# ALGAL BIOREACTORS FOR NUTRIENT REMOVAL AND BIOMASS PRODUCTION DURING THE TERTIARY TREATMENT OF DOMESTIC SEWAGE

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# Abstract

This thesis covers work carried out on algae bioreactors as a tertiary treatment process for wastewater treatment. The process was primarily assessed by the removal of Phosphorus and Nitrogen as an alternative to chemical and bacterial removal. Algal bioreactors would have the added advantage of carbon sequestration and a by-product in the energy rich algal biomass that should be exploited in the existing AD capacity.

Laboratory scale bioreactors were run (4.5-30L) using the secondary treated final effluent from the local Loughborough sewage works. In a preliminary series of experiments several different bioreactor designs were tested. These included both batch feed and continuous flow feed configurations.

The bioreactors were all agitated to keep the algal cells in suspension. The results demonstrated that the most effective and easy to operate was the batch feed process with the algal biomass by-product harvested by simple gravitational settling. Experiments also compared an artificial light source with natural light in outdoor experiments. Outdoor summer light produced greater growth rates but growth could not be sustained in natural UK winter light.

Light intensity is proportional to productivity and algae require a minimum of around $97W/m^2$ to grow, an overcast winter day (the worst case scenario) was typically around $78W/m^2$, however this was only available for a few hours per day during Nov-Jan. The process would be better suited to areas of the world that receive year round sunlight.

It was shown that phosphorus could be totally removed from wastewater by the algae in less than 24 hours depending on other operating variables. With optimisation and addition of more carbon, a HRT of 10-12 hours was predicted to achieve the EU WFD / UWWTD standard. It was further predicted that the process could be economically and sustainably more attractive than the alternatives for small to medium sized works. Biomass

concentrations of between 1-2g/L were found to best achieve these removals and produce the fastest average growth rates of between 125-150mg/L/d. The uptake rates of phosphorus and nitrogen were shown to be dependent on the type of algae present in the bioreactor. Nitrogen removal was shown to be less effective when using filamentous blue-green algae whilst phosphorus removal was almost completely stopped compared to unicellular green algae that achieved a nitrogen uptake of 5.3mg/L/d and phosphorus uptake of 8mg/L/d. Soluble concentrations of Fe, Ni and Zn were also reduced by 60% in the standard 10 hours HRT.

The predominant algae were shown to depend largely on these concentrations of phosphorus and nitrogen, and the strain most suited to that specific nutrient or temperature environment dominated.

Nutrient uptake rates were linked to algal growth rates which correlated with the availability of Carbon as $CO_2$. $CO_2$ was shown to be the limiting factor for growth; becoming exhausted within 10 hours and causing the pH to rise to above 10.5. The literature showed this was a common result and the use of $CO_2$ sparging would more than double performance making this process a good candidate for waste $CO_2$ sequestration. Heat generated from combustion or generators with exhaust $CO_2$ would also be ideal to maintain a year round constant temperature of between 20-25°C within the bioreactors. A number of possible uses for the algal biomass generated were examined but currently the most feasible option is wet anaerobic co-digestion.

Further economic analysis was recommended on the balance between land area and complementary biomass generation for AD. It was also suggested given the interest as algae as a future fuel source, the process could also be adapted for large scale treatment and algal biomass production in areas of the world where land was available.

*Key words*- algae, lagoons, wastewater, phosphorus, nitrogen, biofuel, bioreactor, nutrient removal, eutrophication, sewage, carbon.

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# Reference lists

## List of symbols

| | |
|---|---|
| Ca | Calcium |
| $CaCO_3$ | Calcium Carbonate |
| Cd | Cadmium |
| $CO_2$ | Carbon Dioxide |
| $CO_3^{2-}$ | Carbonate Ion |
| Cr | Chromium |
| Cu | Copper |
| Fe | Iron |
| Hg | Mercury |
| $H^+$ | Hydrogen Ion |
| $H_2CO_3$ | Carbonic Acid |
| $H_2O$ | Water |
| HCl | Hydrochloric acid |
| $HCO_3^-$ | Hydrogen Carbonate Ion |
| $HNO_3$ | Nitric acid |
| N | Nitrogen |
| Na | Sodium |
| Ni | Nickel |
| $NO_3$ | Nitrates |
| $OH^-$ | Hydroxide Ion |
| P | Phosphorus |
| $PO_4$ | Phosphates |
| Pb | Lead |
| Zn | Zinc |

