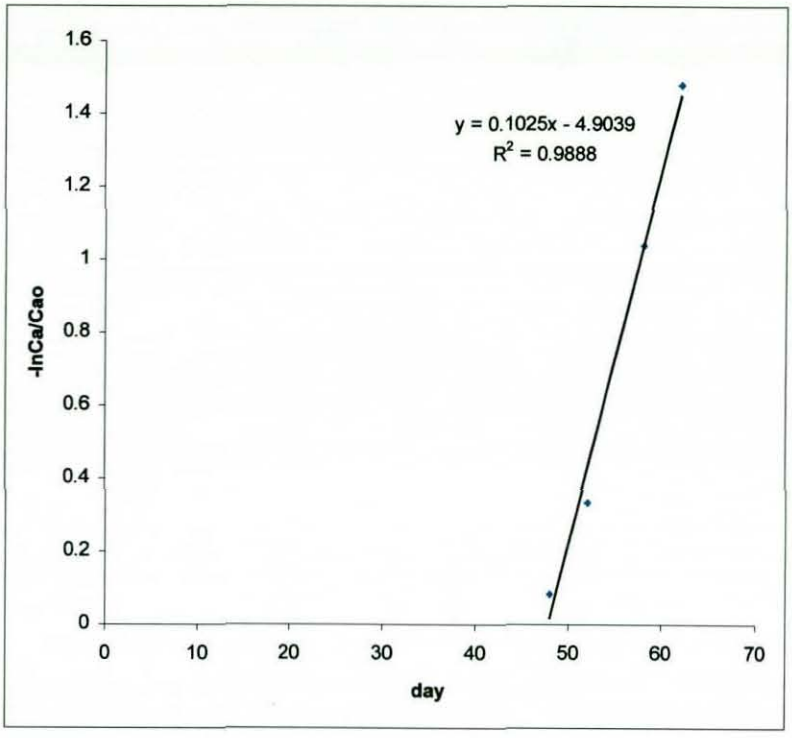
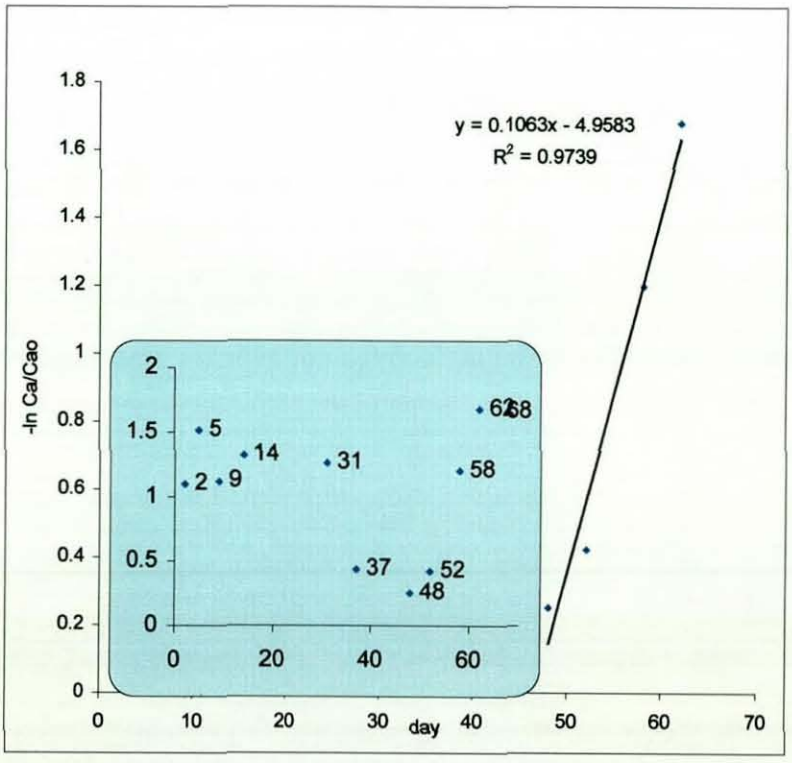


Figure 6.60 First order oil degradation rates normalized to total solids in the Airlift BSTR



6.4.4 INIPOL AMENDED BASIC BSTR

The parameters that were monitored are as listed in Table 5.13 and full results of the sixty-two day run are as in spreadsheets *Appendix N* (p. 311-314). For the Inipol amended BSTR, the ambient temperature range during operation was between 18.8 – 24 C ^{0} and the pH was kept, as in all the reactors, between 6.5 and 7.5 by the addition of sodium hydroxide.

6.4.4.1 RELATIONSHIP BETWEEN SOLIDS, SUBSTRATE AND GROWTH

Most of the relationships previously observed for the Basic BSTR were also noted to hold for the Inipol amended BSTR, although the behavior of the reactors were significantly different. Similar to the Basic BSTR, total solids and total suspended solids were found to be representative of substrate due to most of the oil being associated with solids. In the case of the Inipol amended reactor, the release or dispersion of the oil into the mixed liquor fraction was very closely associated with the dispersion of solids as can be seen in Figure 6.61. Charting the volatile solids to the O&G profile (inset) lends further support that substrate was closely associated with the solids. However, unlike the Basic BSTR, the peaks and plateau of bacterial growth closely coincided with the total solids peak and plateau periods instead of lagging behind, as shown in Figure 6.62 possibly indicating the immediate availability of the substrate.

As also noted in the Basic BSTR, the TC of mixed liquor can be used to represent substrate as shown in Figure 6.63. This may be a useful parameter to monitor for the biodegradation of substrate in place of O&G measurements. In the Basic BSTR it was postulated that dissolved solids could be related to soluble oil fractions. With the Inipol amended BSTR dissolved solids were closely related to the total oil content as shown in Figure 6.64. and therefore it is a possibility that this parameter represents soluble oil fractions; the soluble oil fraction being a constant fraction of total oil. Again, similar to the Basic BSTR, soluble TOC and dissolved solids share a similar pattern at the start then a diverging pattern later when total oil quantities became much lower, shown in Figure 6.65 and inset. For the Inipol amended reactor, one data point for soluble TOC was dropped, as at that point soluble TOC was very high due to a second Inipol addition on day twenty-four. The first time Inipol was added, it was added to the sludge but the second addition was into the mixed liquor and the most immediate effect was a raised soluble TOC fraction which could have come from either the Inipol itself or from

Figure 6.61 Oil loss associated with solids dispersion/substrate closely associated with solids

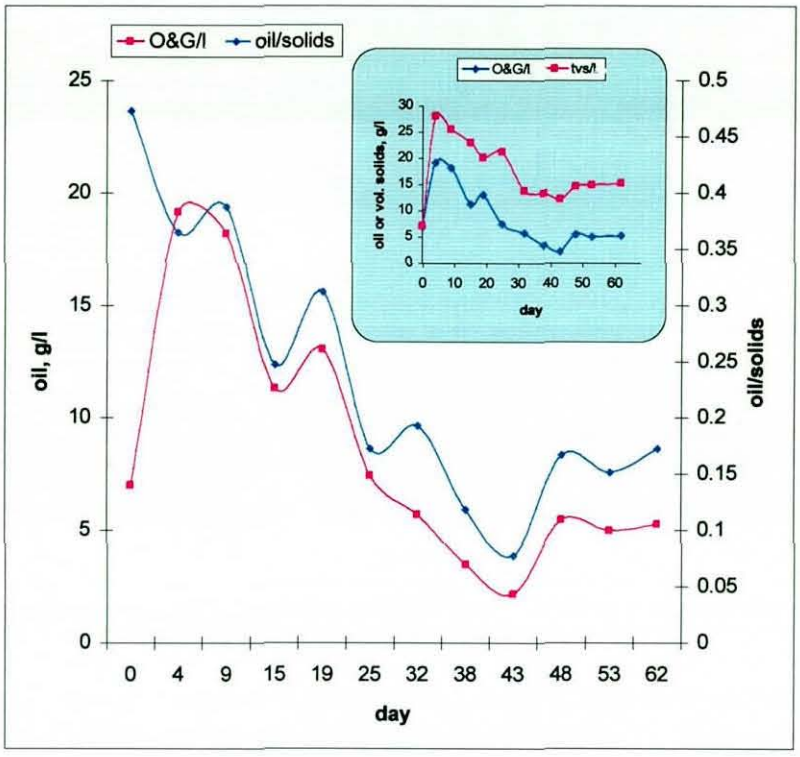


Figure 6.62. Bacterial growth curves in the Inipol ammended BSTR related to TS in mixed liquor.

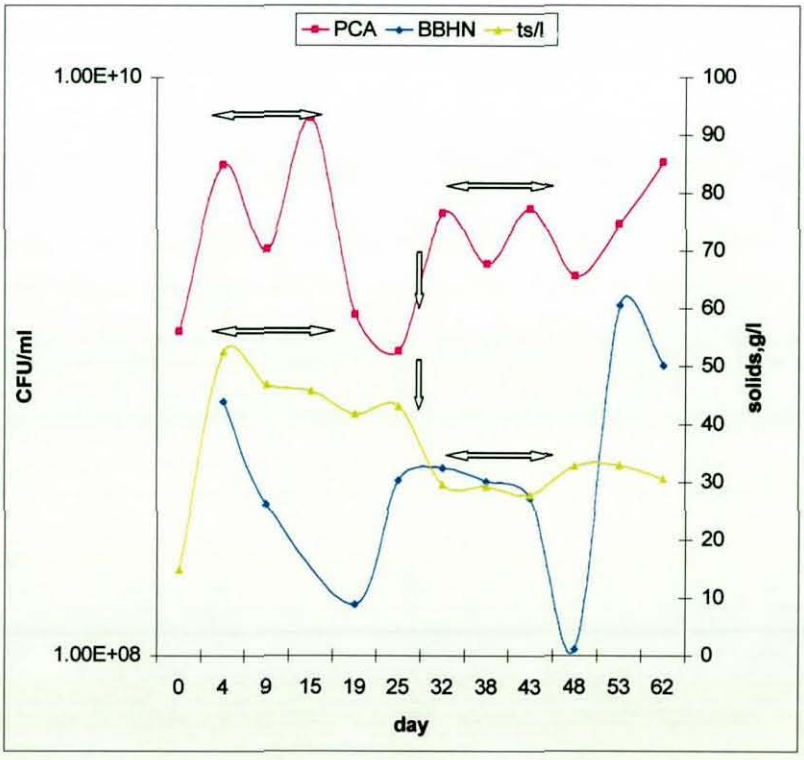


Figure 6.63 TC of mixed liquor representing oil and grease

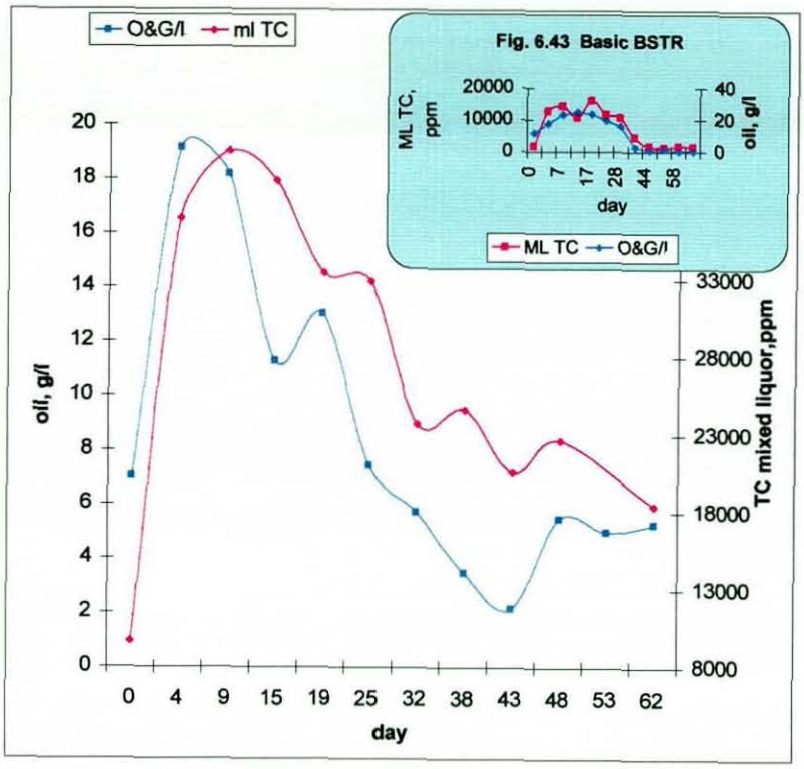
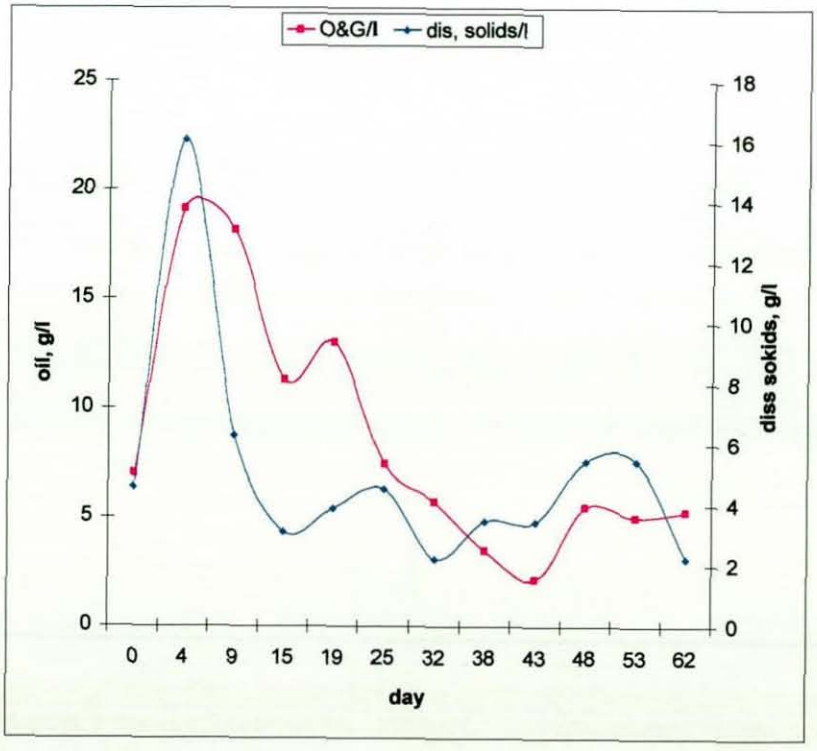


Figure 6.64 Oil and grease and dissolved solids relationship

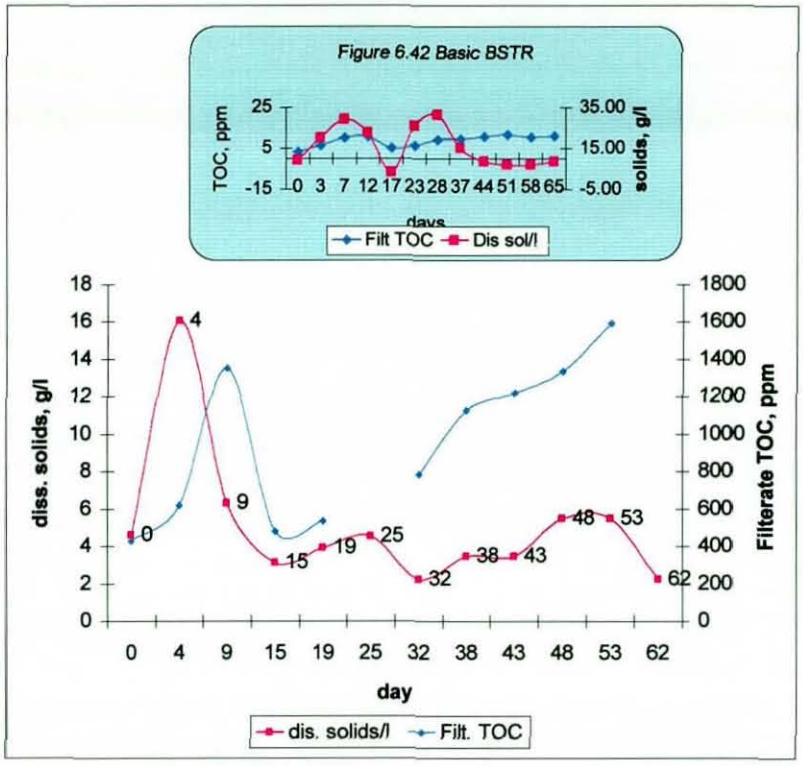


raised solubilities of substrate. The second soluble TOC peak was almost six times higher than the first peak which possibly means, that the increase was not due to an increase in substrate solubility as that would have also happened with the first Inipol addition.

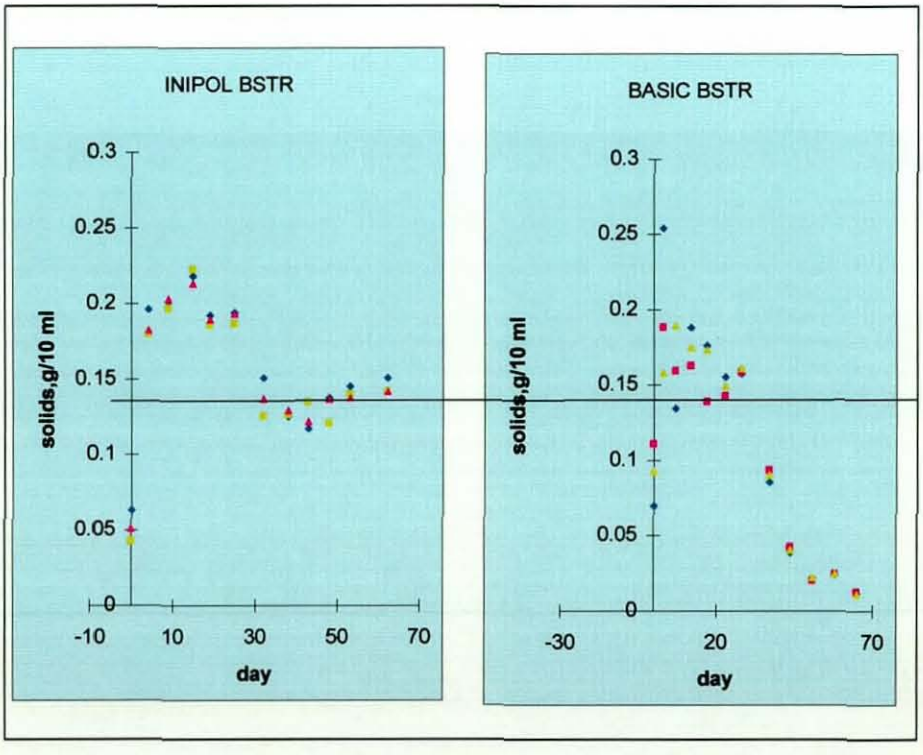
Comparing the Basic BSTR, from the previously discussed results in section 6.3.2 and the Inipol amended BSTR from the above, the higher degree of treatment afforded through the addition of Inipol was due to Inipol effecting a higher solids dispersion into the mixed liquor which occurred at a steady rate through the whole treatment period. In the Basic BSTR, there seemed to be oil dispersed into the mixed liquor only at the start. The oil possibly dispersed with the solids as well as desorbed off solids. The Inipol amended reactor had higher solids in the mixed liquor fraction for most of the treatment compared to the Basic BSTR as shown by the triplicate analysis of the total suspended solids in Figure 6.66 and this possibly contributed to the improved overall extent of degradation (approximately 60 % compared to 45%). However, when the total oil contents lost from the mixed liquor (as measured by subtracting highest oil in mixed liquor with last day oil in mixed liquor) of the Basic BSTR and the Inipol amended BSTR were compared it would seem that the oil loss in the Inipol amended reactor was lower. This is probably because not all the degraded oil from mixed liquor can be accounted for as oil was continuously being desorbed and degraded in between the sampling periods and the discontinuous sampling would not be able to account for actual oil lost for each period. Figure 6.61 gives this indication as oil can sometimes be seen to be increasing.

From the results of the Basic BSTR and the Inipol amended BSTR, it would seem that for the oily sludge to be treated, foremost, the solids have to be dispersed from the sludge mass then oil is either utilized by bacteria through desorption from the solids into the aqueous media or through an attached growth process. While references in literature frequently discussed the desorption, solubilization and dissolution effect of surfactants as previously highlighted in Chapter 3, there were few if any, literature available on surfactant effect on dispersion of solids. The characteristic of oily sludges arising from refinery, platforms, oil tanks are physically different to oil contaminated soil which is the substrate used in most of the research found in literature. The oily sludge resists slurrying into solution, compared to loose soil aggregates and, as previously mentioned, strong stirring promoted agglomeration of the sludge. Inipol addition seemed to reduce this behaviour.

Figure 6.65 Soluble TOC and dissolved solids pattern in the Inipol ammended reactor



6.66 Triplicates of TSS in Inipol ammended BSTR and Basic BSTR



Mixing the sludge with Inipol first before slurrying was shown to improve oil dispersion in an earlier experiment (Experiment 6.3.6.5) and from comparison between the Inipol BSTR and Basic BSTR, this is related to higher solids in the mixed liquor. Possibly, the surfactants in Inipol caused the very fine particulates sorbed with oil to be more evenly distributed by the intercalation of the substrate (hydrophobic tails of surfactant and hydrophopic surface of substrate) into the surfactant matrix, much in the same manner as when HOC is solubilized in micelles in surfactant solution. When the Inipol amended sludge was then slurried, there was a higher and even rate of solids dispersed into the mixed liquor phase. Zhang and Miller, (1992) found that a biosurfactant dramatically enhanced the aqueous dispersion and dissolution of solid octodecane particulates. While their research showed the relationship of increasing solubility of octadecane in surfactant solution, the dispersion was also a factor that lead to the enhanced solubilities. Octadecane was observed to be dispersed into fine particles floating on the liquid surface, instead of the usual large flakes, when surfactant was added. At low biosurfactant concentrations, this enhanced solubility was shown to be a result of the surfactant reducing the surface tension of the liquid while at higher biosurfactant concentrations the authors postulated was due to the dispersion of octadecane into surfactant micelles. The authors do not go beyond this to explain the improved enhanced solubility of octadecane. Chung *et al.* (1993) studied intraparticle diffusion and sorption effects on biodegradation of pollutant diffusing in and out of porous aggregates suspended in a liquid media. The authors determined from their mathematical modeling and experiments that aggregate size determined the diffusion coefficient of the pollutant which in turn determined the biodegradation rates. They determined that the smaller the aggregate sizes, the larger the diffusion coefficient and the higher the degradation rates. Therefore, from this, it is inferred that octodecane showed improved aqueous solubility through the dispersion of the solid particulates into finer particles which then had larger diffusion coefficients and effected better solubilizing rates. The rate of solubilization (or desorption) and its limitation on biodegradation rates has been previously discussed in Chapter 3. A similar research by Volkering (1995) on particle size and the rate of solubilization and its limitation on biodegradation rates were also previously discussed in Chapter 3. While surfactants increase solubilizing capacity of HOCs into solution, giving opportunity for a greater extent of degradation of low solubility compounds, the solubilization rate may be the biodegradation rate determining factor. From the foregoing discussion, it is postulated that Inipol caused a higher dispersion of oil associated solids into the mixed liquor, possibly by the surfactant matrix effects as well as the reduction in surface tension of the mixed liquor. This larger amount of solids have in effect, higher desorption surface areas, which Chung *et al.*

(1993) also demonstrated in his model to have a similar effect as larger diffusion coefficients and this in turn allowed more oil to be desorbed.

Previously discussed in section 6.4.1.1, the Inipol amended BSTR resulted in less oil per mixed liquor solids and part of this was due to the higher solids compared to oil in mixed liquor as shown in Table 6.18. There was also another notably different observation on the Inipol amended BSTR, oil globules floating on the meniscus layer were noted from day one of treatment, to the extent there was almost an unbroken oil layer sitting on top of the water layer when the reactor stirrer and aerator were switched off. Since the pattern of solids and oil in the mixed liquor were the same, it is concluded that, while the solids were dispersed into the mixed liquor portion, oil was concurrently being desorbed from the solids.

In section 6.4.1.2, oil degraders were shown to be inhibited at the start of the treatment. This was postulated to be due to the Inipol solubilizing substrate and making it available for the heterotrophs. Another possibility is that Inipol was providing an alternative carbon source. It can be seen from Figure 6.67 that after the addition of Inipol, soluble TOC, which provides a readily assimilable carbon source, increased considerably, especially after the second addition on day twenty-five. Soluble TOC in the Inipol amended BSTR was possibly a combination of Inipol and solublilized oil from the sludge. After each Inipol addition, oil degraders reduced in numbers or plateaud while heterotrophic counts increased. It can be seen that right before Inipol was added on day twenty-five, heterotrophic counts were reducing while oil degraders were increasing and the increase of oil degraders was halted when Inipol was added and the heterotrophs again recorded an increase. The oil degrader count only noticeably increased when clingage was added on day forty-eight, giving the oil degraders a substrate for which they were competitively advantaged to assimilate. Therefore, it is most likely that when Inipol was added, the heterotrophs used this as substrate which was rapidly used up and only when the Inipol soluble TOC was used up, the inhibition towards the less degradable substrate was lifted and the oil degraders gained the advantage at the end. A plot of bacterial counts against oil remaining in Figure 6.68 showed very poor correlation supporting the conclusion that Inipol was possibly the preferred substrate for the heterotrophs. Further investigations to confirm this would need to include monitoring the loss of Inipol. The heterotrophs were dominant and inhibited the increase in growth of the oil degraders. From a comparison of heterotrophic (PCA) and oil degrader counts (BBHN) between the Basic BSTR (referred as BSTR 1) and the Inipol amended BSTR (referred as BSTR 2), shown in Figure 6.69 it is clearly evident that the heterotroph

Figure 6 .67 Bacterial growth in relation to soluble TOC in mixed liquor in the Inipol amended BSTR

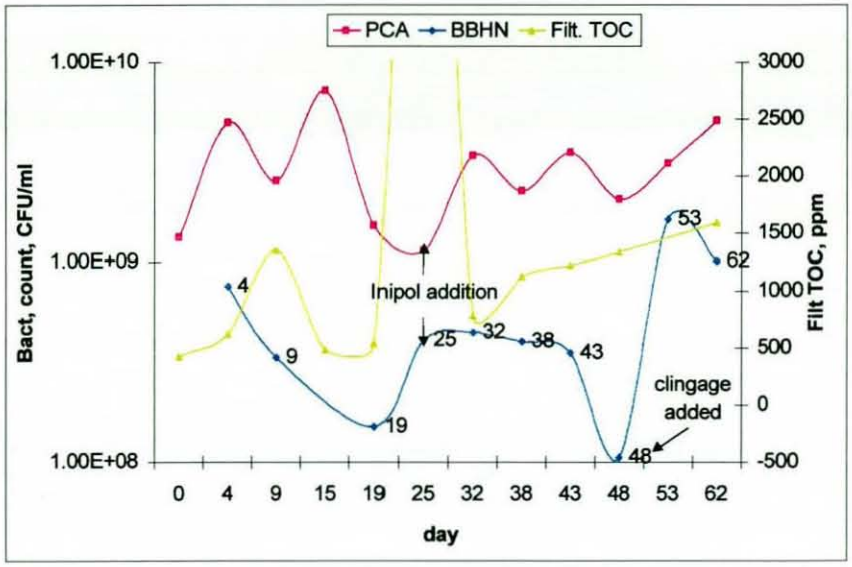


Figure 6.68 Bacterial counts plotted against oil remaining in the Inipol amended BSTR

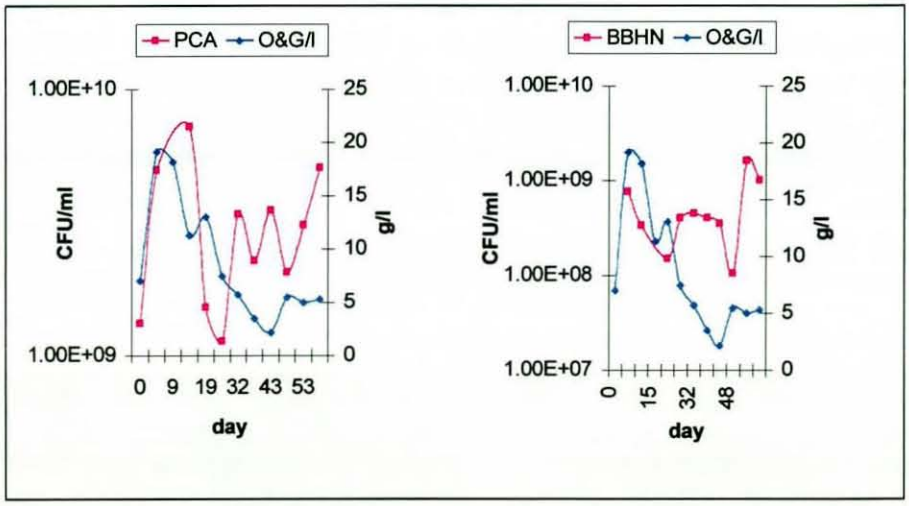
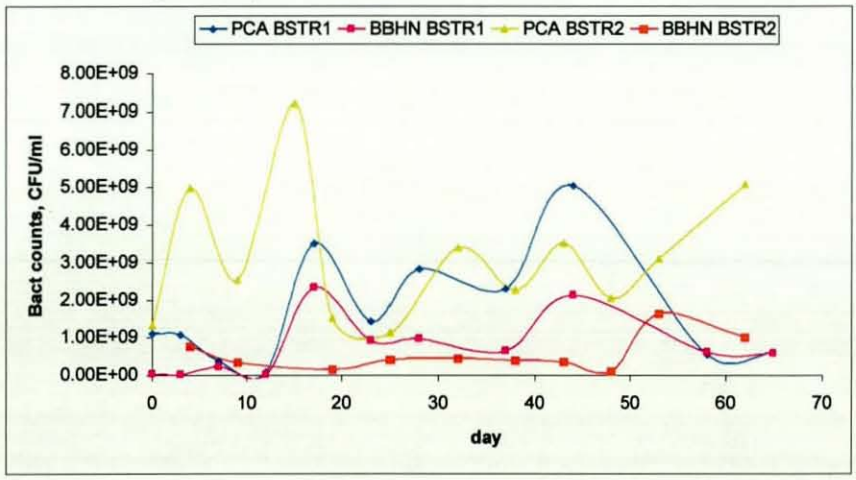


Figure 6.69 Elevated heterotrophic counts in the Inipol amended BSTR (BSTR 2)



counts were elevated well above the heterotrophs in the Basic BSTR, again substantiating that Inipol was used as substrate. However, substantial oil was degraded and removed from the Inipol amended BSTR. While growth increase was inhibited, oil degrader counts remained at approximately 3.5-4.5x10^8 and was possibly responsible for degrading the oil. Substrate loss continued till the end of treatment and increase in substrate mineralization was evidenced by an increase in inorganic carbon as treatment progressed as shown in Figure 6.70. Substantial solids reduction was also seen in the Inipol amended run, another indication of mineralization. Other researchers have noticed a similar trait from the use of surfactants whereby the surfactant was used as substrate but regardless of this, the low solubility substrate was concomitantly degraded (Bury and Miller 1993: Churchill *et al.* (1995b). Churchill *et al.* (1995b) showed that oleic acid in the Inipol formulation served as an alternative carbon source for the growth of the bacteria but the mineralization of phenanthrene was not suppressed. In the research by Liu *et al.* (1995), Brij 30 was shown to be degraded together with naphthalene while Triton X-100 remained essentially the same as start. This resulted in a slightly lower extent of mineralization of naphthalene in Brij 30 solutions compared to Triton X-100, however the authors have noted the difference as not being statistically significant.

6.4.4.2 RATES OF REACTION FOR OIL DEGRADATION

The degradation of oil in the Inipol amended BSTR was also split to two phases as it was seen that there were two different gradients for oil loss as seen at the start and at the end of treatment. The degradation of the oil was seen to occur from the start of the reactor up to day forty-three, when oil actually started to increase in the mixed liquor. Compared to the Basic BSTR, oil degradation was seen to occur earlier and only one early data point (day zero) was dropped in the calculations of the rates for the first degradation phase compared to three to four early data points in the Basic BSTR during which time oil was still increasing (desorbing) into mixed liquor. Refer to Figure 6.71.

The onset of phase two in the Inipol amended BSTR was day forty-three which showed an increase in oil due to more solids being dispersed into the mixed liquor which was an observation noted at the time of the run as well as evidenced by the solids analysis. On day forty-three, the mixed liquor changed to a darker colour as the solids that were sticking onto the glass wall entered the mixed liquor leaving most of the walls clean of solids. This was possibly an indication that surface tension of the liquid had dropped and as a result there was less creeping of the oil onto the glass walls which was also

Figure 6.70 Increase in inorganic carbon as treatment progresses

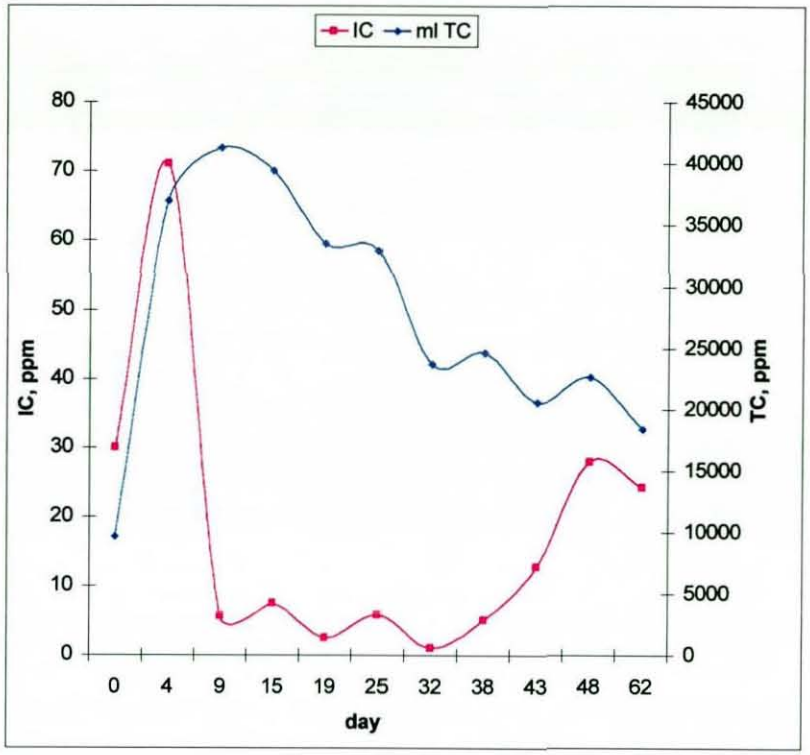
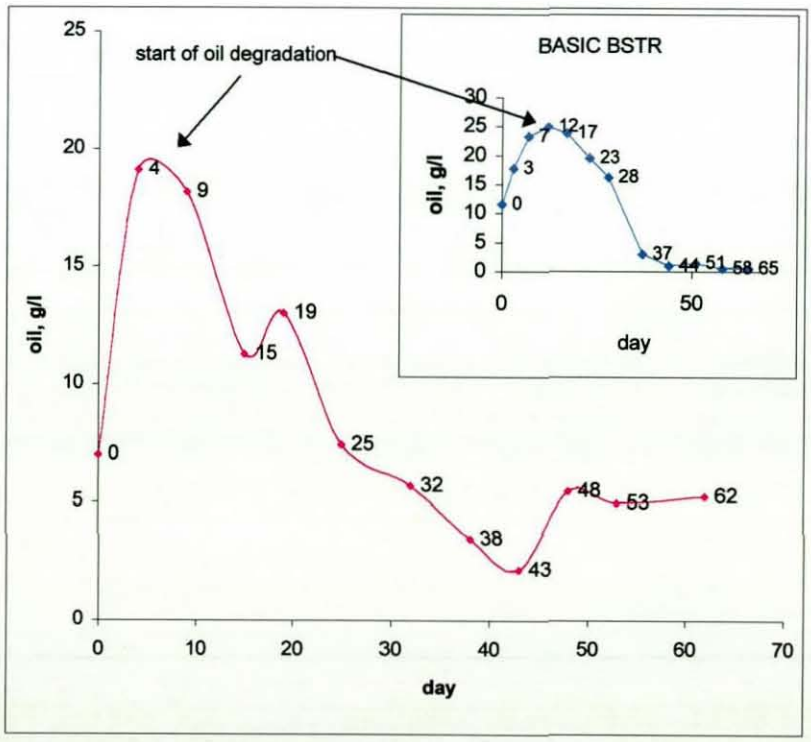


Figure 6. 71 Earlier onset of degradation in the Inipol amended BSTR



observed by Pritchard *et al.* (1992) in their study of Inipol for the USEPA. Also noted on day forty-three was a change in the smell of the reactor which had a more 'bacterial' smell rather than an 'oily' smell. The solids dispersion after day forty-three could be from the action of biosurfactants as there is a steep rise in the number of oil degraders at this time. However, there did not seem to be any more oil being significantly degraded for the almost three week period between day forty-three and day sixty-two when the reactor was stopped.

When the rate of reaction was plotted for the Inipol amended reactor it was shown to fit first order degradation rates but had lower degradation rates compared to the Basic BSTR. This indicates that while the addition of Inipol improved the extent of degradation by improving the mass transfer for the reactor as a whole through improving the solids dispersion, the rate of oil degradation in the mixed liquor was inhibited in the presence of Inipol. However, the addition of Inipol sustained a much longer first phase, thirty-nine days, compared to the Basic BSTR at twenty to twenty-five days and it has been shown that most of the oil degradation occurred in the first phase. The second phase had much lower rates and had very poor fits for both linear and first orders rates of reaction. It is postulated that the use of Inipol as substrate lowered the degradation rate for oil. From the results, it is clear that Inipol caused increased solubilization of oil into mixed liquor, however, the 'rates of solubilization' effect on biodegradation rate could not be compared as the biodegradation rates in the Inipol amended reactor was actually inhibited, though at this point in the research it is postulated to be due to the use of Inipol as a substrate rather than inhibition due to substrate unavailability. However it cannot be discounted either that surfactants may have interfered with the binding or attachment mechanism of bacteria to the hydrophobic oil substrate resulting in the depressed biodegradation rates.

During experimental set-up, the choice of Inipol was in part due to it reportedly enhancing mineralization rates of naphthalene (Churchill 1995b). Therefore, by setting up a control reactor which had no added surfactant affecting solubility enhancement and solubility rate enhancement, the initial degradation rate in the control would be one that is limited by solubility rate of the oil (assuming degradation rate is limited by solubilizing/desorbing rate). The addition of surfactant would then show an increase in the initial degradation rate if desorption rate was limiting. This did not happen. The initial rate was inhibited with the addition of Inipol but the onset of a first order degradation rate was much earlier, likely due to an increase in solubility of the oil substrate. The control i.e. the Basic BSTR, showed delayed oil degradation. The initial

soluble oil concentration in the control reactor was too low and at low substrate concentrations, bacterial cultures may not be able to produce the necessary extracellular enzymes to begin metabolizing the low water soluble compounds for degradation to proceed i.e. it was solubility limited which in effect is a reflection of the rate of solubilization. If the solubilization rates were higher in the control reactor, the first order oil degradation would have initiated earlier as in the Inipol amended run. Therefore, Inipol must have also increased the solubilization rate of the oil as well as increased the solubility of the oil allowing for a longer phase one first order degradation rate and a greater extent of oil degradation. The degradation rate inhibition was most likely due to the metabolism of another carbon source originating from the Inipol.

From Figure 6.72 and 6.73, the rate of degradation in the mixed liquor fitted a first order plot with R^2 value at 0.96, and reaction rate coefficient 0.06 day^{-1} . When the rates were normalized to solids in the mixed liquor, Figure 6.74 & 6.75, only linear order plots were meaningful. The inset Figures 'a' of 6.72 and 6.73 show the fit if the whole period of day four to day sixty-two is fitted to the rate order and inset Figure 'b' shows the bad fit of either rate orders for the second phase. From the foregoing and the previous results for the Basic BSTR, it can be seen that the two reactors behaved fairly different due to the Inipol. From the poorer fit of the first order and linear rate plots when normalized to solids it is concluded there is less correlation of oil degradation with solids in mixed liquor for the Inipol amended BSTR wherease in the Basic BSTR it can be seen that there is a correlation between solids and oil degradation seen from the improved fit in the rate order curves normalized to solids. It is therefore postulated, there could be a greater extent of attached growth process occurring in the Basic BSTR compared to the Inipol amended BSTR where the oil was desorbed off solids prior to being degraded in the aqueous phase. Inipol may have improved dispersion of solids through enmeshing (micellarisation) the solids in a sort of 'matrix' causing more even distribution but in doing so may have prevented the attachment of bacteria to substrate or interfered with the attachment of biosurfactant and bacteria to substrate. Therefore, the improvement in extent of degradation of oil in the Inipol amended BSTR is through increasing the extent of oil solubilizing into the mixed liquor as was seen visually from the amount of oil floating in the mixed liquor as well as the O&G analysis. Any increase in the rate of solubilization was not apparent as the degradation rate was inhibited to below the control reactor but it can be inferred from the other results as discussed above. Even though the rates of oil degradation in the mixed liquor are at about half of the Basic BSTR, the extent of degradation as well as amount of oil per solids showed improvement over the control by having a longer phase one period and by dispersing

Figure 6.72 The fit for linear rate order for oil degradation in Inipol amended BSTR.

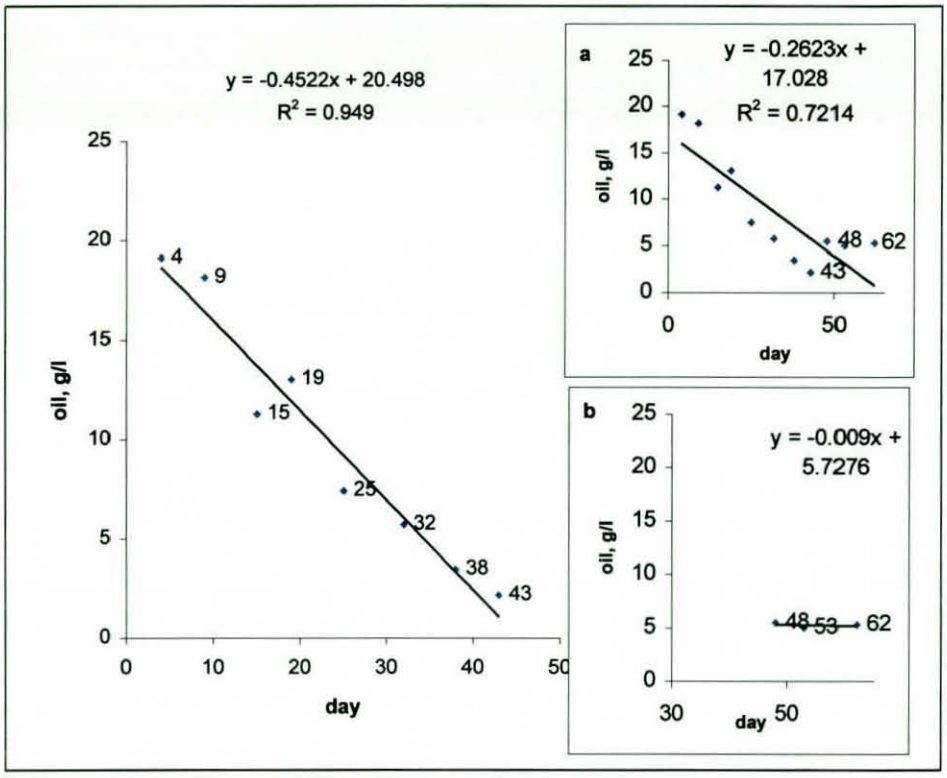


Figure 6.73 The fit for a first order oil degradation in the Inipol amended BSTR

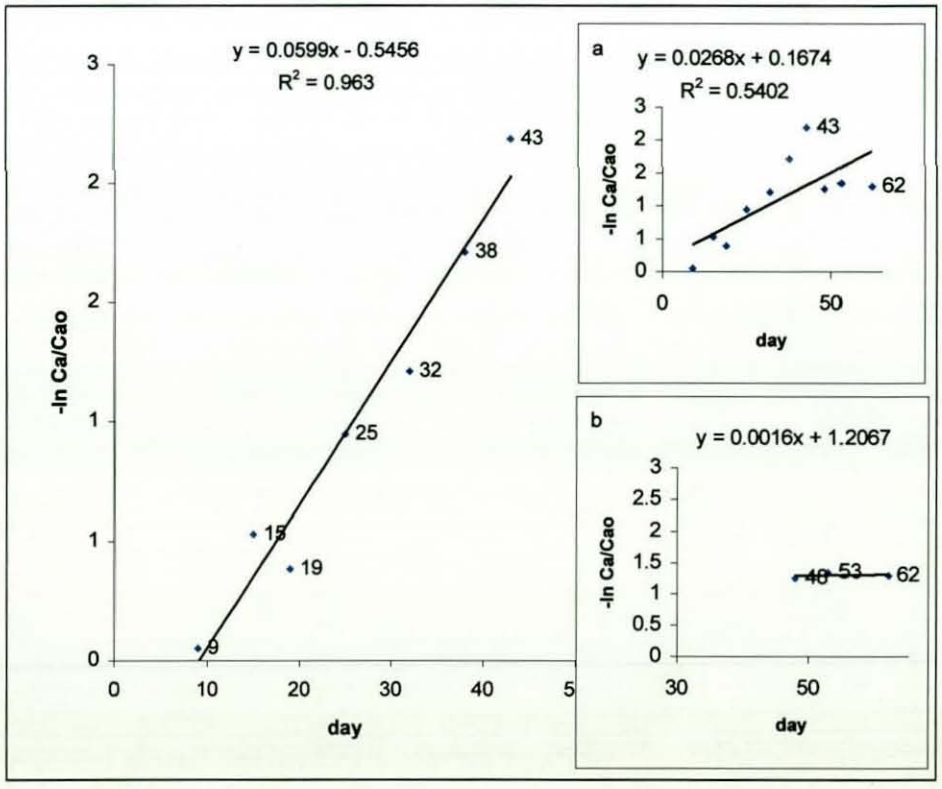


Figure 6.74 Linear degradation rate normalized to solids in mixed liquor

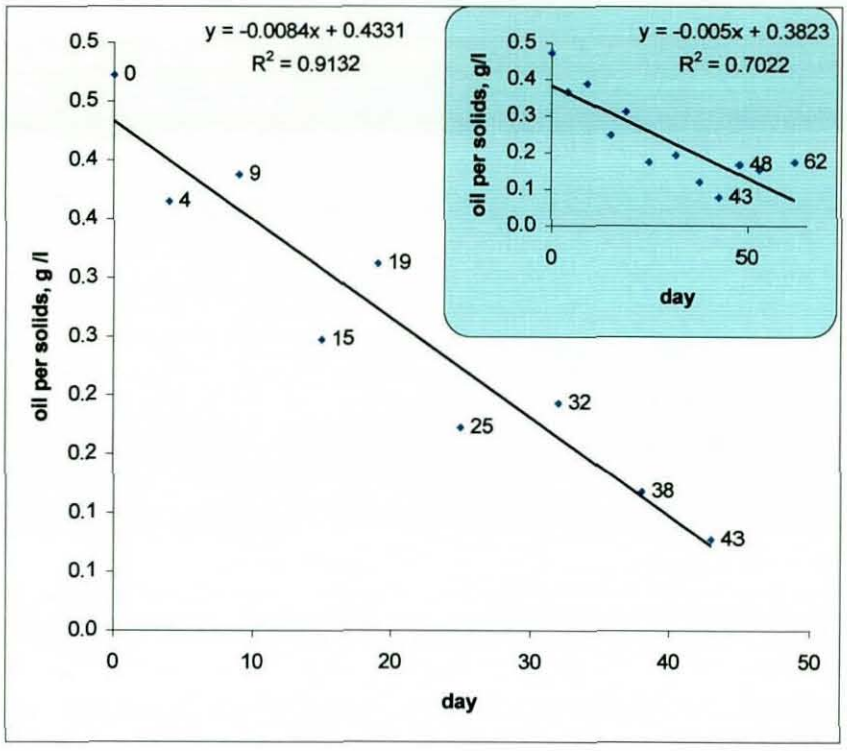
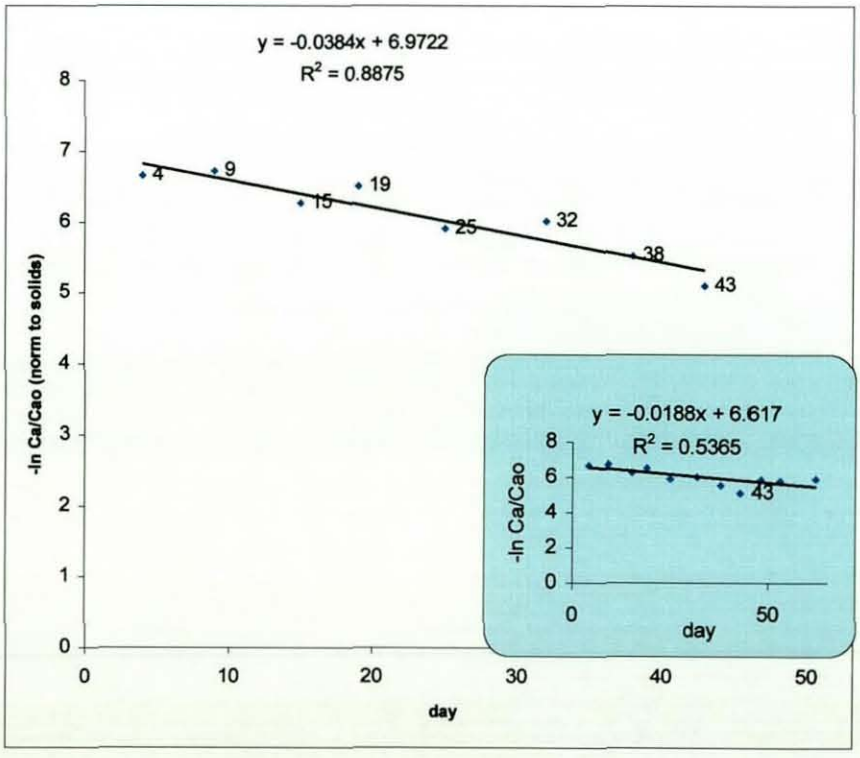


Figure 6.75 First order degradation rate normalized to solids in mixed liquor



the solids better thereby increasing the amount of oil desorbed into the mixed liquor where it was effectively degraded. Volkering *et al.* (1995) noted a similar observation that surfactants increased the duration of the initial exponential growth phase which he coupled to naphthalene utilization.

6.5 DEGRADATION OF PAH IN THE THREE REACTORS

The final PAH quantities in the treated sludge are compared to UTS limits in Table 6.24. The PAH losses were calculated from a difference from baseline samples and the final quantities in the treated sludge. All calculations converting peak areas of the HPLC trace to PAH quantities together with the dilution conversions are in *Appendix* O together with the HPLC chromatograms. The integration of peaks are not shown as peaks were integrated individually manually using the Chemstation software.

Table 6.24 Treated settled sludge PAH quantities compared to UTS limits

Peak no.	Name	Baseline (ppm)	Basic BSTR (ppm)	Airlift BSTR (ppm)	Inipol BSTR (ppm)	UTS (ppm)
4	Fluorene	4.31	*Bdl	Bdl	Bdl	3.4
5	Phenanthrene	32.14	0.58	0.99	1.13	5.6
6	Anthracene	2.31	0.27	0.56	1.06	3.4
7	Fluoranthene	4.71	Bdl	Bdl	Bdl	
8	Pyrene	18.36	16.46	8.1	5.99	8.2
9	Benzo(a)anthracene }	48.1	27.42	10.01	33.13	8.2
10	Chrysene }					
11	Benzo(b)fluorenthene	6.35	6.23	4.34	5.81	
14	Dibenzo(a,h)anthracene	57.11	22.71	18.62	56.64	
15	Benzo(ghi)perelene	7.33	4.18	3.92	8.22	
16	Indeno(1,2,3cd)pyrene	37.45	36.76	21.69	82.79	
	Total PAH	212.1	114.5	68.15	194.1	
	% loss		46	67.87	8.5	

*Below detection limit

From the results, the Airlift BSTR was the most successful in treating PAH closest to UTS limits, with only benzo(a) anthracene and chrysene, which could not be separated as two peaks, barely missing the limits. Due to the set fluorescent detector time table and the slight time drift, which was possibly due to the samples being processed through Empore discs, there was a jump in the baseline right at the time peak 9 and 10 eluted. For this reason, the results for peak 9 and 10 are possibly less accurate. The last two peaks eluting also had problems. Peak no. 15, (benzo(ghi)perylene), is a very broad peak and there was some interference with the solvent peak at this time. The elution of

peak no 16 (indeno(1,2,3cd)perylene) was affected by peak 15. Integrating from base to base was difficult at times for these two peaks, possibly resulting in less accuracy for these two peaks. Peaks 12 and 13 (benzo(k)fluoranthene and benzo(a)pyrene) were not detected at the sample concentrations measured.

The results for the Inipol amended BSTR show that the surfactant significantly retarded the degradation of total PAH. However, the UTS limit for all except benzo(a)anthracene and chrysene were met. The 5 and 6 ring compounds, benzo(b)fluorenthene, dibenzo(a,h)anthracene, benzo(ghi)perelene and indeno(1,2,3cd)pyrene were not significantly degraded and in the case of indeno(1,2,3cd)pyrene, increased significantly. The measurement of the last peak for indeno(1,2,3cd)pyrene had some interference from peak 15 and the results may have less accuracy and this affected the overall total PAH lost. While there may have been some accumulation of the 6 ringed PAH due to its recalcitrance this cannot be positively confirmed until further tests. Churchill *et al.* (1995a) and Churchill *et al.* (1995b) noted that specific PAHS were concomitantly degraded together with Inipol. While oil was substantially lost as shown from the previous section, total PAH lost was only about 9 %. However, the small loss of total PAH was due to the very large area of peak 16. The loss of PAH in the Basic and Airlift BSTR reflect similar quantities as the lost of O&G. The quantities of PAH desorbed into mixed liquor from the bottom solids and subsequently degraded show a similar pattern to the oil loss as shown in the following Figures, 6.76, 6.77 and 6.78.

6.6 IMPLICATIONS OF REACTOR AND PROCESS DESIGN ON RATES AND EXTENT OF OIL DEGRADATION

From the previous results it is clear that operational factors as well as reactor design has implications in the reaction rates as well as extents of degradation. The results of using surfactants have been shown to be variable and is specific to the system (microorganism, concentration of HOC, presence of solids etc.) and surfactant. In this case, the use of Inipol interfered with degradation such that rates of degradation in the mixed liquor were reduced. The mechanism for this is unclear but from the observation that oil was clearly dispersed and heterotrophic counts were elevated, it is postulated that Inipol prevented oil uptake due to the use of Inipol as a competitive substrate or due to Inipol preventing the attachment of the oil to the bacteria. However, while degradation rates of oil in the mixed liquor were lowered the overall amount of oil

Figure 6. 76 Pattern of PAH desorption and degradation in the Basic BSTR

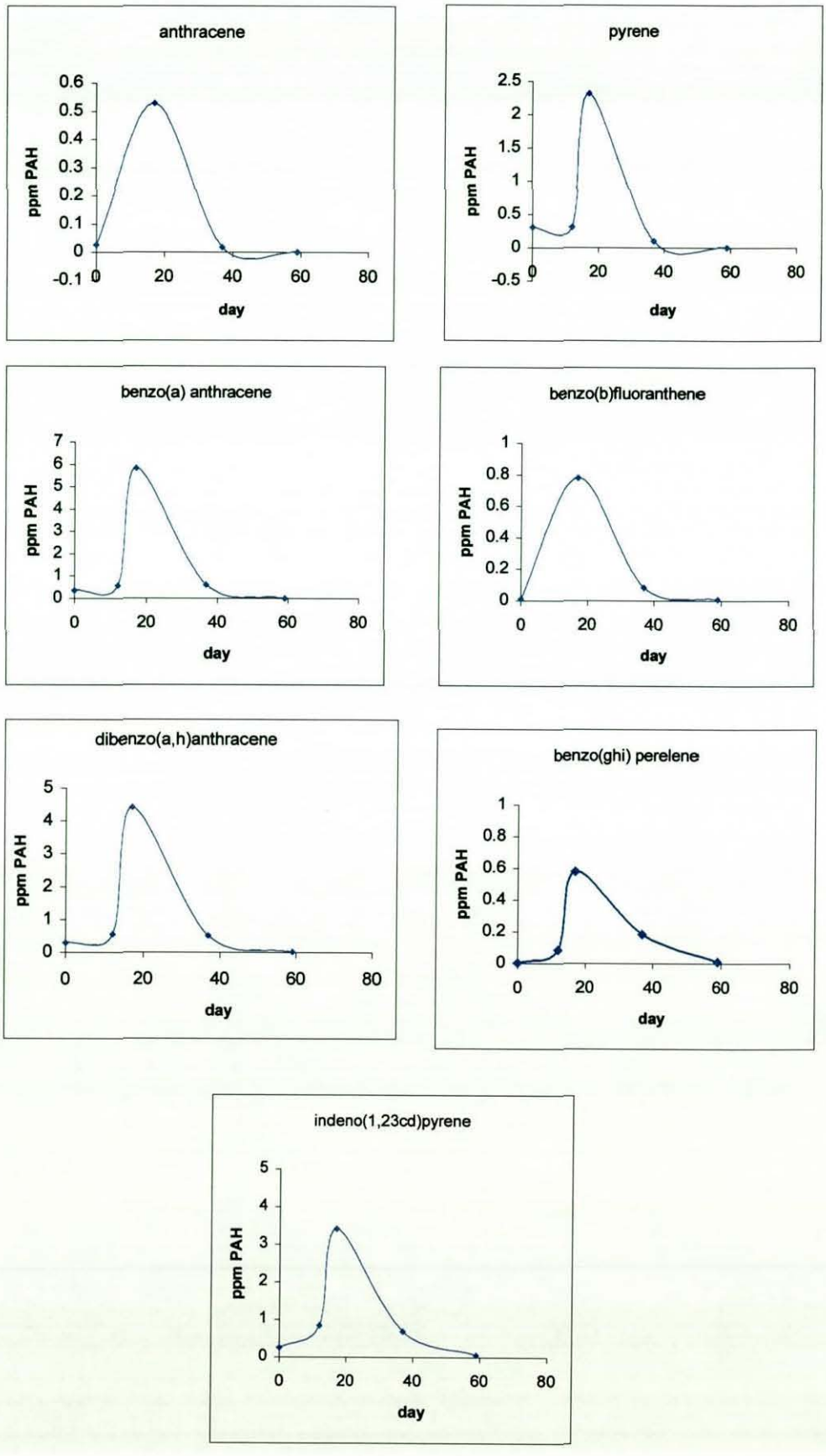


Figure 6. 77 Pattern of PAH desorption and degradation in the Airlift BSTR

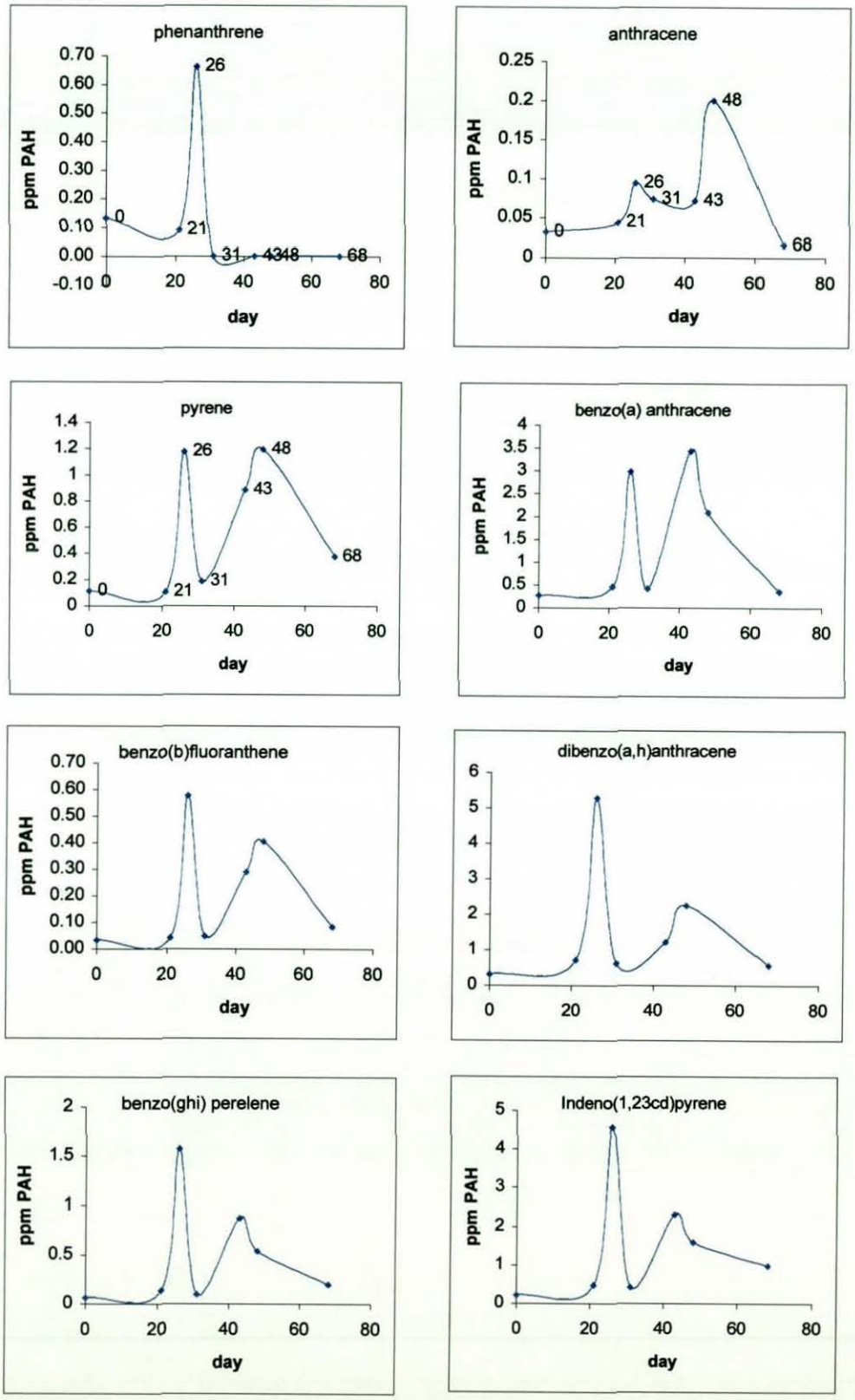
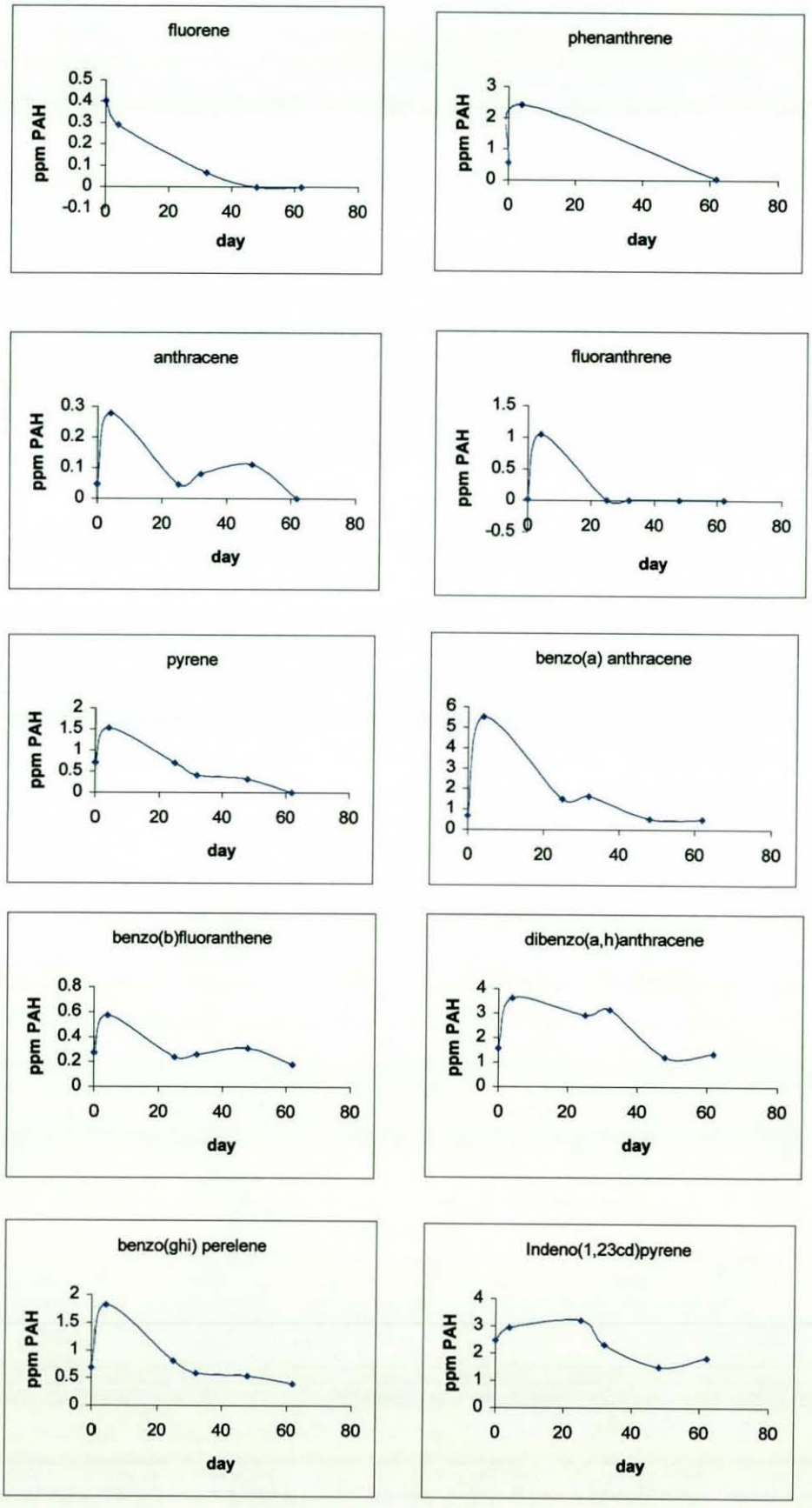


Figure 6.78 Pattern of PAH desorption and degradation in the Inipol Amended BSTR



degraded was higher compared to the Basic BSTR as more oil was desorbed into the mixed liquor by the surfactant, allowing for treatment to proceed in the mixed liquor phase. In the same manner, Inipol also caused less clingage and therefore amount of untreated sludge was reduced. However, degradation of PAH was significantly reduced.

Amount of clingage seemed to be a salient point in the Airlift BSTR design. While this can be overcomed by redesigning the reactor, for example including a sludge return from the top to the bottom of the reactor, it is thought that the ratio of clingage and total sludge treated in a scaled-up reactor would be less significant. The airlift provided better solids dispersion and oil desorption into mixed liquor, seen in the higher TS in the mixed liquor, and resulted in the higher amounts of oil degraded in the sludge. Foaming was more significant in the Airlift BSTR due, in part, to the airlift design which seemed to promote foaming. Possibly, the higher concentration of oil on the floating layer which was due to the airlift pulling the hydrophobic oil droplets into the air bubbles which accumulated on the upper surface area, also concentrated the degrading cultures here. Surfactant production exacerbated the foaming which trapped oil and solids in the foam layer. However, after the foaming, oil loss was enhanced and foaming could be the reason for this. The foam layer had a structure which allowed for close contact between substrate and microorganism while at the same time it was non oxygen limited. Oxygen in liquid is limited to its solubility limit while in the foam layer oxygen is possibly as in air. These two factors, better solids dispersion and surfactant production, led to the higher oil losses in the sludge from the Airlift BSTR.

6.6.1 COMPARISON OF OIL DEGRADATION RATES AND EXTENT

More traditional biological treatment of oily sludges by landfarming have shown rates between 0.02 and 0.60 g hydrocarbon per kg soil per day for heavy oils, sludges, and crudes (Morgan and Watkinson 1989; Bossert and Bartha 1984) . The reactors in this study achieved between 2.3 – 2.7 g/kg mixed liquor solids per day (refer to Table 6.18).

Another research by Yare, (1991) compared the rates and extent of oil degradation in a landfarm set-up and a stirred tank reactor set up, achieved a half life of 311 days for TOC in the landfarm and 22 days in the stirred tank reactor. In Yare's (1991) research, the extent of PAH degradation in the landfarm experiment was higher at 91 % compared to the stirred tank PAH loss at 63%. However treatment time in the stirred tank reactor was much reduced which possibly determined the extent of PAH loss. The reactors in

this research achieved a half life for TC of mixed liquor between 30 days and 55 days. The TC of mixed liquor was shown to be representative of the remaining O&G in the previous discussion on each reactor. Analytical methods play a significant part in how the results are determined as discussed in the literature review, and while Yare's (1991) experimental results, as others, cannot be directly compared with this research, the rates achieved can be seen to be within the same magnitudes/scale. Reactor and process design in this research, as discussed previously, was significant in determining the rates and extent of O&G and PAH loss as summarized and compared to other research in the Table 6.25 below.

Table 6.25: Comparison of rates and extent of oil degradation in this research with other researchers

	TC/TOC/ O&G half life (days)	% oil loss	% PAH loss
Basic BSTR	30 (TC)	45	46
Airlift BSTR	50 (TC)	61	68
Basic Inipol BSTR	55(TC)	60	8.5
*Yare Landfarm	311(TOC)	NR	91
*Yare Reactor	22(TOC)	NR	63
** Bossert and Compeau	38-122 (O&G)	NR	NR
*** Brown & Donnelly	143(O&G)	NR	NR

*Yare (1991)

**Bossert and Compeau (1995) from various studies on bioremediation of crude and crude residuals

***Brown and Donnely (1983) from landfarming of refinery sludge.

NR – not reported

In comparing the half lives between the reactors, while the Basic BSTR shows a much shorter half life, the half life is only for up to a 45 % loss in O&G as that was the limit for the Basic BSTR. Desorption of oil limited the extent of oil degradation. Comparing the zero order reaction rate coefficients of the three reactors, the Basic BSTR gave a k of 0.9, the Airlift BSTR, 0.6 and the Inipol amended BSTR, 0.45. The reduced rates in the Airlift and Inipol amended BSTRs could possibly be due to much higher O&G content in the mixed liquor inhibiting the rates due to toxicity.

6.7 MECHANISM OF HYDROCARBON UPTAKE

From the results, as discussed for all the reactor runs, two mechanisms for hydrocarbon

uptake could be occurring. The pattern of bacterial growth were seen to follow quite closely the solids pattern in the mixed liquor especially in the Airlift BSTR. This could indicate attached growth. Heijnen *et al.* (1992) noted the occurrence of biofilm formation on small-suspended solids in airlifts. The researchers noted the attached growth maintained high active biomass concentrations. The bacterial counts in the Airlift BSTR were maintained high at 10^{8} –10^{10} CFU per ml. Marks *et al.*(1992) noted high solids loading favored biodegradation of PAH which they postulated occurred due to the solids providing an increase in surface area. Keuth and Rehm (1991) in their study of biodegradation of phenanthrene from a contaminated soil, observed specific growth rates increased with increasing amount of solids.

In the Inipol amended BSTR, while solids and bacterial growth patterns were similar, observation of free oil floating in the mixed liquor indicated desorption of oil from the solids. While attached growth could still be occurring, the bacterial cultures needed to be able to take up oil that was either in a separate phase or emulsified in solution. This process may be different to the uptake due to attached growth where both the bacteria and substrate are in direct proximity. The occurrence of free or emulsified oil possibly require motile bacteria or suspended growth.

6.8 PROSPECTS FOR BIOREACTOR TREATMENT OF OILY SLUDGES

The results from all the reactor runs have shown that bioreactor treatment is technically feasible and improvements in the extent of degradation to meet regulatory limits is possible through reactor and process design. As mentioned in the literature review waste are classified as hazardous according to hazardous criteria such as toxicity and oily sludges are regulated due to the toxicity of the PAH compounds. Bioreactor treatment has shown that these sludges can be detoxified by biodegrading the PAH compounds. This is more environmentally sustainable than incineration which uses a lot of energy and adds pollution to the air besides also being more costly. While, the costing for a reactor based treatment system would be quite system specific, there are references in literature as to the cost savings involved between bioreactor treatment and incineration. These include a savings of 60 % or more by the use of bioreactors compared to incineration (Abrishamian *et al.* 1992) and Vail (1991) has quoted the cost to biodegrade oily sludges in a reactor based system to be at US $20/ton compared to incineration at US$1,400/ton. Therefore bioreactor treatment offers a technically feasible, as well as a more cost effective and environmentally sustainable solution, to treating oily sludges than the current method of incineration.

CHAPTER 7
CONCLUSIONS

The results and literature as discussed in Chapter 6, indicate that the oily sludges were treated successfully by aerobic reactor treatment to various extents as determined by measuring the remaining O&G and total PAH contents.

As mentioned in the research objectives, a reactor based treatment was chosen for several reasons and the results from this study have supported the decision i.e.;

- it is technically feasible and the treatment limits achieved depended on the reactor design and operational factors;
- VOC release would be minimal and can be regulated with the use of an activated carbon trap. As mentioned in section 6.4.3.1, trapped VOC, measured by extracting the activated carbon in the exhaust, was minimal at a total of 0.7g;
- Better process control may be achieved by the use of online monitors for TC as the results from this study indicate that this is a good indicator for the O&G ;
- While reactor based treatments would already improve mass transfer rates over those from landfarming and composting activities, the mass transfer in the reactor was also shown to be improved through reactor design and surfactant addition.

Following are the summarized reactor performance and treatment limits for the reactors.

The Basic BSTR, which served to generate results for comparison of reactor design and surfactant addition, achieved a base O&G degradation extent of 45 % and 46% loss of total PAH for a treatment period of sixty-five days. All but pyrene and benzo(a) anthracene reached UTS limits which are more stringent than BDAT limits. The half life for TC in the mixed liquor, was 30 days. In the Basic BSTR, the limit for degradation was reached after forty-five days, whereby after this, degradation was minimal. The extent of oil degradation was much lower in the Basic BSTR due to the limiting mass transfer rates of the oil associated solids into the mixed liquor. The rate coefficient (normalized to volume of mixed liquor) fitted linear rate order at k = 0.8747 day $^{-1}$ (R^2= 0.92).

The Airlift BSTR was shown to improve solids mixing and therefore performance. The experimental results determined the extent of degradation of O&G and PAH was the best at 61% and 68% respectively for a treatment period of sixty-eight days. The TC half life was fifty days to achieve the percentage losses as mentioned. The limits of treatment

may not have been reached by the end of this treatment period, however at the end of this sixty-eight days, the PAH was treated to better than USA's BDAT limits (see Chapter 1). UTS limits were reached for all but benzo(a)anthracene and chrysene. The rate coefficient (normalized to volume of mixed liquor) fitted linear rate order with k = 0.6 day^{-1} (R^2=0.99). While the rate is lower compared to the Basic BSTR and the degradation period was extended compared to the Basic BSTR the final results were better O&G degradation. The lower rates have been attributed to an increase in toxicity of the mixed liquor from the higher oil content. The enhanced removals of O&G and PAH degradation are attributed to better mass transfer of the solids into mixed liquor as well as the enhanced biological surfactant production both effected by the airlift. Elevated levels of surfactant were indicated by foaming and this possibly contributed to the success of the Airlift BSTR.

The second run of the Basic BSTR gave mixed results on the use of the oleophilic fertilizer with surfactants (Inipol EAP 22) to improve degradation rates and extent of degradation. While the extent of oil degradation was improved, reaction rates, as in the Airlift BSTR, was reduced possibly for the same reasons. The extent of oil loss was similar in scale as the Airlift BSTR at 60%, however PAH degradation was retarded at 8.5 %. However, as mentioned previously, the results for total PAH degradation may be skewed by analytical interference. It is likely that the treatment limits were not reached by the time the reactor was stopped at sixty-two days, as oil in the mixed liquor and oil degrader counts were still high ($1x10^{9}$). Reaction rates fitted a first order rate with k = 0.06 day^{-1} (R^2 =0.96). While reaction rates fitted first order rates better, linear order reaction rate coefficient only had a slightly less better fit at R^2 of 0.95 with a k = 0.45 day^{1}. The half life for TC in the Inipol amended BSTR was 55 days. The PAHS were concomitantly degraded with the O&G in this reactor run, however, the larger PAHs (four and five ring compounds) showed more recalcitrance compared to the Airlift BSTR. However, UTS limits were achieved for all except benzo(a)anthracene and chrysene. The addition of surfactant increased the amount of solids as well as effected the desorption of oil into the mixed liquor and this extended the period for phase one degradation (shown in Chapter 6 to be the period of active degradation) which in turn increased the extent (total percentage lost) of oil loss in this reactor compared to the Basic BSTR.

It can also be concluded from the reactor studies, while degradation rates, as inferred from the reaction rate coefficients (plotted to rate orders), may be slightly reduced due to higher oil content in the mixed liquor increasing the toxicity to the bacterial cultures, the

overall treatment extent is improved. This is probably due to substrate's proximity with the microorganisms when the oil associated solids are dispersed into mixed liquor. Hence, solids dispersion from the sludge is critical in ensuring its degradation and this is closely linked with reactor and process design. The Airlift and Inipol amended BSTR were both more successful in reducing O&G than the Basic BSTR due to the higher oil associated solids in the mixed liquor. The presence of the oil associated solids in the mixed liquor phase allowed for both attached growth as well as oil desorption into mixed liquor to take place.

From the monitoring of several performance parameters, it is concluded that, the choice of analytes to indicate breakdown is crucial to determining meaningful results. The parameter TVS and TVSS provides a fast and simple approach to monitor bacterial counts in other waste water treatment but, in these type of reactor studies, are not representative of bacterial counts. However, monitoring bacterial numbers is important in these reactor studies and monitoring heterotrophic counts on PCA has been found to be correlated to oil degradation and this can be used to monitor the degradation process rather than monitoring the hydrocarbon utilizer counts which are more laborious. Monitoring substrate loss is also important and from this study measuring the TC of the mixed liquor was representative of the O&G and with the use of a Total Carbon analyzer machine can be used for quick and instant monitoring of the hydrocarbon substrate in the reactors.

From the preliminary series of shake flask culture experiments conducted to determine the idealized conditions for the reactors, several conclusions can be made. In Experiment D and E, it is concluded that the use of an acclimatized culture with constant exposure to a hydrocarbon substrate led to higher bacterial counts and oil degradation rates. Experiment G determined that higher sludge loadings e.g. twenty percent solids load retarded degradation rates possibly through an increase in toxicity of the mixed liquor. This observation was supported by the later reactor studies whereby improved desorption of oil into the mixed liquor in the Airlift and Inipol amended BSTR resulted in lower reaction rate coefficients.

Finally, the overall aim of this research was to determine if oily sludge could be efficiently degraded in specialized aerobic reactors, to meet with BDAT PAH limits. Reactor as well as process design, in this case the use of surfactants, were shown to have a significant effect on improving the mass transfer rate of the substrate to the microorganisms.

CHAPTER 8
RECOMMENDATIONS FOR FUTURE RESEARCH

From the results of the reactor runs, it is clear that the Airlift BSTR succeeded the most in treating the mixed liquor fraction and solids. The success was also clearly related to the airlift which effectively lifted solids into the mixed liquor fraction improving the overall mass transfer of the reactor. However, due to the sludge's property and the airlift design, clingage was a large factor affecting overall results of the reactor. For a larger scale operations this factor will probably be less of a problem. However, the airlift can be redesigned to reduce the clingage, possibly by increasing the diameter to reduce splashing the sides when the airlift returned the solids to the mixed liquor. To create improve mixing of the larger diameter vessel, baffles can be placed on the side walls. Furthermore, foaming can be a problem in the airlift as it can cause an overspill and this can also be overcome by redesigning the solids return path. From the airlift, the solids can be returned to a 'shower spray nozzle' where it should collapse some of the foam. However, this would need to be tested as observations of the foam layer showed solids were collecting within the foam structure which may resist collapsing. The airlift would need a larger head space to ensure the foam does not overflow. An alternate design would be to allow the foam layer to overflow the top and return to the bottom of the reactor through another bottom feed line. This design would also improve the solids mixing from the bottom of the reactor.

It is also recommended that in future, reactor studies on oil degradation are monitored daily for TC in the mixed liquor as it has been shown that TC is representative of O&G.

Alternatively, the Airlift BSTR could employ surfactants to reduce clingage as surfactants were shown to reduce creeping of the oil associated solids onto the glass vessel walls. The use of surfactants may, however increase the foaming. Foaming in the Inipol amended BSTR was characteristically different compared to the foaming in the Airlift BSTR whereby the foaming with Inipol was not persistent and unstructured. Therefore, surfactant may be beneficial in two ways in the Airlift BSTR. For one, it would reduce the amount of clingage and improve treatment extent. The use of surfactants also showed it prevented the formation of the sticky sludge agglomeration

as well as a better solids dispersion and this would also be applicable in the Airlift BSTR resulting in better mass transfer rates for solids throughout the reactor. However the choice of surfactant has to be determined first to ensure that degradation rates are not significantly reduced. Tests to determine surfactant choice should include the determination of whether it is preferentially utilized at the expense of the hydrocarbon substrate; if it is toxic; if it prevents bacterial attachments etc. Further work should continue to determine the rate inhibition factors observed in the Inipol amended BSTR run. The 3-parameter model by Guerin and Boyd (1992) that determines if rate is inhibited due to substrate unavailability can be used by setting up a control experiment which is soil free to determine the uninhibited rate.

The use of biosurfactants would also be recommended for further work. New methods to improve biosurfactant production and recovery needs to be tested. There are indications that an airlift design with suspended particles in mixed liquor may be inducive for biosurfactant production from comparison of the airlift stirred tank design and a basic stirred tank design. Recent work (UKM personal communication), have shown that surfactants are easily harvested with higher yields in airlift reactors by collecting the foam layer (as opposed to the whole mixed liquor). Trial runs of the Airlift BSTR at 37 ^{0} C also indicated foaming was more intense at elevated temperatures and this can be exploited for surfactant production.

The media for surfactant production should also be tested in the lab. There is some indication from Experiment E on 'primed and unprimed cultures' that bacterial cultures selected for growth on hydrophobic substrates have higher cell yields when grown in hydrocarbon substrates compared to non hydrocarbon substrate. Growth techniques should be tested further to determine surfactant production media for the cultures.

Further work to determine the connection between the lasting emulsification ability of surfactants with biodegradation potential is also recommended for further work.

While the above work is recommended in order to further improve the design and running conditions of the Airlift BSTR, the results of the research already indicate that the Airlift BSTR was successful in treating to reduce the oil content and the PAHs to BDAT limits. Therefore it is recommended that follow up work should include a scale up of the reactor.

Further work to determine the dewatering capability of the treated sludge should also be carried out as this will reduce the amount of treated sludge that will require disposal.

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GLOSSARY

API	American Petroleum Institute
Abs	absorbance
Avg	average
BBH	Bushnell Haas media/agar
BBHN	Bushnell –Haas agar with naphthalene
BOD	biological oxygen demand
BSTR	batch stirred tank reactor
CFU	colony forming unit
Cruc	crucible
Cul	culture
Corr Fact/CF	correction factor
DO	dissolved oxygen
DF	dilution factor
F/M	food to microorganisms ratio
DCM	dichloromethane
DOUR	dissolved oxygen uptake rate
Filt	filtered
TCTFE	trichlorotrifluoroethane
TS	total solids
TVS	total volatile solids
TFS	total fixed solids
TSS	total suspended solids
TVSS	total volatile suspended solids
TFSS	total fixed suspended solids
O&G	oil and grease
TOC	total organic carbon
TC	total carbon
IC	inorganic carbon
ML	mixed liquor
ml	millilitre
MPN	most probable number

PAH	polynuclear aromatic hydrocarbon
PCA	plate count agar
UTS	Universal treatment standards
Ppm	parts per million
W	with
Wt	weight

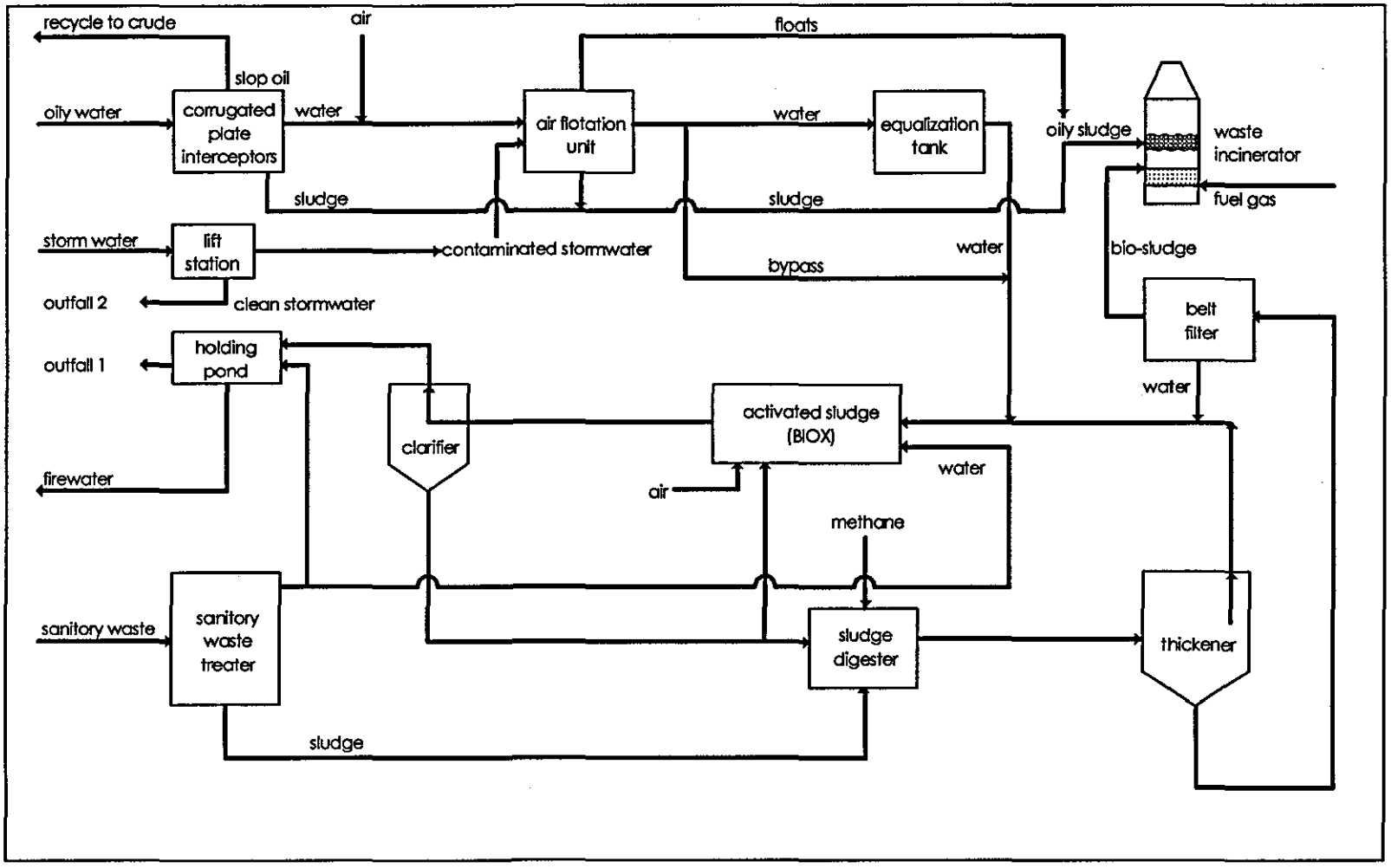
SOURCES AND TREATMENT OF OILY WASTES IN A REFINERY

A waste water treatment plant in a refinery receives several different waste streams. A well run refinery segregates the different streams according to its characteristics and hence their treatment requirements (Figure B1). An example of this is; keeping a storm water drain fully segregated from the oily waste water stream since the storm water drains effluents requiring minimal treatment. Oil contamination in a refinery waste water originates from; desalting, a process where crude is thoroughly mixed with water to remove salts that would interfere with downstream refining operations; stream stripping, part of the distillation process; and also cooling tower blowdowns, product treating, tank drains, sample drains and equipment washdown. The largest volumetric contributions are cooling tower blowdown and contaminated storm water.