| | |
|---|---|
| nm | Nanometres |
| μm | Microns |
| ppm | Parts per million |

# List of abbreviations

| | |
|---|---|
| ABP | Algae Based Pond |
| AD | Anaerobic Digestion |
| BNR | Biological Nutrient Removal |
| BOD | Biological Oxygen Demand |
| BPR | Biological Phosphorus Removal |
| CHP | Combined Heat and Power |
| COD | Chemical Oxygen Demand |
| DAF | Dissolved Air Floatation |
| DBP | Duckweed Based Pond |
| DIC | Dissolved Inorganic Carbon |
| DO | Dissolved Oxygen |
| DOC | Dissolved Organic Carbon |
| EC | Electro-conductivity, µs/cm |
| HRAP | High Rate Algal Pond |
| HRT | Hydraulic Retention Time |
| IC | Inorganic Carbon |
| ICP | Inductively Coupled Plasma analyser |
| OLR | Organic Loading Rate |
| PAO | Phosphorus Accumulating Organism |
| RAS | Return Activated Sludge |
| ROC | Renewable Obligation Certificate |
| SBR | Sequencing Batch Reactor |
| STW | Sewage Treatment Works |
| SVO | Straight Vegetable Oil |
| TSS | Total suspended solids |
| TDS | Total dissolved solids |
| TVS | Total volatile solids |
| TOC | Total Organic Carbon |
| UWWTD | Urban Wastewater Treatment Directive |
| VFA | Volatile Fatty Acid |
| WFD | Water Framework Directive |
| WHO | World Health Organisation |

| WSP | Waste Stabilization Pond |
| WWT | Wastewater Treatment |

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# 1. Introduction

## 1.1 The problem

With the phenomenal increase in urban population and industrial growth, some of the major problems of a basic nature confronting mankind are those of quality and quantity of food, drinking water, disposal of sewage, industrial wastes, $CO_2$ / other toxic emissions and fuel shortages. Algal bioreactors / HRAPs using suitably controlled algal growth can provide potential solutions to some of these sustainability issues / problems.

Algae are synonymous with eutrophic natural waters. STW discharge final effluent to the local water course. This effluent contains a high concentration of essential growth nutrients, P, N and other trace elements. The STW have been targeted to reduce the nutrients released to meet the EU water directive UWWD 1991 & WFD 2003 set standards, which over time become more stringent. The removal of nutrients, especially P is a difficult and costly process and can involve the use of unsustainable chemicals.

Eutrophication is frequently a result of nutrient pollution such as the release of sewage effluent and run-off from fertilizers into natural waters. Eutrophication generally promotes excessive plant growth, in turn; these mass blooms usually result in large scale decay and a rapid increase in BOD. This is likely to cause severe reductions in water quality.

Eutrophication has moved up the agenda as a result of the WFD and there is much debate as to the most appropriate ways of controlling nutrient releases into the environment. Nutrient removal at the sewage works is not as successful as hoped. This is especially true of removing P. Enrichment in activated sludge is difficult to control especially with large variable amounts of storm water, chemical removal is unsustainable.

## 1.2. Why is P and N removal important?

The removal of N from wastewater is desirable for other reasons:

- As free ammonia it is non-polar and toxic to fish and other aquatic organisms; the EU limit is 0.5mg/L as $NH_3$.

- Ammonia is also an O consuming compound and therefore exerts a risk of O depletion.

- Along with P, N is a plant nutrient and consequently contributes to eutrophication.

- As the nitrate ion it can combine with haemoglobin, a potential hazard to infants. It may also be reduced to mutagenic nitrosamines in the gastrointestinal tract. The EU limit is 50mg/L as $NO_3$.

Both P and N are now regulated with the prospect of lower permissible concentrations in the future.