Treatment processes in a refinery waste water treatment plant are similar to other industrial and domestic sources inasmuch as that the aim is phase separation. Suspended solids are separated by settling and dissolved organics are partially converted to settleable solids. The main difference of these wastes is the high amounts of floating or emulsified oil. Oil and grease for combined, untreated refinery waste water is typically 42 to 2,000 mg/l. Oil has a unique characteristic in that it is lighter than water. This characteristic allows for the recovery of the easily separable, floating oil layer through physical means. However, oil may also be dispersed as emulsion droplets, as colloidal particles and as individual dissolved molecules.

Oily solids and sludges are also formed as the oils become bound with inorganic and organic particulates which settle to the bottom of the various waste water treatment units. These sludges accumulate in the various units in the waste water treatment plant. These wastes have to be removed and ultimately disposed of as solid wastes. Other sources of sludge arise from tank cleanings, accumulated sediments from oxidation ponds, oil contaminated soils and spent lubricants that cannot be economically reprocessed.

Figure B1 : Refinery waste water treatment system



In order to better understand how these sludges arise and their variable characteristics, the units generating these sludges will be briefly reviewed. The propriety treatment unit actually used for treatment in each refinery waste water treatment plant will vary widely. However most waste water treatment systems rely on a few common components as described below. The treatment units are also usually linked in the following sequence.

Components of a treatment system are as follows:

1. Removal of floating oil through units such as skimmers, API gravity separators, and corrugated plate interceptors.
2. Removal of suspended solids and further removal of emulsified oil by chemical sedimentation or air floatation in units such as dissolved air floatation and induced air floatation.
3. Reduction of colloidal and soluble BOD. Some type of bio-chemical oxidation will be required as a secondary treatment to reduce BOD. Examples of the treatment units in use are oxidation ponds, activated sludge facilities, trickling filters and rotating biological contractors.
4. Removal of non-degradable organics. Example of this type of treatment is the use of activated carbon adsorption in holding tanks or ponds.
5. Final holding pond. This serves several purposes and is a feature of most refineries. In some instances it serves as surge pond for storm water runoffs. The holding pond can also dilute and equalize fluctuating effluents quality. It is often used deliberately to reprocess off specification effluent. Floating aerators may be provided in the basin to provide a positive dissolved oxygen content in the treated effluent.

Ideally the best system for waste treatment, i.e. retention times, chemical dosing and aeration are designed according to detailed plant design. Strategic use of in-plant pretreatment for individual waste streams may prevent the off-set of the overall waste water treatment systems. Strongly contaminated waste streams are best pretreated at source prior to feeding back to the waste water treatment plant.

FUNDAMENTALS OF THE UNIT PROCESSES

Each treatment unit, although performs a similar function such as, gravity separation of floating oil, by API separator or Corrugated Plate Interceptors (CPI), the fundamentals

of the processes are different and the resulting efficiencies for oil removal vary as does the type of sludge generated. Following is a brief description of the fundamentals of the unit processes as well as their efficiencies for oil removal and type of sludge generated.

1. Unit process for gravity separation of oil

Gravity separation performance is highly dependent on the laminar flow of the influent. In order for oil droplets to rise, the fluid flow must be laminar and too much turbulence is disruptive. Consequently, volumetric load has a pronounced effect on a separators performance. Gravity separators yield, oil that is recycled into the refining process and water, free from floating oil, but still containing emulsified oil.

a) API separator

The API consists of long rectangular concrete tanks which include flow distribution baffles and oil skimmers. API separators are designed to remove floating oil by skimming (Figure B2). In an API, oil droplets have a rise distance up to several feet. Maximum throughput is proportional to its surface area.

The retention baffle prevents the surface oil from spilling out of the cell. Settled sludge at the bottom of the cell are moved by either a sludge rake or scrappers towards a sludge hopper. Water, largely free from floating oil underflows into the effluent flume over the effluent weir. Inputs to the separators may contain several percent oil. The general design criterion for an API is to separate oil globules larger than 150 microns. Typical water qualities from API's are oil content in the range 50-100 mg/l. Solids from API separators may equal 40 - 80 mg/l on weight water processed and is removed as 3-5 % sludge. The disadvantage of an API is that it suffers from shock loadings under storm conditions resulting in turbulent flow conditions and re-entrainment of previously separated (but not skimmed) oil. The process also generates sludge that is heavier than water. The sludge is made up of mineral sediments with substantial amounts of adhering oil.

Figure B2 : Schematic view of an API Separator

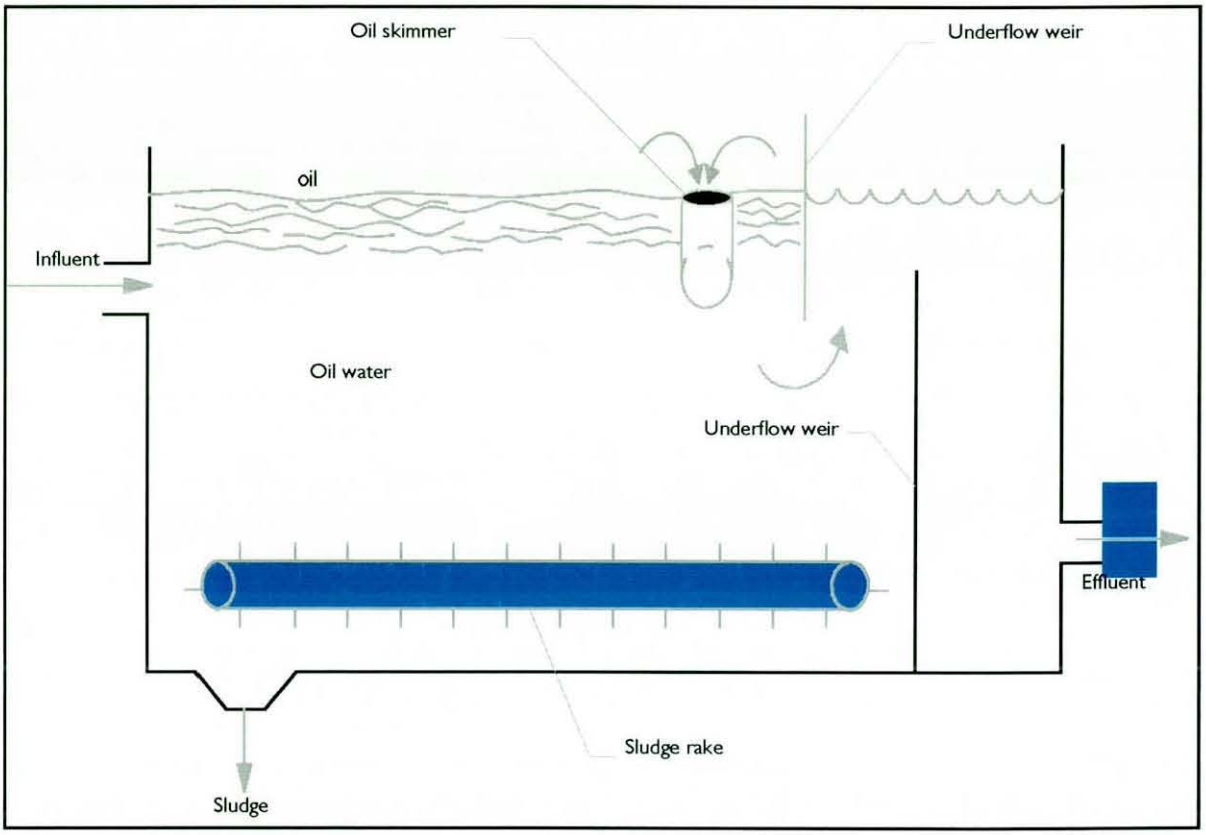
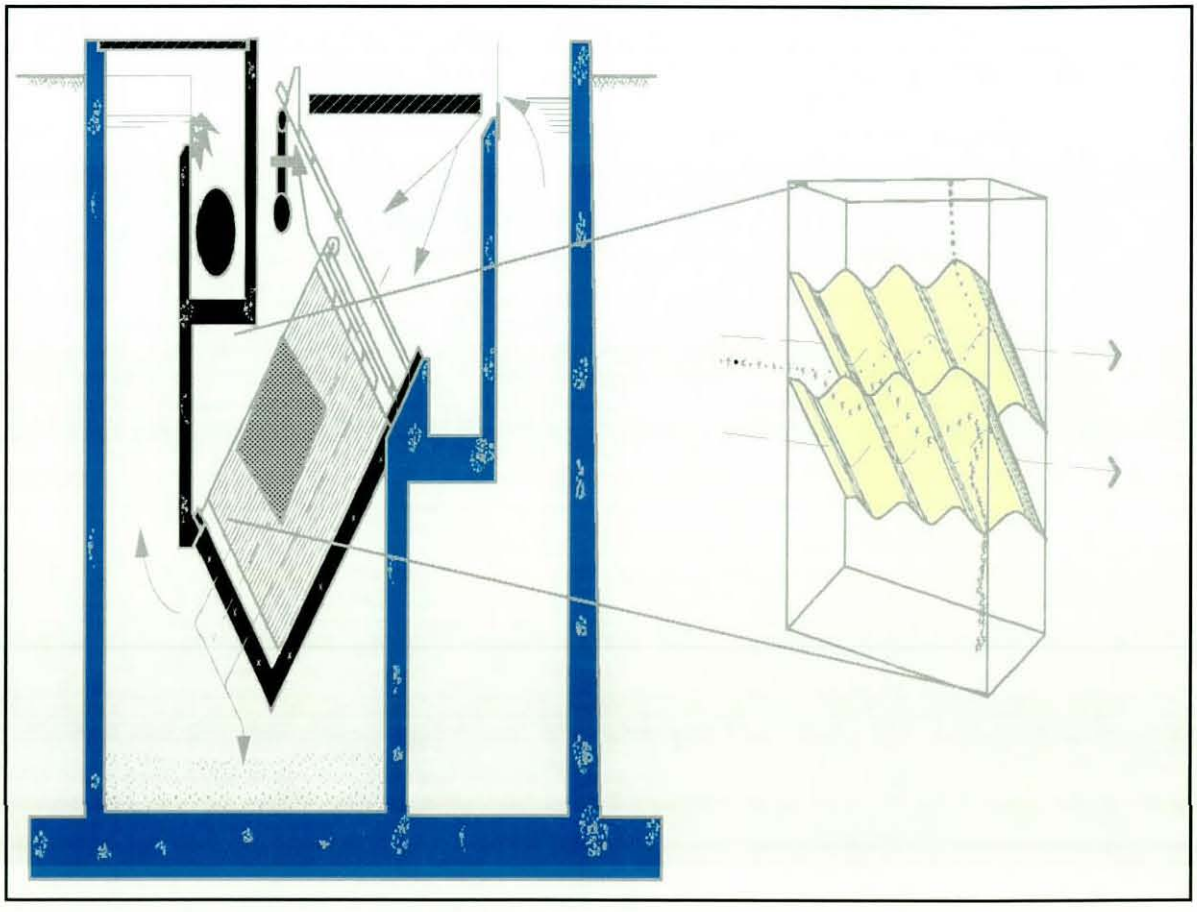


Figure B3 : Corrugated Plate Interceptors (CPI) Inset : Cross flow Interceptors



b. Corrugated Plate Interceptors (CPI)

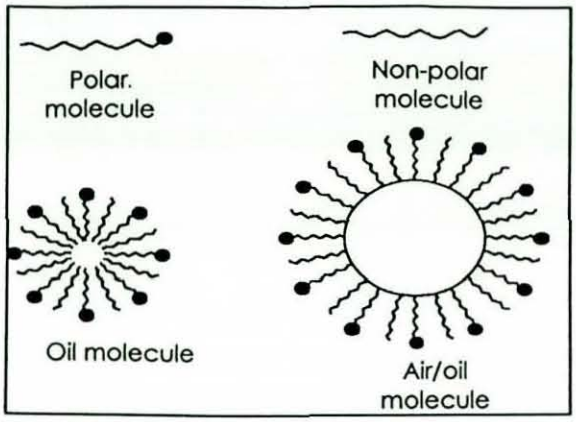
CPI's employ parallel plates to assist the oil-water separation. The plates are corrugated to increase surface area upon which the oil globules coalesce. Oily water stream travels in a downward direction through the plate pack which is made up of corrugated metal or plastic plates assembled in a parallel arrangement and set inclined at 45 ^{0} (Figure B3). The oil droplets rise counter current against the direction of flow and solids settle out cocurrent with the direction of flow. As the droplets coalesce on the plate surface, they increase in size and flow upwards until they attain enough buoyancy and begin to rise. Slop oil collected in the vessel's upper section is returned to the crude oil feed system. Solids are removed from the bottom at the sludge pit. CPI units have limits for throughput and oil loading that defines performance efficiencies. A CPI unit's throughput is proportional to the total plate area and unit volume. An API separators maximum throughput is proportional to the surface area and because of this, a CPI unit has a higher throughput. Plate separators are normally designed to remove droplets larger than 60 microns. Removal of 60 micron oil droplets will generally result in an effluent quality of 10-30 mg/l oil and grease. A disadvantage of CPI is the parallel flow of incoming effluent , which causes the solids, moving concurrent with the flow having less time on the plate pack compared to the countercurrent oil droplets. Hence a poorer separation of solids. This was overcome by the introduction of the Cross Flow Interceptor (CFI)(inset B3). In a CFI, the plates are aligned at 45 ^{0} across the direction of flow.

2. Unit Process for Air Floatation

The waste stream from gravity separators still contain emulsified oil - usually up to 100 mg/l which is still to high for effective biological removal. The oil interferes with oxygen transfer and coalesces within the system. Much of the oil can be removed by flotation. These processes convert microscopic oil droplets into microscopic air/oil bubbles.

The hydrophilic and hydrophobic parts of the oil promote micelle formation on microemulsion that have a non polar oleophilic interior. Air dissolved in the water will accumulate in the interior of the oil particle because of the polarity gradient and diffusion. This process creates air/oil bubbles that have lower densities than oil micelles and microemulsion. Air/oil bubbles then rise to surface much more quickly than do

Fig B4 : Colloidal dispersion of oil in wastewater air flotation process



emulsion droplets. Adding surfactants or a chemical coagulant can improve the separation efficiency of air flotation units. The coagulants are usually cationic polyelectrolytes which promote the coagulation of fine emulsion droplets into larger separable solids. These subsequently will coalesce to form a single oil phase.

Oils recovered from a floatation unit can be recycled to crude feed. However, if a surfactant has been used, the 'floats' generally are not recycled to crude as surfactants in a desalter or crude distillation column can have adverse effects on operations. The 'float' is removed by a rotating skimmer on the top for disposal. Any heavy sediments that may be present in the air floatation unit, settle to the bottom and are removed as a wet oily sludge by a submerged rotating scrapper arm.

a. Dissolved Air Floatation (DAF)(Figure B 5)

Air floatation can be achieved by dissolving air at 4-6 atmospheres upstream of the flotation tank. When pressure is released by entering the DAF tank, the dissolved air forms small air bubbles and air/oil bubbles rise to the surface where a rotating skimmer collects the oil. DAF units downstream a CPI unit can achieve 10-25 mg/l effluent oil and grease, provided influent oil contamination can be kept below 160mg/l.

b. Induced Air Floatation (IAF) (Figure B 6)

Alternatively, air can be introduced into the waste water by an aspirator device or by a fine hole diffuser. The air/water contact occurs at atmospheric pressure. The compressed air may be dispersed throughout the waste by a high speed impeller or the IAF may be applied in stages for increased removal efficiencies. The resulting air bubbles float emulsified oil and other particulates to the surface where a rotating skimmer removes the oil float.

Figure B5 : Dissolved Air Flotation

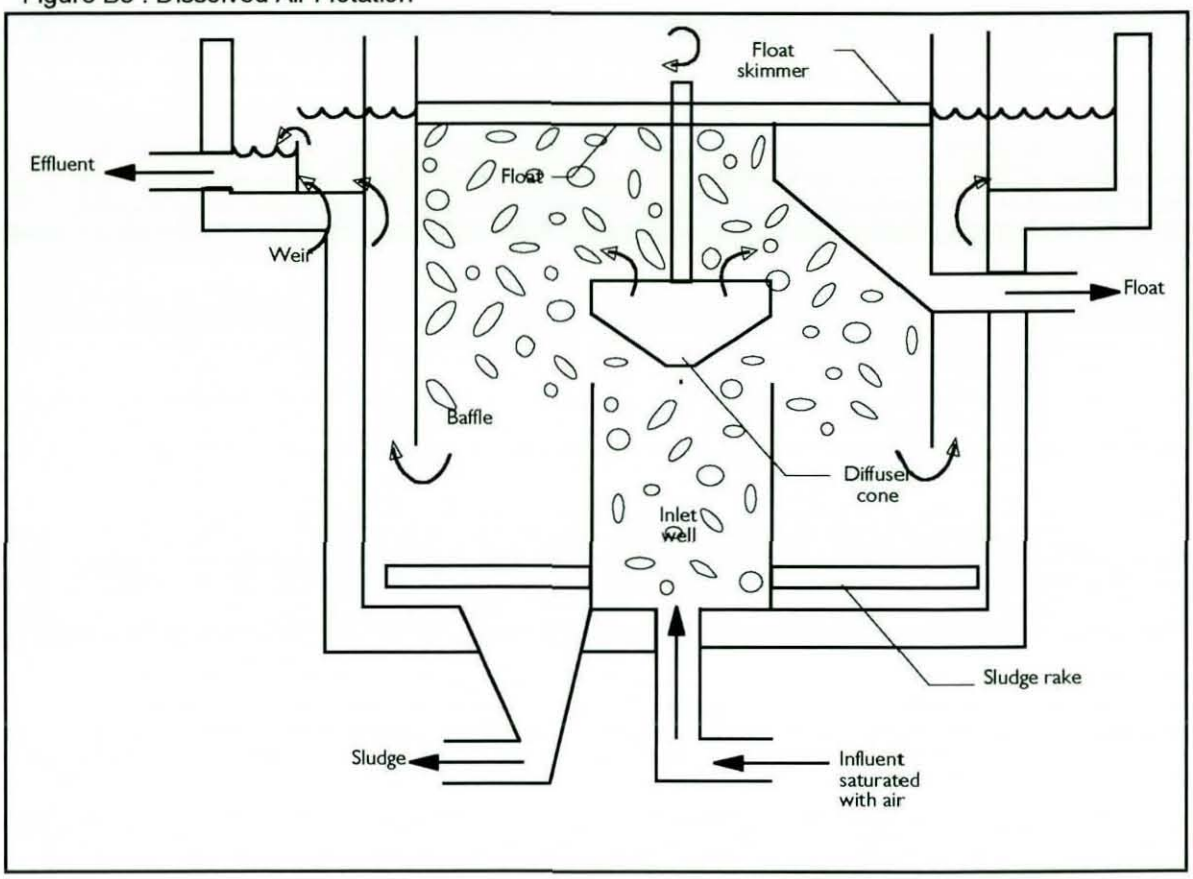
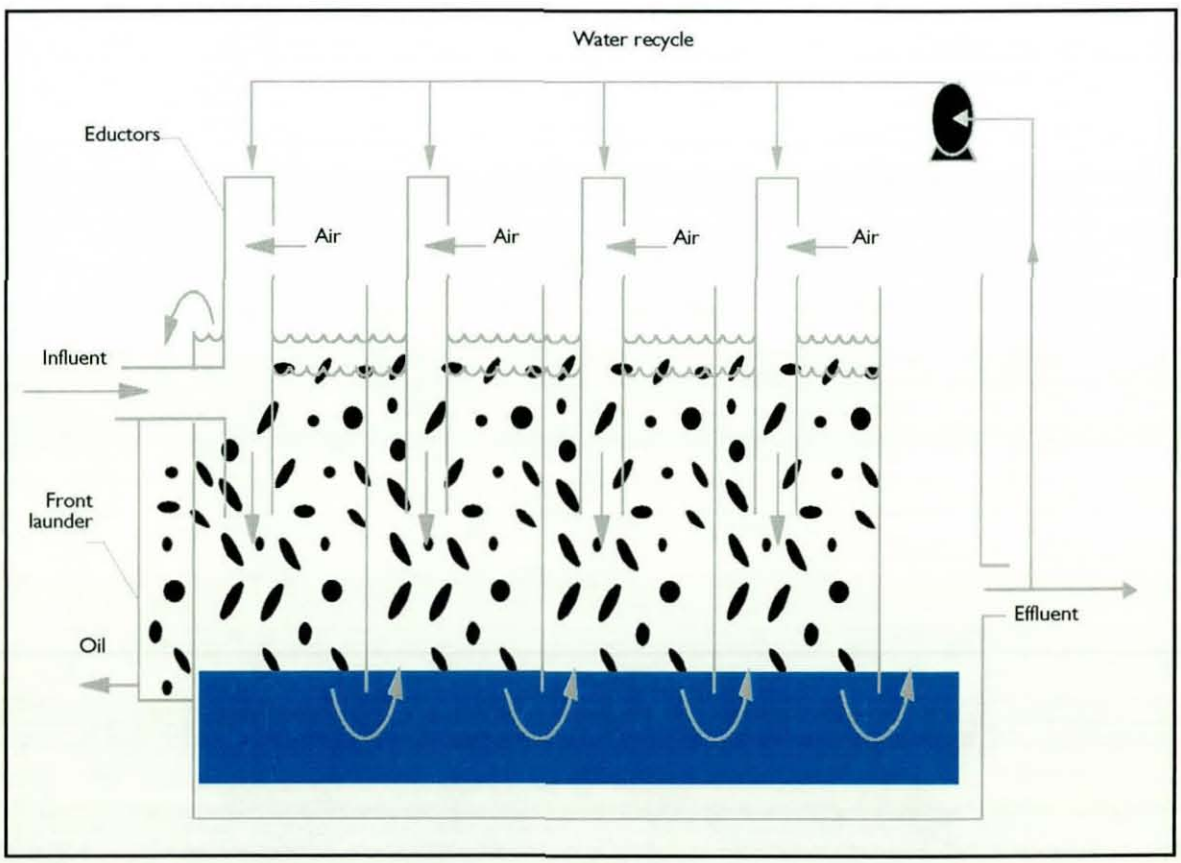


Figure B6 : Induced Air Flotation



Depending on specific design, destruction removal efficiencies may be comparable in the DAF and IAF.

3. Unit process for biological treatment

Biological treatment includes oxidation ponds, trickling filters, rotating biological contractors and activated sludge. The principal aim of biological treatment is to reduce the BOD of refinery effluents in general but also to remove specific toxic substances. The removal is accomplished in part by the complete oxidation of the organics to mineral end products and to a larger extent by conversion of dissolved organics to flocculated and settleable microbial biomass.

a. Oxidation ponds, in its simplest form, is a shallow lagoon sized to retain effluents from half a day to several days providing opportunity for microbial degradation of dissolved organics. Aeration may be augmented by floating or fixed aerators. Oxidation pond bottoms are often anaerobic including silt, microbial biomass and undegraded organics which tend to settle and accumulate.

b. Trickling Filters and Rotating Biological Contractors

The trickling filter was extensively used by early refiners due to its inexpensiveness. It is now rarely used as clogging of the filter beds necessitated the periodic removal of the filter beds. This is an expensive process as well as it yielded voluminous and noxious filter bed material that needed disposal.

A newer fixed film system employed with some success during the last decade at some refineries as an alternative to trickling filters or activated sludge units are the rotating biological contractors (RBC). (Figure B7 and B8) RBC's are free of the clogging problems that are inherent in trickling filters and they are able to handle effluents high in alcohols and ketones well. RBC's adapt well to load fluctuations and have moderate operating costs and do not produce aerosols.

c. Activated Sludge Treatment

The activated sludge process involves contacting and mixing aqueous wastes with microbial sludges in a system supplied with vigorous mixing and with a means for recycling previously synthesized microbial sludge. Good settling of sludge floc is crucial

Figure B 7 :Conventional RBC with mechanical drive and optional input

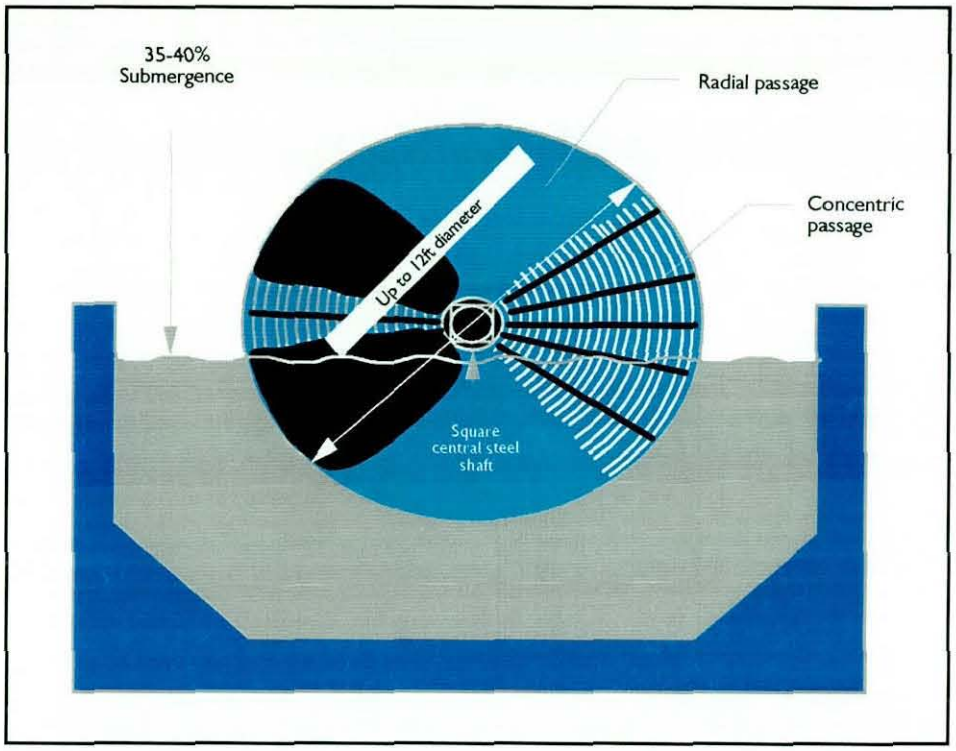
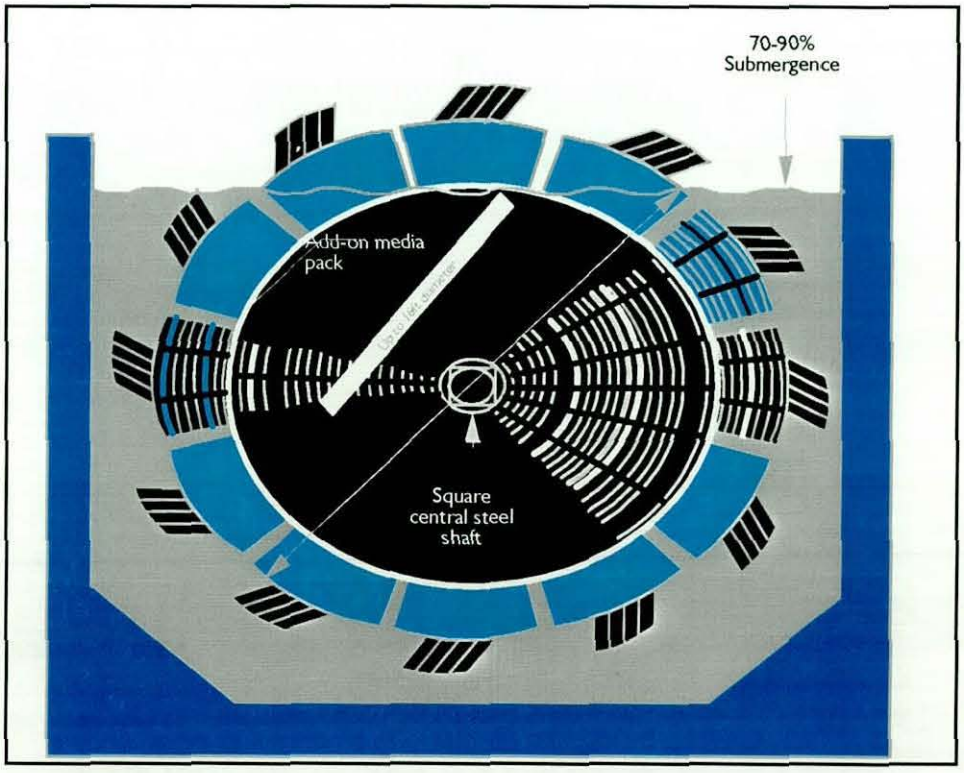


Figure B 8 : Submerged type RBC equipped with air capture cups
(air is used to both rotate and aerate the biodisc)



to the operation of a successful activated sludge treatment system. Poor settling sludge is known as bulking and results in poor removal of dissolved organics and also a high discharge rate of organic solids (floc). Bulking in activated sludge plants when treating a refinery's waste water may be due to the very nature of refinery effluents, that is, the association of the hydrocarbon with the floc itself can cause bulking as can the imbalance of nutrients. While nitrates and sulphates are present in process waters, phosphorus is not and may limit microbial growth and activity. A common solution would be to add effluents from boiler cooling towers that contain phosphates, to the process water. Another cause of bulking is the presence of filamentous organisms such as *Nocardia*, *Microthrix* and *Rhodoccocus*. New activated sludge sytems incorporate methods to prevent bulking such as sequential batch, integral anoxic and selector zones and high DO and low F/M ratios. Ultimate products of the activated sludge process are microbial biomass associated with humuslike substances and residual hydrocarbon. Some of the settled sludge is recycled into the system and the excess is removed.

Table 1 summarizes the oil and grease concentration expected at various parts in a refinery waste water treatment plant . Higher concentrations may be observed for overloads or system upsets.

Table 1 : Oil and grease concentration at various points in a refinery wastewater treatment system

EFFLUENT STREAM	CONCENTRATION RANGE(mg/l)
Oily water sewer	42 to 2,000
API separator	30 to 300
CPI	25 to 100
Air Flotation	5 to 50
Biological Oxidation	0 to 5

Sludge may collect at the bottom (or top) of any treatment unit in the waste water treatment plant. Other sources of sludge from a refinery may come from tank cleanings, desalter bottoms, tar residues from distillation, contaminated soil etc.

APPENDIX B

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APPENDIX C

WASTE CATEGORY NUMBER	DESCRIPTION
USA	
F037	Any petroleum refinery sludge generated from gravitational separation of oil/water/solids during the storage or treatment of process wastewater and oily cooling waters
F038	Any petroleum refinery sludge generated from the physical and/or chemical separation of oil/water/solids in process and oily cooling waters
K048	Dissolved air flotation sludge from the petroleum refining industry (PRI)
K049	Slop oil emulsion solids from the PRI
K050	Heat exchanger bundle cleaning sludge from the PRI
K051	API separator sludge from the PRI
K052	Tank bottoms (leaded) from the PRI
U019	Benzene
U022	Benzo (a) pyrene
U063	Di-benzo-(a,h)-anthracene
U064	Di-benzo-(a,i)-pyrene
U120	Fluoranthene
U165	Napthalene
MALAYSIA	
N014	Oil Tanker sludge
N015	Oil water mixture such as ballast water
N016	Sludge from oil storage tanks
S011	Waste oil or oily sludge from waste water treatment plant of oil refinery or crude oil terminals
S014	Oil or sludge from refinery maintenance operation
S021	Tar or tarry residues from oil refinery or petroleum plant

WASTE CATEGORY NUMBER	DESCRIPTION
UK	WASTES FROM PETROLEUM REFINING, NATURAL GAS PURIFICATION AND PYROLYTIC TREATMENT OF COAL
05	Oily sludges and solid wastes
0501	Tank bottom sludges
050103	Oil spills
050105	Other tars
050108	OIL WASTES (except edible oils,0500 and 1200)
13	BILGE OILS
1304	Bilge oils from inland navigation
130401	Bilge oils from jetty sewers
130402	Bilge oils from other navigation
130403	OIL/WATER SEPARATOR CONTENTS
1305	Oil/water separator solids
130501	Oil/water separator sludges
130502	Intercepter sludge
130503	Desalter sludges or emulsions
130504	Other emulsions
130505	OIL WASTES NOT OTHERWISE SPECIFIED
1306	Oil wastes not other wise specified

GROWTH RATE AND SUBSTRATE UTILIZATION (DEGRADATION) IN A BATCH REACTOR

The specific growth rate can be expressed by the equation of Monod:

$$\mu = \frac{\mu_m \quad S}{K_s + S} \qquad (2\text{-}1)$$

Where;

μ = specific growth rate, time $^{-1}$

μ_m = maximum value of μ, time $^{-1}$

S = residual growth-limiting substrate concentration, mass/unit volume

K_s = half-velocity constant, substrate concentration at one-half maximum growth rate, mass/unit volume

The effect of substrate concentration on the specific growth rate is shown in Figure D1.

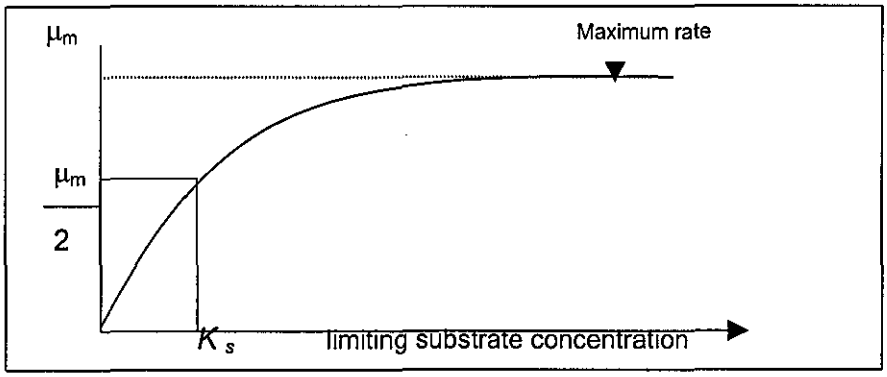


Figure.D1 : Plot showing the effects of a limiting nutrient on the specific growth rate

The measurement of substrate and biomass with time will enable the quantification of K_s and μ.

In reactor slurry studies, the rate of growth of bacterial cells can be defined by the following relationship:

$$r_g = \mu X \qquad (2.2)$$

where r_g = rate of bacterial growth, mass/unit volume.time

μ= specific growth rate, time -1

X = concentration of microorganisms, mass/unit volume

Because the change in mass for bacterial growth, *dX/dt*, is equal to the rate of bacterial growth in batch cultures, r_g , the following relationship is also valid for a batch reactor:

$$\frac{dX}{dt} = \mu X \qquad (2.3)$$

If the value of μ from Eq. 2.1 is substituted in Eq. 2.3 the resulting expression for the rate of growth is:

$$r_g = \frac{\mu_m XS}{K_s + S} \qquad (2.4)$$

As shown in Fig 2, growth is coupled to substrate utilization. The Monod equation is similarly used to express biodegradation or substrate utilization rates:

$$\frac{dS}{dt} = - \frac{kXS}{K_s + S} \qquad (2.5)$$

Where:

S = substrate concentration., mg/l

X = biomass concentration, mg/l

K_s = the half velocity coefficient, mg/l

t = the time in hours

k = the maximum specific substrate utilization rate, l/hr

APPENDIX E

MEDIA RECIPES

BUSHNELL HAAS MEDIA

Ingredients per liter

Magnesium sulphate	0.2 g
Calcium chloride	0.02 g
Monopotassium phosphate	1g
Dipotassium phosphate	1 g
Ammonium nitrate	1 g
Ferric chloride	0.05 g

In 1 l of demineralised water, final pH at 7.0 ±2 at 25 ^{0} C

To make BBH agar, 15 g purified agar (Difco Ultrapure) was added to 1 l BBH media

PHOSPHATE BUFFERED SALINE SOLUTION

Sodium chloride	8 g
Pottassium hydrogen phosphate	0.34
Dipottassium hydrogen phosphate	1.21

In 1 l demineralised water

PHOSPHATE LIMITED PROTEOSE PEPTONE GLUCOSE-AMMONIUM (PPGAS) MEDIA

Ammonium chloride	0.02M
Pottassium chloride	0.02 M
Tris Hydrochloric acid	0.12 M
Glucose	0.5%
Proteose peptone	1 %
Magnesium sulphate	0.0016M

Final pH 7.2

KAY'S MINIMAL MEDIA

Ammonium hydrogen phosphate	0.3%
Dipottasium hydrogen phophate	0.2%
Glucose	0.2%
Ferrous sulphate	0.5 mg Fe/l
Magnesium sulphate	0.1%

SLUDGE CHARACTERISTICS

sludge characteristics	no of duplicates	
dry solids fraction	3	0.742
percentage oil and grease per dry solids	6	33.11
percentage asphaltene content (by method IP143)	2	0.75
percentage fixed solids (combustion at 550 ^{0} C)	3	60.21
percentage volatiles	3	39.74
Heavy metals (per dry wt kg)	2	
Copper		38
Cadmium		2
Chromium		34
Aluminium		159
Silver		3
Zinc		58
Lead		184
Mercury		0.26
Selenium		4
Nickel		412
Barium		5
Iron		38,030

SLUDGE CHARACTERISTICS

Sludge Heavy Metal Analysis by ICP

0.5351 gm dry weight sample digested twice with Aqua Regia
Filtered and brought to 100 ml volume in graduated bottle.
Measured with ICP model: Thermo Jarrel Ash - Atomscan 16

Average of 4 readings

Element	Wavelength	ppm	Alternate wlgth	ppm	avg	Corr Factor	per kg sludge
Copper	*324.861*	0.2057	*327.391*	0.1987	**0.2022**	0.02022	**38.**
Cadmium	*228.808*	0.0033	*214.4*	0.0134	**0.00835**	0.000835	**2.**
Chromium	*267.77*	0.1741	*205.5*	0.1882	**0.18115**	0.018115	**34.**
Aluminium	*309.266*	0.6973	*167.7*	1.009	**0.85315**	0.085315	**159.**
Silver	*328.11*	0.0221	*3280*	0.008	**0.01505**	0.001505	**3.**
Zinc	*213.854*	0.3189	*202.5*	0.305	**0.31195**	0.031195	**58.**
Lead	*182.201*	1.85	*220.351*	0.1201	**0.98505**	0.098505	**184.**
Mercury	*185.011*	0.0018	*194.2*	0.001	**0.0014**	0.00014	**0.26**
Selenium	*204.022*	0.04	*196*	0.001	**0.0205**	0.00205	**4.**
Nickel	*221.645*	2.21	*231.6*	2.202	**2.206**	0.2206	**412.**
Barium	*493.405*	0.0253	*455.4*	0.0245	**0.0249**	0.00249	**5.**
Iron	*238.202*	200.2	*259.9*	206.8	**203.5**	20.35	**38030.**

FLUORESCENCE EXITATION AND EMISSION SPECTRA

Table A: Most intense peak for an uncorrected fluorescence spectral characteristics of PAH in 80:20 acetonitrile/water

PAH	**Fluorescence excitation spectra*	*Fluorescence emission spectra*
Naphthalene	220	324
Acenapthene	339	not specified
Fluorene	268	303
Phenanthrene	249	345
Anthracene	250	350
Fluoranthene	284	463
Pyrene	333	370
Benzo(a)anthracene	285	384
Chrysene	263	360
Benzo(b)fluoranthene	296	433
Benzo(k)fluoranthene	304	407
Benzo(a)pyrene	297	404
Dibenzo(a,h)anthracene	295	392
Benzo (ghi)perelene	295	404
Indeno(1,2,3-cd)pyrene	239	496

Source
A. Bjorseth (1983). Handbook of Polycyclic Aromatic Hydrocarbons, Marcel Dekker, pg. 220-221.

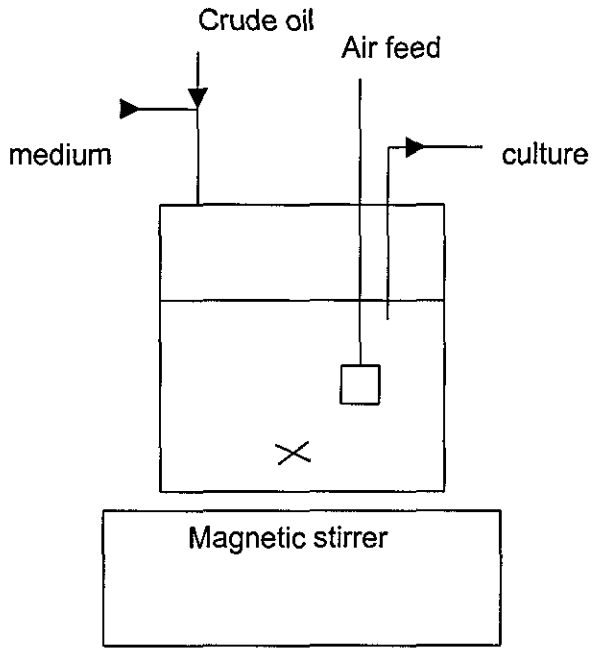
APPENDIX H

GROWTH OF CULTURES IN A CHEMOSTAT

Cultures were grown in a 5 l Erlenmeyer Flask placed on a magnetic stirrer for mixing. Air was supplied with the use of an air pump which was fed through a tube with a stone diffuser at the end.

Working volume = 2800 ml
HRT = 96 hrs
Pump set to 1.92 ml /min running for 30 mins for every hour in 12 hrs per day
Volume changed per day = 691.2 ml
Media BBH with 1 % yeast extract and 1-5 % topped crude oil

The chemostat was placed in the warm room with constant room temperature at 37 ^{0} C. Crude oil was syringed through a separate inlet as mixing it in the feed ultimately caused all the oil to be coated in the tubes.



INIPOL EAP 22 CHARACTERISTICS

Physical characteristicsIc EAP 22	
Appearance	clear liquid
Specific gravity @ 20 ° C	0.996
Viscosity @ 20 ° C	250 cPo
Pour point	11 ° C
Flash point	100 ° C
Chemical Constituents	
Blend of surfactants and butylglycol	
Nitrogen and phosphorus	

Manufacturer's address

CECA S.A.
12, Place de l'Iris
La Defense 2
92400 Courbevoie
Hauts-de-Seine (FRANCE)

OIL AND GREASE DETERMINATION

SLUDGE

O&G extraction with TCTFE and DCM

No	Sludge sample	Sludge dsf	dry wt	Solvent	Flask	Flask w oil	oil	% O&G/dry wt
1	17.82	0.7674	13.6751	TCTFE	145.70	150.03	4.33	31.66
2	17.00	0.7674	13.0458	TCTFE	147.80	151.68	3.88	29.74
3	15.15	0.7546	11.4322	TCTFE	164.77	168.09	3.32	29.04
4	15.17	0.7546	11.4473	TCTFE	170.14	173.46	3.32	29.00
5	17.14	0.7674	13.153	DCM	196.00	200.40	4.40	33.45
6	15.01	0.7546	11.3265	DCM	167.52	171.52	4.00	35.32
7	15.19	0.7546	11.4624	DCM	171.37	175.37	4.00	34.90
8	15.06	0.7546	11.3643	DCM	161.25	164.87	3.62	31.85
							Avg TCTF	29.86
							Avg DCM	33.88

O&G extraction 4 hrs VS 16 hours with DCM

No	Lgth of Ext.(hr)	Spike	Qty	Cruc	Cruc w oil	Oil	% recovered	
1	4	crude oil	1.02	57.97	58.65	0.68	66.67	
2	4	crude oil	1.16	57.22	57.97	0.75	64.66	
3	16	crude oil	1.80	57.99	59.15	1.16	64.44	
4	16	crude oil	1.80	57.25	58.41	1.16	64.44	
						Avg 4 hr	65.66	
						Avg 16 hr	64.44	

ML

O&G extraction of ML using method 5.1.3.1

No	Spike	Qty	Cruc	Cruc w oil	Oil	% recovered	
1	crude oil	0.1748	88.0597	88.1763	0.1166	66.70	
2	crude oil	0.1787	101.366	101.4847	0.1185	66.31	
3	crude oil	0.1770	101.494	101.6125	0.1188	67.12	
4	Extr. O&G	0.3604	27.8117	28.1178	0.3061	84.93	
5	Extr. O&G	0.3522	41.8779	42.1756	0.2977	84.53	
6	Extr. O&G	0.3048	39.3924	39.6578	0.2654	87.07	
					Avg crude	66.71	
					Avg ext O&	85.51	

HPLC DETERMINATION FOR PAH

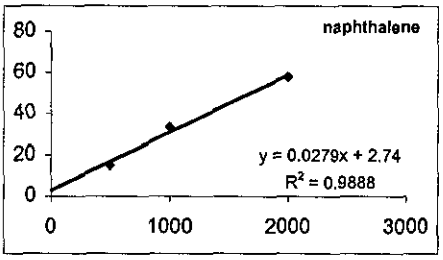
SPREADSHEET USED FOR CALIBRATION FOR UVD

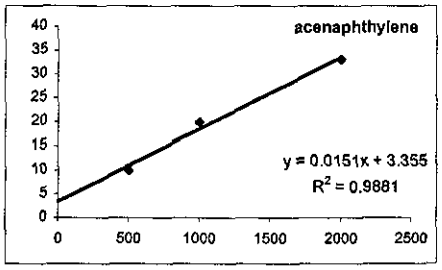
PAH calibration standards at, 500, 1000, 2000 ug/l
Using Columns Vydac and Lichros
Chromatograms Suhana PAH123 and 124 (Vydac) and Suhana PAH110 (also used for FLD calib)

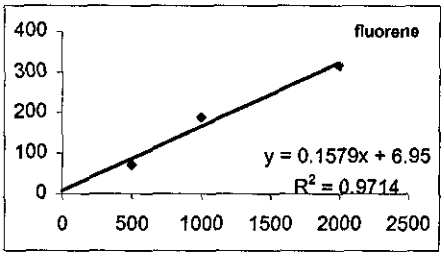
Peak no	Name\Conc(ug/l)	500	1000	2000
1	naphthalene	14.995	33.25	57.76
2	acenapthylene	9.93	19.85	33
3	acenapthene	not integrated		
4	fluorene	70.26	188.32	314.94
5	phenanathrene	195.3	365.65	710.42
6	anthracene	410	749.42	1312.89
7	fluoranthene	45.61	95.84	169.44
8	pyrene	40.96	79.46	138.07
9	benzo(a) anthracene	105.55	236.46	426.48
10	chrysene	177.67	398.34	715.48
11	benzo (b)fluoranthene	140.98	246.48	446.64
12	Benzo(k) fluoranthene	101.27	175.21	314.24
13	Benzo (a) pyrene	140.98	246.48	314.24
14	di benzo(a,h) anthracene	16.94	57.037	99.725
15	Benzo (ghi) perelene	66.54	91.442	159.4
16	Indeno(1,2,3,cd)pyrene	173	198.52	363
3	ACENAPTHENE	200	5000	22,000
	(single std)	12.62	320	1370

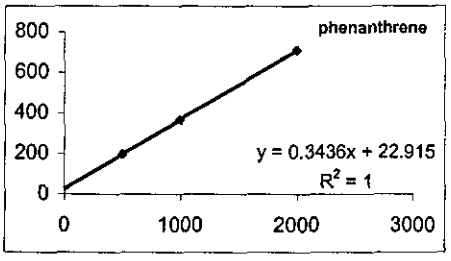
HPLC DETERMINATION FOR PAH

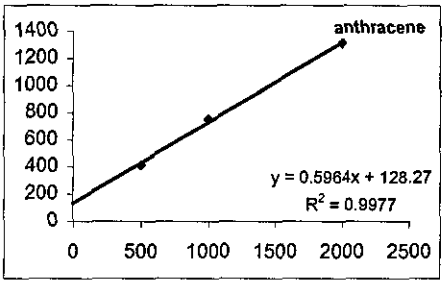
PAH CALIBRATION, 500, 1000, 2000 ug/l for UVD

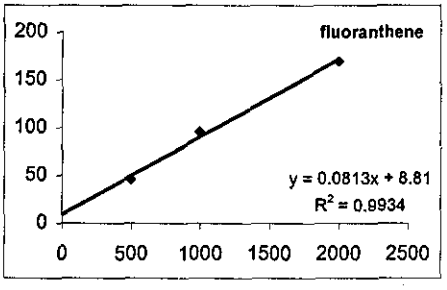


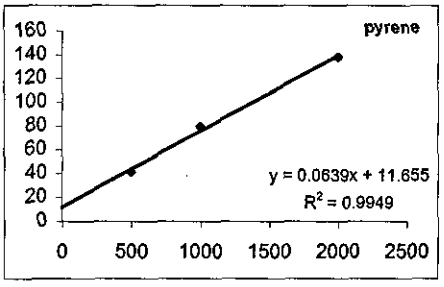


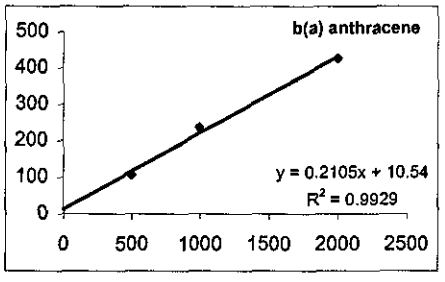


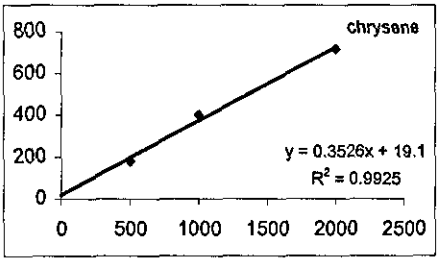


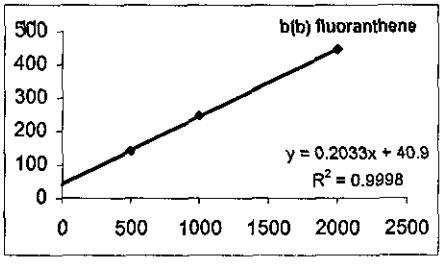




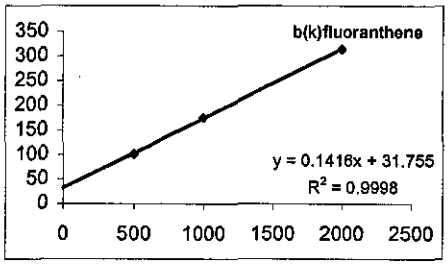


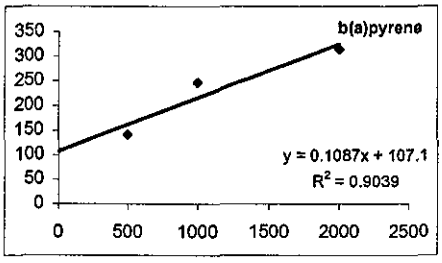


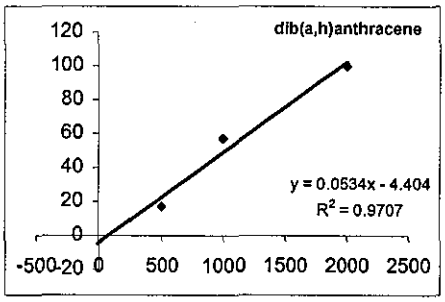


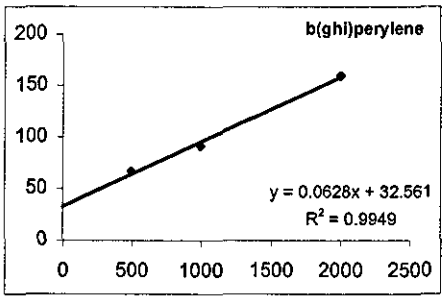


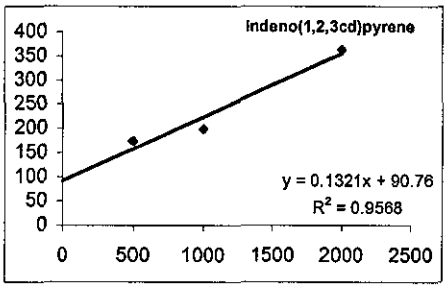
APPENDIX J

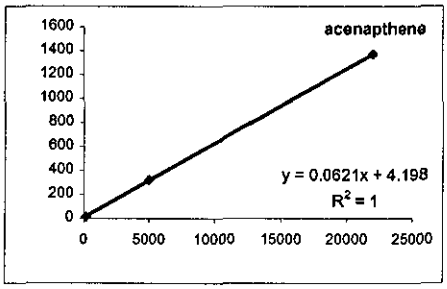

b(k)fluoranthene
y = 0.1416x + 31.755
R² = 0.9998
350
300
250
200
150
100
50
0
0 500 1000 1500 2000 2500


b(a)pyrene
y = 0.1087x + 107.1
R² = 0.9039
350
300
250
200
150
100
50
0
0 500 1000 1500 2000 2500

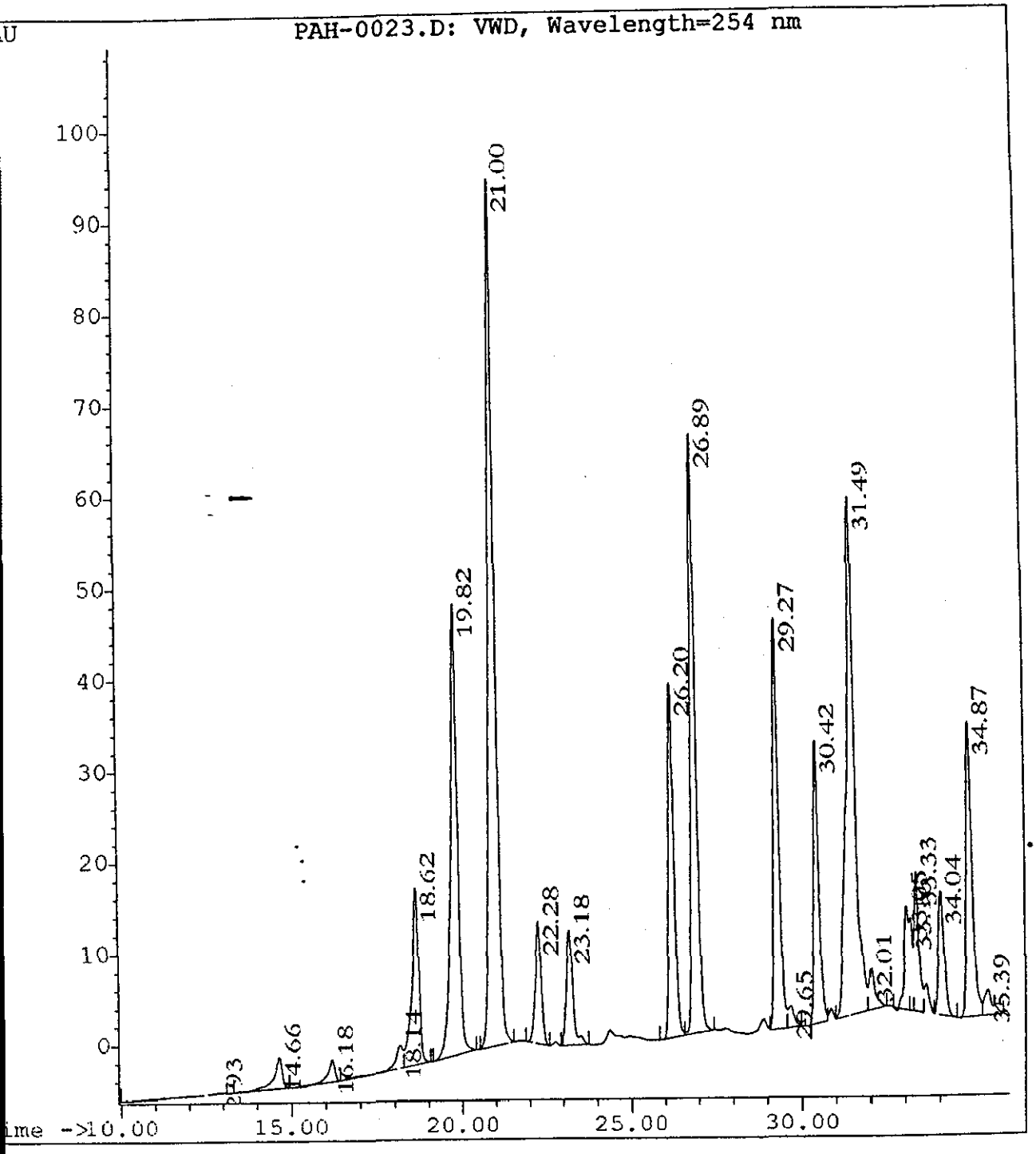

dib(a,h)anthracene
y = 0.0534x - 4.404
R² = 0.9707
120
100
80
60
40
20
0
-500-20 0 500 1000 1500 2000 2500


b(ghi)perylene
y = 0.0628x + 32.561
R² = 0.9949
200
150
100
50
0
0 500 1000 1500 2000 2500

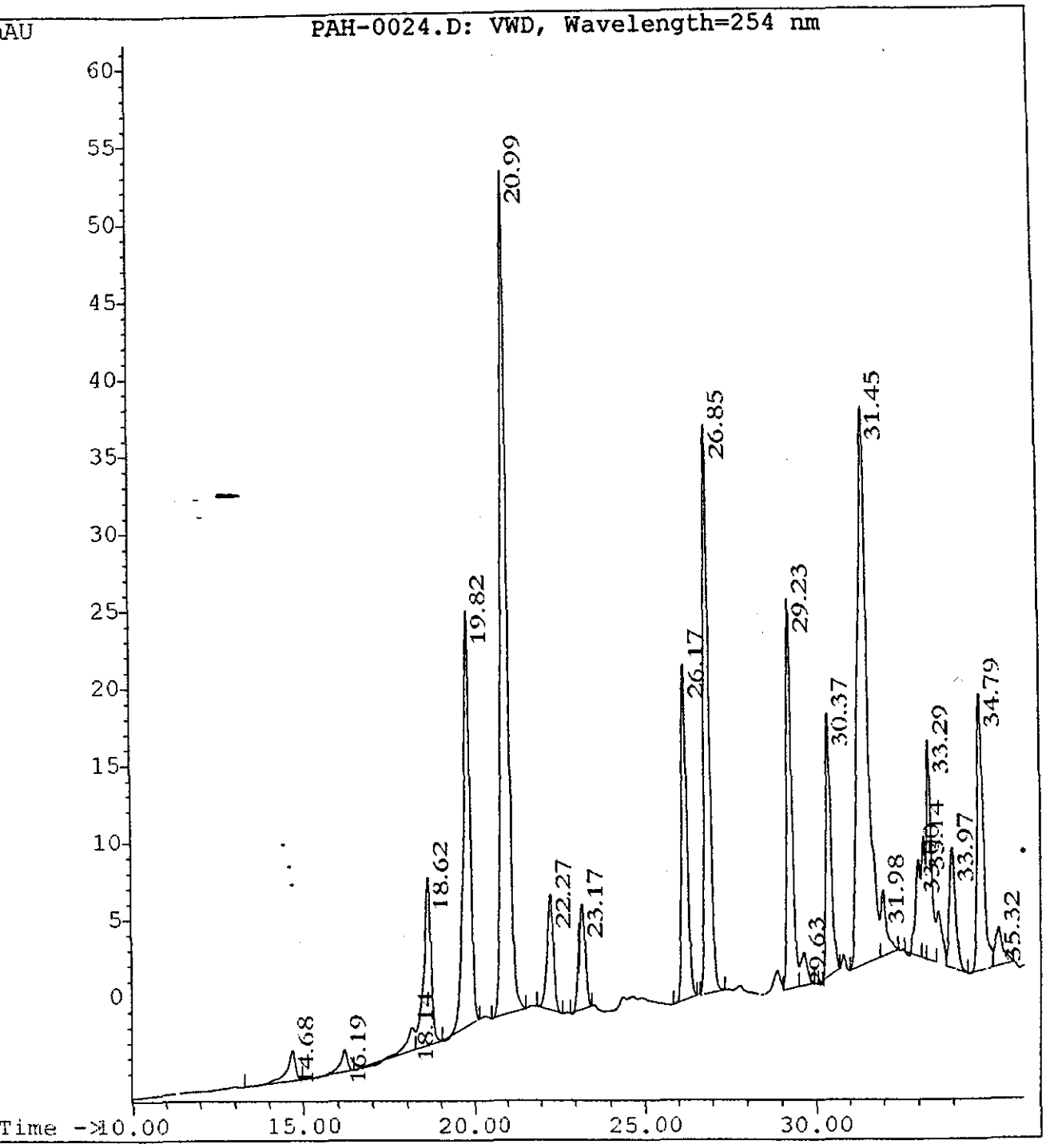

indeno(1,2,3cd)pyrene
y = 0.1321x + 90.76
R² = 0.9568
400
350
300
250
200
150
100
50
0
0 500 1000 1500 2000 2500


acenapthene
y = 0.0621x + 4.198
R² = 1
1600
1400
1200
1000
800
600
400
200
0
0 5000 10000 15000 20000 25000

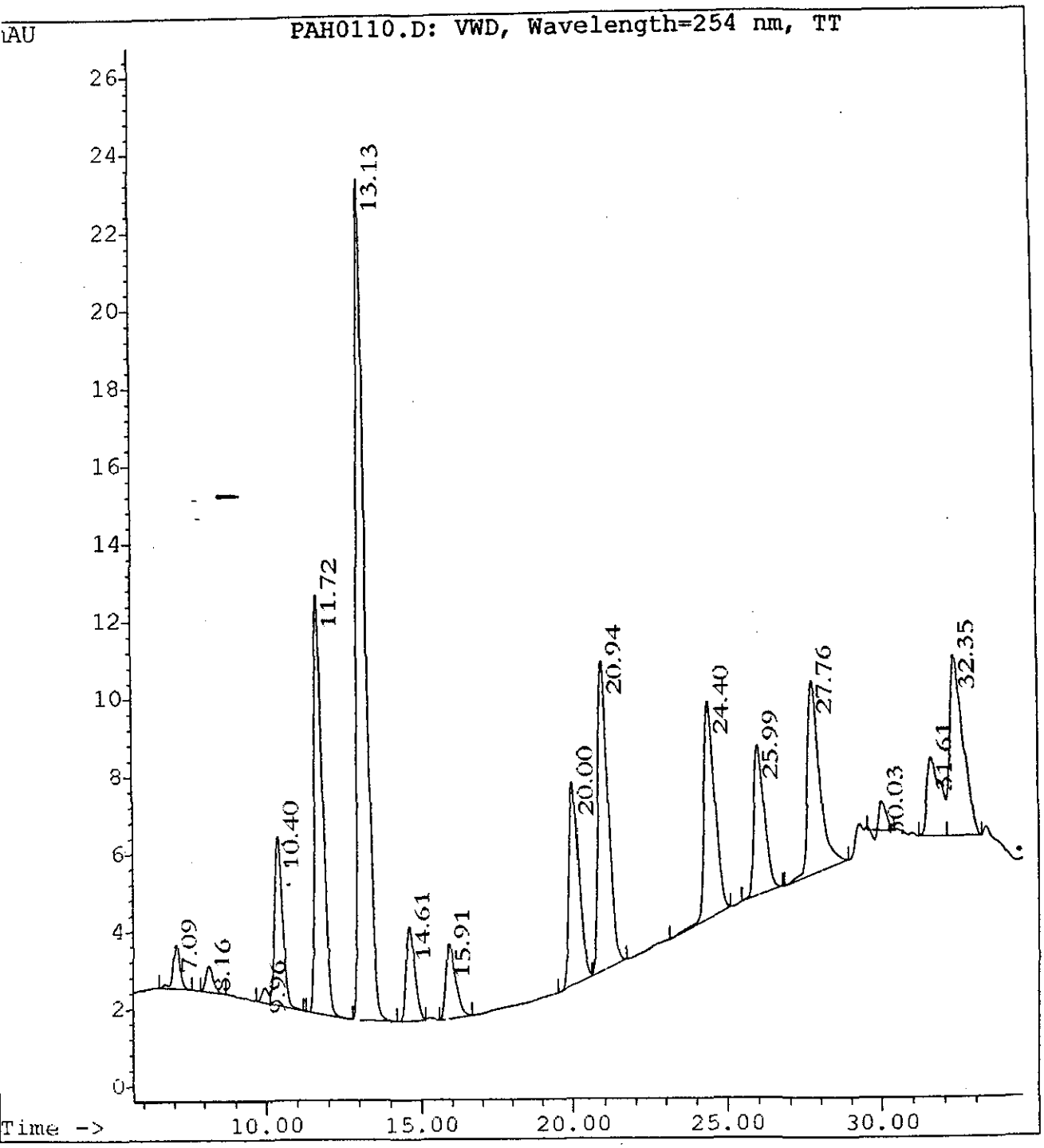
File: A:\SUHANA\PAH-0023.D
Operator: SUHANA
Date Acquired: Mon Apr 19 12:48:49 1993
Method File Name: SUPAH9.M
Sample Name: PAH.STD.2000mg/l
Misc Info:
Bottle Number: 1



File: A:\SUHANA\PAH-0024.D
Operator: SUHANA
Date Acquired: Mon Apr 19 13:47:01 1993
Method File Name: SUPAH9.M
Sample Name: PAH.STD.1000mg/1
Misc Info:
Bottle Number: 1



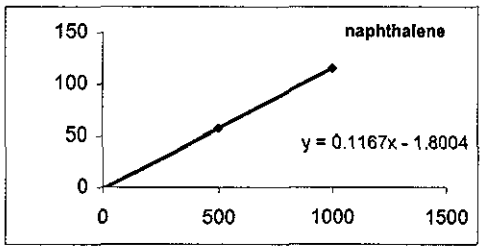
File: A:\PAH0110.D
Operator: suhana
Date Acquired: 13/9/96 18:37:27
Method File Name: SUPAHS.M
Sample Name: pah 500
Misc Info:
Bottle Number: 1

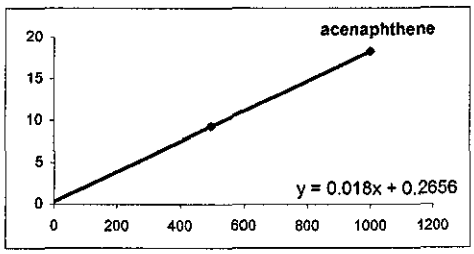


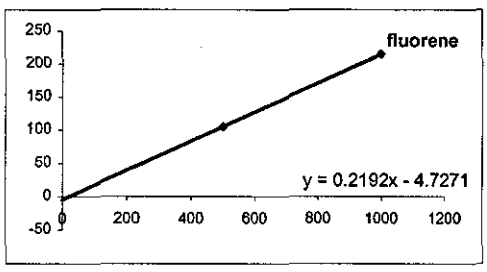
HPLC DETERMINATION FOR PAH

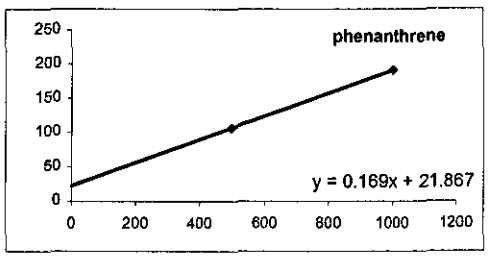
SPREADHEET USED FOR CALIBRATION FOR FLD										
	CALIBRATION	FLD	500 and 1000 mg/l							
	conc. ug/l	500	500	1000	1000	after mpore	window	Av area	Av area	
Peak	Chromatogram	110	112	108	109		for RT	500	1000	
1	naphthalene	7.16	7.141	7.126	7.132	7.222	7.14-7.22			
	area	55.9207	57.1949	107.832	122			56.56	114.92	
2	acenapthylene	NF								
	area									
3	acenapthene	10.038	10.038	9.999	10.007		9.99 - 10.04			
	area	9.2906	9.2654	18.0135	18.5673			9.28	18.29	
4	fluorene	10.464	10.474	10.422	10.373	10.651	10.37 - 10.65			
	area	105.006	104.704	199.907	228.9672					
5	phenanathrene	11.798	11.802	11.754	11.765	11.981	11.75 - 11.98			
	area	105.2291	107.5035	152.1947	229.5366			106.37	190.87	
6	anthracene	13.207	13.225	13.155	13.158	13.411	13.15- 13.41			
	area	230.6513	213.5656	454.5117	522.6537			222.11	488.58	
7	fluoranthene	14.692	14.715	14.6333	14.65		14.63 - 14.69			
	area	33.7898	34.023	67.2255	77.4385			33.91	72.33	
8	pyrene	15.975	16.002	15.922	15.929	16.382	15.92 - 16.002			
	area	80.2669	83.2208	158.8453	180.7686			81.74	169.81	
9	benzo(a) anthracene	20.087	20.101	19.991	20.0015		19.99 - 20.08			
	area	62.33739	60.7158	111.3582	128.1229			61.53	119.74	
10	chrysene	20.995	21.037	20.927	20.946	19.743	19.74 - 21.037			
	area	46.8953	46.1055	86.2071	98.4224			46.50	92.31	
11	benzo (b)fluoranthene	24.465	24.513	24.381	24.397	25.143	24.38 - 25.14			
	area	84.1085	85.5863	160.8949	187.0126			84.85	173.95	
12	Benzo(k) fluoranthene	26.065	26.118	25.977	25.983	26.801	25.97 - 26.11			
	area	457.2804	461.7476	858.4127	982.7369			459.51	920.57	
13	Benzo (a) pyrene	27.82	27.882	27.729	27.748	28.563	27.72 - 28.56			
	area	115.1611	103.241	230.4175	265.0154			109.20	247.72	
14	di benzo(a,h) anthracen	30.112	30.168	30.005	30.014	30.867	30.00 - 30.87			
	area	49.2795	55.8201	82.592	74.9516			52.55	78.77	
15	Benzo (ghi) perelene	31.678	31.733	31.584	31.584	32.447	31.58 - 32.45			
	area	357.484	360.168	683.031	778.837			358.83	730.93	
16	Indeno(1,2,3,cd)pyrene	32.425	32.5	32.31	32.33	33.492	32.31 - 32.42			
	area	54.1104	53.4146	103.678	118.08			53.76	110.88	
		1846.812	1836.356	3475.123	4013.113			1841.58	3744.12	
	Spike -Decaf	4000	40,000	40,000			13.38 -13.44	4,000	av area for 40,000	
	after anthracene	13.44	13.37	13.378				4.95	26.27	
		4.95	26.86	25.699						

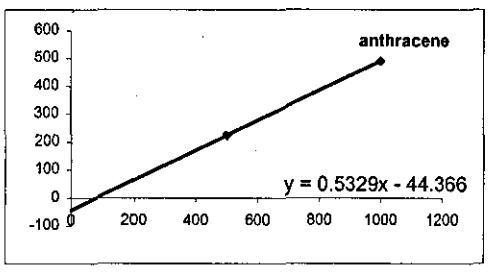
FLD calibration 500 and 1000 ug/l for FLD

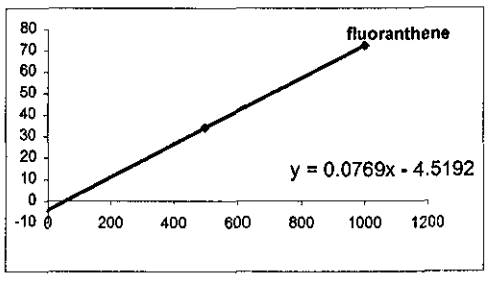


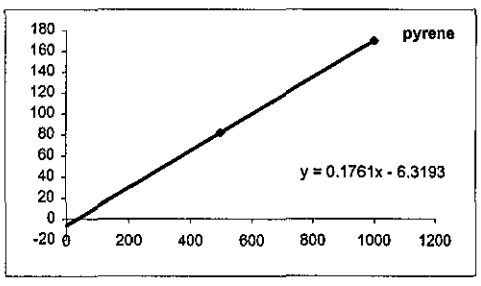


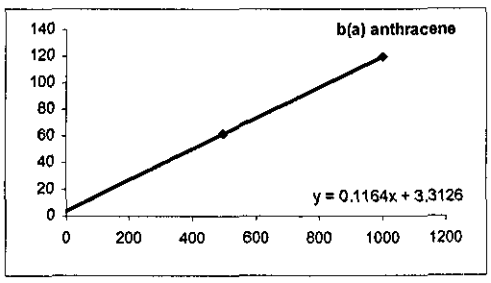


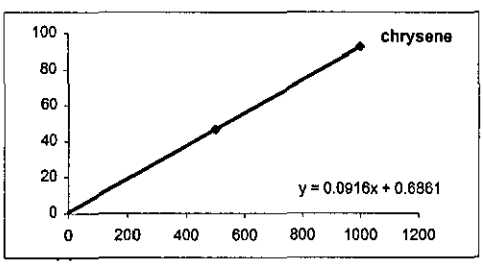


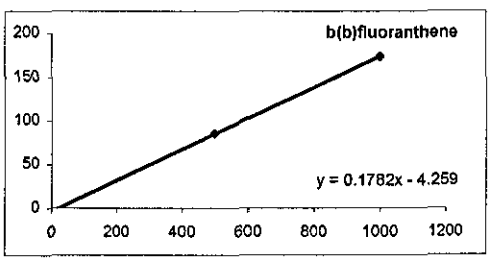




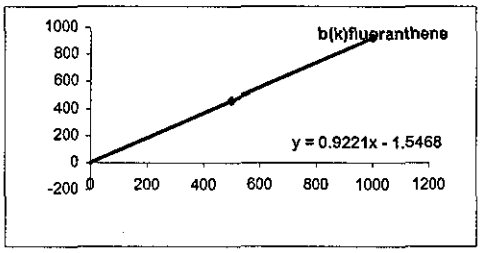


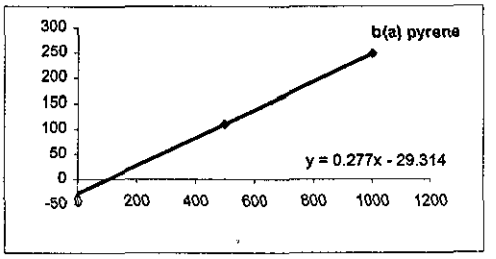


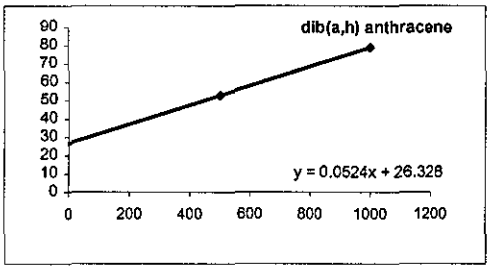


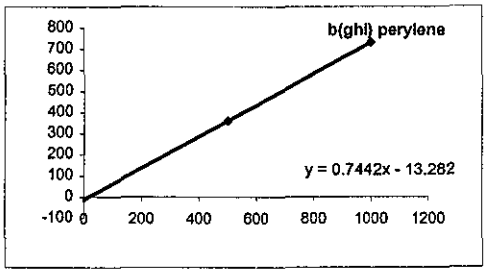


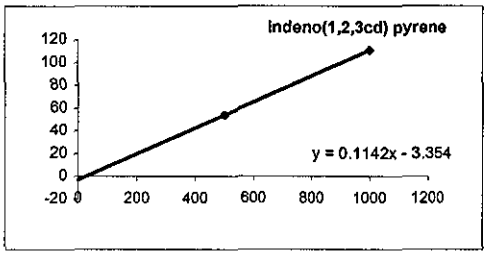
APPENDIX J

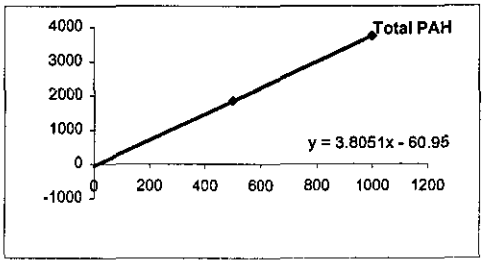

b(k)fluoranthene
y = 0.9221x - 1.5468
1000
800
600
400
200
0
-200
0
200
400
600
800
1000
1200

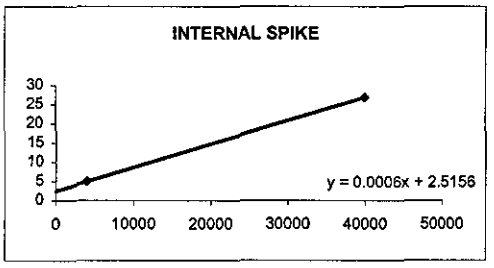

b(a) pyrene
y = 0.277x - 29.314
300
250
200
150
100
50
0
-50
0
200
400
600
800
1000
1200


dib(a,h) anthracene
y = 0.0524x + 26.328
90
80
70
60
50
40
30
20
10
0
0
200
400
600
800
1000
1200


b(ghi) perylene
y = 0.7442x - 13.282
800
700
600
500
400
300
200
100
0
-100
0
200
400
600
800
1000
1200


Indeno(1,2,3cd) pyrene
y = 0.1142x - 3.354
120
100
80
60
40
20
0
-20
0
200
400
600
800
1000
1200


Total PAH
y = 3.8051x - 60.95
4000
3000
2000
1000
0
-1000
0
200
400
600
800
1000
1200


INTERNAL SPIKE
y = 0.0006x + 2.5156
30
25
20
15
10
5
0
0
10000
20000
30000
40000
50000

MICROBIAL INOCULA SOURCE

LFBBHO

		filter	105	550	ts	std dev	tvs	std dev	tfs	std dev	tfs/ts	tvs/ts		cruc wt	w oil	oil	std dev	oil/ts	oil/tfs	oil g/l
	1	0.1976	0.2071	0.2011	0.0095	0.0009	0.0060	0.0025	0.0035	0.0034	0.3684	0.63		42.7443	42.7676	0.0233	0.0017	0.61	1.66	
day 0	2	0.1968	0.2079	0.2055	0.0111		0.0024		0.0087		0.7838	0.22		42.729	42.7552	0.0262		0.59	0.75	
	3	0.2021	0.2117	0.2045	0.0096		0.0072		0.0024		0.2500	0.75		44.6416	44.6649	0.0233		0.61	2.43	
	avg				0.0101		0.0052		0.0049		0.4674	0.532598				0.0243				
	g/l				0.5033		0.2600													1.213333
		cruc wt	105	550	ts		tvs		tfs		tfs/ts	tvs/ts		cruc wt	w oil	oil		oil/ts	oil/tfs	oil g/l
day1	1	0.2029	0.2148	0.205	0.0119	0.0004	0.0098	0.0004	0.0021	0.0001	0.1765	0.82		17.8309	17.8525	0.0216	0.0014	0.45	2.57	
	2	0.203	0.2145	0.2052	0.0115		0.0093		0.0022		0.1913	0.81		17.1689	17.209	0.0201		0.44	2.28	
	3	0.2008	0.212	0.203	0.0112		0.0090		0.0022		0.1964	0.80		19.4466	19.4695	0.0229		0.51	2.60	
					0.0115		0.0094									0.0215				1.076667
	g/l				0.5767		0.4683													
		cruc wt	105	550	ts		tvs		tfs		tfs/ts	tvs/ts		cruc wt	w oil	oil		oil/ts	oil/tfs	
day 2	1	0.2022	0.2132	0.2045	0.0110	0.0006	0.0087	0.0005	0.0023	0.0001	0.2091	0.79								
	2	0.202	0.2137	0.2044	0.0117		0.0093		0.0024		0.2051	0.79								
	3	0.1993	0.2114	0.2018	0.0121		0.0096		0.0025		0.2066	0.79								
					0.0116		0.0092		0.0024											
	g/l				0.5800		0.4600		0.1200											
		cruc wt	105	550	ts		tvs		tfs		tfs/ts	tvs/ts		cruc wt	w oil	oil		oil/ts	oil/tfs	
day 3	1	0.2004	0.2096	0.203	0.0092	0.0064	0.0066	0.0071	0.0026	0.0008	0.2826	0.72		42.744	42.7639	0.0199	0.0015	0.54	1.91	
	2	0.2016	0.2226	0.2027	0.0210		0.0199		0.0011		**0.0524**	**0.95**		42.7296	42.7465	**0.0169**		**0.20**	**3.84**	
	3	0.1995	0.2105	0.2017	0.0110		0.0088		0.0022		0.2000	0.80		44.6412	44.6599	0.0187		0.43	2.13	
					0.0137		0.0118		0.0020							0.0185				
	g/l				0.6867		0.5883		0.0983											0.925
		cruc wt	105	550	ts		tvs		tfs		tfs/ts	tvs/ts		cruc wt	w oil	oil		oil/ts	oil/tfs	
day 4	1	0.2006	0.2108	0.2024	0.0102	0.0002	0.0084	0.0001	0.0018	0.0002	0.1765	0.82								
	2	0.1973	0.2075	0.1993	0.0102		0.0082		0.0020		0.1961	0.80								
	3	0.1968	0.2074	0.199	0.0106		0.0084		0.0022		0.2075	0.79								
					0.0103		0.0083		0.0020											
	g/l				0.5167		0.4167		0.1000											
		cruc wt	105	550	ts		tvs		tfs		tfs/ts	tvs/ts		cruc wt	w oil	oil		oil/ts	oil/tfs	
day 5	1	0.2014	0.2114	0.2041	0.0100	0.0003	0.0073	0.0003	0.0027	0.0001	0.2700	0.73		17.1106	17.1289	0.0183	0.0016	0.46	1.69	
	2	0.2029	0.2134	0.2055	0.0105		0.0079		0.0026		0.2476	0.75		17.1903	17.2118	0.0215		0.51	2.07	
	3	0.2026	0.2129	0.2051	0.0103		0.0078		0.0025		0.2427	0.76		16.3385	16.3578	0.0193		0.47	1.93	
					0.0103		0.0077		0.0026							0.0197				
	g/l				0.5133		0.3833		0.1300											0.985
MPN at start		1.00E+09			avg std dev	0.0014	avg std dev	0.0018	avg std dev	0.0008						avg std dev	0.0016			
turbidity		65NTU																		

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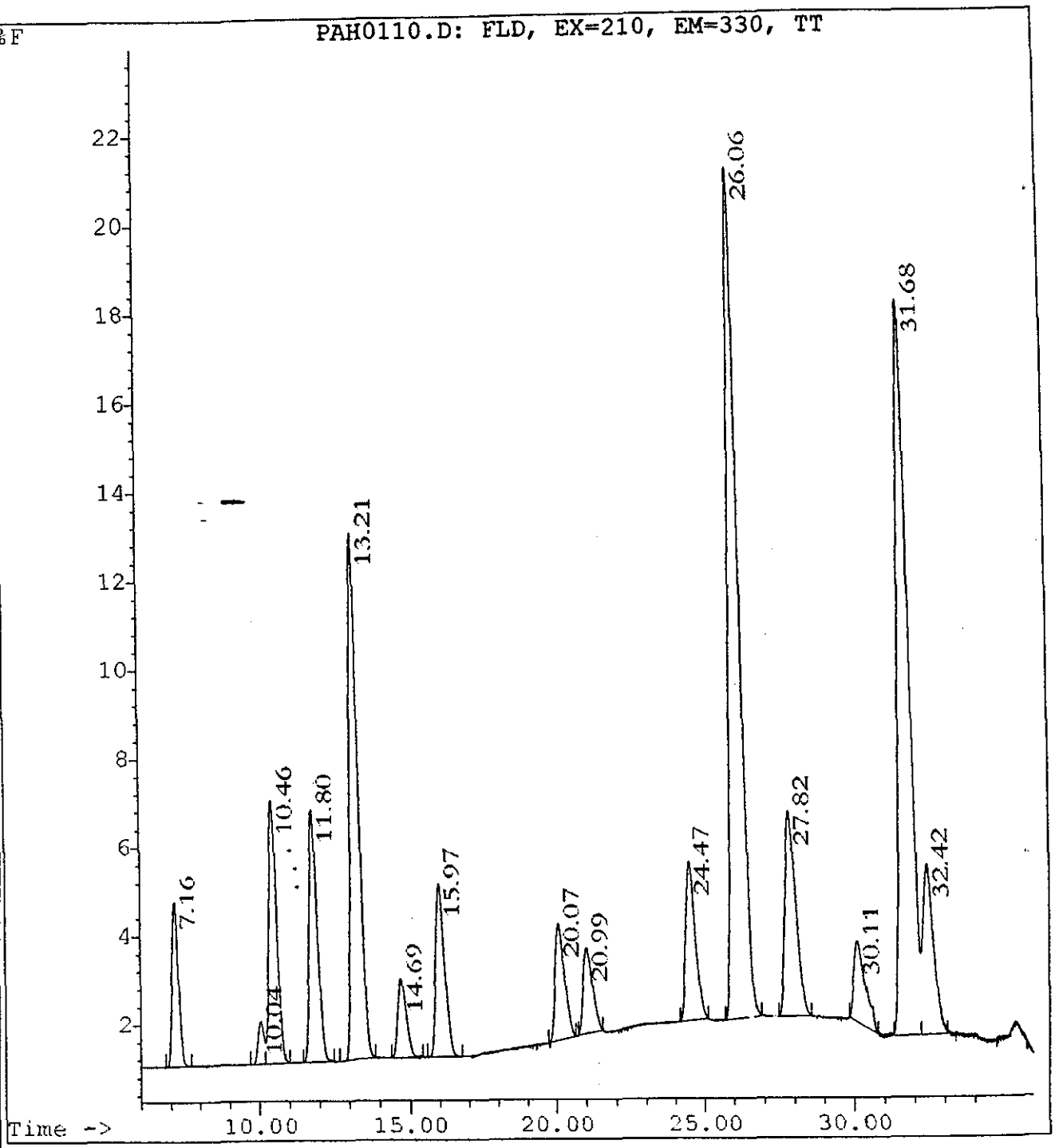
Sample Name: pah 500
Misc Info:
Bottle Number: 1



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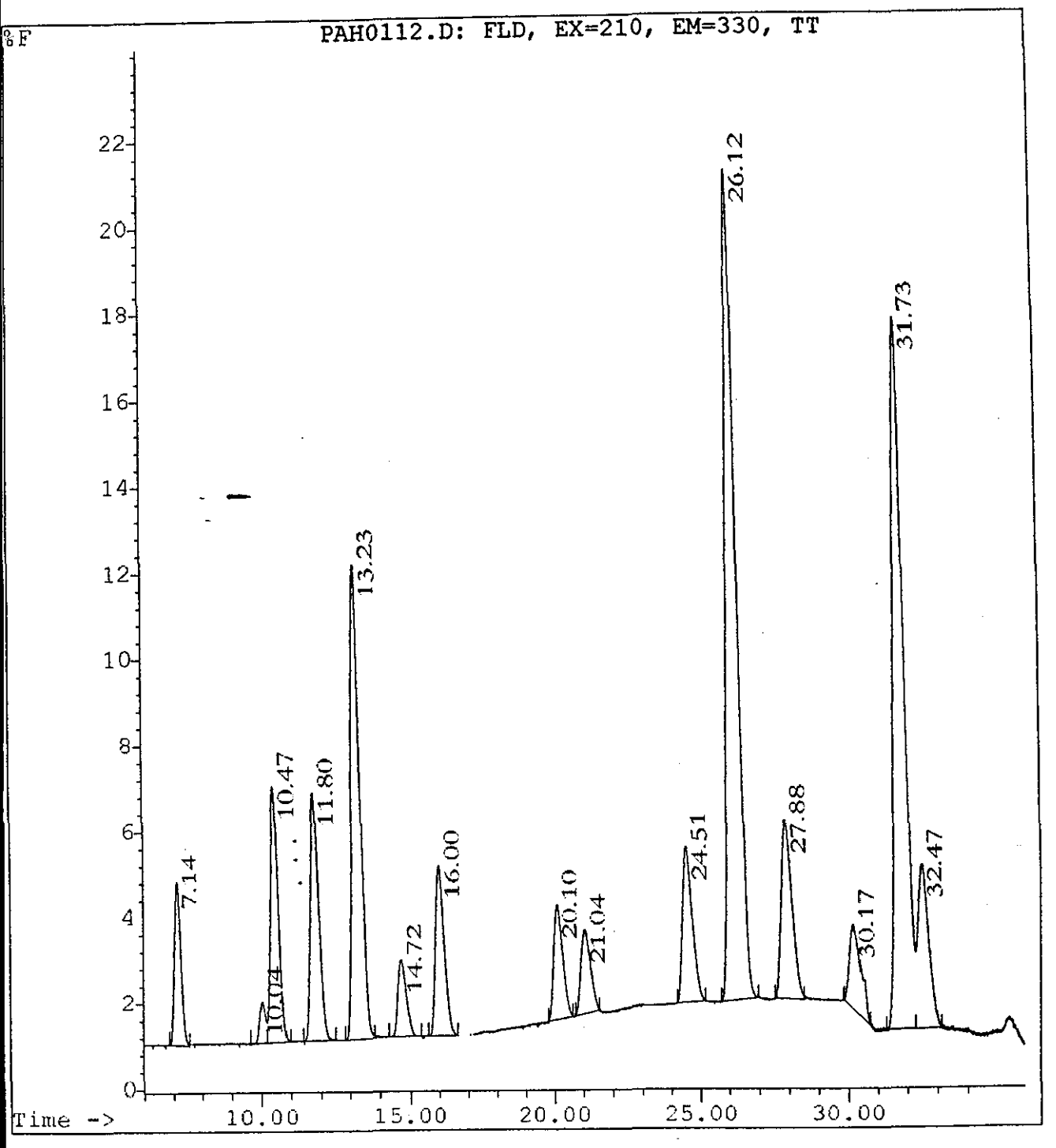
Sample Name: pah 500
Misc Info:
Bottle Number: 1