The human population and volume of wastewater is increasing along with the implementation of ever stricter discharge standards. The cost of resources such as chemicals and electricity are increasing in cost at a faster rate than inflation and no longer sustainable. Innovation is necessary to provide cheap, clean, sustainable and more efficient wastewater treatment processes.
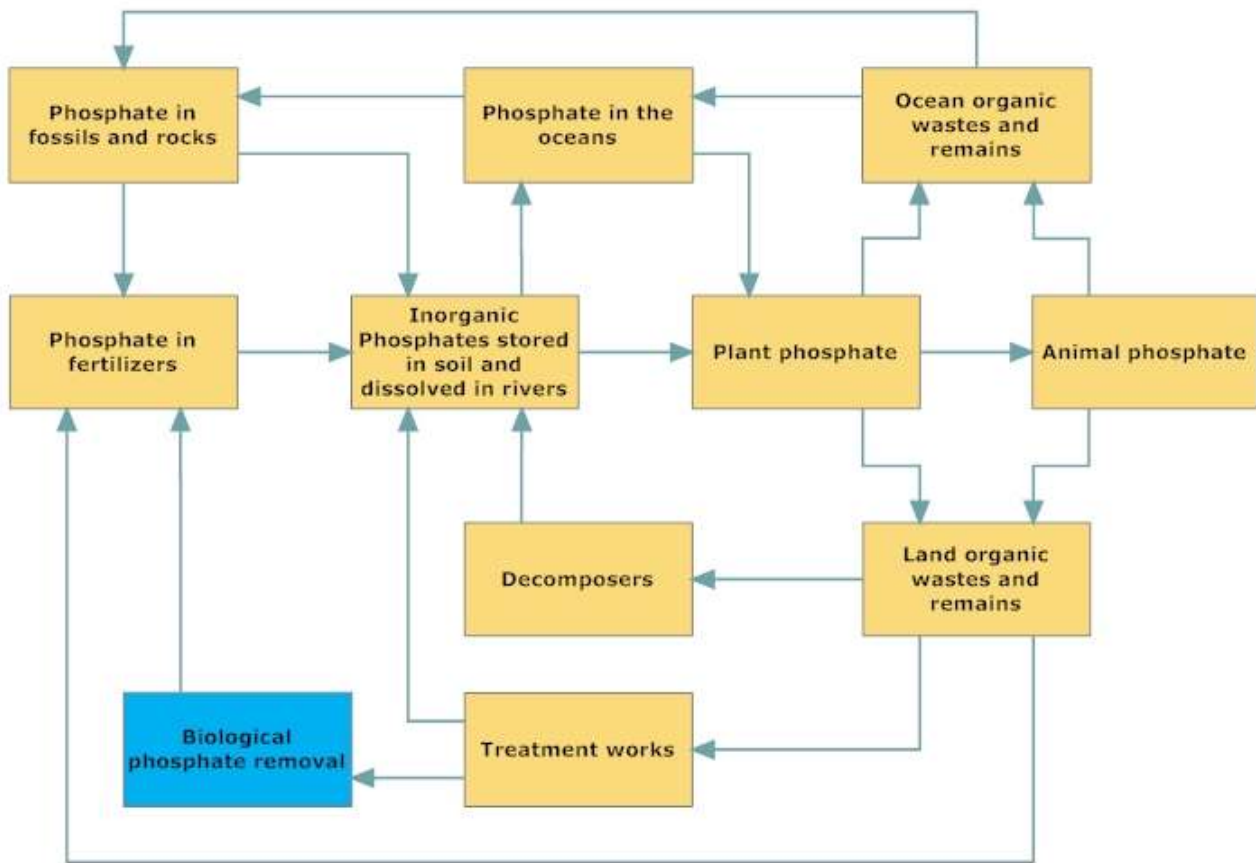
**Figure 1 - Showing the P cycle**

## 1.3. Benefits of using a biological method

- A biological method is natural and does not require the use of unsustainable chemicals
- Once the system has been set up, the running costs are lower
- Instead of having a process to remove just P or just N, the biological process can remove both P and N whilst simultaneously sequestering C, reducing other trace nutrients and providing oxygen for improved BOD reduction through an algae/bacteria co-symbiotic relationship
- A biomass by-product is created that can be used as an energy source or fertiliser
- The water utilities are familiar with bio-processes

## 1.4. The solution and motivation for work

Biological processes are usually more sustainable since they operate at normal temperatures and pressure although usually slow. Microscopic algae could be used for nutrient removal through controlled, accelerated eutrophication as a tertiary treatment process. It has been investigated to determine whether it could replace existing processes and is possible in the UK climate.

The aims were therefore to determine whether algae could be grown in sewage effluent to concentrate plant nutrients, N and P and other trace elements whilst sequestering C. The surplus algal biomass could be converted into biogas by anaerobic digestion and / or used as a nutrient rich fertiliser.

There is sufficient evidence to provide an initial motivation for a controlled investigation on algal bioreactors. The idea is based on recreating a natural process that achieves the same result. Algae have already been shown to be successful at growing in a range of different stages of a smaller wastewater treatment process.

Along with the motivation from an economic and sustainability view point, I am personally motivated by a passionate interest in environmental management and biological processes. This was a great opportunity to combine research on an exciting area of biology with a number of interlinking modern environmental issues.