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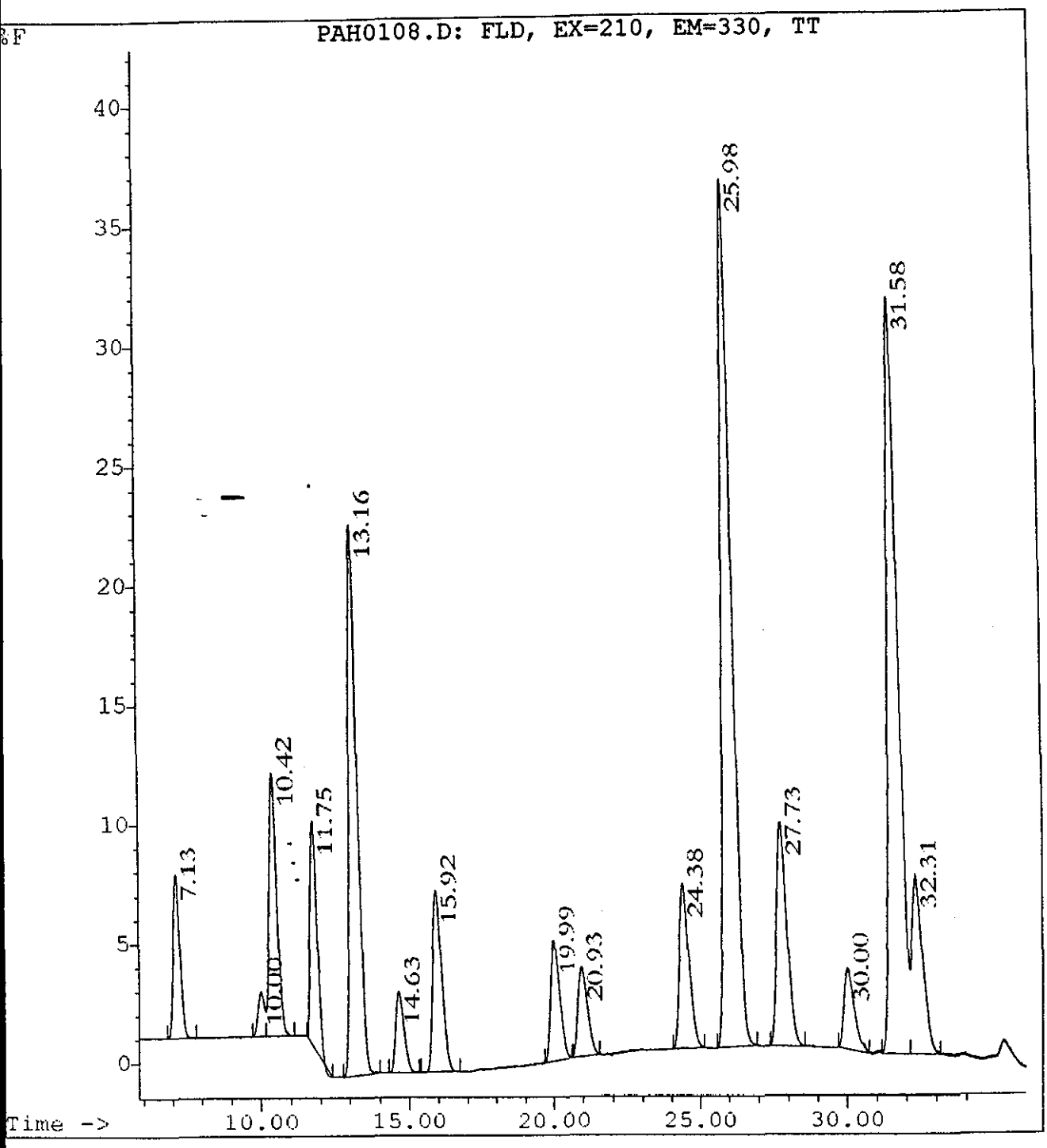
Sample Name: pah 100
Misc Info:
Bottle Number: 1



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Sample Name: pah 100
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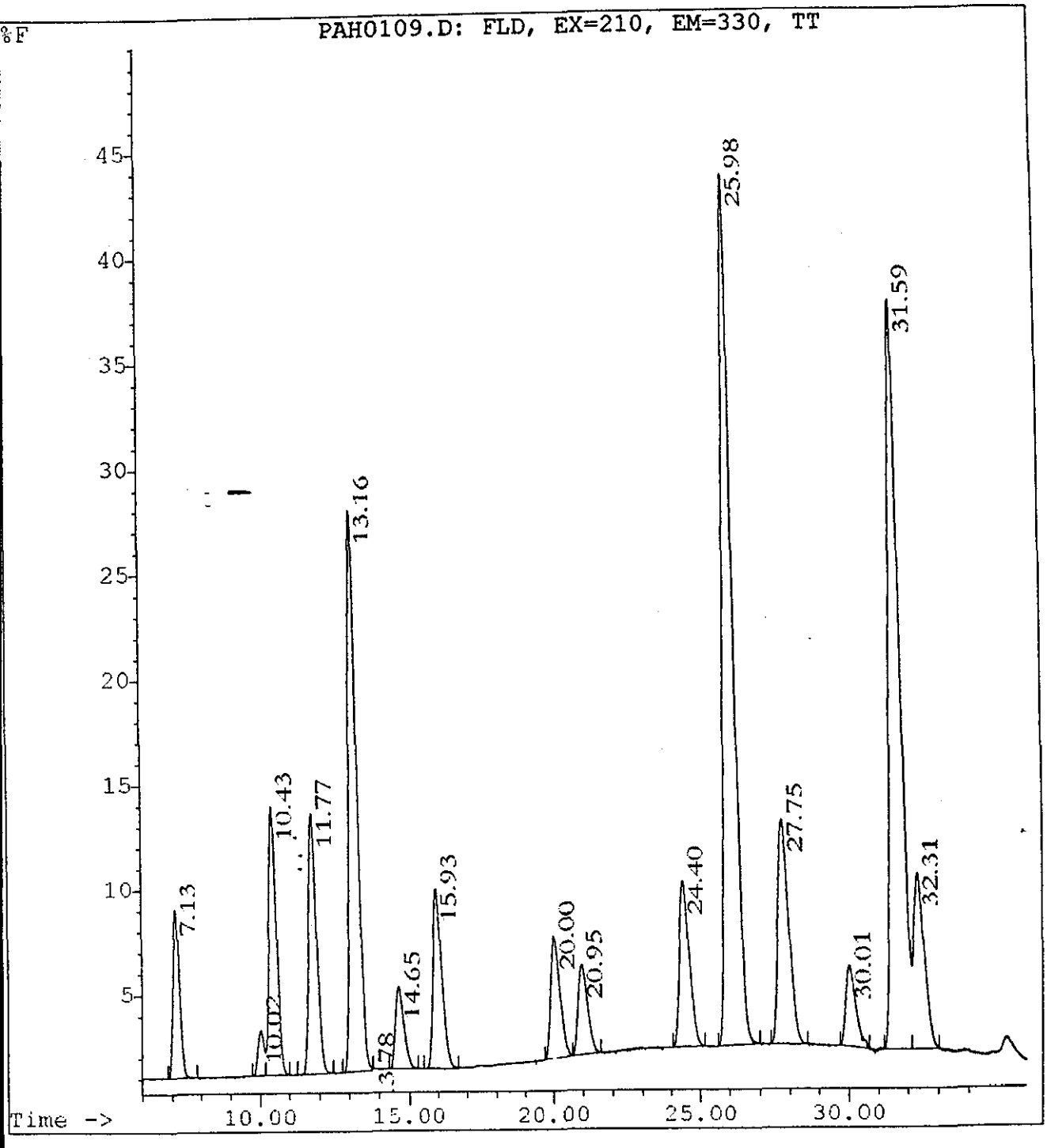


MICROBIAL INOCULA SOURCE

LFN

		filter	105	550	ts	std dev	tvs	std dev	tfs	std dev	tfs/ts	tvs/ts		cruc wt	w oil	oil	std dev	oil/ts	oil/tfs	oil g/l
	1	0.2011	0.2195	0.204	0.0184	0.0008	0.0155	0.0007	0.0029	0.0002	0.16	0.84		41.7079	41.7336	0.0257	0.0028	0.35	2.22	
day 0	2	0.1998	0.2175	0.2029	0.0177		0.0146		0.0031		0.18	0.82		63.1603	63.1833	0.0230		0.32	1.85	
	3	0.1982	0.2151	0.2009	0.0169		0.0142		0.0027		0.16	0.84		54.0504	54.0706	0.0202		0.30	1.87	
	avg				0.0177		0.0148		0.0029							0.0230				1.01
	g/l				0.8833		0.7383													
		cruc wt	105	550	ts		tvs		tfs		tfs/ts	tvs/ts		cruc wt	w oil	oil		oil/ts	oil/tfs	oil g/l
day1	1	0.1994	0.2205	0.2019	0.0211	0.0032	0.0186	0.0027	0.0025	0.0005	0.12	0.88		16.3361	16.355	0.0189	0.0014	0.22	1.89	
	2	0.2007	0.227	0.2038	0.0263		0.0232		0.0031		0.12	0.88		21.0526	21.0703	0.0177		0.17	1.43	
	3	0.1978	0.2183	0.2	0.0205		0.0183		0.0022		0.11	0.89		19.9374	19.9578	0.0204		0.25	2.32	
					0.0226		0.0200		0.0026							0.0190				1.02
	g/l				1.1317		1.0017													
		cruc wt	105	550	ts		tvs		tfs		tfs/ts	tvs/ts		cruc wt	w oil	oil		oil/ts	oil/tfs	
day 2	1	0.2001	0.221	0.2021	0.0209	0.0016	0.0189	0.0011	0.0020	0.0006	0.10	0.90								
	2	0.1999	0.2229	0.203	0.0230		0.0199		0.0031		0.13	0.87								
	3	0.2022	0.2262	0.2051	0.0240		0.0211		0.0029		0.12	0.88								
					0.0226		0.0200		0.0027											
	g/l				1.1317		0.9983													
		cruc wt	105	550	ts		tvs		tfs		tfs/ts	tvs/ts		cruc wt	w oil	oil		oil/ts	oil/tfs	
day 3	1	0.1993	0.2164	0.2013	0.0171	0.0006	0.0151	0.0003	0.0020	0.0005	0.12	0.88		41.7073	41.7266	0.0193	0.0021	0.28	2.41	
	2	0.2046	0.2225	0.2075	0.0179		0.0150		0.0029		0.16	0.84		63.1599	63.1817	0.0218		0.30	1.88	
	3	0.2005	0.2172	0.2026	0.0167		0.0146		0.0021		0.13	0.87		54.0505	54.0739	0.0234		0.35	2.79	
					0.0172		0.0149		0.0023							0.0215				1.17
	g/l				0.8617		0.7450													
		cruc wt	105	550	ts		tvs		tfs		tfs/ts	tvs/ts		cruc wt	w oil	oil		oil/ts	oil/tfs	
day 4	1	0.204	0.224	0.207	0.0200	0.0003	0.0170	0.0007	0.0030	0.0004	0.15	0.85								
	2	0.2106	0.2307	0.2129	0.0201		0.0178		0.0023		0.11	0.89								
	3	0.1973	0.2178	0.1995	0.0205		0.0183		0.0022		0.11	0.89								
					0.0202		0.0177		0.0025											
	g/l				1.0100		0.8850													
		cruc wt	105	550	ts		tvs		tfs		tfs/ts	tvs/ts		cruc wt	w oil	oil		oil/ts	oil/tfs	
day 5	1	0.1997	0.2179	0.2024	0.0182	0.0003	0.0155	0.0003	0.0027	0.0001	0.15	0.85		22.663	22.679	0.0160	0.0021	0.22	1.48	
	2	0.2015	0.2203	0.2043	0.0188		0.0160		0.0028		0.15	0.85		19.9386	19.9588	0.0202		0.27	1.80	
	3	0.2006	0.2192	0.2033	0.0186		0.0159		0.0027		0.15	0.85		17.7761	17.7947	0.0186		0.25	1.72	
					0.0185		0.0158		0.0027							0.0183				0.93
	g/l				0.9267		0.7900													
					avg std dev	0.0011	avg std dev	0.0009	avg std dev	0.0004						avg std dev	0.0020			
MPN at start		1.00E+10																		
Turbidity		69.5 NTU																		

MICROBIAL INOCULA SOURCE

ASN

		filter	105	550	ts	std dev	tvs	std dev	tfs	std dev	tfs/ts	tvs/ts		cruc wt	w oil	oil	std dev	oil/ts	oil/tfs	oil g/l
	1	0.1993	0.2205	0.2029	0.0212	0.0011	0.0176	0.0009	0.0036	0.0008	0.17	0.83		42.9437	42.954	0.0103	0.0130	0.12	0.72	
day 0	2	0.2023	0.2243	0.2051	0.022		0.0192		0.0028		0.13	0.87		41.9768	42.0116	0.0348		0.40	3.11	
	3	0.2002	0.22	0.2023	0.0198		0.0177		0.0021		0.11	0.89		45.9572	45.9872	0.03		0.38	3.67	
	avg				0.021		0.018167									0.025033				1.5
	g/l				1.0500		0.9083													
		cruc wt	105	550	ts		tvs		tfs		tfs/ts	tvs/ts		cruc wt	w oil	oil		oil/ts	oil/tfs	oil g/l
day1	1(20 ml)	0.2013	0.2297	0.2046	0.0284	0.0083	0.0251	0.0069	0.0033	0.0015	0.12	0.88								
	2 (10 ml)	0.1983	0.2121	0.1994	0.0138		0.0127		0.0011		0.08	0.92								
	3. (10 ml)	0.2021	0.2162	0.2026	0.0141		0.0136		0.0005		0.04	0.96								
in 10 ml					0.014		0.0129													
g/l					1.4000		1.2900													
		cruc wt	105	550	ts		tvs		tfs		tfs/ts	tvs/ts		cruc wt	w oil	oil		oil/ts	oil/tfs	
day 2	1(20 ml)	0.2003	0.2285	0.2036	0.0282	0.0076	0.0249	0.0067	0.0033	0.0012	0.12	0.88								
	2(20 ml)	0.1998	0.2265	0.2043	0.0267		0.0222		0.0045		0.17	0.83								
	3 (10 ml)	0.1988	0.2132	0.201	0.0144		0.0122		0.0022		0.15	0.85								
in 10 ml					0.0141		0.01194													
g/l					1.4100		1.1940													
		cruc wt	105	550	ts		tvs		tfs		tfs/ts	tvs/ts		cruc wt	w oil	oil		oil/ts	oil/tfs	
day 3	1(20 ml)	0.1998	0.2257	0.2044	0.0259	0.0081	0.0213	0.0061	0.0046	0.0020	0.18	0.82		42.9436	42.9656	0.022	0.0003	0.20	1.67	
	2 (10 ml)	0.203	0.2148	0.2037	0.0118		0.0111		0.0007		0.06	0.94		41.9766	41.9981	0.0215		0.20	1.19	
	3. (10 ml)	0.206	0.2181	0.2076	0.0121		0.0105		0.0016		0.13	0.87		45.9571	45.9786	0.0215		0.37	2.44	
in 10 ml					0.0123		0.0108									0.021667				1.075
g/l					1.2300		1.0800													
		cruc wt	105	550	ts		tvs		tfs		tfs/ts	tvs/ts		cruc wt	w oil	oil		oil/ts	oil/tfs	
day 4	1(20 ml)	0.199	0.2194	0.202	0.0204	0.0062	0.0174	0.0052	0.003	0.0010	0.15	0.85		17.8321	17.8496	0.0175	0.0006	0.21	1.46	
	2 (10 ml)	0.2013	0.213	0.203	0.0117		0.01		0.0017		0.15	0.85		18.194	18.2113	0.0173		0.37	2.54	
	3. (10 ml)	0.2022	0.2105	0.2032	0.0083		0.0073		0.001		0.12	0.88		21.2074	21.2259	0.0185		0.58	4.62	
in 10 ml					0.010067		0.008667				0.137613	0.862387				0.017767				0.925
g/l					1.0067		0.8667													
		cruc wt	105	550	ts		tvs		tfs		tfs/ts	tvs/ts		cruc wt	w oil	oil		oil/ts	oil/tfs	
day 5	1(20 ml)	0.199	0.221	0.2033	0.022	0.0056	0.0177	0.0037	0.0043	0.0019	0.20	0.80								
	2 (10 ml)	0.2007	0.2135	0.2017	0.0128		0.0118		0.001		0.08	0.92								
	3. (10 ml)	0.2061	0.2179	0.207	0.0118		0.0109		0.0009		0.08	0.92								
in 10 ml					0.011867		0.010517				0.116617	0.883383								
g/l					1.1867		1.0517													
					avg std dev	0.0062	avg std dev	0.0049	avg std dev	0.0014						avg std dev	0.0046			
MPN on day 0		1.00E+11																		
turbidity		67 NTU																		

MICROBIAL INOCULA SOURCE

OSN

		filter	105	550	ts	std dev	tvs	std dev	tfs	std dev	tfs/ts	tvs/ts		cruc wt	w oil	oil	std dev	oil/ts	oil/tfs	oil g/l
	1	0.2017	0.2166	0.204	0.0149	0.0004	0.0126	0.0004	0.0023	0.0003	0.15	0.85		43.9433	43.9727	0.0294		0.49	3.20	
day 0	2	0.2025	0.2167	0.2048	0.0142		0.0119		0.0023		0.16	0.84		46.0085	46.0433	0.0348		0.61	3.78	
	3	0.2008	0.2155	0.2037	0.0147		0.0118		0.0029		0.20	0.80								
	avg				0.0146		0.0121									0.0321				1.605
	g/l				0.7300		0.6050													
		cruc wt	105	550	ts		tvs		tfs		tfs/ts	tvs/ts		cruc wt	w oil	oil		oil/ts	oil/tfs	oil g/l
day1		0.2001	0.2193	0.2018	0.0192	0.0024	0.0175	0.0019	0.0017	0.0005	0.09	0.91								
		0.2065	0.2294	0.2089	0.0229		0.0205		0.0024		0.10	0.90								
		0.208	0.2316	0.2106	0.0236		0.021		0.0026		0.11	0.89								
					0.0219		0.019667													
g/l					1.0950		0.9833													
		cruc wt	105	550	ts		tvs		tfs		tfs/ts	tvs/ts		cruc wt	w oil	oil		oil/ts	oil/tfs	
day 2		0.1985	0.2194	0.201	0.0209	0.0048	0.0184	0.0049	0.0025	0.0003	0.12	0.88		45.7808	45.8085	0.0277	0.0031	0.32	1.82	
		0.2006	0.2127	0.2032	0.0121		0.0095		0.0026		0.21	0.79		43.9447	43.9745	0.0298		0.36	2.98	
		0.2012	0.2208	0.2032	0.0196		0.0176		0.002		0.10	0.90		46.0088	46.0325	0.0237		0.27	1.97	
					0.01753		0.015167									0.0271				1.3533
g/l					0.8767		0.7583													
		cruc wt	105	550	ts		tvs		tfs		tfs/ts	tvs/ts		cruc wt	w oil	oil		oil/ts	oil/tfs	
day 3		0.2019	0.2237	0.2057	0.0218	0.0007	0.018	0.0004	0.0038	0.0007	0.17	0.83								
		0.2055	0.226	0.208	0.0205		0.018		0.0025		0.12	0.88								
		0.1999	0.2216	0.2029	0.0217		0.0187		0.003		0.14	0.86								
					0.02133		0.018233													
g/l					1.0667		0.9117													
		cruc wt	105	550	ts		tvs		tfs		tfs/ts	tvs/ts		cruc wt	w oil	oil		oil/ts	oil/tfs	
day 4		0.2036	0.2135	0.2048	0.0099		0.0087		0.0012		0.12	0.88		18.8065	18.8263	0.0198	0.0064	0.50	4.12	
		0.1996	0.2085	0.2	0.0089		0.0085		0.0004		0.04	0.96		21.0541	21.0749	0.0208		0.58	13.00	
														19.4486	19.4578	0.0092				
in 10 ml					0.0094		0.0086													1.015
g/l					0.9400		0.8600													
					avg std dev	0.002	avg std dev	0.0019	avg std dev	0.0005										
MPN on day 0		1.00E+11																		
turbidity		90 NTU																		

EXPERIMENT E
PRIMED VS UNPRIMED CULTURES

DAYS	0	1	2	3	4	5
TS Cul A	0.2235	0.2184	0.2333	0.2194	0.2218	0.2216
	0.2171	0.2175	0.2336	0.2235	0.2169	0.2256
	0.2197	0.2103	0.2308	0.23	0.2178	0.2138
avg	0.2201	0.2154	0.2326	0.2243	0.2188	0.2203
std dev	0.0032	0.0044	0.0015	0.0053	0.0026	0.0060
VSS Cul A	0.0214	0.0163	0.0312	0.0173	0.0197	0.0195
	0.015	0.0154	0.0315	0.0214	0.0148	0.0235
	0.0176	0.0082	0.0287	0.0279	0.0157	0.0117
avg	0.0180	0.0133	0.0305	0.0222	0.0167	0.0182
std dev	0.0032	0.0044	0.0015	0.0053	0.0026	0.0060
TS Cul B	0.2226	0.2226	0.2365	0.2375	0.237	0.2372
	0.2248	0.2295	0.2344	0.2363	0.2266	0.2339
	0.2151	0.2248	0.2382	0.2415	0.2307	0.2332
avg	0.2208	0.2256	0.2364	0.2384	0.2314	0.2348
std dev	0.0051	0.0035	0.0019	0.0027	0.0052	0.0021
VSS Cul B	0.0205	0.0205	0.0344	0.0354	0.0349	0.0351
	0.0227	0.0274	0.0323	0.0342	0.0245	0.0318
	0.013	0.0227	0.0361	0.0394	0.0286	0.0311
avg	0.0187	0.0235	0.0343	0.0363	0.0293	0.0327
std dev	0.0051	0.0035	0.0019	0.0027	0.0052	0.0021

MINERAL SALT REQUIREMENT

	1X					2X			
	HRS	filter 105	filter 450	tvs			filter 105	filter 450	tvs
		0.212	0.2042	0.0078		0	0.2163	0.2079	0.0084
		0.2074	0.2	0.0074			0.216	0.2075	0.0085
		0.2069	0.2012	0.0057			0.209	0.2032	0.0058
				0.0070					0.0076
day1	25	0.2158	0.2063	0.0095		1	0.2129	0.2044	0.0085
		0.213	0.2034	0.0096			0.2145	0.2042	0.0103
		0.2095	0.201	0.0085			0.2145	0.2071	0.0074
				0.0092					0.0087
	30	0.2189	0.208	0.0109		2	0.2181	0.2028	0.0153
		0.213	0.2019	0.0111			0.2212	0.2033	0.0179
		0.2087	0.1987	0.01			0.2181	0.2049	0.0132
				0.0107					0.0155
	36	0.2223	0.2032	0.0191		3	0.2224	0.2075	0.0149
		0.2147	0.2017	0.013			0.2189	0.2041	0.0148
		0.2163	0.2027	0.0136			0.2216	0.208	0.0136
				0.0152					0.0144
day2	45	0.2205	0.2053	0.0152		4	0.2267	0.2091	0.0176
		0.2162	0.2021	0.0141			0.2231	0.2079	0.0152
		0.2176	0.2027	0.0149			0.2208	0.2098	0.011
				0.0147					0.0146
	52	0.2164	0.2027	0.0137		5	0.2248	0.2097	0.0151
		0.2168	0.1993	0.0175			0.2248	0.2084	0.0164
		0.2134	0.1992	0.0142			0.2175	0.2024	0.0151
				0.0151					0.0155
	59	0.2181	0.2045	0.0136					
		0.2144	0.2002	0.0142					
		0.2159	0.2021	0.0138					
				0.0139					
day 3	70	0.2186	0.2018	0.0168					
		0.217	0.1988	0.0182					
		0.2204	0.2036	0.0168					
				0.0173					
	83	0.2335	0.2142	0.0193					
		0.2232	0.2067	0.0165					
		0.2234	0.2095	0.0139					
				0.0166					
day 4	94	0.2206	0.2046	0.016					
		0.2214	0.2046	0.0168					
		0.2346	0.2167	0.0179					
				0.0169					
day 5	121	0.2314	0.21	0.0214					
		0.2347	0.2113	0.0234					
		0.2352	0.2063	0.0289					
				0.0246					

SLUDGE LOADING RATE

5 % load

pH 7															
day 0	wt cruc	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil	O&G/ts	O&G/tfs	MPN
1	42.7448	42.8122	42.7838	0.0674	0.0284	0.0390	0.58	0.42	1.0000	68.4101	68.6694	0.2593	0.9618	1.6622	1.00E+10
2	44.6416	44.7505	44.7070	0.1089	0.0435	0.0654	0.60	0.40	2.0000	64.1175	64.4623	0.3448	0.7816	1.3180	pH7
3	47.3236	47.4192	47.3774	0.0956	0.0418	0.0538	0.56	0.44	3.0000	57.9783	58.3763	0.3980	1.0408	1.8494	
avg				0.0906	0.0379	0.0527	0.58	0.42				0.3340	0.9214	1.5836	
day 3															
pH 5	wt cruc	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil	O&G/ts	O&G/tfs	MPN
1	42.9428	43.0221	43.0004	0.0793	0.0217	0.0576	0.73	0.27	1.0000	56.1629	58.5735	0.4106	1.2945	1.7821	1.00E+10
2	45.9566	46.0313	46.0106	0.0747	0.0207	0.0540	0.72	0.28	2.0000	65.5783	65.9321	0.3538	1.1841	1.6380	pH5
3	43.9422	44.0200	43.9974	0.0778	0.0228	0.0552	0.71	0.29	3.0000	61.6813	62.0460	0.3647	1.1719	1.6517	
avg				0.0773	0.0217	0.0556	0.72	0.28				0.3764	1.2178	1.6923	
day 6															
pH 4	wt cruc	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil	O&G/ts	O&G/tfs	MPN
1	42.7449	42.8560	42.8102	0.1111	0.0458	0.0653	0.59	0.41	1.0000	68.4101	68.7384	0.3283	0.7387	1.2569	1.00E+10
2	43.1742	43.3012	43.2486	0.1270	0.0526	0.0744	0.59	0.41	2.0000	64.1175	64.4100	0.2925	0.5758	0.9829	pH4
3	47.3236	47.4944	47.4439	0.1708	0.0505	0.1203	0.70	0.30	3.0000	57.9783	58.3362	0.3579	0.5239	0.7438	
avg				0.1363	0.0496	0.0867	0.63	0.37				0.3262	0.5984	0.9411	
day 9															
pH 5	wt cruc	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil	O&G/ts	O&G/tfs	MPN
1	58.1612	58.2657	58.2303	0.1045	0.0354	0.0691	0.66	0.34	1.0000	66.4078	66.7195	0.3117	0.7457	1.1277	1.00E+11
2	65.5772	65.7513	65.6924	0.1741	0.0589	0.1152	0.66	0.34	2.0000	64.1169	64.3902	0.2733	0.3924	0.5931	pH5
3	61.6798	61.8225	61.7520	0.1427	0.0705	0.0722	0.51	0.49	3.0000	57.9793	58.3056	0.3263	0.5717	1.1298	
avg				0.1404	0.0549	0.0855	0.61	0.39				0.3038	0.5699	0.9502	
day 12															
pH 6	wt cruc	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil	O&G/ts	O&G/tfs	MPN
1	42.9443	43.1276	43.0749	0.1833	0.0527	0.1306	0.71	0.29	1.0000	58.1584	58.4765	0.3181	0.4339	0.6089	1.00E+11
2	45.9580	46.1222	46.0592	0.1642	0.0630	0.1012	0.62	0.38	2.0000	65.5739	65.8720	0.2981	0.4539	0.7364	pH6
3	43.9435	44.1397	44.0543	0.1962	0.0854	0.1108	0.56	0.44	3.0000	61.6795	61.9437	0.2642	0.3366	0.5961	
				0.1812	0.0670	0.1142	0.63	0.37				0.2935	0.4081	0.6472	
day 15															
pH 6	wt cruc	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil	O&G/ts	O&G/tfs	MPN
1	42.7449	42.8854	42.8365	0.1405	0.0489	0.0916	0.65	0.35	1.0000	68.4078	68.6579	0.2501	0.4450	0.6826	1.00E+11
2	43.1742	43.3260	43.2653	0.1518	0.0407	0.1111	0.73	0.27	2.0000	64.1169	64.3783	0.2614	0.4305	0.5882	pH6
3	47.3236	47.4938	47.4439	0.1702	0.0499	0.1203	0.71	0.29	3.0000	57.9793	58.2175	0.2382	0.3499	0.4950	
avg				0.1542	0.0465	0.1077	0.70	0.30				0.2499	0.4085	0.5886	
day 18															
pH 6	wt cruc	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil	O&G/ts	O&G/tfs	MPN
1	52.3121	52.6542	52.5602	0.3421	0.0940	0.2481	0.73	0.27		58.1581	58.4254	0.2673	0.1953	0.2693	1.00E+11
2	61.4003	61.6940	61.6150	0.2937	0.0790	0.2147	0.73	0.27		65.5745	65.8495	0.2750	0.2341	0.3202	pH6
3	54.7380	55.0657	54.9261	0.3277	0.1396	0.1881	0.57	0.43		61.6789	61.8595	0.1806	0.1378	0.2400	
avg				0.3212	0.1042	0.2170	0.68	0.32				0.2410	0.1876	0.2777	
day 21															
pH 6	wt cruc	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil	O&G/ts	O&G/tfs	MPN
1	42.9430	43.1405	43.0681	0.1975	0.0714	0.1261	0.64	0.36		68.4076	68.6554	0.2478	0.3137	0.4913	1.00E+11
2	45.9570	46.1705	46.0902	0.2135	0.0803	0.1332	0.62	0.38		64.1165	64.3780	0.2615	0.3062	0.4908	pH6
3	43.9429	44.2876	44.1461	0.3447	0.1415	0.2032	0.59	0.41		57.9781	58.2855	0.3074	0.2229	0.3782	
				0.2519	0.0977	0.1542	0.62	0.38				0.2722	0.2809	0.4534	

SLUDGE LOADING RATE

5% load

ts/day	0	3	6	9	12	15	18	21
1	0.0674	0.0793	0.1111	0.1045	0.1833	0.1405	0.3421	0.1975
2	0.1099	0.0747	0.1270	0.1741	0.1642	0.1518	0.2937	0.2135
3	0.0956	0.0778	0.1708	0.1427	0.1962	0.1702	0.3277	0.3447
avg ts	0.0910	0.0773	0.1363	0.1404	0.1812	0.1542	0.3212	0.2519
std dev	0.0216	0.0023	0.0309	0.0349	0.0161	0.0150	0.0249	0.0808
ts/l	18.1933	15.4533	27.2600	28.0867	36.2467	30.8333	64.2333	50.3800
tvs								
1	0.0284	0.0217	0.0458	0.0354	0.0527	0.0489	0.0940	0.0714
2	0.0435	0.0207	0.0526	0.0589	0.0630	0.0407	0.0790	0.0803
3	0.0418	0.0226	0.0505	0.0705	0.0854	0.0499	0.1396	0.1415
avg tvs	0.0379	0.0217	0.0496	0.0549	0.0670	0.0465	0.1042	0.0977
std dev	0.0083	0.0010	0.0035	0.0179	0.0167	0.0050	0.0316	0.0382
MPN	1.00E+10	1.00E+10	1.00E+10	1.00E+11	1.00E+11	1.00E+11	1.00E+11	1.00E+11
tfs								
1	0.039	0.0576	0.0653	0.0691	0.1306	0.0916	0.2481	0.1261
2	0.0664	0.054	0.0744	0.1152	0.1012	0.1111	0.2147	0.1332
3	0.0538	0.0552	0.1203	0.0722	0.1108	0.1203	0.1881	0.2032
avg tfs	0.0531	0.0556	0.0867	0.0855	0.1142	0.1077	0.2170	0.1542
std dev	0.0137	0.0018	0.0295	0.0258	0.0150	0.0147	0.0301	0.0426
tfs/l	10.6133	11.1200	17.3333	17.1000	22.8400	21.5333	43.3933	30.8333
fs/ts								
1	0.58	0.73	0.59	0.66	0.71	0.65	0.73	0.64
2	0.6	0.72	0.59	0.66	0.62	0.73	0.73	0.62
3	0.56	0.71	0.7	0.51	0.56	0.71	0.57	0.59
avg fs/ts	0.58	0.72	0.63	0.61	0.63	0.7	0.68	0.62
vs/ts								
1	0.42	0.27	0.41	0.34	0.29	0.35	0.27	0.36
2	0.4	0.28	0.41	0.34	0.38	0.27	0.27	0.38
3	0.44	0.29	0.3	0.49	0.44	0.29	0.43	0.41
avg vs/ts	0.42	0.28	0.37	0.39	0.37	0.3	0.32	0.38
O&G								
1	0.2593	0.4106	0.3283	0.3117	0.3181	0.2501	0.2673	0.2478
2	0.3448	0.3538	0.2925	0.2733	0.2981	0.2614	0.275	0.2615
3	0.398	0.3647	0.3579	0.3263	0.2642	0.2382	0.1806	0.3074
avg O&G	0.334	0.3764	0.3262	0.3038	0.2935	0.2499	0.241	0.272
std dev	0.0700	0.0301	0.0327	0.0274	0.0272	0.0116	0.0524	0.0312
O&G/l	16.7	18.82	16.31	15.19	14.675	12.495	12.05	13.6
O&G/TS								
1	0.96	1.29	0.74	0.75	0.43	0.45	0.2	0.31
2	0.78	1.18	0.58	0.39	0.45	0.43	0.23	0.31
3	1.04	1.17	0.52	0.57	0.34	0.35	0.14	0.22
avg o&g/ts	0.92	1.22	0.6	0.57	0.41	0.41	0.19	0.28
O&G/TFS								
1	1.66	1.78	1.26	1.13	0.61	0.68	0.27	0.49
2	1.3	1.64	0.98	0.59	0.74	0.59	0.32	0.49
3	1.85	1.65	0.74	1.13	0.6	0.49	0.24	0.38
avg o&g/fs	1.57	1.7	0.94	0.95	0.65	0.59	0.28	0.45

SLUDGE LOADING RATE

10% load

day 0	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts	O&G	cruc wt	w oil	oil	oil/ts	oil/tfs	MPN
1	42.0312	42.1677	42.1093	0.1365	0.0584	0.0781	0.57	0.43	1	62.7988	63.5576	0.7578	5.5516	9.7029	1.00E+10
2	43.1742	43.4123	43.2815	0.2381	0.1308	0.1073	0.45	0.55	2	70.4502	71.0788	0.6286	2.6401	5.8583	
3	41.7084	41.8169	41.7843	0.1085	0.0326	0.0759	0.70	0.30	3	62.6012	63.4178	0.8166	7.5263	10.7589	
avg				0.1610	0.0739	0.0871	0.57	0.43				0.7433	5.2393	8.7733	
day 3															
ph 5	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil	oil/ts	oil/tfs	MPN
1	54.0506	54.2884	54.2401	0.2378	0.0483	0.1895	0.80	0.20	1	62.6993	63.5258	0.8265	3.4756	4.3615	1.00E+11
2	41.9757	42.1847	42.1365	0.2090	0.0482	0.1608	0.77	0.23	2	69.7185	70.4682	0.7497	3.5871	4.6623	
3	46.0071	46.1764	46.1264	0.1693	0.0500	0.1193	0.70	0.30	3	67.8759	68.4630	0.7871	4.6491	6.5977	
avg				0.2054	0.0488	0.1565	0.76	0.24				0.7878	3.9039	5.2071	
day 6															
pH 4	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil	oil/ts	oil/tfs	MPN
1	42.0312	42.3126	42.1655	0.2814	0.1471	0.1343	0.48	0.52	1	62.8003	63.3427	0.5424	1.9275	4.0387	1.00E+10
2	44.6416	44.8811	44.8133	0.2395	0.0678	0.1717	0.72	0.28	2	70.4483	71.2025	0.7542	3.1491	4.3925	
3	41.7084	41.9950	41.8631	0.2866	0.1319	0.1547	0.54	0.46	3	59.9470	60.6355	0.6885	2.4023	4.4505	
avg				0.2692	0.1156	0.1536	0.58	0.42				0.6617	2.4930	4.2939	
day 9															
pH 4	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil	oil/ts	oil/tfs	MPN
1	62.7002	62.9748	62.8438	0.2746	0.1310	0.1436	0.52	0.48	1	62.8003	63.3719	0.5716	2.0816	3.9805	1.00E+11
2	69.7188	70.0181	69.8720	0.2993	0.1461	0.1522	0.51	0.49	2	70.4483	71.0050	0.5567	1.8602	3.6577	
3	67.6792	68.0848	67.8975	0.4056	0.1873	0.2183	0.54	0.46	3	59.9470	60.4918	0.5448	1.3432	2.4956	
avg				0.3262	0.1548	0.1714	0.52	0.48				0.5577	1.7637	3.3779	
day 12															
pH 6	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil	oil/ts	oil/tfs	MPN
1	54.0518	54.4492	54.2649	0.3974	0.1843	0.2131	0.54	0.46	1	62.6980	63.1481	0.4501	1.1326	2.1122	1.00E+11
2	41.9765	42.3472	42.1420	0.3707	0.2052	0.1655	0.45	0.55	2	69.7172	70.1552	0.4380	1.1815	2.6465	
3	46.0071	46.3786	46.2135	0.3715	0.1651	0.2064	0.56	0.44	3	67.6741	68.2409	0.5668	1.5257	2.7461	
avg				0.3799	0.1849	0.1950	0.51	0.49				0.4850	1.2800	2.5016	
day 15															
pH 6	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil	oil/ts	oil/tfs	MPN
1	42.0312	42.5276	42.3154	0.4964	0.2122	0.2842	0.57	0.43	1	62.8003	63.2031	0.4028	0.8114	1.4173	1.00E+11
2	44.6416	45.0934	44.9278	0.4518	0.1656	0.2862	0.63	0.37	2	70.4483	70.9020	0.4537	1.0042	1.5853	
3	41.7084	42.4030	42.2226	0.6946	0.1804	0.5142	0.74	0.26	3	59.9470	60.4209	0.4739	0.6823	0.9218	
avg				0.5478	0.1861	0.3615	0.65	0.35				0.4435	0.8326	1.3081	
day 18															
pH 6	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil	oil/ts	oil/tfs	MPN
1	44.9727	45.3415	45.1654	0.3688	0.1761	0.1927	0.52	0.48	1	62.6983	63.1213	0.4230	1.1470	2.1951	1.00E+11
2	41.1753	41.7171	41.4953	0.5418	0.2218	0.3200	0.59	0.41	2	69.7171	70.1218	0.4047	0.7470	1.2647	
3	57.2361	57.7048	57.4691	0.4687	0.2157	0.2530	0.54	0.46	3	67.6715	68.0459	0.3744	0.7988	1.4798	
				0.4598	0.2045	0.2552	0.55	0.45				0.4007	0.8976	1.6466	
day 21															
pH6	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil	oil/ts	oil/tfs	MPN
1	54.0507	54.4972	54.3572	0.4465	0.1400	0.3065	0.69	0.31	1	62.8003	63.1296	0.3293	0.7375	1.0744	1.00E+09
2*	41.9762	43.0039	42.8052	1.0277	0.1987	0.8290	0.81	0.19	2	70.4585	71.2768	0.8183	0.7962	0.9871	
3	46.0079	46.3148	46.2070	0.3069	0.1078	0.1991	0.65	0.35	3	59.9458	60.2760	0.3302	1.0759	1.6585	
				0.5937	0.1488	0.4449	0.71	0.29				0.4926	0.8699	1.2400	

SLUDGE LOADING RATE

10% load

ts	0	3	6	9	12	15	18	21
1	0.1365	0.2378	0.2814	0.2746	0.3974	0.4964	0.3688	0.4465
2	0.2381	0.2090	0.2395	0.2983	0.3707	0.4518	0.5418	0.5139
3	0.1085	0.1693	0.2866	0.4056	0.3715	0.6946	0.4687	0.3069
avg ts	0.1610	0.2054	0.2692	0.3262	0.3799	0.5476	0.4598	0.4224
std dev	0.0682	0.0344	0.0258	0.0698	0.0152	0.1292	0.0868	0.1056
ts/l	32.2067	41.0733	53.8333	65.2333	75.9733	109.5200	91.9533	84.4867
tvs								
1	0.0584	0.0483	0.1471	0.1310	0.1843	0.2122	0.1761	0.1400
2	0.1308	0.0482	0.0678	0.1461	0.2052	0.1656	0.2218	0.0993
3	0.0326	0.0500	0.1319	0.1873	0.1651	0.1804	0.2157	0.1078
avg tvs	0.0739	0.0488	0.1156	0.1548	0.1849	0.1861	0.2045	0.1157
std dev	0.0509	0.0010	0.0421	0.0291	0.0201	0.0238	0.0248	0.0215
tvs/l	14.7867	9.7667	23.1200	30.9600	36.9733	37.2133	40.9067	23.1400
MPN	1.00E+10		1.00E+10	1.00E+11	1.00E+11	1.00E+11	1.00E+11	1.00E+09
tfs								
1	0.0781	0.1895	0.1343	0.1436	0.2131	0.2842	0.1927	0.3065
2	0.1073	0.1608	0.1717	0.1522	0.1655	0.2862	0.32	0.4146
3	0.0759	0.1193	0.1547	0.2183	0.2064	0.5142	0.253	0.1991
avg tfs	0.09	0.16	0.15	0.17	0.20	0.36	0.26	0.31
std dev	0.0175	0.0353	0.0187	0.0409	0.0258	0.1322	0.0637	0.1078
tfs/l	17.42	31.31	30.71	34.27	39.00	72.31	51.05	61.35
fs/ts								
1	0.57	0.8	0.48	0.52	0.54	0.57	0.52	0.69
2	0.45	0.77	0.72	0.51	0.45	0.63	0.59	0.81
3	0.7	0.7	0.54	0.54	0.56	0.74	0.54	0.65
avg fs/ts	0.57	0.76	0.58	0.58	0.51	0.65	0.55	0.71
vs/ts								
1	0.43	0.2	0.52	0.48	0.46	0.43	0.48	0.31
2	0.55	0.23	0.28	0.49	0.55	0.37	0.41	0.19
3	0.3	0.3	0.46	0.46	0.44	0.26	0.46	0.35
avg vs/ts	0.43	0.24	0.42	0.48	0.49	0.35	0.45	0.29
O & G								
1	0.7578	0.8265	0.5424	0.5716	0.4501	0.4028	0.423	0.33
2	0.6286	0.7497	0.7542	0.5567	0.438	0.4537	0.4047	0.409
3	0.8166	0.7871	0.6885	0.5448	0.5668	0.4739	0.3744	0.33
avg O&G	0.7343	0.7878	0.6617	0.5577	0.4850	0.4435	0.4007	0.3563
std dev	0.0962	0.0384	0.1084	0.0134	0.0711	0.0366	0.0245	0.0456
O&G/l	36.72	39.39	33.09	27.89	24.25	22.17	20.04	17.82
O & G/TS								
1	1.39	0.87	0.48	0.52	0.28	0.20	0.29	0.18
2	0.66	0.90	0.79	0.47	0.30	0.25	0.19	0.20
3	1.88	1.16	0.60	0.34	0.38	0.17	0.20	0.27
avg o&g/ts	1.14	0.96	0.61	0.43	0.32	0.20	0.22	0.21
O & G/FS								
1	2.43	1.09	1.01	1.00	0.53	0.35	0.55	0.27
2	1.46	1.17	1.10	0.91	0.66	0.40	0.32	0.25
3	2.69	1.65	1.11	0.62	0.69	0.23	0.37	0.41
avg o&g/fs	2.11	1.26	1.08	0.81	0.62	0.31	0.39	0.29

APPENDIX J

SLUDGE LOADING RATE

20% load

day 0	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts	O&G	cruc wt	w oil	oil	oil/ts	oil/tfs	oil/tvs
1	52.3305	52.6607	52.4918	0.3302	0.1689	0.1613	0.49	0.51	1	17.8337	18.7141	0.8804			
2	61.4150	61.6760	61.5303	0.2610	0.1457	0.1153	0.44	0.56	2	17.7755	18.5718	0.7963			
3	54.8939	55.2145	55.0912	0.3206	0.1233	0.1973	0.62	0.38	3	22.6623	23.4063	0.7440			
avg				0.3039	0.1460	0.1580	0.52	0.48				0.8069	1.3274	2.5540	5.5280
day 3	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil	oil/ts	oil/tfs	
1	30.2253	30.9020	30.6352	0.6767	0.2668	0.4099	0.61	0.39	1	17.7755	18.4926	0.7171			
2	44.6414	45.6348	45.1409	0.9934	0.4939	0.4995	0.50	0.50	2	22.6623	23.6360	0.9737			
3	41.7086	42.3127	42.1429	0.6041	0.1698	0.4343	0.72	0.28	3	18.8085	19.8322	1.0237			
avg				0.7581	0.3102	0.4479	0.61	0.39				0.9048	0.5968	1.0101	2.9172
day 6	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil	oil/ts	oil/tfs	
1	61.4150	61.7334	61.6565	0.3184	0.0769	0.2415	0.76	0.24	1	17.7754	18.4151	0.6397			
2	54.0499	54.4910	54.3542	0.4411	0.1368	0.3043	0.69	0.31	2	22.6624	23.3807	0.7183			
3	43.9423	44.3773	44.2723	0.4350	0.1050	0.3300	0.76	0.24	3	18.8082	19.5554	0.7472			
avg				0.3982	0.1062	0.2919	0.74	0.26				0.7017	0.8812	1.2019	6.6056
day9	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil	oil/ts	oil/tfs	
1	46.0071	46.4209	46.2788	0.4138	0.1421	0.2717	0.66	0.34	1	30.2246	30.9423	0.7177			
2	45.9577	46.5622	46.3677	0.6045	0.1945	0.4100	0.68	0.32	2	44.6414	45.3500	0.7086			
3	41.9770	42.4523	42.3238	0.4753	0.1285	0.3468	0.73	0.27	3	41.7083	42.3975	0.6892			
avg				0.4979	0.1550	0.3428	0.69	0.31				0.7052	0.7082	1.0284	4.5485
day12	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil	oil/ts	oil/tfs	
1	52.3305	52.8694	52.6802	0.5389	0.2092	0.3297	0.61	0.39	1	30.2260	30.8384	0.6124			
2	61.4150	62.0810	61.9130	0.6660	0.1680	0.4980	0.75	0.25	2	44.6426	45.1553	0.5127			
3	54.8939	55.3087	55.1810	0.4148	0.1277	0.2871	0.69	0.31	3	43.9431	44.5533	0.6102			
avg				0.5399	0.1683	0.3716	0.68	0.32				0.5784	0.5357	0.7783	3.4369
day 15	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil	oil/ts	oil/tfs	
1	63.1594	63.7969	63.4316	0.6375	0.3653	0.2722	0.43	0.57	1	42.7305	43.2429	0.5124			
2	54.0499	54.4333	54.2818	0.3834	0.1515	0.2319	0.60	0.40	2	30.2268	30.7906	0.5638			
3	42.9425	43.5140	43.1835	0.5715	0.3305	0.2410	0.42	0.58	3	41.7110	42.2547	0.5437			
avg				0.5308	0.2824	0.2484	0.48	0.52				0.5400	0.5086	1.0870	1.9118
day 18	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil	oil/ts	oil/tfs	
1	42.7477	43.2283	42.9531	0.4806	0.2752	0.2054	0.43	0.57	1	50.2828	50.9572	0.6744			
2	43.1757	43.4854	43.3975	0.3097	0.0879	0.2218	0.72	0.28	2	47.0370	47.5685	0.5315			
3	47.3226	47.9504	47.6883	0.6278	0.2621	0.3657	0.58	0.42	3	48.0010	48.4333	0.4323			
avg				0.4727	0.2084	0.2843	0.58	0.42				0.5461	0.5776	1.0330	2.6203
day 21	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil	oil/ts	oil/tfs	
1	41.9770	42.8733	42.3995	0.8963	0.4738	0.4225	0.47	0.53	1	63.1594	63.6032	0.4438			
2	45.9570	46.4588	46.2787	0.4998	0.1781	0.3217	0.64	0.36	2	42.9425	43.3989	0.4564			
3	46.0071	47.0054	46.5841	0.9983	0.4213	0.5770	0.58	0.42	3	43.9423	44.4449	0.5026			
avg				0.7981	0.3577	0.4404	0.56	0.44				0.4676	0.2929	0.5309	1.3071

SLUDGE LOADING RATE

20% load

ts /day	0	3	6	9	12	15	18	21
1	0.3302	0.6767	0.3184	0.4138	0.5389	0.6375	0.4806	0.8963
2	0.2610	0.9934	0.4411	0.6045	0.6660	0.3834	0.3097	0.4998
3	0.3206	0.6041	0.4350	0.4753	0.4148	0.5715	0.6278	0.9983
avg ts	0.3039	0.7581	0.3982	0.4979	0.5399	0.5308	0.4727	0.7981
std dev	0.0375	0.2070	0.0691	0.0973	0.1256	0.1318	0.1592	0.2633
ts/l	60.7867	151.6133	79.6333	99.5733	107.9800	106.1600	94.5400	159.6267
tvs								
1	0.1689	0.2668	0.0769	0.1421	0.2092	0.3653	0.2752	0.4738
2	0.1457	0.4939	0.1368	0.1945	0.1680	0.1515	0.0879	0.1781
3	0.1233	0.1698	0.1050	0.1285	0.1277	0.3305	0.2621	0.4213
avg tvs	0.1460	0.3102	0.1062	0.1550	0.1683	0.2824	0.2084	0.3577
std dev	0.0228	0.1663	0.0300	0.0348	0.0408	0.1147	0.1046	0.1578
vs/l	29.1933	62.0333	21.2467	31.0067	33.6600	56.4867	41.6800	71.5467
tfs								
1	0.1613	0.4099	0.2415	0.2717	0.3297	0.2722	0.2054	0.4225
2	0.1153	0.4995	0.3043	0.41	0.498	0.2319	0.2218	0.3217
3	0.1973	0.4343	0.33	0.3468	0.2861	0.241	0.3657	0.577
avg tfs	0.1580	0.4479	0.2919	0.3428	0.3713	0.2484	0.2643	0.4404
std dev	0.0411	0.0463	0.0455	0.0692	0.1119	0.0211	0.0882	0.1286
fs/l	31.5933	89.5800	58.3867	68.5667	74.2533	49.6733	52.8600	88.0800
avg fs/ts	0.52	0.61	0.74	0.69	0.68	0.48	0.58	0.56
avgt vs/ts	0.48	0.39	0.26	0.31	0.32	0.52	0.42	0.44
O & G								
1	0.8804	0.7171	0.6397	0.7177	0.6124	0.5124	0.6744	0.4438
2	0.7963	0.9737	0.7183	0.7086	0.5127	0.5638	0.5315	0.4564
3	0.744	1.0237	0.7472	0.6892	0.6102	0.5437	0.4323	0.5026
avg O&G	0.8069	0.9048	0.7017	0.7052	0.5784	0.5400	0.5461	0.4676
std dev	0.0688	0.1645	0.0556	0.0146	0.0569	0.0259	0.1217	0.0310
O&G/l	40.3450	45.2417	35.0867	35.2583	28.9217	26.9983	27.3033	23.3800
O&G/ts	1.33	0.6	0.88	0.71	0.54	0.51	0.58	0.29
O&G/tfs	2.6	1.01	1.2	1.03	0.78	1.09	1.03	0.53
O&G/tvs	5.53	2.92	6.606	4.548	3.437	1.912	2.62	1.307

EXPERIMENT H
Abiotic Control Experiment for 10 % solids load

day 0	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts	O&G	cruc wt	w oil	oil	oil/ts	oil/tfs
1	58.1597	58.2862	58.2573	0.1265	0.0289	0.0976	0.77	0.23	1	42.7448	44.1955	1.4507	2.87	3.72
2	65.5763	65.7161	65.696	0.1398	0.0201	0.1197	0.86	0.14	2	43.1743	44.7088	1.5345	2.74	3.20
3	61.6812	61.8136	61.7886	0.1324	0.025	0.1074	0.81	0.19	3	47.3226	48.6807	1.3581	2.56	3.16
avg				0.1329	0.0247	0.1082	0.81	0.19				1.4478	2.72	3.35
day 3														
	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil	oil/ts	oil/tfs
1	42.0304	42.1676	42.1411	0.1372	0.0265	0.1107	0.81	0.19	1	62.7982	64.1399	1.3417	2.44	3.03
2	44.6426	44.7812	44.7559	0.1386	0.0253	0.1133	0.82	0.18	2	70.4489	72.0376	1.5887	2.87	3.51
3	41.7091	41.8317	41.8065	0.1226	0.0252	0.0974	0.79	0.21	3	59.9456	61.3127	1.3671	2.79	3.51
avg				0.1328	0.0257	0.1071	0.81	0.19				1.4325	2.70	3.34
day 6														
	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil	oil/ts	oil/tfs
1	54.051	54.1953	54.1696	0.1443	0.0257	0.1186	0.82	0.18	1	62.6966	64.0028	1.3062	2.26	2.75
2	41.976	42.1405	42.115	0.1645	0.0255	0.139	0.84	0.16	2	69.715	71.027	1.312	1.99	2.36
3	46.0078	46.1361	46.1131	0.1283	0.023	0.1053	0.82	0.18	3	67.6682	69.0641	1.3959	2.72	3.31
avg				0.1457	0.0247	0.1209	0.83	0.17				1.338	2.30	2.77
day 9														
	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil	oil/ts	oil/tfs
1	44.9689	45.076	45.0546	0.1071	0.0214	0.0857	0.80	0.20	1	62.7989	64.2631	1.4642	3.42	4.27
2	41.1723	41.3019	41.2811	0.1296	0.0208	0.1088	0.84	0.16	2	70.4522	71.6505	1.1983	2.31	2.75
3	57.233	57.3748	57.3518	0.1418	0.023	0.1188	0.84	0.16	3	59.9454	61.2792	1.3338	2.35	2.81
avg				0.1262	0.0217	0.1044	0.83	0.17				1.3321	2.64	3.19
day 12														
	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil	oil/ts	oil/tfs
1	54.0498	54.2098	54.1847	0.16	0.0251	0.1349	0.84	0.16	1	62.6948	64.0044	1.3096	2.05	2.43
2	41.9753	42.0954	42.0693	0.1201	0.0261	0.094	0.78	0.22	2	69.7129	70.9522	1.2393	2.58	3.30
3	46.0071	46.1815	46.1594	0.1744	0.0221	0.1523	0.87	0.13	3	67.6676	69.008	1.3404	1.92	2.20
avg				0.1515	0.0244	0.1271	0.84	0.16				1.2964	2.14	2.55
day 15														
	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil	oil/ts	oil/tfs
1	44.9707	45.1423	45.1224	0.1716	0.0199	0.1517	0.88	0.12	1	62.8003	64.2326	1.4323	2.09	2.36
2	41.1745	41.3365	41.3169	0.162	0.0196	0.1424	0.88	0.12	2	70.4495	71.7125	1.263	1.95	2.22
3	57.2349	57.358	57.3393	0.1231	0.0187	0.1044	0.85	0.15	3	59.9465	61.2895	1.343	2.73	3.22
avg				0.1522	0.0194	0.1328	0.87	0.13				1.3461	2.21	2.55
day 18														
	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil	oil/ts	oil/tfs
1	54.0521	54.2062	54.1828	0.1541	0.0234	0.1307	0.85	0.15	1	62.6973	64.0954	1.3981	2.27	2.67
2	41.9765	42.1329	42.1084	0.1564	0.0245	0.1319	0.84	0.16	2	69.715	70.9373	1.2223	1.95	2.32
3	46.009	46.1666	46.1383	0.1576	0.0283	0.1293	0.82	0.18	3	67.6695	68.9618	1.2923	2.05	2.50
				0.1560	0.0254	0.1306	0.84	0.16				1.3042	2.09	2.50
day 21														
	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil	oil/ts	oil/tfs
1	42.0299	42.1615	42.1377	0.1316	0.0238	0.1078	0.82	0.18	1	48.8189	50.0033	1.1844	2.25	2.75
2	57.2344	57.3747	57.349	0.1403	0.0257	0.1146	0.82	0.18	2	50.2822	51.5077	1.2255	2.18	2.67
3	41.7093	41.8358	41.8083	0.1265	0.0275	0.099	0.78	0.22	3	48.0007	49.4005	1.3998	2.77	3.53
				0.1328	0.0257	0.1071	0.81	0.19				1.2699	2.39	2.96

ABIOTIC CONTROL EXPERIMENT FOR 10%

SOLIDS LOAD

day	0	3	6	9	12	15	18	21
ts 1	0.1265	0.1372	0.1443	0.1071	0.16	0.1716	0.1541	0.1316
ts 2	0.1398	0.1386	0.1645	0.1296	0.1201	0.162	0.1564	0.1403
ts 3	0.1324	0.1226	0.1283	0.1418	0.1744	0.1231	0.1576	0.1265
avg ts	0.1329	0.1328	0.1457	0.1262	0.1515	0.1522	0.1560	0.1360
std dev	0.0067	0.0089	0.0181	0.0176	0.0281	0.0257	0.0018	0.0070
ts/l	26.5800	26.5600	29.1400	25.2333	30.3000	30.4467	31.2000	27.2000
Vs 1	0.0289	0.0265	0.0257	0.0214	0.0251	0.0199	0.0234	0.0238
vs 2	0.0201	0.0253	0.0255	0.0208	0.0261	0.0196	0.0245	0.0257
vs 3	0.025	0.0252	0.023	0.023	0.0221	0.0187	0.0283	0.0275
avg vs	0.0247	0.0257	0.0247	0.0217	0.0244	0.0194	0.0254	0.0248
std dev	0.0044	0.0007	0.0015	0.0011	0.0021	0.0006	0.0026	0.0019
vs/l	4.94	5.14	4.94	4.346667	4.886667	3.88	5.08	4.96
fs 1	0.0976	0.1107	0.1186	0.0857	0.1349	0.1517	0.1307	0.1078
fs2	0.1197	0.1133	0.139	0.1088	0.094	0.1424	0.1319	0.1146
fs3	0.1074	0.0974	0.1053	0.1188	0.1523	0.1044	0.1293	0.099
avg fs	0.1082	0.1071	0.1209	0.1044	0.1271	0.1328	0.1306	0.1112
std dev	0.0111	0.0085	0.0170	0.0170	0.0299	0.0251	0.0013	0.0078
fs/l	21.64	21.42	24.18	20.88667	25.41333	26.56667	26.12	22.24
day	0	3	6	9	12	15	18	21
oil 1	1.4507	1.3417	1.3062	1.4642	1.3096	1.4323	1.3981	1.1844
oil 2	1.5345	1.5887	1.312	1.1983	1.2393	1.263	1.2223	1.2255
oil 3	1.3581	1.3671	1.3959	1.3338	1.3404	1.343	1.2923	1.3998
avg oil	1.4478	1.4325	1.338	1.3321	1.2964	1.3461	1.3042	1.2699
std dev	0.0882	0.1359	0.0502	0.1330	0.0518	0.0847	0.0885	0.1144
oil/l	72.39	71.625	66.9	66.605	64.82	67.305	65.21	63.495
total abiotic oil loss			8.9 g/l					
oil at start in 25 ml flask			1.4478					
oil at end in 25 ml flask			1.2699					
a loss of			0.1779					
loss %			0.123					

APPENDIX J

EXTRACTION AND MEASUREMENT OF O&G IN THE PRESENCE OF SURFACTANT

FTIR standard calibration curve for O&G extraction method development

Range of standard weights prepared from extracted O&G from sludge, dissolved in 10 ml TCTFE

No.	1	2	3	4	5	6
g	0.0064	0.0702	0.0748	0.2036	0.2398	0.3159

Std no 1	0.0064
peak, (cm-1)	absorption
2957.5	0.068
2928.9	0.1073
2857.6	0.056
total abs	0.2313

Std no 2	0.0702
peak, (cm-1)	absorption
2957.7	0.4449
2928.9	0.8375
2857.4	0.4424
total abs	1.7248

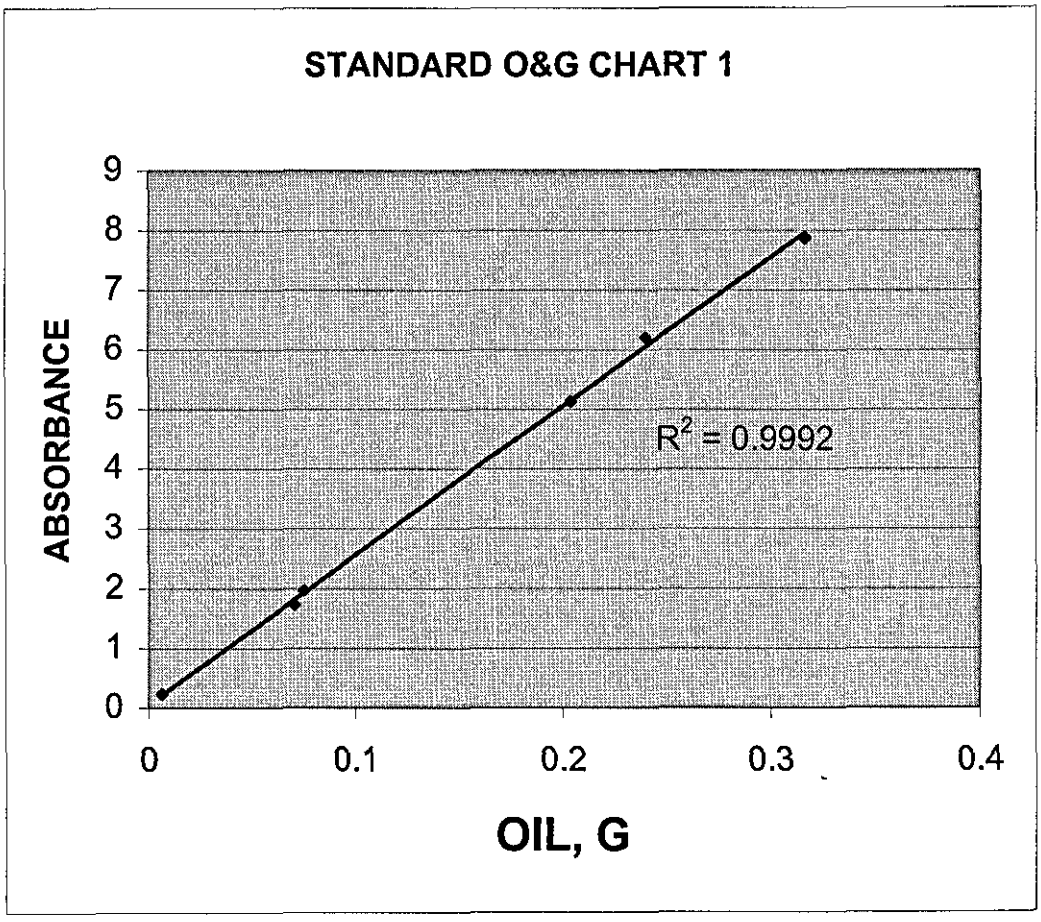
Std no 3	0.0748
peak, (cm-1)	absorption
2957.5	0.5126
2928.6	0.9548
2857.1	0.496
total abs	1.9639

Std no 4	0.2036
peak, (cm-1)	absorption
2957.6	1.2813
2928.4	2.5759
2857.2	1.2738
total abs	5.131

Std no 5	0.2398
peak, (cm-1)	absorption
2957.5	1.4562
2930.5	3.276
2857.1	1.4534
total abs	6.1956

Std no 6	0.3159
peak, (cm-1)	absorption
2957.5	2.1598
2928.6	3.5876
2857.1	2.1196
total abs	7.867

oil, g	0.0064	0.0702	0.0748	0.2036	0.2398	0.3159
abs cm -1	0.2313	1.7248	1.9639	5.131	6.1956	7.867



APPENDIX J

EXTRACTION AND MEASUREMENT OF O&G IN THE PRESENCE OF SURFACTANT

Determination of O&G using FTIR absorption from samples with added surfactants

samples in 10 ml TCTFE		amt in subsample
1B	Brij 35 and 0.7184 g oil	0.1197
2B	Brij 35 and 0.7318 g oil	0.122
3S	Sapogenat and 0.7039 g oil	0.1173
4S	Sapogenat and 0.7159 g oil	0.1193
5I	Inipol and 0.7050 g oil	0.1175
6I	Inipol and 0.7320 g oil	0.122

sample 1B	0.1197 g oil
peak, (cm-1)	absorption
2957.5	0.7405
2928.6	1.3655
2857.1	0.7118
total abs	2.8178

sample 2B	0.122g oil
peak, (cm-1)	absorption
2957.5	0.7551
2928.6	1.433
2857.1	0.7367
total abs	2.9248

sample 3S	0.1173 g oil
peak, (cm-1)	absorption
2957.5	0.779
2928.6	1.4662
2857.1	0.7578
total abs	3.003

sample 4S	0.1193 g oil
peak, (cm-1)	absorption
2957.5	0.7449
2928.6	1.4189
2857.1	0.729
total abs	5.131

sample 5I	.1175 g oil
peak, (cm-1)	absorption
2957.5	0.8671
2928.6	1.6485
2857.1	0.8674
total abs	3.383

sample 6L	0.122 g oil
peak, (cm-1)	absorption
2957.5	0.8506
2929.6	1.6099
2857.1	0.8497
total abs	3.3102

Conversion of absorbance to oil content based on calibration standard curve 1

sample	oil	abs	extrapolate O&G		% recovery
1B	0.1197	2.8178	0.112 g		93.57
2B	0.122	2.9248	0.116 g		95.08
3S	0.1173	3.003	0.118 g		100.6
4S	0.1193	5.131	0.114 g		95.56
5I	0.1175	3.383	0.120 g		102.13
6I	0.122	3.3102	0.124 g		101.64

SELECTION OF HIGHEST DEGRADATION RATE ENHANCER SURFACTANT

day	surf		cruc/filter	105	550	ts	vs	fs	vs/ts	fs/ts	avg vs	vs/fs	inorganic C	
0	brij 35	1	0.1841	2.3914	1.6143	2.2073	0.7771	1.4302	0.3521	0.6479	0.81	0.5434		tc
		2	0.1911	2.4572	1.6157	2.2661	0.8415	1.4246	0.3713	0.6287		0.5907		
	sapogena	3	0.1934	2.4552	1.6962	2.2618	0.759	1.5028	0.3356	0.6644	0.78	0.5051		
		4	0.1931	2.4245	1.6255	2.2314	0.799	1.4324	0.3581	0.6419		0.5578		
	inipol	5	0.1948	2.367	1.588	2.1722	0.769	1.4032	0.3540	0.6460	0.78	0.5480		
		6	0.1927	2.4335	1.6336	2.2408	0.8	1.4408	0.3570	0.6430		0.5552		
	control	7	0.1911	2.4302	1.6449	2.2391	0.7853	1.4538	0.3507	0.6493	0.79	0.5402		
		8	0.1913	2.4109	1.3805	2.2196	1.0304	1.1892	0.4642	0.5358		0.8665		
12	brij 35		42.0413	43.9886	43.5367	1.9473	0.4519	1.4954	0.2321	0.7679	0.43	0.3022	3.1	13,500
		2	58.1632	59.9684	59.5618	1.8052	0.4056	1.3986	0.2252	0.7748		0.2907	6.5	
	sapogena	3	47.3361	49.1924	48.8088	1.8563	0.3836	1.4727	0.2066	0.7934	0.44	0.2605	1	17,000
		4	70.4533	72.4129	71.9251	1.9596	0.4878	1.4718	0.2489	0.7511		0.3314	1.7	
	inipol	5	43.1861	45.0906	44.6608	1.9045	0.4298	1.4747	0.2257	0.7743	0.43	0.2914	59	14,000
		6	61.6833	63.5481	63.1584	1.8648	0.3897	1.4751	0.2090	0.7910		0.2642	35.7	
	control	7	42.754	44.5899	44.1273	1.8359	0.4626	1.3733	0.2520	0.7480	0.46	0.3369	3.1	12,000
		8	41.9807	43.8699	43.2193	1.8892	0.6506	1.2386					3.9	

day	surf		cruc	105	550	ts	vs	fs	vs/ts	fs/ts	avg vs	vs/fs		
2	brij 35	1	58.1595	lost							1.59			
		2	70.4501	72.8324	71.9141	2.3823	0.9183	1.464	0.3855	0.6145		0.6273		
	sapogena	3	61.6825	64.4436	63.2384	2.7611	1.2052	1.5559	0.4365	0.5635	1.28	0.7746		
		4	62.603	65.4119	64.0474	2.8089	1.3645	1.4444	0.4858	0.5142		0.9447		
	inipol	5	42.0389	44.3773	43.5123	2.3384	0.865	1.4734	0.3699	0.6301	0.86	0.5871		
		6	47.3342	49.7164	48.8711	2.3822	0.8453	1.5369	0.3548	0.6452		0.5500		
	control	7	43.1833	45.4561	44.6528	2.2728	0.8033	1.4695	0.3534	0.6466	0.84	0.5466		
		8	42.7541	45.0779	44.205	2.3238	0.8729	1.4509	0.3756	0.6244		0.6016		

day	surf		cruc	105	550	ts	vs	fs	vs/ts	fs/ts	avg vs	vs/fs		
5	brij 35	1	50.4418	52.6368	52.0596	2.195	0.5772	1.6178	0.2630	0.7370	0.6289	0.3568		
		2	47.0361	49.2828	48.6022	2.2467	0.6806	1.5661	0.3029	0.6971		0.4346		
	sapogena	3	44.3619	46.9654	45.9263	2.6035	1.0391	1.5644	0.3991	0.6009	1.0736	0.6642		
		4	49.1714	51.7601	50.652	2.5887	1.1081	1.4806	0.4281	0.5719		0.7484		
	inipol	5	65.68	67.8224	67.1242	2.2424	0.6982	1.5442	0.3114	0.6886	0.6952	0.4521		
		6	67.7615	69.967	69.2748	2.2055	0.6922	1.5133	0.3139	0.6861		0.4574		
	control	7	57.242	59.4359	58.7747	2.1939	0.6612	1.6327	0.3014	0.6986	0.6541	0.4314		
		8	41.7172	43.8662	43.2193	2.149	0.6469	1.5021	0.3010	0.6990		0.4307		

day	surf		cruc	105	550	ts	vs	fs	vs/ts	fs/ts	avg vs	vs/fs		
8	brij 35	1	53.1732	55.5571	54.9504	2.3839	0.9067	1.4772	0.3803	0.6197	0.9183	0.6138		
		2	42.9649	45.3963	44.4665	2.4314	0.9298	1.5016	0.3824	0.6176		0.6192		
	sapogena	3	45.9603	48.7508	47.4702	2.7905	1.2806	1.5099	0.4589	0.5411	1.2746	0.8481		
		4	43.9475	46.7295	45.4609	2.782	1.2686	1.5134	0.4560	0.5440		0.8382		
	inipol	5	48.8207	51.1297	50.3319	2.309	0.7978	1.5112	0.3455	0.6545	0.7941	0.5279		
		6	49.7044	51.9905	51.2001	2.2861	0.7904	1.4957	0.3457	0.6543		0.5284		
	control	7	47.6235	49.8581	49.0812	2.2346	0.7769	1.4577	0.3477	0.6523	0.7791	0.5330		
		8	54.0477	56.3361	55.5548	2.2884	0.7813	1.5071	0.3414	0.6586		0.5184		

SELECTION OF HIGHEST DEGRADATION RATE ENHANCER

Surfactant selection

DAY 0

sample 1B	
peak, (cm-1)	absorption
2957.5	0.7924
2928.6	1.5426
2857.1	0.8124
total abs	3.1474

sample 2B	
peak, (cm-1)	absorption
2957.5	1.0169
2928.6	1.8583
2857.1	1.0354
total abs	3.9106

sample 3S	
peak, (cm-1)	absorption
2957.5	0.6807
2928.6	1.3349
2857.1	0.6996
total abs	2.7152

sample 4S	
peak, (cm-1)	absorption
2957.5	0.6596
2928.6	1.3022
2857.1	0.6765
total abs	2.6383

sample 5I	
peak, (cm-1)	absorption
2957.5	0.7306
2928.6	1.4323
2857.1	0.7584
total abs	2.9212

sample 6L	
peak, (cm-1)	absorption
2957.5	0.9239
2929.7	1.8185
2857.1	0.9562
total abs	3.6986

sample 7C	
peak, (cm-1)	absorption
2957.5	0.5833
2928.6	0.9784
2858.1	0.4966
total abs	2.0583

sample 8C	
peak, (cm-1)	absorption
2957.5	0.7312
2928.6	1.4496
2857.1	0.7614
total abs	2.9422

Conversion of absorbance to oil content based on calibration standard curve 2

sample	total abs	average	extrapolate O&G (g)		Corr Fact	O&G (g)
1B	3.1474					
2B	3.9106	3.529	0.158		1.06	0.16748
3S	2.7152					
4S	2.6383	2.6767	0.125		1.02	0.1275
5I	2.9212					
6I	3.6986	3.3099	0.15		0.98	0.147
7C	2.0583					
8C	2.9422	2.5003	0.115			0.115

DAY 2

sample 1B	
peak, (cm-1)	absorption
2957.9	0.6733
2928.6	1.2949
2857.1	0.6757
total abs	2.6444

sample 2B	
peak, (cm-1)	absorption
2957.5	0.703
2928.6	1.355
2857.1	0.7106
total abs	2.765

sample 3S	
peak, (cm-1)	absorption
2957.9	0.7084
2928.6	1.3357
2857.1	0.7133
total abs	2.7574

sample 4S	
peak, (cm-1)	absorption
2957.9	0.6841
2928.6	1.3179
2857.1	0.6894
total abs	2.6914

sample 5I	
peak, (cm-1)	absorption
2957.9	0.7378
2928.6	1.4053
2857.1	0.7444
total abs	2.8874

sample 6I	
peak, (cm-1)	absorption
2957.9	0.7597
2928.6	1.4389
2857.1	0.7691
total abs	2.96777

sample 7C	
peak, (cm-1)	absorption
2957.9	0.7298
2928.6	1.3368
2857.1	0.6998
total abs	2.7664

sample 8C	
peak, (cm-1)	absorption
2956.8	0.8054
2928.6	1.4752
2857.1	7708
total abs	3.0514

Conversion of absorbance to oil content based on calibration standard curve 2

sample	total abs	extrapolate O&G (g)		Corr Fact	O&G (g)
1B	2.6444	0.12			
2B	2.765	0.128	0.124	1.06	0.1314
3S	2.7574	0.128			
4S	2.6914	0.125	0.1265	1.02	0.129
5I	2.8874	0.134			
6I	2.9677	0.136	0.135	0.98	0.1323
7C	2.7664	0.128			
8C	3.0514	0.133	0.1305		0.1305

DAY 5

sample 1B	
peak, (cm-1)	absorption
2957.5	0.5837
2928.6	1.0621
2856.9	0.5474
total abs	2.1932

sample 2B	
peak, (cm-1)	absorption
2956.8	0.629
2928.6	1.1667
2856.9	0.604
total abs	2.3997

sample 3S	
peak, (cm-1)	absorption
2956.8	0.6003
2928.6	1.067
2856.9	0.5541
total abs	2.214

sample 4S	
peak, (cm-1)	absorption
2956.8	0.5471
2928.6	0.937
2856.9	0.4749
total abs	1.959

sample 5I	
peak, (cm-1)	absorption
2956.8	0.6951
2928.6	1.2102
2856.9	0.6329
total abs	2.5382

sample 6L	
peak, (cm-1)	absorption
2956.8	0.7454
2928.6	1.3942
2856.9	0.7492
total abs	2.8888

sample 7C	
peak, (cm-1)	absorption
2956.8	0.697
2929.6	1.2738
2856.9	0.6574
total abs	2.6282

sample 8C	
peak, (cm-1)	absorption
2956.8	0.7691
2929.6	1.4874
2856.9	0.7723
total abs	3.0288

Conversion of absorbance to oil content based on calibration standard curve 2

sample	total abs	avg	extrapolate O&G (g)		Corr Fact	O&G (g)
1B	2.1932					
2B	2.3997	2.29645	0.105		1.06	0.1113
3S	2.214					
4S	1.959	2.0865	0.1		1.02	0.102
5I	2.5382					
6I	2.8888	2.7135	0.125		0.98	0.1225
7C	2.6282					
8C	3.0288	2.8285	0.13			0.13

DAY 8

sample 1B	
peak, (cm-1)	absorption
2957.5	0.8107
2928.6	1.3343
2857.1	0.7033
total abs	2.8483

sample 2B	
peak, (cm-1)	absorption
2957.5	0.7486
2928.6	1.1849
2857.1	0.6201
total abs	2.5536

sample 3S	
peak, (cm-1)	absorption
2957.5	0.6261
2928.6	1.0401
2857.1	0.5409
total abs	2.2071

sample 4S	
peak, (cm-1)	absorption
2957.5	0.6726
2928.6	1.1186
2858.1	0.5699
total abs	2.3611

sample 5I	
peak, (cm-1)	absorption
2957.5	0.7196
2928.6	1.1959
2858.1	0.6069
total abs	2.5224

sample 6L	
peak, (cm-1)	absorption
2957.5	0.5762
2928.6	0.9356
2858.1	0.4788
total abs	1.9906

sample 7C	
peak, (cm-1)	absorption
2957.5	0.8187
2928.6	1.6125
2857.1	0.837
total abs	3.2682

sample 8C	
peak, (cm-1)	absorption
2957.5	0.7189
2929.6	1.1738
2858.1	0.5906
total abs	2.4833

Conversion of absorbance to oil content based on calibration standard curve 2

sample	total abs	Avg	extrapolate O&G (g)		Corr Fact	O&G (g)
1B	2.8483					
2B	2.5536	2.70095	0.125		1.06	0.1325
3S	2.2071					
4S	2.3611	2.2841	0.105		1.02	0.1071
5I	2.5224					
6I	1.9906	2.2565	0.103		0.98	0.1009
7C	3.2682					
8C	2.4833	2.87575	0.135			0.135

DAY 12

sample 1B	
peak, (cm-1)	absorption
2957.5	0.7262
2928.6	1.2747
2857.1	0.6559
total abs	2.6569

sample 2B	
peak, (cm-1)	absorption
2957.5	0.5462
2928.6	0.8657
2858.1	0.4392
total abs	1.8506

sample 3S	
peak, (cm-1)	absorption
2957.5	0.6479
2928.6	1.0081
2858.1	0.5046
total abs	2.1606

sample 4S	
peak, (cm-1)	absorption
* CENTRIFUGE	
BOTTLE	
BROKE	
total abs	

sample 5I	
peak, (cm-1)	absorption
2957.5	0.8012
2929.7	1.3365
2858.1	0.7157
total abs	2.8534

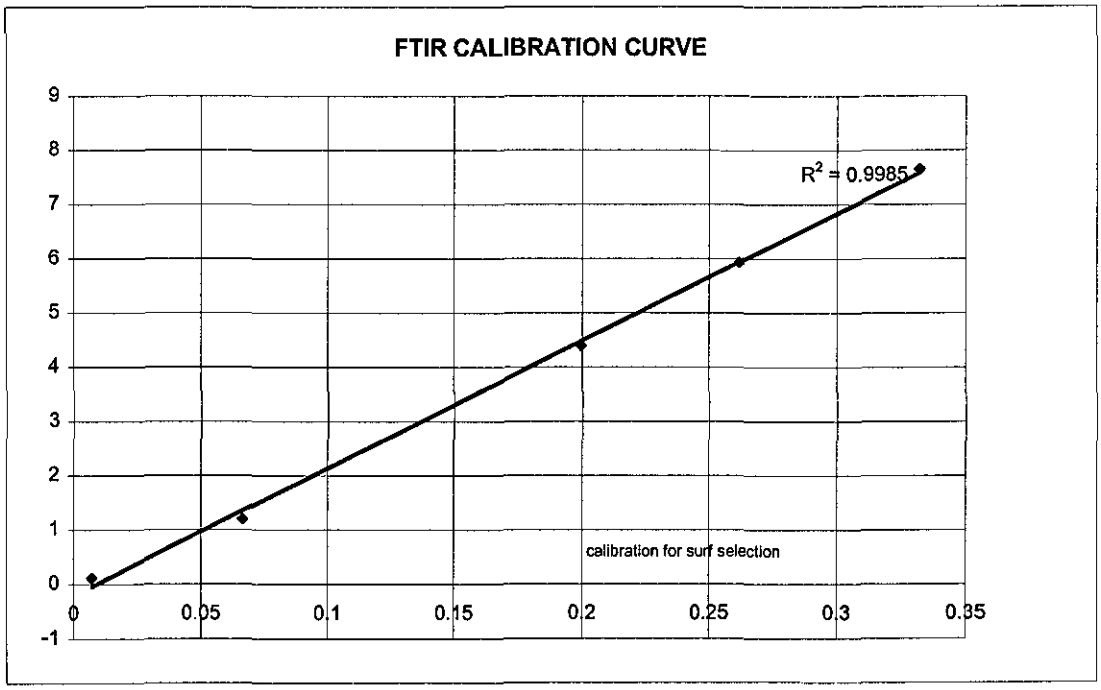
sample 6I	
peak, (cm-1)	absorption
2957.5	0.5172
2928.6	0.8015
2857.1	0.4049
total abs	1.7236

sample 7C	
peak, (cm-1)	absorption
2957.5	0.5341
2928.6	0.8281
2858.1	0.4141
total abs	1.7763

sample 8C	
peak, (cm-1)	absorption
2957.5	0.58
2928.6	0.9088
2857.1	0.4566
total abs	1.9454

Conversion of absorbance to oil content based on calibration standard curve 2

sample	total abs	avg	extrapolate O&G (g)		Corr Fact	O&G (g)
1B	2.6569					
2B	1.8506	2.25375	0.105		1.06	0.1113
3S	2.1606					
4S	*	2.1066	0.1		1.02	0.102
5I	2.8534					
6I	1.7236	2.2885	0.108		0.98	0.1058
7C	1.7763					
8C	1.9454	1.86085	0.0902			0.0902



TESTING THE ADDITION OF SURFACTANT TO SLUDGE OR MIXED LIQUOR

sample 1A inipot 1	
peak, (cm-1)	absorption
2958.5	0.4243
2929.1	8151
2857.5	0.4407
total abs	1.6801

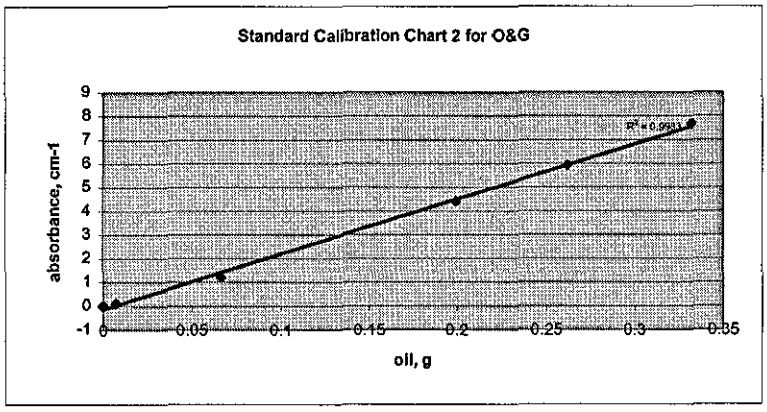
sample 1B it2	
peak, (cm-1)	absorption
2958.3	0.3563
2929	0.6768
2857.5	0.3702
total abs	1.4033

sample 2A it3b	
peak, (cm-1)	absorption
2959.6	0.1106
2928.4	0.2008
2858.1	0.1116
total abs	0.423

sample 2B it4b	
peak, (cm-1)	absorption
2958.5	0.251
2929.7	0.463
2858.1	0.2634
total abs	0.9774

Conversion of absorbance to oil content based on calibration std 2

sample	total abs	extrapolate O&G (g)		Avg O&G (g)
1A	1.6801	0.083		0.0755
1B	1.4033	0.068		
2A	0.423	0.028		0.0385
2B	0.9774	0.049		



APPENDIX K

TRACER STUDIES ON THE AIR LIFT STR USING LITHIUM CHLORIDE

tracer #	% solids	mixing	volume	LiCl	Std.	Cm	Ct	Cm/Ct	time eq
			(l)	(g)		(mg/l)	(mg/l)		
28	0	stir	3.8	0.227	12	10	9.76	1.02	200 sec
25	0	stir	3.7	0.228	9	10.6	10.05	1.05	200 sec
24	0	air	3.7	0.225	9	10	9.95	1.01	80 sec
27	0	air	3.8	0.227	12	10	9.76	1.02	80 sec
16	0	both	3.8	0.104	3	4.2	4.46	0.94	120 sec
29	0	both	3.8	0.226	13	10.15	9.74	1.04	100 sec
35	5	stir	3.7	0.226	15	11.2	9.99	1.12	300 sec
31	5	stir	3.8	0.227	14	11.5	9.78	1.18	300 sec
34	5	air	3.7	0.231	16	10.5	10.22	1.03	100 sec
32	5	air	3.7	0.227	15	10	10.05	1.00	100 sec
36	5	both	3.7	0.232	16	10.7	10.27	1.04	80 sec
33	5	both	3.5	0.239	16	12	11.16	1.08	40 sec
39	10	stir	3.6	0.2320	18	12	10.549	1.14	230 sec
40	10	stir	3.6	0.227	18	11.8	10.299	1.15	300 sec
42	10	air	3.6	0.23	18	11.63	10.42	1.12	240 sec
41	10	air	3.6	0.227	18	11.63	10.3	1.13	200 sec
37	10	both	3.5	0.2290	17	10	10.80	0.93	100 sec
38	10	both	3.5	0.226	17	11.4	10.676	1.07	100 sec

EQUILIBRIUM CHECK
Lithium chloride is injected to a volume of water in a magnetically stirred beaker. The assumption is made that mixing is instantaneous and there is no dead space. Equilibrium is reached at Cm/Ct of 0.97 - 0.99.