## 1.5. About the project

The project studied the environmental parameters required to optimise this waste-algae-energy process by considering algal growth rate as a function of light, temperature, biomass concentration, nutrient concentration, $CO_2$ and pH.

A set of experiments was run to test the P, N and C removal capabilities and growth rate of different strains of algae under varying light and temperature conditions. These

experiments also report on tests on how the algae can be removed from the wastewater before discharge. All the experiments studied the design of a photo-bioreactor / HRAP system that was suitable for use within an operating sewage treatment works.

A considerable amount of research is currently being carried out in the field of algal biomass cultivation as an alternative to traditional crops for the creation of biofuel. The majority of the research into algal biomass production is from five main areas.

- Small scale, more control, higher value products
    - Industrial bioreactors:

- Large scale, less control, cheaper
    - Large scale saline oceans or pools
    - Naturally eutrophic lakes and water courses
    - Tanks or ponds, HRAPs
    - Sewage lagoons

Each method has its own advantages, which will be considered in the literature review, but this project will be dealing specifically with using industrial bioreactors for experimentation and investigating HRAPs for scale up, both using wastewater. The use of wastewater as a medium for growing algae has a number of economic and environmentally sustainable benefits:

- Already high in nutrients, no need for additional fertiliser
- Not in competition for use with land based crops, industries or human consumption
- Located in and around sewage treatment works that may not be suitable for other land use

Due to the relatively small volume available it is not seen as having a significant impact on global fuel production; other methods would be needed for this, i.e. large scale desert based lagoons.

The project should be judged as an engineering and scientific extension to sewage treatment. It has looked at existing work investigating algal lagoons which have been used in sewage treatment in warm countries for some time. Using experimentally based methods, it expanded on the key objectives previously listed to address the possibilities for extension to the existing knowledge base.

## 1.6. Design requirements for the photo-bioreactor system

A bioreactor is to support a biologically active culture. Its type is defined by its mode of operation, they can be batch or continuous flow. Bioreactor design is a complex engineering task but under these engineered optimum conditions the microorganisms or cells are able to perform their desired function with greater efficiency.

Quantitative assessment of the bioreactor's environmental conditions ($CO_2$, Oxygen, nutrient concentration, flow rates, lighting, temperature, pH, and agitation speed/circulation rate) need to be monitored, reported and controlled to allow comparisons.

The system should minimize costs (capital investment, operational and maintenance costs and other costs derived from economic and environmental restrictions) whilst optimizing performance to achieve acceptable EU WFD standards for the treated effluent and reach maximum algal yields.

Algal bioreactor performance is based on a number of variables; some are difficult to control such as ambient temperature, solar radiation and wastewater quality. Other variables can be included into the design of a new system such as size of plant, channel width, pond depth (governing light penetration), design discharge, pump capacities and

agitation method. The final factors affecting performance are the control variables such as biomass concentration, HRT and $CO_2$ sparging for pH control. The overall performance will be based on, the effluent quality, the removal of nutrients C, N and P, algae production rates and any revenue generated by the algal biomass.

Summary of design requirements:

- To Remove P and N from wastewater using algae. Primarily to focus on reducing the concentration of P to meet EU standards of a maximum of 1mg/L (95%).
- Minimising energy usage.
- Minimise land area usage.
- Have a method of removing decaying algal cells from the system to prevent re-mineralisation of nutrients into solution.
- Ensure the greatest penetration of light into the fluid to maximise photosynthesis.
- The ability to meet the optimum temperature for performance of the system by being able to utilise waste heat and control the bioreactor temperature.
- Minimise HRT to reduce both capital and running costs

A species of algae that would be ideal for this process would possess the following characteristics:

- Ability to grow on sewage
- Effective at removing both P and N
- Relatively high growth rate
- Easily harvested
- Not largely affected by the presence of bacteria
- Could withstand some extreme environmental condition, i.e. high temperature, pH or salinity to prevent invasion from other species

## 1.7. Structure of the thesis

This introduction to the project is followed by a section containing background information about the topics discussed throughout this thesis. This is provided to fill basic knowledge gaps on the subject required for understanding the issues directly related to this thesis. Section 3 then provides a critical review of the existing literature on nutrient removal by algal bioreactors and HRAPs along with the integral topics directly associated with this project. It shows information about the research that has already been carried out and identifies knowledge gaps to provide a direction to advance the current scientific understanding. It also enables a platform for comparison with existing results.