TRACER STUDIES ON THE BASIC STR USING LITHIUM CHLORIDE

10% SOLIDS

tracer #	stir spee	air	volum	Li	LiCl	Li	Std.	li bs + Ct	Ct	Cm	Cm/C	time eq
		l/min	(l)	baselin	(g)	baseline			(mg/l)	(mg/l)		
1	150	2	8	10.38	0.499	10.19	1	10.38+10.19	20.57	20.05	0.97	20 sec
2	250	2	8	0	0.508	0	2	10.38	10.38	11.70	1.13	30 sec
3	150	4	8	0	0.5	0	5	10.21	10.21	10.80	1.06	20 sec
4	250	4	8	0	0.51	0	4	10.43	10.43	10.70	1.03	30 sec
4b	250	4	8	10.7	0.49	10.01	4	10.7+10.01	20.71	19.70	0.95	40 sec
5	150	6	8	10.8	0.501	10.24	5	10.8+10.24	21.04	19.60	0.93	50 sec
6	250	6	8	10	0.493	10.08	6	10+10.08	20.08	20.00	1.00	20 sec
7	36	4	8	0	0.503	0	7	10.29	10.29	11.90	1.16	60 sec

APPENDIX L

Basic BSTR

day 0										O&G		
	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil
1	42.037	42.2378	42.1863	0.2008	0.0515	0.1493	0.74	0.26	1	41.7417	41.883	0.1413
2	47.3322	47.5079	47.4583	0.1757	0.0496	0.1261	0.72	0.28	2			
3	43.1811	43.3502	43.3036	0.1691	0.0466	0.1225	0.72	0.28	3	27.2655	27.3345	0.0690
avg				0.1819	0.0492	0.1326	0.73	0.27				

day 3											
	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil
1	45.9603	46.3637	46.1388	0.4034	0.2249	0.1785	0.44	0.56	1	27.5448	27.696	0.1512
2	43.9465	44.359	44.1295	0.4125	0.2295	0.183	0.44	0.56	2	41.9929	42.1774	0.1845
3	46.0117	46.3928	46.17	0.3811	0.2228	0.1583	0.42	0.58	3	28.0241	28.2218	0.1977
avg				0.3990	0.2257	0.1733	0.43	0.57				0.1778

day 7											
	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil
1	68.4155	68.811	68.6074	0.3955	0.2036	0.1919	0.49	0.51	1	42.2901	42.4799	0.1898
2	62.4802	62.9474	62.7044	0.4672	0.243	0.2242	0.48	0.52	2	41.6668	41.9413	0.2745
3	64.1222	64.6186	64.3025	0.4964	0.3161	0.1803	0.36	0.64	3	27.2134	27.4479	0.2345
avg				0.4530	0.2542	0.1988	0.44	0.56				0.2329

day 12											
	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil
1	44.9777	45.3564	45.1532	0.3787	0.2032	0.1755	0.46	0.54	1	40.9877	41.272	0.2843
2	41.1801	41.6019	41.3588	0.4218	0.2431	0.1787	0.42	0.58	2	41.4377	41.6627	0.225
3	41.7144	42.1155	41.9054	0.4011	0.2101	0.191	0.48	0.52	3	26.7328	26.973	0.2402
avg				0.4005	0.2188	0.1817	0.45	0.55				0.2498

day 17											
	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil
1	58.161	58.3586	58.2573	0.1976	0.1013	0.0963	0.49	0.51	1	41.7482	41.9835	0.2353
2	68.4139	68.6091	68.5121	0.1952	0.097	0.0982	0.50	0.50	2	41.2343	41.4888	0.2545
3	61.6824	61.8751	61.7716	0.1927	0.1035	0.0892	0.46	0.54	3	27.4474	27.6715	0.2241
avg				0.1952	0.1006	0.0946	0.48	0.52				0.2380

day 23											
	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil
1	65.5755	65.9439	65.7831	0.3684	0.1608	0.2076	0.56	0.44	1	41.074	41.3115	0.2375
2	62.4802	62.9193	62.7103	0.4391	0.209	0.2301	0.52	0.48	2	40.1037	40.2677	0.164
3	64.1178	64.5394	64.3328	0.4216	0.2066	0.215	0.51	0.49	3	38.8871	39.0715	0.1844
avg				0.4097	0.1921	0.2176	0.53	0.47				0.1953

day 28											
	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil
1	42.0363	42.564	42.2668	0.5277	0.2972	0.2305	0.44	0.56	1	39.2977	39.4582	0.1605
2	47.3308	47.785	47.5399	0.4542	0.2451	0.2091	0.46	0.54	2	27.8958	28.0391	0.1433
3	43.1808	43.6091	43.3886	0.4283	0.2205	0.2078	0.49	0.51	3	41.734	41.9163	0.1823
				0.4700	0.2543	0.2158	0.46	0.54				0.1620

day 37											
	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil
1	44.9764	45.2104	45.1364	0.234	0.074	0.16	0.68	0.32	1	41.6156	41.6474	0.0318
2	41.1792	41.417	41.3422	0.2378	0.0748	0.163	0.69	0.31	2	27.0218	27.0496	0.0278
3	41.7144	41.9596	41.8829	0.2452	0.0767	0.1685	0.69	0.31	3	26.572	26.6016	0.0296
				0.2390	0.0752	0.1638	0.69	0.31				0.0297

day 44											
	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil
1	42.0368	42.1648	42.119	0.128	0.0458	0.0822	0.64	0.36	1	41.6156	41.6474	0.0318
2	47.332	47.4579	47.412	0.1259	0.0459	0.08	0.64	0.36	2	27.0218	27.0496	0.0278
3	43.1815	43.2993	43.2576	0.1178	0.0417	0.0761	0.65	0.35	2	27.0218	27.0496	0.0278
				0.1239	0.0445	0.0794	0.64	0.36				0.0291

day 51											
	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil
1	65.5754	65.6601	65.6214	0.0847	0.0387	0.046	0.54	0.46	1	38.9583	38.9748	0.0165
2	62.4802	62.5734	62.531	0.0932	0.0424	0.0508	0.55	0.45	2	27.5923	27.6066	0.0143
3	64.1178	64.2071	64.1664	0.0893	0.0407	0.0486	0.54	0.46	3	39.3163	39.329	0.0127
				0.0891	0.0406	0.0485	0.54	0.46				0.0145

day 58											
	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil
1	42.7472	42.8429	42.8026	0.0957	0.0403	0.0554	0.58	0.42	1	25.8721	25.8766	0.0045
2	44.6475	44.736	44.698	0.0885	0.038	0.0505	0.57	0.43	2	41.6822	41.6875	0.0053
3	57.2372	57.3288	57.2884	0.0916	0.0404	0.0512	0.56	0.44	3	38.9786	38.9852	0.0066
				0.0919	0.0396	0.0524	0.57	0.43				0.0055

day 65											
	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil
1	44.9763	45.0746	45.035	0.0983	0.0396	0.0587	0.60	0.40	1	38.8598	38.865	0.0052
2	41.1792	41.2621	41.2295	0.0829	0.0326	0.0503	0.61	0.39	2	27.1715	27.1772	0.0057
3	41.7135	41.8164	41.7758	0.1029	0.0406	0.0623	0.61	0.39	3	41.3975	41.4021	0.0046
				0.0947	0.0376	0.0571	0.60	0.40				0.0052

	oil/ts	oil/tfs	bacterial counts		filter	105	550	tss	tvss	tfss	fs/ts
	0.78	1.07	PCA	1.10E+09	0.1923	0.2621	0.2421	0.0698	0.02	0.0498	0.71
	0.00	0.00	bbhn	>3e7	0.1911	0.3018	0.2757	0.1107	0.0261	0.0846	0.76
	0.38	0.52			0.1896	0.2822	0.2565	0.0926	0.0257	0.0669	0.72
	0.00	0.00									

	oil/ts	oil/tfs	bacterial counts		filter	105	550	tss	tvss	tfss	fs/ts
	0.38	0.87	PCA	1.08E+09	0.1917	0.4458	0.2718	0.2541	0.174	0.0801	0.32
	0.46	1.06	bbhn	1.32E+07	0.1909	0.3791	0.2636	0.1882	0.1155	0.0727	0.39
	0.50	1.14			0.1925	0.3503	0.2558	0.1578	0.0945	0.0633	0.40
	0.45	1.02									

	oil/ts	oil/tfs	bacterial counts		Filter	105	550	tss	tvss	tfss	fs/ts
	0.42	0.95	PCA	3.80E+08	0.19	0.3243	0.2549	0.1343	0.0894	0.0649	0.48
	0.61	1.38	bbhn	2.20E+08	0.1906	0.3499	0.2556	0.1593	0.0943	0.065	0.41
	0.52	1.18			0.1908	0.3801	0.2681	0.1893	0.112	0.0773	0.41
	0.52	1.17									

	oil/ts	oil/tfs	bacterial counts		filter	105	550	tss	tvss	tfss	fs/ts
	0.71	1.56	PCA	9.15E+07	0.1903	0.3785	0.2694	0.1882	0.1091	0.0791	0.42
	0.56	1.24	BBHN	2.00E+07	0.1912	0.3539	0.2676	0.1627	0.0863	0.0764	0.47
	0.60	1.32			0.191	0.366	0.2726	0.175	0.0934	0.0816	0.47
	0.62	1.37									

	oil/ts	oil/tfs	bacterial counts		filter	105	550	tss	tvss	tfss	fs/ts
	1.21	2.49	PCA	3.51E+09	0.191	0.3671	0.2754	0.1761	0.0917	0.0844	0.48
	1.30	2.69	BBHN	2.33E+09	0.1886	0.3272	0.2591	0.1386	0.0681	0.0705	0.51
	1.15	2.37			0.1918	0.3652	0.2742	0.1734	0.091	0.0824	0.48
	1.22	2.52									

	oil/ts	oil/tfs	bacterial counts		filter	105	550	tss	tvss	tfss	fs/ts
	0.58	1.09	PCA	1.43E+09	0.1918	0.3471	0.2849	0.1553	0.0622	0.0931	0.60
	0.40	0.75	BBHN	9.20E+08	0.1939	0.3363	0.2764	0.1424	0.0599	0.0825	0.58
	0.45	0.85			0.1922	0.34415	0.279	0.15195	0.06515	0.0868	0.57
	0.48	0.90									

	oil/ts	oil/tfs	bacterial counts		filter	105	550	tss	tvss	tfss	fs/ts
	0.34	0.17	PCA	2.83E+09	0.1913	0.3486	0.2769	0.1573	0.0717	0.0856	0.54
	0.30	0.17	BBHN	9.75E+08	0.1902	0.3487	0.2763	0.1585	0.0724	0.0861	0.54
	0.39	0.22			0.1915	0.3524	0.2835	0.1609	0.0689	0.092	0.57
	0.34	0.19									

	oil/ts	oil/tfs	bacterial counts		filter	105	550	tss	tvss	tfss	fs/ts
	0.13	0.19	PCA	2.29E+09	0.1903	0.276	0.2514	0.0857	0.0246	0.0611	0.71
	0.12	0.17	BBHN	6.50E+08	0.1937	0.2868	0.2592	0.0931	0.0276	0.0655	0.70
	0.12	0.18			0.1921	0.2824	0.2545	0.0903	0.0279	0.0624	0.69
	0.12	0.18									

	oil/ts	oil/tfs	bacterial counts		filter	105	550	tss	tvss	tfss	fs/ts
	0.26	0.40	PCA	5.04E+09	0.1913	0.2299	0.216	0.0386	0.0139	0.0247	0.64
	0.22	0.35	BBHN	2.13E+09	0.1897	0.232	0.2163	0.0423	0.0157	0.0266	0.63
	0.22	0.35			0.1919	0.2325	0.2165	0.0406	0.016	0.0246	0.61
	0.24	0.37									

	oil/ts	oil/tfs	bacterial counts		filter	105	550	tss	tvss	tfss	fs/ts
	0.19	0.34	not done		0.1922	0.2132	0.2011	0.021	0.0121	0.0089	0.42
	0.16	0.30			0.1933	0.2135	0.2032	0.0202	0.0103	0.0099	0.49
	0.14	0.26			0.1933	0.2153	0.2026	0.022	0.0127	0.0093	0.42
	0.16	0.30									

	oil/ts	oil/tfs	bacterial counts		filter	105	550	tss	tvss	tfss	fs/ts
	0.05	0.09	PCA	5.47E+08	0.1902	0.2143	0.2016	0.0241	0.0127	0.0114	0.47
	0.06	0.10	BBHN		0.1949	0.2193	0.2066	0.0244	0.0127	0.0117	0.48
	0.07	0.13			0.192	0.2168	0.2041	0.0248	0.0127	0.0121	0.49
	0.06	0.10									

	oil/ts	oil/tfs	bacterial counts		filter	105	550	tss	tvss	tfss	fs/ts
	0.05		PCA	6.20E+08	0.1924	0.2154	0.2038	0.023	0.0116	0.0114	0.50
	0.06		BBHN		0.1904	0.2153	0.2031	0.0249	0.0122	0.0127	0.51
	0.05				0.192	0.214	0.2031	0.022	0.0109	0.0111	0.50
	0.05										

APPENDIX L

BSTR RUN 1												
day	0	3	7	12	17	23	28	37	44	51	58	65
ts 1	0.2008	0.4034	0.3955	0.3787	0.1976	0.3684	0.5277	0.234	0.128	0.0847	0.0957	0.0983
2	0.1757	0.4125	0.4672	0.4218	0.1952	0.4391	0.4542	0.2378	0.1259	0.0932	0.0885	0.0829
3	0.1691	0.3811	0.4964	0.4011	0.1927	0.4216	0.4283	0.2452	0.1178	0.0893	0.0916	0.1029
avg ts	0.1819	0.3990	0.4530	0.4005	0.1952	0.4097	0.4701	0.2390	0.1239	0.0891	0.0919	0.0947
std dev	0.0167	0.0162	0.0519	0.0216	0.0025	0.0368	0.0516	0.0057	0.0054	0.0043	0.0036	0.0105
std dev %	9.19	4.05	11.46	5.38	1.26	8.99	10.97	2.38	4.35	4.78	3.93	11.06
ts/l	18.19	39.90	45.30	40.05	19.52	40.97	47.01	23.90	12.39	8.91	9.19	9.47
tvs 1	0.0515	0.2249	0.2036	0.2032	0.1013	0.1608	0.2972	0.074	0.0458	0.0387	0.0403	0.0396
2	0.0496	0.2295	0.243	0.2431	0.097	0.209	0.2237	0.0748	0.0459	0.0424	0.038	0.0326
3	0.0466	0.2228	0.3161	0.2101	0.1035	0.2066	0.2205	0.0767	0.0417	0.0407	0.0404	0.0406
avg tvs	0.0492	0.2257	0.2542	0.2188	0.1006	0.1921	0.2471	0.0752	0.0445	0.0406	0.0396	0.0376
std dev	0.0025	0.0034	0.0571	0.0213	0.0033	0.0272	0.0434	0.0014	0.0024	0.0019	0.0014	0.0044
std dev %	5.02	1.52	22.45	9.75	3.29	14.14	17.56	1.85	5.39	4.56	3.43	11.59
tvs/l	4.92	22.57	25.42	21.88	10.06	19.21	24.71	7.52	4.45	4.06	3.96	3.76
tfs 1	0.1493	0.1785	0.1919	0.1755	0.0963	0.2076	0.2305	0.16	0.0822	0.046	0.0554	0.0587
2	0.1261	0.183	0.2242	0.1787	0.0982	0.2301	0.2091	0.163	0.08	0.0508	0.0505	0.0503
3	0.1225	0.1583	0.1803	0.191	0.0892	0.215	0.2078	0.1685	0.0761	0.0486	0.0512	0.0623
avg tfs	0.1326	0.1733	0.1988	0.1817	0.0946	0.2176	0.2158	0.1638	0.0794	0.0485	0.0524	0.0571
std dev	0.0145	0.0132	0.0227	0.0082	0.0047	0.0115	0.0127	0.0043	0.0031	0.0024	0.0027	0.0062
std dev %	10.97	7.59	11.44	4.50	5.02	5.27	5.91	2.63	3.89	4.96	5.06	10.78
tfs/l	13.26	17.33	19.88	18.17	9.46	21.76	21.58	16.38	7.94	4.85	5.24	5.71
fs/ts												
1	0.74	0.44	0.49	0.46	0.49	0.56	0.44	0.68	0.64	0.54	0.58	0.6
2	0.72	0.44	0.48	0.42	0.5	0.52	0.46	0.69	0.64	0.55	0.57	0.61
3	0.72	0.42	0.36	0.48	0.46	0.51	0.49	0.69	0.65	0.54	0.56	0.6
avg fs/ts	0.73	0.43	0.44	0.45	0.48	0.53	0.46	0.69	0.64	0.54	0.57	0.6
vs/ts												
1	0.26	0.56	0.51	0.54	0.51	0.44	0.56	0.32	0.36	0.46	0.42	0.4
2	0.28	0.56	0.52	0.58	0.5	0.48	0.54	0.31	0.36	0.45	0.43	0.39
3	0.28	0.58	0.64	0.52	0.54	0.49	0.51	0.31	0.35	0.46	0.44	0.39
avg vs/ts	0.27	0.57	0.56	0.55	0.52	0.47	0.54	0.31	0.36	0.46	0.43	0.4
O&G			day	0	5	11	16	25	32	39	46	53
1	0.1413	0.1512	0.1898	0.2843	0.2353	0.2375	0.1605	0.0318	0.0115	0.0165	0.0045	0.0052
2	0.1384	0.1845	0.2745	0.225	0.2545	0.164	0.1433	0.0278	0.0077	0.0143	0.0053	0.0057
3	0.069	0.1977	0.2345	0.2402	0.2241	0.1844	0.1823	0.0296	0.0097	0.0127	0.0066	0.0046
avg O&G	0.1162	0.1778	0.2329	0.2498	0.2379	0.1953	0.162	0.0297	0.0096	0.0145	0.0055	0.0052
std dev	0.0409	0.0240	0.0424	0.0308	0.0154	0.0379	0.0195	0.0020	0.0019	0.0019	0.0011	0.0006
std dev %	35.22	13.48	18.19	12.33	6.46	19.43	12.06	6.75	19.80	13.16	19.27	10.59
O&Gg/l	11.62	17.78	23.29	24.98	23.79	19.53	16.2	2.97	0.96	1.45	0.55	0.52
cum oil loss			cum oil loss	0	0.0119	0.0545	0.0878	0.2201	0.2402	0.2353	0.2414	0.2434
avg o&g/ts	0.64	0.4456	0.5141	0.6237	1.2188	0.4767	0.3447	0.1243	0.0775	0.1627	0.0598	0.0549
avg o&g/fs	1.026	1.026	1.1715	1.3748	2.5148	0.8975	0.7507	0.1813	0.1209	0.299	0.105	0.0912
tss 1	0.0698	0.2541	0.1343	0.1882	0.1761	0.1553	0.1573	0.0857	0.0386	0.021	0.0241	0.0116
2	0.1107	0.1882	0.1593	0.1627	0.1386	0.1424	0.1585	0.0931	0.0423	0.0202	0.0244	0.0122
3	0.0926	0.1578	0.1893	0.175	0.1734	0.1493	0.1609	0.0903	0.0406	0.022	0.0248	0.0109
avg tss	0.091	0.2	0.161	0.1753	0.1627	0.149	0.1589	0.0897	0.0405	0.0211	0.0244	0.0116
std dev	0.0205	0.0492	0.0275	0.0128	0.0209	0.0065	0.0018	0.0037	0.0019	0.0009	0.0004	0.0007
std dev %	22.52	24.61	17.10	7.27	12.85	4.33	1.15	4.17	4.57	4.27	1.44	5.61
tss/l	9.10	20.00	16.10	17.53	16.27	14.90	15.89	8.97	4.05	2.11	2.44	1.16
tvss 1	0.02	0.174	0.0694	0.1091	0.0917	0.0622	0.0717	0.0246	0.0139	0.0121	0.0127	0.0116
2	0.0261	0.1155	0.0943	0.0863	0.0681	0.0599	0.0724	0.0276	0.0157	0.0103	0.0127	0.0122
3	0.0257	0.0943	0.112	0.0934	0.091	0.0625	0.0689	0.0279	0.016	0.0127	0.0127	0.0109
avg tsvs	0.0239	0.1279	0.0919	0.0963	0.0836	0.0615	0.071	0.0267	0.0152	0.0117	0.0127	0.0116
std dev	0.0034	0.0413	0.0214	0.0117	0.0134	0.0014	0.0019	0.0018	0.0011	0.0012	0.0000	0.0007
std dev %	14.28	32.27	23.29	12.12	16.06	2.31	2.61	6.83	7.47	10.68	0.00	5.61
tsvs/l	2.39	12.79	9.19	9.63	8.36	6.15	7.10	2.67	1.52	1.17	1.27	1.16
tfss 1	0.0498	0.0801	0.0791	0.0844	0.0931	0.0856	0.0856	0.0611	0.0247	0.0089	0.0114	0.0114
2	0.0846	0.0727	0.0764	0.0705	0.0825	0.0861	0.0861	0.0655	0.0266	0.0099	0.0117	0.0127
3	0.0669	0.0773	0.0816	0.0824	0.0868	0.092	0.092	0.0624	0.0246	0.0093	0.0121	0.0111
avg tsfs	0.0671	0.0767	0.079	0.0791	0.0875	0.0879	0.0879	0.063	0.0253	0.0094	0.01173	0.01173
std dev	0.0174	0.0037	0.0026	0.0075	0.0053	0.0036	0.0036	0.0023	0.0011	0.0005	0.0004	0.0009
std dev %	25.93	4.87	3.29	9.50	6.09	4.05	4.05	3.59	4.45	5.35	2.99	7.25
tsfs/l	6.71	7.67	7.90	7.91	8.75	8.79	8.79	6.30	2.53	0.94	1.17	1.17
ssfs/tss												
1	0.7135	0.3152	0.4832	0.4203	0.4793	0.5995	0.5442	0.713	0.6399	0.4238	0.473	0.4957
2	0.7642	0.3863	0.408	0.4696	0.5087	0.5794	0.5432	0.7035	0.6288	0.4901	0.48	0.51
3	0.7225	0.4011	0.4083	0.4663	0.4752	0.5712	0.5718	0.691	0.6059	0.4227	0.4879	0.5045
avg	0.73	0.37	0.43	0.45	0.49	0.58	0.55	0.70	0.62	0.45	0.48	0.50
dis solids	9.09	19.90	29.20	22.52	3.25	26.07	31.12	14.93	8.34	6.80	6.75	8.31
per liter												
BACT #												
PCA	1.10E+09	1.08E+09	3.80E+08	9.15E+07	3.51E+09	1.43E+09	2.83E+09	2.29E+09	5.04E+09		5.47E+08	6.20E+08
BBHN	3.00E+07	1.32E+07	2.20E+08	2.00E+07	2.33E+09	9.20E+08	9.75E+08	6.50E+08	2.13E+09		6.10E+08	5.73E+08
TOC												
filterate	273	587	941	992	473	572	849	878	883.68	1090	962	1052
ML	1,140	9,125	10,310	7,615	11,885	8,560	7,885	3,296	1,545	957	1,115	991

Calculations for TC of Mixed Liquor of Reactors sampled by 'boat' of TOC machine
and TC and IC for filterate or dissolved solids in mixed liquor

Reactor - BASIC BSTR

DAY	DATE	ML TC	Std dev	CF	DF	Total TC ML	Vol Filt.	Dilution	DF	CF	TC filterate	std dev	Total TC	IC filtrate	Total IC	TOC Filterate
		(a)		(b)		(a x bxc)			Vol filt/dil.		(y)		(y x DF x CF)	(z)	(z x DF x CF)	(TC - IC)
day 0	29-Aug	227.9	*6.942*	1.386	5	1579	15	**66**	4.40	1.071	**64.01**	***1.006***	**301.6**	**0.758**	**3.6**	298.1
day 3	1-Sep	1825	*12.62*	1.386	5	12647	15	**119**	7.93	1.071	**76.36**	***1.144***	**648.8**	**3.204**	**27.2**	621.6
day 7	5-Sep	2062	*8.153*	1.386	5	14290	15	**60**	4.00	1.071	**236.9**	***0.802***	**1014.9**	**1.642**	**7.0**	1007.8
day 12	10-Sep	1523	*1.106*	1.386	5	10554	15	**46**	3.07	1.071	**327.5**	***0.847***	**1075.6**	**3.412**	**11.2**	1064.4
day 17	15-Sep	2377	*13.58*	1.347	5	16009	15	**45**	3.00	1.071	**161.2**	**0.123**	**517.9**	**3.375**	**10.8**	507.1
day 23	21-Sep	1712	*6.123*	1.347	5	11530	15	**60**	4.00	1.071	**144.8**	***0.984***	**620.3**	**1.753**	**7.5**	612.8
day 28	26-Sep	1577	*9.986*	1.347	5	10621	15	72	4.80	1.071	177	*0.992*	**909.9**	0	**0.0**	909.9
day 37	5-Oct	659.3	*6.638*	1.347	5	4440	15	**43**	2.87	1.071	**310**	***0.644***	**951.8**	**3.453**	**10.6**	941.2
day 44	12-Oct	230.9	*4.768*	1.347	5	1555	15	**59**	3.93	1.071	**251.5**	***0.246***	**1059.5**	**2.697**	**11.4**	1048.1
day 51	19-Oct	191.4	*1.172*	1.347	5	1289	15	**45**	3.00	1.071	**367.1**	***0.51***	**1179.5**	**3.798**	**12.2**	1167.3
day 58	26-Oct	223.1	*0.125*	1.347	5	1503	15	**38**	2.53	1.071	**395.3**	***0***	**1072.5**	**10.47**	**28.4**	1044.1
day 65	2-Nov	198.2	*1.878*	1.347	5	1335	15	**52**	**3.47**	**1.071**	**304**	***0.614***	**1128.7**	**10.47**	**38.9**	1089.8

BASIC BSTR MASS BALANCE

At start	Sludge at 1090 g wet weight				
Cruc	wsludge	at 105 C	sludge	dry sludge	DSF
42.0367	46.6088	45.1483	4.5721	3.1116	0.6806
47.3315	51.9275	50.4282	4.5960	3.0967	0.6738
43.1792	46.7272	45.5773	3.5480	2.3981	0.6759
				avg	0.6767
Sludge dry solids load is at 737.603 g Oil load, at 33.11 % O&G per dry weight, is at 224.22 g					

At end

BEAKER	wsludge	sludge	add sampled sludge		
266.9	1230.72	963.82	1006.3		
Cruc	wsludge	at 105 C	sludge	dry sludge	DSF
42.03	50.43	47.75	8.4	5.72	0.6810
47.32	56.97	53.79	9.65	6.47	0.6705
42.18	55.92	51.13	13.74	8.95	0.6514
				avg	0.6676
				total dry solids	671.8059

O&G settled solids			
sludge	dry sludge	oil	% oil
14.41	9.6201	1.88	19.5424
14.98	10.0006	1.93	19.2987
14.14	9.4399	1.83	19.3859
		avg	19.4090
		std dev	0.1235
		Total	130.3975

solids ML			
cruc	w dry solids	Volume	6895ml
44.9762	45.06	dry solids	solids g/l
41.18	41.26	0.0838	8.38
41.7149	41.79	0.08	8
		0.0751	7.51
		avg	7.9633
		TOTAL	55.16 g

O&G ML			
cruc	w oil		
38.8598	38.865	oil	oilg/l
27.1715	27.1772	0.0052	0.52
41.3975	41.4021	0.0057	0.57
		0.0046	0.46
		avg	0.5167
		std dev	0.0551
		Total	3.5626

APPENDIX M

AIRLIFT BSTR

day 0 pH 7.2

	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts
1	44.9771	45.0852		0.1081				
2	41.1904	41.3795		0.1891				
3	41.7144	41.8252		0.1108				
avg				0.138				

O&G	cruc wt	w oil	oil	oil/ts		bact#	1.00E+07	1.00E+08	1.00E+09	avg	control
1	20.3839	20.409	0.0251			PCA	123,130	13,13,9	1,1,1	1.15E+08	0,0,0
2	19.7993	19.8094	0.0101		28 days	BBHN	96,86	-		9.10E+07	0,1,0
3	20.0635	20.0743	0.0108								

	filter	105	550	tss	tvss	tfss	fs/ts	dissolved solids
0	0.1898	0.2513	0.2381	0.0615	0.0132	0.0483	0.79	0.0594
	0.1912	0.2745	0.2583	0.0833	0.0162	0.0671	0.81	
	0.1903	0.2754	0.2588	0.0851	0.0166	0.0685	0.80	
				0.0766	0.0153	0.0613		

day 2 pH 7.2

	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts
1	42.7488	42.8949		0.1461				
2	65.58	65.7		0.12				
3	36.8646	36.9773		0.1127				
avg				0.1263				

	cruc wt	w oil	oil	oil/ts		bact#	1.00E+07	1.00E+08	1.00E+09	avg	control
1	20.0115	20.0194	0.0079			PCA	TNTC	168,193,167	15,20,16	1.74E+09	0,0,0
2	20.3673	20.3749	0.0076		25 days	BBHN	TNTC	71,69,60	21,9,12	1.03E+09	0,0,0
3	20.2724	20.2747	0.0023								
			0.0059	0.05							

	filter	105	550	tss	tvss	tfss	fs/ts	dissolved solids
2	0.1913	0.307	0.2869	0.1157	0.0201	0.0956	0.83	0.0184
	0.1917	0.293	0.2739	0.1013	0.0191	0.0822	0.81	
	0.1923	0.2989	0.2786	0.1066	0.0203	0.0863	0.81	
				0.1079	0.0198	0.0880		

day 5 pH 7.2

	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts
1	44.9743	45.1166	45.092	0.1423	0.0246	0.1177		
2	41.1785	41.3157	41.2913	0.1372	0.0244	0.1128		
3	41.7136	41.8926	41.8624	0.179	0.0302	0.1488		
avg				0.1528	0.0264	0.1284		

	cruc wt	w oil	oil	oil/ts	mousse	bact#	1.00E+07	1.00E+08	1.00E+09	avg
1	20.2541	20.2588	0.0047		solids	PCA		102,85,103	10,11,12	1.03E+09
2	20.255	20.26	0.005		0.8537	BBHN		77,48,56	7,8,3	6.02E+08
3	20.7958	20.8003	0.0045							
			0.0047	0.03						

	Filter	105	550	tss	tvss	tfss	fs/ts	dissolved solids
5	0.1926	0.3141	0.2933	0.1215	0.0208	0.1007	0.83	0.0183
	0.1911	0.3518	0.3268	0.1607	0.025	0.1357	0.84	
	0.1928	0.3141	0.2928	0.1213	0.0213	0.1	0.82	
				0.1345	0.0224	0.1121		

day 9 ph 7.1

	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts
1	42.7456	42.8983	42.8722	0.1527	0.0261	0.1266		0.17
2	36.8586	37.0588	37.021	0.2002	0.0378	0.1624		0.19
3	65.5728	65.7374	65.7094	0.1646	0.028	0.1366		0.17
avg				0.1725	0.0306	0.1419		

	cruc wt	w oil	oil	oil/ts		bact#	1.00E+07	1.00E+08	1.00E+09	avg
1	20.7282	20.7361	0.0079			PCA		62,73,68	9,-,8	7.83E+08
2	19.9267	19.9341	0.0074		31 days	BBHN		25,32,28	3,1,2	2.59E+08
3	20.9681	20.9765	0.0084							
			0.0079	0.05						

	filter	105	550	tss	tvss	tfss	fs/ts	dissolved solids
9	0.1905	0.3402	0.3166	0.1497	0.0237	0.126	0.84	0.0322
	0.188	0.3221	0.2994	0.1341	0.0227	0.1114	0.83	
	0.1905	0.3276	0.3045	0.1371	0.0231	0.114	0.83	
				0.1403	0.0232	0.1171		

day 14 pH 7.0 temp 13

	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts
1	44.9758	45.1328	45.1065	0.157	0.0263	0.1307	0.83	0.17
2	41.1786	41.3521	41.3243	0.1736	0.0278	0.1457	0.84	0.16
3	41.7145	41.9145	41.8839	0.2	0.0306	0.1694	0.85	0.15
avg				0.1768	0.0282	0.1486	0.84	0.16

	cruc wt	w oil	oil	oil/ts		bact#	1.00E+07	1.00E+08	1.00E+09	avg
1	20.3328	20.3398	0.007			PCA		60,59,65	4,9,12	7.23E+08
2	20.1413	20.1476	0.0063		21 days	BBHN	oil	21,36,24		2.70E+08
3	19.6367	19.6431	0.0064							
			0.0066	0.04						

	filter		550	tss	tvss	tfss	fs/ts	dissolved solids
14	0.1894	0.3325	0.3086	0.1431	0.0239	0.1192	0.83	0.0216
	0.1924	0.3444	0.3198	0.152	0.0248	0.1272	0.84	
	0.1896	0.3602	0.333	0.1706	0.0272	0.1434	0.84	
				0.1552	0.0253	0.1299		

stopped run due to spill, top up sludge, restart.

restart

day 21 temp 15

	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts
1	42.7479	42.962	42.918	0.2141	0.044	0.1701	0.79	0.21
2	47.3319	47.576	47.5286	0.2441	0.0474	0.1967	0.81	0.19
3	36.869	37.0807	37.0362	0.2117	0.0445	0.1672	0.79	0.21
avg				0.2233	0.0453	0.178	0.80	0.20

	cruc wt	w oil	oil	oil/ts		bact#	1.00E+07	1.00E+08	1.00E+09	avg
1	20.5447	20.5832	0.0385			PCA		45,40,43	10,6,14	2.66E+08
2	19.8783	19.9169	0.0386			BBHN		lab accident		
3	20.0643	20.1022	0.0379							
			0.0383	0.17						

	filter	105	550	tss	tvss	tfss	fs/ts	dissolved solids
21	0.1918	0.3471	0.2849	0.1553	0.0622	0.0931	0.60	0.0743
	0.1939	0.3363	0.2764	0.1424	0.0599	0.0825	0.58	
	0.1922	0.3415	0.279	0.1493	0.0625	0.0868	0.58	
				0.1490	0.0615	0.0875		

mousse (5 m (5 mis)

day 24

	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts
1	44.9762	45.8377	45.1637	0.8615	0.674	0.1875	0.22	0.78
2	41.1885	41.8709	41.3664	0.6824	0.5045	0.1779	0.26	0.74
3	41.7141	42.4043	41.8987	0.6902	0.5056	0.1846	0.27	0.73
				0.7447	0.5614	0.1833	0.25	0.75

	cruc wt	w oil	oil	oil/ts
1	20.0048	20.5555	0.5509	
2	20.2957	20.7783	0.4826	
3	20.0764	20.5006	0.4242	
			0.4859	0.65

day 26 H 7 temp 22

	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts
1	42.0371	42.2946	42.2089	0.2575	0.0857	0.1718	0.67	0.33
2	43.18	43.4913	43.3978	0.3113	0.0935	0.2178	0.70	0.30
3	57.2386	57.5164	57.4274	0.2778	0.089	0.1888	0.68	0.32
				0.2822	0.0894	0.1928	0.68	0.32

	cruc wt	w oil	oil	oil/ts		bact#	1.00E+07	1.00E+08	1.00E+09	avg
1	20.2267	20.3503	0.1236			PCA		138,134,146	12,16,14	1.40E+09
2	20.2588	20.356	0.0972			BBHN		7,32,12		1.70E+08
3	20.5197	20.6369	0.1172							
			0.1127	0.40						

	filter	105	550	tss	tvss	tfss	fs/ts	dissolved solids
26	0.1896	0.4515	0.3805	0.2619	0.071	0.1909	0.73	0.0371
	0.192	0.4274	0.3585	0.2354	0.0689	0.1665	0.71	
	0.1906	0.4285	0.355	0.2379	0.0735	0.1644	0.69	
				0.2451	0.0711	0.1739		

obser-nature of foam changed, higher solids in ML...higher oil.

day 31 pH 7 temp 21 — foam layer less thick(less solid) sides of reactor clean. N & P checked not limiting.

	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts
1	44.975	45.1431	45.1003	0.1681	0.0428	0.1253	0.75	0.25
2	41.1795	41.3834	41.3315	0.2039	0.0519	0.152	0.75	0.25
3	41.7145	41.9155	41.8665	0.201	0.049	0.152	0.76	0.24
				0.191	0.0479	0.1431	0.75	0.25
foam	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts
	42.7485	42.8393	42.7731	0.0908	0.0662	0.0246	0.27	0.73

	cruc wt	w oil	oil	oil/ts		bact#	1.00E+07	1.00E+08	1.00E+09	avg
1	20.1273	20.152	0.0247			PCA		49,47,34	3,4,6	4.30E+08
2	20.4152	20.4343	0.0191			BBHN		17,17,18	4,2,0	1.85E+08
3	19.4986	19.5195	0.0209							
			0.0216	0.11						
	cruc wt	w oil	oil	oil/ts						
1	20.3416	20.3918	0.0502							
2	20.8757	20.975	0.0993							
			0.0748	0.82						

	filter	105	550	tss	tvss	tfss	fs/ts	dissolved solids
31	0.1925	0.3657	0.3246	0.1732	0.0411	0.1321	0.76	0.0313
	0.1902	0.3427	0.3058	0.1525	0.0369	0.1156	0.76	
	0.1902	0.3436	0.3069	0.1534	0.0367	0.1167	0.76	
				0.1597	0.0382	0.1215		

day 37 pH 7 temp 22 added 5N NaOH t0 ph 7.2

	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts
1	54.0452	54.3118	54.2388	0.2666	0.073	0.1936	0.73	0.27
2	46.0093	46.3136	46.2364	0.3043	0.0772	0.2271	0.75	0.25
3	45.7778	46.0406	45.9664	0.2628	0.0742	0.1886	0.72	0.28
				0.2779	0.0748	0.2031	0.73	0.27

	cruc wt	w oil	oil	oil/ts		bact#	1.00E+07	1.00E+08	1.00E+09	avg
1	20.5333	20.6056	0.0723			PCA		74,71,69	7,4,5	6.22E+08
2	20.8342	20.9055	0.0713			BBHN		5,6,-	4,2,1	1.45E+08
3	20.635	20.7068	0.0718							
			0.0718	0.26						

	filter	105	550	tss	tvss	tfss	fs/ts	dissolved solids
37	0.197	0.4861	0.4066	0.2891	0.0795	0.2096	0.73	0.0242
	0.1962	0.4321	0.369	0.2359	0.0631	0.1728	0.73	
	0.1953	0.4314	0.3646	0.2361	0.0668	0.1693	0.72	
				0.2537	0.0698	0.1839		

day 43 pH6.9 temp 22 Clingage put back in

	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts
1	65.5764	65.8723	65.7652	0.2959	0.1071	0.1888	0.64	0.36
2	67.7568	68.0664	67.9527	0.3098	0.1137	0.1961	0.63	0.37
3	57.2384	57.5266	57.4224	0.2882	0.1042	0.184	0.64	0.36
				0.298	0.1083	0.1896	0.64	0.36

	cruc wt	w oil	oil	oil/ts		bact#	1.00E+07	1.00E+08	1.00E+09	avg
1	20.4376	20.5879	0.1503			PCA		106,91,-	8,10,10	9.59E+08
2	20.2813	20.41	0.1287			BBHN		6,4,4	0,2,-	7.30E+07
3	19.8063	19.9394	0.1331							
			0.1374	0.46						

	filter	105	550	tss	tvss	tfss	fs/ts	dissolved solids
43	0.1943	0.4506	0.3635	0.2562	0.087	0.1692	0.66	0.0255
	0.1939	0.4684	0.3761	0.2745	0.0923	0.1822	0.66	
	0.1939	0.4807	0.3808	0.2868	0.0999	0.1869	0.65	
				0.2725	0.0931	0.1794		

day 48 ph 7.2 temp 20 mousse layer changed from last week. thick and does not break.

	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts
1	42.0358	42.209	42.168	0.1732	0.041	0.1322	0.76	0.24
2	47.3316	47.6612	47.572	0.3296	0.0892	0.2404	0.73	0.27
3	43.1808	43.4298	43.3607	0.249	0.0691	0.1799	0.72	0.28
				0.2693	0.0664	0.1842	0.74	0.26

	cruc wt	w oil	oil	oil/ts		bact#	1.00E+08	1.00E+09	1.00E+10	avg
1	19.9903	20.0918	0.1015			PCA	tntc	418,316,371	66,53,52	4.69E+10
2	20.3556	20.4651	0.1095			BBHN		146,160,155	11,7,15	1.30E+10
3	19.664	19.7644	0.1004							
			0.1038	0.36						

	filter	105	550	tss	tvss	tfss	fs/ts	dissolved solids
48	0.1963	0.4388	0.3749	0.2425	0.0639	0.1786	0.74	0.0233
	0.1951	0.4848	0.4094	0.2895	0.0752	0.2143	0.74	
	0.1954	0.896	0.5858	0.5006	0.1102	0.3904	0.78	
				0.2660	0.0831	0.2611		

day 52 pH 7 temp 21

	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts
1	54.0475	54.3097	54.2362	0.2622	0.0735	0.1887	0.72	0.28
2	46.0111	46.2689	46.1948	0.2578	0.0741	0.1837	0.71	0.29
3	45.7798	46.0624	45.9822	0.2826	0.0802	0.2024	0.72	0.28
				0.2675	0.0759	0.1916	0.72	0.28

	cruc wt	w oil	oil	oil/ts		bact#	1.00E+08	1.00E+09	1.00E+10	avg
1	19.8269	19.9038	0.0769			PCA		5,3,9	1,0,0	4.45E+08
2	20.3722	20.4448	0.0726			BBHN		4,1,1	0,0,0	2.00E+08
3	19.8961	19.9891	0.093	0.33						
			0.0808							

	filter	105	550	tss	tvss	tfss	fs/ts	dissolved solids
52	0.195	0.4174	0.3574	0.2224	0.06	0.1624	0.73	0.0155
	0.1919	0.4433	0.377	0.2514	0.0663	0.1851	0.74	
	0.1913	0.4737	0.3979	0.2824	0.0758	0.2066	0.73	
				0.2521	0.0674	0.1847		

day 58 pH 7.2 temp 21 not much mousse left

	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts
1	41.1813	41.4583	41.3958	0.277	0.0625	0.2145	0.77	0.23
2	41.7162	41.9858	41.9206	0.2696	0.0652	0.2044	0.76	0.24
3	42.7509	43.0654	42.9732	0.3145	0.0922	0.2223	0.71	0.29
				0.2870	0.0733	0.2137	0.75	0.25

	cruc wt	w oil	oil	oil/ts		bact#	1.00E+08	1.00E+09	1.00E+10	avg
1	20.5678	20.5983	0.0305			PCA		17,10,17	8,1,0	4.50E+09
2	20.3925	20.4286	0.0361			BBHN		5,0,1	2,0,0	2.00E+08
3	20.0875	20.1407	0.0532	0.17						
			0.0399							

	filter	105	550	tss	tvss	tfss	fs/ts	dissolved solids
58	0.1929	0.4939	0.4211	0.301	0.0728	0.2282	0.76	0.0307
	0.1937	0.4097	0.3613	0.216	0.0484	0.1676	0.78	
	0.1933	0.4452	0.3908	0.2519	0.0544	0.1975	0.78	
				0.2563	0.0585	0.1978		

day 62 pH 7.2 temp 21

	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts
1	42.0396	42.3039	42.2478	0.2643	0.0561	0.2082	0.79	0.21
2	47.3346	47.5129	47.4728	0.1783	0.0401	0.1382	0.78	0.22
3	43.1836	43.638	43.5259	0.4644	0.1121	0.3423	0.75	0.25
				0.2990	0.0694	0.2296	0.77	0.23

	cruc wt	w oil	oil	oil/ts		bact#	1.00E+07	1.00E+08	1.00E+09	avg
1	19.5619	19.5867	0.0248			PCA		68,73,69	2,11,6	6.60E+08
2	20.4769	20.5603	0.0834			BBHN		35,19,22	2,2,2	2.25E+08
3	20.5508	20.5773	0.0265	0.06						
			0.0257							

	filter	105	550	tss	tvss	tfss	fs/ts	dissolved solids
62	0.1934	0.4563	0.4025	0.2619	0.0528	0.2091	0.80	0.0002
	0.1932	0.4141	0.3676	0.2209	0.0465	0.1744	0.79	
	0.1921	0.6056	0.5012	0.4135	0.1044	0.3091	0.75	
				0.2986	0.0679	0.2309		

day 68 pH7.2 temp 20

	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts
1	65.5793	65.8428	65.7764	0.2635	0.0664	0.1971	0.75	0.25
2	67.76	67.968	67.9152	0.208	0.0528	0.1552	0.75	0.25
3	57.2411	57.5325	57.4459	0.2914	0.0866	0.2048	0.70	0.30
				0.2543	0.0686	0.1857	0.73	0.27

	cruc wt	w oil	oil	oil/ts		bact#	1.00E+07	1.00E+08	1.00E+09	avg
1	20.5611	20.5811	0.02			PCA		47,42,47	7,5	4.50E+08
2	19.9928	20.0178	0.025			BBHN				
3	20.0126	20.0348	0.0222	0.08						
			0.0257							

	filter	105	550	tss	tvss	tfss	fs/ts	dissolved solids
	0.1965	0.3879	0.3408	0.1914	0.0471	0.1443	0.75	0.2543
	0.1931	0.3878	0.3402	0.1947	0.0476	0.1471		
	0.183	0.3883	0.3347	0.2033	0.0516	0.1517		

APPENDIX M

AIRLIFT															
day	0	2	5	9	14	21	26	31	37	43	48	52	58	62	68
ts	0.1081	0.1461	0.1423	0.1527	0.157	0.2141	0.2575	0.1681	0.2666	0.2959	0.1732	0.2622	0.277	0.2643	0.2635
2	0.1891	0.12	0.1372	0.2002	0.1735	0.2441	0.3113	0.2039	0.3043	0.3098	0.3296	0.2578	0.2696	0.1783	0.208
3	0.1106	0.1127	0.179	0.1646	0.2	0.2117	0.2778	0.201	0.2628	0.2882	0.249	0.2826	0.3145	0.4544	0.2914
avg ts	0.1094	0.1263	0.1528	0.1725	0.1768	0.2233	0.2822	0.1910	0.2779	0.298	0.2693	0.2675	0.287	0.299	0.2543
std dev		0.0176	0.0228	0.0247	0.0217	0.0181	0.0272	0.0199	0.0229	0.0109		0.0132	0.0241	0.1413	0.0425
std dev %		13.90	14.92	14.33	12.27	8.08	9.63	10.41	8.26	3.67		4.95	8.39	47.25	16.69
ts/l	21.88	25.26	30.56	34.50	35.36	44.66	56.44	38.20	55.58	59.60	57.86	53.50	57.40	59.80	50.86
tvs 1			0.0246	0.0261	0.0263	0.044	0.0857	0.0428	0.073	0.1071	0.041	0.0735	0.0625	0.0561	0.0664
2			0.0244	0.0378	0.0278	0.0474	0.0935	0.0519	0.0772	0.1137	0.0892	0.0741	0.0652	0.0401	0.0528
3			0.0302	0.028	0.0306	0.0445	0.089	0.049	0.0742	0.1042	0.0691	0.0802	0.0922	0.1121	0.0866
avg tvs			0.0264	0.0306	0.0282	0.0453	0.0894	0.0479	0.0748	0.1083	0.0792	0.0759	0.0733	0.0694	0.0686
std dev			0.0033	0.0063	0.0022	0.0018	0.0039	0.0046	0.0022	0.0049		0.0037	0.0164	0.0378	0.0170
std dev %			12.47	20.52	7.74	4.05	4.38	9.70	2.89	4.50		4.88	22.41	54.48	24.79
tvs/l			5.28	6.12	5.64	9.08	17.88	9.58	14.96	21.66	15.84	15.18	14.66	13.88	13.72
tfs 1			0.1177	0.1266	0.1307	0.1701	0.1718	0.1253	0.1936	0.1888	0.1322	0.1887	0.2145	0.2082	0.1971
2			0.1128	0.1624	0.1457	0.1967	0.2178	0.152	0.2271	0.1961	0.2404	0.1837	0.2044	0.1382	0.1552
3			0.1488	0.1366	0.1694	0.1672	0.1888	0.152	0.1886	0.184	0.1799	0.2024	0.2223	0.3423	0.2048
avg tfs			0.1264	0.1419	0.1486	0.1780	0.1928	0.1431	0.2031	0.1896	0.2102	0.1916	0.2137	0.2296	0.1857
std dev			0.0195	0.0185	0.0195	0.0163	0.0233	0.0154	0.0209	0.0061		0.0097	0.0090	0.1037	0.0267
std dev %			15.45	13.02	13.13	9.13	12.06	10.77	10.31	3.21		5.05	4.20	45.17	14.37
tfs/l			25.28	28.38	29.72	35.60	38.56	28.62	40.62	37.92	42.04	38.32	42.74	45.92	37.14
fs/ts 1					0.83	0.79	0.67	0.75	0.73	0.64	0.76	0.72	0.77	0.79	0.75
2					0.84	0.81	0.7	0.75	0.75	0.63	0.73	0.71	0.76	0.78	0.75
3					0.85	0.79	0.68	0.76	0.72	0.64	0.72	0.72	0.71	0.75	0.7
avg fs/ts					0.84	0.80	0.68	0.75	0.73	0.64	0.74	0.72	0.75	0.77	0.73
vs/ts 1				0.17	0.17	0.21	0.33	0.26	0.27	0.36	0.24	0.28	0.23	0.21	0.25
2				0.19	0.16	0.19	0.3	0.25	0.25	0.37	0.27	0.29	0.24	0.22	0.25
3				0.17	0.15	0.21	0.32	0.24	0.28	0.36	0.28	0.28	0.29	0.25	0.3
avg vs/ts					0.16	0.20	0.32	0.25	0.27	0.36	0.26	0.28	0.25	0.23	0.27
day	0	2	5	9	14	21	26	31	37	43	48	52	58	62	68
O&G						restart	foam changed	slides clean		change in	mousse thick		no mousse		
1	0.0251	0.0079	0.0047	0.0079	0.007	0.0385	0.1236	0.0247	0.0723	0.1503	0.1015	0.0769	0.0305	0.0248	0.02
2	0.0101	0.0076	0.005	0.0074	0.0063	0.0386	0.0972	0.0191	0.0713	0.1287	0.1095	0.0726	0.0361		0.025
3	0.0108	0.0023	0.0045	0.0084	0.0064	0.0379	0.1172	0.0209	0.0718	0.1331	0.1004	0.093	0.0532	0.0265	0.0222
avg O&G	0.0153	0.0059	0.0047	0.0079	0.0066	0.0383	0.1127	0.0216	0.0718	0.1374	0.1038	0.0808	0.0399	0.0257	0.0224
std dev	0.0085	0.0032	0.0003	0.0005	0.0004	0.0004	0.0138	0.0029	0.0005	0.0114	0.0050	0.0108	0.0118	0.0012	0.0025
std dev %	55.33	53.39	5.35	6.33	5.74	0.99	12.22	13.24	0.70	8.31	4.79	13.31	29.64	4.68	11.19
per l	1.53	0.59	0.47	0.79	0.66	3.83	11.27	2.16	7.18	13.74	10.38	8.08	3.99	2.57	2.24
cum oil loss		0.94	1.06	0.74	0.87			9.11	4.09		3.36	5.66	9.75	11.17	11.5
oil loss		0.94	0.12	-0.32	0.13		-7.44	9.11	5.02		3.36	2.3	4.09	1.42	0.33
avg o&g/ts	0.1399	0.0467	0.0308	0.0458	0.0373	0.1715	0.3994	0.1131	0.2584	0.4611	0.3588	0.3021	0.1390	0.0860	0.0881
avg o&g/fs			0.0372	0.0557	0.0444	0.2152	0.5845	0.1509	0.3535	0.7247	0.4938	0.4217	0.1867	0.1119	0.1206
foam							day 24	day 31							
ts							0.7447	0.0908							
tvs							0.5614	0.0662							
tfs							0.1833	0.0246							
oil							0.4859	0.0748							
tss	0	2	5	9	14	21	26	31	37	43	48	52	58	62	68
1	0.0615	0.1157	0.1215	0.1497	0.1431	0.1553	0.2619	0.1732	0.2891	0.2562	0.2425	0.2224	0.301	0.2619	0.1914
2	0.0833	0.1013	0.1607	0.1341	0.152	0.1424	0.2354	0.1525	0.2359	0.2745	0.2895	0.2895	0.216	0.2209	0.1947
3	0.0851	0.1066	0.1213	0.1371	0.1706	0.1493	0.2379	0.1534	0.2361	0.2868	0.5006	0.2824	0.2519	0.4135	0.2033
avg tss	0.0766	0.1079	0.1345	0.1403	0.1552	0.149	0.2451	0.1597	0.2537	0.2725	0.266	0.2521	0.2563	0.2414	0.1965
std dev	0.0131	0.0073	0.0227	0.0083	0.0140	0.0065	0.0146	0.0117	0.0307	0.0154		0.0369	0.0427	0.1015	0.0061
std dev %	17.15	6.75	16.87	5.90	9.04	4.33	5.97	7.33	12.08	5.65		14.62	16.65	42.03	3.13
TSS/l	15.32	21.58	26.9	28.06	31.04	29.8	49.02	31.94	50.74	54.5	53.2	50.42	51.26	48.28	39.3
tvss	0.0132	0.0201	0.0208	0.0237	0.0239	0.0622	0.071	0.0411	0.0795	0.087	0.0639	0.06	0.0728	0.0528	0.0471
2	0.0162	0.0191	0.025	0.0227	0.0248	0.0599	0.0689	0.0369	0.0631	0.0923	0.0752	0.0663	0.0484	0.0465	0.0476
3	0.0166	0.0203	0.0213	0.0231	0.0272	0.0625	0.0735	0.0367	0.0668	0.0999	0.1102	0.0758	0.0544	0.1044	0.0516
avg tvss	0.0153	0.0198	0.0224	0.0232	0.0253	0.0615	0.0711	0.0382	0.0698	0.0931	0.07	0.0674	0.0585	0.0497	0.0488
std dev	0.0019	0.0006	0.0023	0.0005	0.0017	0.0014	0.0023	0.0025	0.0086	0.0065		0.0080	0.0127		0.0025
std dev %	12.15	3.25	10.24	2.17	6.74	2.31	3.24	6.50	12.32	6.96		11.80	21.73		5.05
TVSS/l	3.06	3.96	4.48	4.64	5.06	12.3	14.22	7.64	13.96	18.62	14	13.48	11.7	9.94	9.76
tfss	0.0483	0.0956	0.1007	0.126	0.1192	0.0931	0.1909	0.1321	0.2096	0.1692	0.1786	0.1624	0.2282	0.2091	0.1443
2	0.0671	0.0822	0.1357	0.1114	0.1272	0.0825	0.1665	0.1156	0.1728	0.1822	0.2143	0.1851	0.1676	0.1744	0.1471
3	0.0685	0.0863	0.1	0.114	0.1434	0.0868	0.1644	0.1167	0.1693	0.1869	0.3904	0.2066	0.1975	0.3091	0.1517
avg tfss	0.0613	0.088	0.1121	0.1171	0.1299	0.0875	0.1739	0.1216	0.1839	0.1794	0.1965	0.1847	0.1978	0.1918	0.1477
std dev	0.0113	0.0069	0.0204	0.0078	0.0123	0.0053	0.0147	0.0092	0.0223	0.0092		0.0221	0.0303		0.0037
std dev %	18.40	7.80	18.21	6.65	9.49	6.09	8.47	7.59	12.14	5.11		11.97	15.32		2.53
TFSS/l	12.26	17.6	22.42	23.42	25.98	17.5	34.78	24.3	36.78	35.88	39.3	36.94	39.56	38.36	29.54
diss solids	0.0328	0.0184	0.0183	0.0322	0.0216	0.0743	0.0371	0.0313	0.0242	0.0255	0.0233	0.0154	0.0307	0.0576	0.0578
p/l	6.56	3.68	3.66	6.44	4.32	14.86	7.42	6.26	4.84	5.1	4.66	3.08	6.14	11.52	11.56
DAYS	0	2	5	9	14	21	26	31	37	43	48	52	58	62	68
PCA	1.15E+08	1.74E+09	1.03E+09	7.63E+08	7.23E+08	2.65E+08	1.40E+09	4.30E+08	6.22E+08	9.59E+08	4.69E+10	5.80E+08	4.50E+09	8.60E+08	4.50E+08
BBH	9.10E+07	1.03E+09	6.02E+08	2.59E+08	2.70E+08		1.70E+08	1.85E+08	1.45E+08	7.30E+07	1.30E+10	2.00E+08	2.00E+08	2.25E+08	
filterate															
TC	605	547	371	401	258	474	311	368	451	378	472	562	631	844	1036
IC	25.2	11.2	10.0	8.9	8.9	6.9	3.8	3.3	0.0	50.7	19.7	27.0	8.3	17.4	33.7
TOC	580	536	361	392	251	467	308	365	451	328	453	535	623	827	1002
mf TC	3744	2754	3179	3737	3805	8126	24,242	11,864	21,489	12,823	34,969	11,828	9132	9781	10,007

Sample calculations for TC of Mixed Liquor of Reactors sampled by 'boat' of TOC machine
and TC and IC for filterate or dissolved solids in mixed liquor

Reactor -Airlift BSTR (of 3-5 samples)

		ML TC	Std dev	CF	Total TC ML	Vol Filt.	Dilution	DF	CF	TC filterate	std dev	Total TC	IC filtrate	Total IC	TOC Filterate
		(a)		(b)	(a x b)			Vol filt/dil.		(y)		(y x DF x CF)	(z)	(z x DF x CF)	(TC - IC)
day 0	27-Apr	1356	*3.13*	2.761	3744	15	75	5.00	1.11	109	*2.438*	605.0	4.546	25.2	579.7
day 2	29-Apr	1145	*5.702*	2.405	2754	15	72	4.80	1.11	102.6	*0.426*	546.7	2.094	11.2	535.5
day 5	2-May	1322	*1.72*	2.405	3179	15	76	5.1	1.128	64.87	*2.968*	370.7	1.743	10.0	360.8
day 9	6-May	1554	*2.355*	2.405	3737	15	69	4.6	1.128	77.2	*0.736*	400.6	1.719	8.9	391.7
day 14	11-May	1582	*0.968*	2.405	3805	15	77	5.1	1.128	44.55	*0.52*	258.0	1.2	6.9	251.0
day 21	18-May	3379	*1.136*	2.405	8126	15	94	6.3	1.128	66.99	*0.778*	473.5	0.977	6.9	466.6
day 26	23-May	10080	*5.075*	2.405	24242	15	68	4.5	1.128	60.89	*1.634*	311.4	0.745	3.8	307.6
day 31	28-May	4933	*0.572*	2.405	11864	15	75	5.0	1.128	65.27	*0.355*	368.1	0.588	3.3	364.8
day 37	3-Jun	8927	*0.642*	2.405	21469	15	62	4.1	1.128	*96.8*	*0.483*	451.3	0		451.3
day 43	9-Jun	5332	*5.806*	2.405	12823	15	85	5.7	0.917	72.81	*0.822*	378.3	9.757	50.7	327.6
day 48	14-Jun	14540	*3.025*	2.405	34969	15	70	4.7	0.917	110.4	*1.327*	472.4	4.603	19.7	452.7
day 52	18-Jun	4918	*3.275*	2.405	11828	15	78	5.2	0.917	117.9	*1.02*	562.2	5.662	27.0	535.2
day 58	24-Jun	3797	*3.854*	2.405	9132	15	67	4.5	0.917	154	*0.199*	630.8	2.026	8.3	622.5
day 62	28-Jun	4067	*0.767*	2.405	9781	15	80	5.3	0.917	172.6	*0.662*	844.1	3.565	17.4	826.7
day 68	4-Jul	4161	*0.965*	2.405	10007	15	90	6.0	0.917	188.3	*0.987*	1036.0	6.129	33.7	1002.3

APPENDIX M

Airlift spreadsheet at start and end

At start	Sludge at 493.3 g wet weight				
Cruc	wsludge	at 105 C	sludge	dry sludge	DSF
44.9700	50.6100	49.2100	5.6400	4.2400	0.7518
41.1800	46.6800	45.3000	5.5000	4.1200	0.7491
41.7100	46.1800	45.0700	4.4700	3.3600	0.7517
				avg	0.7508

Sludge dry solids load is at 370.1937 g

Wet sludge	dry sludge	oil	O&G p dry wt
12.0500	9.0483	3.0859	34.1000
10.4100	7.8169	2.6277	33.6400
10.9900	8.2524	2.7296	33.0800
		avg	33.6100
		std dev	0.5108

Oil load, at 33.61 % O&G per dry weight, is at 124.4221g

at end
bottom solids

BEAKER	wsludge	sludge			
455.25	1022.06	566.75			
Cruc	wsludge	at 105 C	sludge	dry sludge	DSF
62.6042	66.4438	64.1757	3.8396	1.5715	0.4093
41.7176	45.5941	43.2843	3.8765	1.5667	0.4042
42.7526	46.8833	44.3956	4.1307	1.643	0.3978
				avg	0.4037
			total dry solids		228.797

O&G settled solids			
sludge	dry sludge	oil	% oil
9.9986	4.0374	0.4576	11.33
9.9641	4.0235	0.3949	9.81
10.1461	4.097	0.427	10.42
		avg	10.52
		std dev	0.7647
		Total	24.0694

Clingage

BEAKER	wsludge	sludge			
118.83	203.73	84.9			
Cruc	wsludge	at 105 C	sludge	dry sludge	DSF
58.1624	64.5058	61.4843	6.3434	3.3219	0.5237
70.4528	73.8782	72.2243	3.4254	1.7715	0.5172
61.6858	68.2297	65.0702	6.5439	3.3844	0.5172
				avg	0.5193
			total dry solids		44.0886

O&G clingage			
sludge	dry sludge	oil	% oil
4.9579	2.5746	1.3087	50.83
5.0027	2.5979	1.3058	50.26
5.0555	2.6253	1.3262	50.52
		avg	50.54
		std dev	0.28
		Total	22.2824

solids ML		Volume	2.25	O&G ML			
cruc	w dry solids	dry solids	solids g/l	cruc	w oil	oil	oilg/l
42.0375	42.1195	0.082	8.2	20.6632	20.6748	0.0116	1.16
47.3331	47.4197	0.0866	8.66	20.3424	20.3523	0.0099	0.99
43.1824	43.2647	0.0823	8.23	20.4229	20.4358	0.0129	1.29
		avg	8.3633			0.0115	1.1467
		TOTAL	18.8174			std dev	0.1504
						Total	2.5875

APPENDIX N

	BSTR RUN 2 WITH INIPOL																								
day 0	9.8.96	No foam buildup. Globules of sludge on top.					pH 7.2	temp 23 C		O&G															
	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil	oil/ts	oil/tfs	bacterial counts				filter	105	550	tss	tvss	tfss	fs/ts
1	65.5798	65.7248	65.6564	0.1450	0.0684	0.0766	0.53	0.47	1	20.1619	20.2098	0.0479	0.32	0.62		1.00E+08	1.00E+09	avg	0.1841	0.2317	0.2077	0.0476	0.0240	0.0236	0.50
2	61.6842	61.7994	61.7469	0.1152	0.0525	0.0627	0.54	0.46	2	20.5313	20.6387	0.1074	0.23	0.43	PCA	117,113,103	18,13,15	1.32E+09	0.1909	0.2542	0.2210	0.0633	0.0332	0.0301	0.48
3	62.6057	62.7896	62.6989	0.1839	0.0907	0.0932	0.51	0.49	3	20.1222	20.1767	0.0545	0.37	0.70	bbhn	lab acc			0.1917	0.2340	0.2117	0.0423	0.0223	0.0200	0.47
avg				0.1480	0.0705	0.0775	0.52	0.48					0.31	0.58								0.0511	0.0265	0.0246	0.48
day 4	13.8.96	globules of oil on top. no foam, oily layer floating.					pH 7.2	temp 20.5 C																	
	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil	oil/ts	oil/tfs					filter	105	550	tss	tvss	tfss	fs/ts
1	48.8218	49.2820	49.0254	0.4602	0.2566	0.2036	0.44	0.56	1	20.4406	20.6587	0.2181	0.42	0.89	bacterial counts				0.1917	0.3623	0.2679	0.1706	0.0944	0.0762	0.45
2	49.7068	50.3475	50.0188	0.6407	0.3287	0.3120	0.49	0.51	2	20.4122	20.5927	0.1805	0.34	0.74		1.00E+08	1.00E+09	avg	0.1917	0.3878	0.2777	0.1961	0.1101	0.086	0.44
3	47.6243	48.0979	47.8422	0.4736	0.2557	0.2179	0.46	0.54	3	19.4795	19.6549	0.1754	0.33	0.72	PCA	347,365	65,67,61	4.97E+09	0.1918	0.3713	0.2697	0.1795	0.1016	0.0779	0.43
avg				0.5248	0.2803	0.2445	0.47	0.54				0.1913	0.36	1.02	bbhn	72,85	9,6,7	7.58E+08				0.1821	0.1020	0.0800	0.44
day 9	18.8.96						pH 7.2	temp 22																	
	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil	oil/ts	oil/tfs					Filter	105	550	tss	tvss	tfss	fs/ts
1	50.4440	50.9055	50.6466	0.4615	0.2589	0.2026	0.44	0.56	1	20.0599	20.2250	0.1651	0.35	0.77	bacterial counts				0.1897	0.3998	0.2867	0.2101	0.1131	0.097	0.46
2	47.0380	47.5177	47.2588	0.4797	0.2589	0.2208	0.46	0.54	2	20.4995	20.6848	0.1853	0.40	0.87		1.00E+08	1.00E+09	avg	0.1887	0.3900	0.2822	0.2013	0.1078	0.0935	0.46
3	44.3636	44.8278	44.5800	0.4642	0.2478	0.2164	0.47	0.53	3	19.9796	20.1741	0.1945	0.42	0.91	PCA	281,289,279	28,23,18	2.53E+09	0.1918	0.3884	0.2829	0.1966	0.1055	0.0911	0.46
avg				0.4685	0.2552	0.2133	0.46	0.54				0.1816	0.52	1.17	bbhn	8,58	lab acc	3.30E+08				0.2027	0.1088	0.0939	0.46
day 15	24.8.96						pH 7.0	temp 22C																	
	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil	oil/ts	oil/tfs					filter	105	550	tss	tvss	tfss	fs/ts
1	48.8216	49.2586	49.0331	0.4370	0.2255	0.2115	0.48	0.52	1	20.5600	20.6789	0.1189	0.26	0.52	bacterial counts				0.1898	0.3884	0.2862	0.1986	0.1022	0.0964	0.49
2	49.7045	50.1607	49.9449	0.4562	0.2158	0.2404	0.53	0.47	2	20.1546	20.2529	0.0983	0.22	0.43		1.00E+08	1.00E+09	avg	0.1898	0.4078	0.2978	0.2180	0.1100	0.1080	0.50
3	47.6244	48.1024	47.8576	0.4780	0.2448	0.2332	0.49	0.51	3	20.7808	20.9020	0.1212	0.27	0.53	PCA	NA	71,73,72	7.20E+09	0.1924	0.4148	0.3008	0.2224	0.1140	0.1084	0.49
avg				0.4571	0.2287	0.2284	0.50	0.50				0.1128	0.25	0.49	BBHN	lab acc	lab acc					0.2130	0.1087	0.1043	0.49
day 19	28.8.96						pH 7.0	temp 18C																	
	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil	oil/ts	oil/tfs					filter	105	550	tss	tvss	tfss	fs/ts
1	41.7196	42.1701	41.9497	0.4505	0.2204	0.2301	0.51	0.49	1	20.1516	20.2805	0.1289	0.31	0.60	bacterial counts				0.1929	0.3835	0.2937	0.1906	0.0898	0.1008	0.53
2	42.7555	43.1778	42.9720	0.4223	0.2058	0.2165	0.51	0.49	2	19.8342	19.9560	0.1218	0.29	0.56		1.00E+08	1.00E+09	avg	0.1918	0.3840	0.2931	0.1922	0.0909	0.1013	0.53
3	57.2454	57.6261	57.4480	0.3807	0.1781	0.2026	0.53	0.47	3	19.9031	20.0429	0.1398	0.33	0.65	PCA	145,142,140	14,15,9	1.51E+09	0.1933	0.3786	0.2947	0.1853	0.0839	0.1014	0.55
avg				0.4178	0.2014	0.2164	0.52	0.48				0.1302	0.31	0.60	BBHN	17,13	lab acc	1.50E+08				0.1894	0.0882	0.1012	0.53
day 25	3.9.96	added Na OH. Added 26.4 mls Inipol. Sampled before additio						temp 22	pH 6.8																
	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil	oil/ts	oil/tfs					filter	105	550	tss	tvss	tfss	fs/ts
1	70.4560	70.8837	70.6745	0.4277	0.2092	0.2185	0.51	0.49	1	20.0832	20.1561	0.0729	0.17	0.33	bacterial counts				0.1912	0.3899	0.3001	0.1987	0.0898	0.1089	0.55
2	67.7647	68.2093	67.9905	0.4446	0.2188	0.2258	0.51	0.49	2	20.6128	20.6983	0.0855	0.20	0.39		1.00E+08	1.00E+09	avg	0.1923	0.3864	0.2954	0.1941	0.0910	0.1031	0.53
3	59.9500	60.3717	60.1624	0.4217	0.2093	0.2124	0.50	0.50	3	20.2653	20.3299	0.0646	0.15	0.30	PCA	121,115,124	7,15,10	1.13E+09	0.1930	0.3791	0.2886	0.1861	0.0905	0.0956	0.51
avg				0.4313	0.2124	0.2189	0.51	0.49				0.0743	0.17	0.34	BBHN	22,41,51	4,5,4	4.05E+08				0.1930	0.0904	0.1025	0.53
day 32	10.9.96	samples less grity and not sticking to glass, no visible change in reactor.							temp 20	pH 6.8 added NAoh															
day 28	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil	oil/ts	oil/tfs					filter	105	550	tss	tvss	tfss	fs/ts
1	65.5811	65.8849	65.7435	0.3038	0.1414	0.1624	0.53	0.47	1	20.3274	20.3724	0.045	0.15	0.07	bacterial counts				0.1947	0.3286	0.2658	0.1339	0.0628	0.0711	0.53
2	61.6845	61.9728	61.8390	0.2883	0.1338	0.1545	0.54	0.46	2	20.6938	20.7572	0.0634	0.21	0.10		1.00E+08	1.00E+09	avg	0.1927	0.3435	0.2727	0.1508	0.0708	0.08	0.53
3	62.6058	62.9003	62.7618	0.2945	0.1385	0.1560	0.53	0.47	3	20.5112	20.5739	0.0627	0.21	0.10	PCA	270,290,308	45,32,39	3.38E+09	0.1909	0.3160	0.2570	0.1251	0.059	0.0661	0.53
				0.2955	0.1379	0.1576	0.53	0.47				0.0570	0.19	0.09	BBHN	54,38,	4,5,4	4.45E+08				0.1366	0.0642	0.0724	0.53
day 38	16/9/96	a lot of clingage went in durin			op up				temp 20	pH 7															
	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil	oil/ts	oil/tfs					filter	105	550	tss	tvss	tfss	fs/ts
1	42.0407	42.3338	42.2005	0.2931	0.1333	0.1598	0.55	0.45	1	20.7786	20.8222	0.0436	0.15	0.27	bacterial counts				0.1915	0.3274	0.2654	0.1359	0.062	0.0739	0.54
2	47.3354	47.6283	47.4953	0.2929	0.1330	0.1599	0.55	0.45	2	20.6346	20.6637	0.0291	0.10	0.18		1.00E+08	1.00E+09	avg	0.1930	0.3183	0.2608	0.1253	0.0575	0.0678	0.54
3	43.1847	43.4773	43.3457	0.2926	0.1316	0.1610	0.55	0.45	3	20.4628	20.4939	0.0311	0.11	0.19	PCA	224,225,230	180,195,211	2.26E+09	0.1936	0.3196	0.2623	0.126	0.0573	0.0687	0.55
				0.2929	0.1326	0.1602	0.55	0.45				0.0346	0.12	0.22	BBHN	dried out	estimate	4.00E+08				0.1291	0.0589	0.0701	0.54
day 43	21/9/96	looks like more solids have entered ML. Bits of solids in samples. Reactor smells bacterial									temp 19.	pH 7.0													
	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil	oil/ts	oil/tfs					filter	105	550	tss	tvss	tfss	fs/ts
1	41.7154	41.9930	41.8710	0.2776	0.1220	0.1556	0.56	0.44	1	20.1158	20.1371	0.0213	0.08	0.14	bacterial counts				0.192	0.3056	0.2577	0.1136	0.0479	0.0657	0.58
2	42.7510	43.0276	42.9070	0.2766	0.1206	0.1560	0.56	0.44	2							1.00E+08	1.00E+09	avg	0.1922	0.3092	0.2585	0.117	0.0507	0.0663	0.57
3	57.2393	57.5184	57.3964	0.2791	0.1220	0.1571	0.56	0.44	2						PCA	262,252,278	33,46,50	3.50E+09	0.1916	0.3261	0.268	0.1345	0.0581	0.0764	0.57
				0.2778	0.1215	0.1562	0.56	0.44							BBHN	74,48,18	4,1	3.50E+08				0.1217	0.0522	0.0695	0.57
day 48	26/9/96	open reactor replaced clingage then sampled.					temp 22	pH 7																	
	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil	oil/ts	oil/tfs					filter	105	550	tss	tvss	tfss	fs/ts
1	70.4500	70.7724	70.6290	0.3224	0.1434	0.1790	0.56	0.44	1	20.1088	20.1635	0.0547	0.17	0.45	bacterial counts				0.1939	0.3457	0.2806	0.1518	0.0651	0.0867	0.57
2	67.7588	68.0879		0.3291			0.00	0.00	2	20.3640	20.4169	0.0529	0.16	0.44		1.00E+08	1.00E+09	avg	0.1926	0.3302	0.2697	0.1376	0.0605	0.0771	0.56
3	59.9446	60.2765	60.1275	0.3319	0.1490	0.1829	0.55	0.45	3	20.3773	20.4338	0.0565	0.17	0.47	PCA	172,166,201	28,33,20	2.05E+09	0.193	0.3135	0.2628	0.1205	0.0507	0.0698	0.58
				0.3278	0.0975	0.1208	0.37	0.30				0.0547	0.17	0.45	BBHN	00,91,5	4,9,9	1.50E+08				0.1368	0.0588	0.0779	0.57
day 53	1/10/96	temp 24	pH 7.2																						
	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil	oil/ts	oil/tfs					filter	105	550	tss	tvss	tfss	fs/ts
1	48.8185	49.1486	48.9994	0.3301	0.1492	0.1809	0.55	0.45	1	19.8763	19.9280	0.0517	0.16	0.29	bacterial counts				0.1976	0.3248	0.2721	0.1272	0.0527	0.0745	0.59
2	49.7022	50.0229	49.8754	0.3207	0.1475	0.1732	0.54	0.46	2	19.7987	19.8519	0.0532	0.16	0.29		1.00E+08	1.00E+09	avg	0.1928	0.338	0.272	0.1452	0.066	0.0792	0.55
3	47.6218	47.9585	47.8102	0.3367	0.1483	0.1884	0.56	0.44	3	20.4044	20.4489	0.0445	0.14	0.25	PCA	277,315,304	33,31,32	3.09E+09	0.1927	0.3321	0.2721	0.1394	0.06	0.0794	0.57
				0.3292	0.1483	0.1808	0.55	0.45				0.0498	0.15	0.28	BBHN	170,120,160	20,19,14	1.63E+09				0.1373	0.0596	0.0777	0.57
day 62	10/10/96	temp 24	pH7.0																						
	cruc wt	105	550	ts	tvs	tfs	tfs/ts	tvs/ts		cruc wt	w oil	oil	oil/ts	oil/tfs					filter	105	550	tss	tvss	tfss	fs/ts
1	48.8285	49.1650	48.9936	0.3365	0.1714	0.1651	0.49	0.51	1	49.9006	49.9538	0.0532	0.17		bacterial counts				0.1899	0.3231	0.2657	0.1332	0.0574	0.0758	0.57
2	49.7303	50.0269	49.8701	0.2966	0.1568	0.1398	0.47	0.53	2	51.8553	51.9056	0.0503	0.16			1.00E+08	1.00E+09	avg	0.1884	0.3391	0.2724	0.1507	0.0667	0.084	0.56
3	47.6231	47.9074	47.7785	0.2843	0.1289	0.1554	0.55	0.45	3	49.5634	49.6179	0.0545	0.18		PCA	471,424,469	56,51,61	5.07E+09	0.1899	0.3311	0.2683	0.1412	0.0628	0.0784	0.56
				0.3058	0.1524	0.1534	0.50	0.50				0.0527	0.17		BBHN	71,111,142	16,8,4	1.01E+09				0.1417	0.0623	0.0794	0.56

APPENDIX N

ISTR RUN												
day	0	4	9	15	19	25	32	38	43	48	53	62
ts 1	0.1450	0.4602	0.4615	0.4370	0.4505	0.4277	0.3038	0.2931	0.2776	0.3224	0.3301	0.3365
2	0.1152	0.6407	0.4797	0.4562	0.4223	0.4446	0.2883	0.2929	0.2766	0.3291	0.3207	0.2966
3	0.1839	0.4736	0.4642	0.4780	0.3807	0.4217	0.2945	0.2926	0.2791	0.3319	0.3367	0.2843
avg ts	0.1480	0.5248	0.4685	0.4571	0.4178	0.4313	0.2955	0.2929	0.2778	0.3278	0.3292	0.3058
std dev	0.0345	0.1006	0.0098	0.0205	0.0351	0.0119	0.0078	0.0003	0.0013	0.0049	0.0080	0.0273
std dev %	23.28	19.16	2.10	4.49	8.40	2.75	2.64	0.09	0.45	1.49	2.44	8.92
ts/l	14.80	52.48	46.85	45.71	41.78	43.13	29.55	29.29	27.78	32.78	32.92	30.58
tvs 1	0.0684	0.2566	0.2589	0.2255	0.2204	0.2092	0.1414	0.1333	0.1220	0.1434	0.1492	0.1714
2	0.0525	0.3287	0.2589	0.2158	0.2058	0.2188	0.1338	0.1330	0.1206		0.1475	0.1568
3	0.0907	0.2557	0.2478	0.2448	0.1781	0.2093	0.1385	0.1316	0.1220	0.1490	0.1483	0.1289
avg tvs	0.0705	0.2803	0.2552	0.2287	0.2014	0.2124	0.1379	0.1326	0.1215	0.1462	0.1483	0.1524
std dev	0.0192	0.0419	0.0064	0.0148	0.0215	0.0055	0.0038	0.0009	0.0008	0.0040	0.0009	0.0216
std dev %	27.22	14.94	2.51	6.45	10.67	2.60	2.78	0.68	0.67	2.71	0.57	14.17
tvs/l	7.05	28.03	25.52	22.87	20.14	21.24	13.79	13.26	12.15	14.62	14.83	15.24
tfs 1	0.0766	0.2036	0.2026	0.2115	0.2301	0.2185	0.1624	0.1598	0.1556	0.1790	0.1809	0.1651
2	0.0627	0.3120	0.2208	0.2404	0.2165	0.2258	0.1545	0.1599	0.1560		0.1732	0.1398
3	0.0932	0.2179	0.2164	0.2332	0.2026	0.2124	0.1560	0.1610	0.1571	0.1829	0.1884	0.1554
avg tfs	0.0775	0.2445	0.2133	0.2284	0.2164	0.2189	0.1576	0.1602	0.1562	0.1810	0.1808	0.1534
std dev	0.0153	0.0589	0.0095	0.0150	0.0138	0.0067	0.0042	0.0007	0.0008	0.0028	0.0076	0.0128
std dev %	19.70	24.09	4.45	6.59	6.35	3.06	2.66	0.42	0.50	1.52	4.20	8.32
tfs/l	7.75	24.45	21.33	22.84	21.64	21.89	15.76	16.02	15.62	18.10	18.08	15.34
avg fs/ts	0.5236	0.4659	0.4553	0.4997	0.5180	0.5075	0.5333	0.5469	0.5623	0.5522	0.5492	0.5016
avg vs/ts	0.4764	0.5341	0.5447	0.5003	0.4820	0.4925	0.4667	0.4527	0.4374	0.4460	0.4505	0.4984
						add inipol	effect inipol			clingage return		
O&G	0	4	9	15	19	25	32	38	43	48	53	62
1	0.0479	0.2181	0.1651	0.1189	0.1289	0.0729	0.0450	0.0436		0.0547	0.0517	0.0532
2	0.1074	0.1805	0.1853	0.0983	0.1218	0.0855	0.0634	0.0291		0.0529	0.0532	0.0503
3	0.0545	0.1754	0.1945	0.1212	0.1398	0.0646	0.0627	0.0311		0.0565	0.0445	0.0545
avg O&G	0.0699	0.1913	0.1816	0.1128	0.1302	0.0743	0.0570	0.0346		0.0547	0.0498	0.0527
std dev	0.0326	0.0233	0.0150	0.0126	0.0091	0.0105	0.0104	0.0079		0.0018	0.0047	0.0022
std dev %	46.64	12.19	8.28	11.18	6.96	14.16	18.29	22.71		3.29	9.34	4.08
O&G/l	6.9933	19.1300	18.1600	11.2800	13.0200	7.4300	5.7000	3.4600		5.4700	4.9800	5.2667
cum oil loss			0.0097	0.0785	0.0611	0.1170	0.1343	0.1567		0.1366	0.1415	0.1386
per liter			0.97	7.85	6.11	11.7	13.43	15.67		13.66	14.15	13.86
avg o&g/ts	0.4725	0.3645	0.3876	0.2468	0.3116	0.1723	0.1929	0.1181		0.1669	0.1513	0.1722
avg o&g/fs	0.9024	0.7824	0.8514	0.4939	0.6017	0.3394	0.3617	0.2160		0.3022	0.2754	0.3433
tss	0	4	9	15	19	25	32	38	43	48	53	62
1	0.0476	0.1706	0.2101	0.1986	0.1906	0.1987	0.1339	0.1359	0.1136	0.1518	0.1272	0.1332
2	0.0633	0.1961	0.2013	0.218	0.1922	0.1941	0.1508	0.1253	0.117	0.1376	0.1452	0.1507
3	0.0423	0.1795	0.1966	0.2224	0.1853	0.1861	0.1251	0.126	0.1345	0.1205	0.1394	0.1412
avg tss	0.0511	0.1821	0.2027	0.213	0.1894	0.193	0.1366	0.1291	0.1217	0.1366	0.1373	0.1417
std dev	0.0109	0.0129	0.0069	0.0127	0.0036	0.0064	0.0131	0.0059	0.0112	0.0157	0.0092	0.0088
std dev %	21.37	7.11	3.38	5.95	1.91	3.30	9.56	4.59	9.22	11.47	6.69	6.18
tss/l	10.22	36.42	40.54	42.6	37.88	38.6	27.32	25.82	24.34	27.32	27.46	28.34
tvss 1	0.024	0.0944	0.1131	0.1022	0.0898	0.0898	0.0628	0.062	0.0479	0.0651	0.0527	0.0574
2	0.0332	0.1101	0.1078	0.11	0.0909	0.091	0.0708	0.0575	0.0507	0.0605	0.066	0.0667
3	0.0223	0.1016	0.1055	0.114	0.0839	0.0905	0.059	0.0573	0.0581	0.0507	0.06	0.0628
avg tsvs	0.0265	0.102	0.1088	0.1087	0.0882	0.0904	0.0642	0.0589	0.0522	0.0588	0.0596	0.0623
std dev	0.0059	0.0079	0.0039	0.0060	0.0038	0.0006	0.0060	0.0027	0.0053	0.0074	0.0067	0.0047
std dev %	22.13	7.70	3.58	5.52	4.27	0.67	9.38	4.51	10.10	12.51	11.18	7.50
tsvs/l	5.3	20.4	21.76	21.74	17.64	18.08	12.84	11.78	10.44	11.76	11.92	12.46
tfss 1	0.0236	0.0762	0.097	0.0964	0.1008	0.1089	0.0711	0.0739	0.0657	0.0867	0.0745	0.0758
2	0.0301	0.086	0.0935	0.108	0.1013	0.1031	0.08	0.0678	0.0663	0.0771	0.0792	0.084
3	0.02	0.0779	0.0911	0.1084	0.1014	0.0956	0.0661	0.0687	0.0764	0.0698	0.0794	0.0784
avg tsfs	0.0246	0.08	0.0939	0.1043	0.1012	0.1025	0.0724	0.0701	0.0695	0.0779	0.0777	0.0794
std dev	0.0051	0.0052	0.0030	0.0068	0.0003	0.0067	0.0070	0.0033	0.0060	0.0085	0.0028	0.0042
std dev %	20.81	6.55	3.16	6.53	0.32	6.51	9.72	4.70	8.65	10.88	3.57	5.28
tsfs/l	4.92	16	18.78	20.86	20.24	20.5	14.48	14.02	13.9	15.58	15.54	15.88
ssfs/tss	0.48	0.44	0.46	0.49	0.53	0.53	0.53	0.54	0.57	0.57	0.57	0.56
ssvs/tss	0.52	0.56	0.54	0.51	0.47	0.47	0.47	0.46	0.43	0.43	0.43	0.44
dis solids	4.58	16.06	6.31	3.11	3.90	4.53	2.23	3.47	3.44	5.46	5.46	2.24
per liter												
BACT #	0	4	9	15	19	25	32	38	43	48	53	62
PCA	1.32E+09	4.97E+09	2.53E+09	7.20E+09	1.51E+09	1.13E+09	3.38E+09	2.26E+09	3.50E+09	2.05E+09	3.09E+09	5.07E+09
BBHN		7.58E+08	3.33E+08		1.50E+08	4.05E+08	4.45E+08	4.00E+08	3.50E+08	1.05E+08	1.63E+09	1.01E+09
						add NaOH	add NaOH					
pH	7.2	7.2	7.2	7	7	6.8	6.8	7	7	7	7	7
temp	23	20.5	22	22	18	22	20	20	19.5	22	24	24

Reactor - Inipol EAP 22 Amended STR

Sample calculations for TC of Mixed Liquor of Reactors sampled by 'boat' of TOC machine
and TC and IC for filterate or dissolved solids in mixed liquor

											(of 3-5 samples)				
Day	**Date**	**ML TC**	***Std dev***	**CF**	**Total TC ML**	**Vol Filt**	**Dilution**	**DF**	**CF**	**TC filterate**	***std dev***	**Total TC**	**IC filtrate**	**Total IC**	**TOC Filterate**
		(a)		(b)	(a x b)			Vol filt/dil.		(y)		(y x DF x CF)	(z)	(z x DF x CF)	(TC - IC)
day 0	9-Aug	3592	*1.131*	2.405	8639	15	60	4.00	0.917	124.6	*0.961*	457.0	8.192	30.0	427.0
day 3	12-Aug	17270	*8.733*	2.405	41534										
day 4	13-Aug	15350	*1.185*	2.405	36917	15	75	5.0	0.917	150.4	*1.322*	689.6	15.48	71.0	618.6
day 9	18-Aug	17150	*1.813*	2.405	41246	15	54	3.6	0.917	411.4	*1.698*	1358.1	1.712	5.7	1352.5
day 15	24-Aug	16370	*0.97*	2.405	39370	15	38	2.5	0.917	210.2	*1.452*	488.3	3.242	7.5	480.8
day 20	29-Aug	13900	*0.83*	2.405	33430	15	27	1.8	0.917	325.7	*1.054*	537.6	1.536	2.5	535.1
day 25	**3-Sep**	**13670**	***2.562***	**2.405**	**32876**	**15**	**80**	**5.3**	**0.917**	**1560**	***1.216***	**7629.4**	**1.194**	**5.8**	**7623.6**
day 32	10-Sep	9848	*2.784*	2.405	23684	15	26	1.7	0.917	494.4	*0.927*	785.8	0.629	1.0	784.8
day 38	16-Sep	10230	*2.222*	2.405	24603	15	30	2.0	0.917	616.8	*0.994*	1131.2	2.761	5.1	1126.1
day 43	21-Sep	8569	*1.933*	2.405	20608	15	38	2.5	0.939	518	*0.236*	1232.2	5.4	12.8	1219.4
day 48	26-Sep	9414	*0.623*	2.405	22641	15	43	2.9	0.939	506.6	*1.939*	1363.7	10.42	28.0	1335.6
day 62	10-Oct	7637	*1.949*	2.405	18367	15	33	2.2	0.939	781.8	*1.622*	1615.0	11.75	24.3	1590.8

Inipol BSTR

sludge	1063.4 g wet weight				
Cruc	wsludge	at 105 C	sludge	dry sludge	DSF
42.0407	46.7556	45.5932	4.7149	3.5525	0.753462
47.3354	52.0107	50.8503	4.6753	3.5149	0.751802
43.1847	48.362	47.0826	5.1773	3.8979	0.752883
					0.752716

OIL AT 33.11% DRY WEIGHT
Load 1063.4 g x dsf x oil%
dry solids load is at 800.42 g
oil at 33.11% dry solids is at 265.02

at end

BEAKER	wsludge	sludge
457.34	1411.54	954.2
Reactor	w residue	sludge
2989.4	3131.7	142.3
BEAKER	w clingage	sludge
116.53	129.4	12.87
TOTAL		1109.37

Cruc	wsludge	at 105 C	sludge	dry sludge	DSF
42.0414	45.8035	44.0148	3.7621	1.9734	0.5245
47.3344	51.297	49.4071	3.9626	2.0727	0.5231
43.1841	47.913	45.6682	4.7289	2.4841	0.5253
				avg	0.5243
			total dry solids		581.6427

O&G bottom solids			
sludge	dry sludge	oil	% oil
7.8763	4.129544	0.6971	16.8808
7.7081	4.041357	0.6548	16.20248
7.686	4.02977	0.6778	16.81982
		avg	16.6344
		std dev	0.3753
		Total	96.7272

solids ML			
cruc	w dry solids	Volume	5,930
70.448	70.5709	dry solids	solids g/l
67.7573	67.8844	0.1229	12.29
59.944	60.0681	0.1271	12.71
		0.1241	12.41
		avg	12.47
		TOTAL	73.95

O&G ML			
cruc	w oil		
19.7123	19.7387	oil	oilg/l
20.124	20.1299	0.0264	2.64
20.3233	20.3391	0.0059	0.59
		0.0158	1.58
		0.0160	1.6033
		std dev	1.4496
		Total	9.5076