Once the knowledge gaps have been identified from the literature review, a concise summary of the aims and objectives are listed.

The methodology section describes the experiments carried out, the different reactors used, the light and temperature conditions used, the procedures used and details specific information about the standard methods used for testing. Following this is a detailed analysis of the results gathered from all the experiments, how they link together and compare with previous work to derive conclusions listed in the next section. After the conclusions is a section that draws all the findings together to describe a recommended design for an optimal performance pilot plant. This is accompanied by a critical summary of the work carried out and suggested recommendations for further work. The thesis closes with a bibliography of references and appendix.

## 1.8. Hypothesis

Nutrient removal from wastewater can be achieved more sustainably and more effectively using a biological process of algal growth in bioreactors than it can by using existing methods. Algal growth will sequester Carbon and improve effluent quality by reducing other pollutants. The biomass by-product can be used as a fuel source.

# 2. Background Reading

## 2.1. An introductory guide to the algae

### 2.1.1. Introduction to algae

Cryptogamia is a class of plant lacking both seeds and flowers of which algae is a division. All algae lack leaves, roots, flowers, seeds and other organ structures that characterize higher plants. Algae are autrophic microscopic plants that require only inorganic nutrients to grow. In aquatic environments, they are the baseline of food webs. Algae range from single-cell organisms to multicellular organisms, some with complex differentiated forms for example the marine seaweeds. The study of marine and freshwater algae is called phycology.

All types of algae differ from photosynthetic bacteria. Photosynthetic bacteria do not have chloroplasts or any membrane bound organelles, instead photosynthesis takes place directly within the cell and no oxygen is evolved.

Cyanobacteria (blue-green algae), are Prokaryotic algae similar to bacteria. They conduct photosynthesis on specialized cytoplasmic membranes called thylakoid membranes, rather than in the chloroplast organelles. These are no longer strictly classed as algae; as they were traditionally in old text books which are now regarded as outdated. The term algae is restricted to Eukaryotic organisms.

Eukaryotic algae conduct photosynthesis within membrane-bound structures (organelles) called chloroplasts. Chloroplasts in plants and eukaryotic algae have evolved from cyanobacteria. Cyanobacteria cells average around 5µm in diameter and are therefore smaller than Eukaryotic algae which average around 12-22µm.

Diatoms, also known as Bacillariophytes are unicellular, microscopic Eukaryotic algae that can be between 5µm and 5mm. A characteristic feature of diatom cells is that they are encased within a unique cell wall made of silica (hydrated silicon dioxide) called a frustule.

Approximately 100,000 known species exist with more than 400 new specimens being described each year (Danielo, 2005). The microscopic forms that live suspended in the water column are called phytoplankton.

Algae are distinguished from other protozoa in that they are photoautotrophic although this is not a hard and fast distinction as some groups contain members that are mixotrophic, deriving energy both from photosynthesis and uptake of organic carbon either by osmotrophy (the uptake of dissolved organic compounds by osmosis for nutrition), myzotrophy (piercing the cell wall/membrane and sucking out the cellular contents to digest), or phagotrophy (wherein particles are enveloped by the cell membrane and internalized for digestion).

Most algal cells are only slightly denser than water; which gives rise to their slow settling rate. A stationary cell will be at a disadvantage because it will absorb the nutrients in its contact layer; for the cell to remain bathed in a continuing supply of dissolved nutrients it must keep moving. The natural tendency for algae is to sink. This can guarantee movement throughout the medium but can be lethal as cells fall out of the lighted zone. Some species are shaped in such a way as to help reduce rates of fall. A greater ratio of surface area to volume including hair-like cell outgrowths is just one of many naturally evolved attributes. Buoyancy can be generated from gas products as with the blue-green algae.

Some species of algae are so rich in oil that it accounts for over 50% of their mass, thus providing both buoyancy and a nutrient store. The National Renewable Energy Laboratory (NREL) has selected approximately 300 species of both fresh-water and salt-water algae to be stored in their species collection at the Marine Bio-products Engineering Center (MarBEC). These oil rich strains are part of an international culture bank available for research. There is also interest in algae as a source of other by-products such as bio-ethanol and biogas.

## 2.1.2. Diversity of algal species

Freshwater algae are a very diverse group of plants found in all moist situations. An abundance of the more common unicellular algal forms are predominantly found in low-lying quiet waters such as pools, ditches and lakes. Algae can exist under very varied temperature conditions. In temperate and arctic climates many can survive prolonged freezing, even when in their ordinary vegetative condition. Numerous healthy algae do not suffer ice damage from low temperatures and when the ice melts, can re-grow. In Arctic/Antarctic regions or mountainous areas like the Alps or Andes there are snow-floras that mainly consist of algae that pass their entire existence in snow and ice. These are known as Cryoplankton and contain a red pigment which gives rise to the term "red snow". Algae also occur in hot springs. They can survive in hot water and hot vapour up to a temperature of 94.5°C. Some of these species become encrusted with carbonate of lime or silica. These robust algae that exist at both low and high temperatures, with a few exceptions, are species of Myxophyceae and Bacillariales genera.

Freshwater algae species have a strong cell wall to resist osmotic pressure and some can also withstand exposure to salt water but few algae can be isolated near the mouths of rivers where there is a frequent change in the salinity. Species of algae can grow in all conditions of varying salinity, from 3.6ppm to 40000ppm and up to 100% in the dry season at Laguna Madre, Texas. Saltwater algae have three main habitats: Living in suspension in open waters, growing on Littoral rocks; twice daily covered and uncovered by tides and growing on Sublittoral rocks; the region below the low water mark. More algae live in coastal regions as these areas are normally more nutrient rich than out at sea. The oceans however contain the largest mass of algae. They are thought to be responsible for 70% of all biogenic $CO_2$ fixation ($120 \times 10^{12}$ tonnes/year) and for about 73-87% of the net global production of oxygen. Much of the $CO_2$ is sequestered and precipitates either as organic matter to be fossilized or as calcite. Ocean algae are normally blue-green algae due to their small size.

## 2.1.3. Classification of algal species

There are many different types and species of algae that range in size and organisation. Chlorophyll is present in all photosynthetic representatives of the algae. The chlorophyll pigment is borne in distinct cell organelles called chloroplast and the colour provides the observer a means of grouping the algae into classes. The combinations of photosynthetic pigments present have an important role in algal classification. There are eight main divisions of freshwater algae which are classified according to their colour and other differences. Algae can be various colours and a single variety may change colour depending on the phase of growth it is in. These colour differences arise due to the proportions of different auxiliary photosynthetic pigments present in addition to the green chlorophylls. Some pigments do not mask the Chlorophyll-a that is present in all algae; here the Chlorophyll-a is dominant and the algae will be green (Chlorophyta and Euglenophyta). The green algae are the largest and most successful group of algae from which the embryophytes (higher plants) evolved. The green reflectance colour represents the section of the spectrum (green-550nm) where the photosynthetic rate is the lowest (purple-420nm and red-660nm, being the highest). If Carotenoids are dominant the algae will be yellow-brown, yellow-golden or yellow-green. This is the case with Chrysophyta, Pyrrophyta, Cryptophyta, Bacillariophyta and Xanthophyta. All these pigments are lipid soluble compounds; unlike the entirely different water soluble pigments found in Cyanobacteria (blue-green algae). These mask the chlorophylls and other pigments giving the cells a blue-green or red colour.

Another characteristic that can be used for classification is how the excess organic matter stored as a food reserve created as a result of photosynthesis. Starch, glycogen, glycoproteins, paramylum, leucosin, oils and fats can all be found.

The chloroplast traps and utilizes light energy to convert $CO_2$ to Carbohydrate. Chlorophyll-a is the most common primary pigment involved in photosynthesis and is the one pathway by which the absorbed radiation is converted to chemical energy. The efficiency of the chloroplast as a light trapping organelle is directly related to the surface area it presents to

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the incident of light. Typically algae have a very large surface area to volume ratio to aid efficiency. This is not only important for trapping light but the cell surface is also means for mass transfer of both nutrients and dissolved gases. A high efficiency gives rise to intensive rates of metabolism and rapid multiplication of algal cells (under optimal conditions, in excess of 300g/m$^3$/d.

As with most biochemistry, the biochemistry of algal cells is of a very complex nature. The cells adaptations to changes in conditions can be rapid due to the intense rates of cell division. They can, as eukaryotes, quickly adapt to different conditions compared to the prokaryotes which must rely on mutation. For a good review of the general characteristics of algal physiology, ecology and taxonomy refer to Harris (1978).

### 2.1.4. Cyanobacteria

Blue-green algae are also frequently found in still freshwaters. In temperate lakes, they form dense populations after the water column has become stratified at the end of spring and the low density warm epilimnion is established above the cold deeper hypolimnion.

Due to their different pigment, blue-green algae can use lower light intensities more effectively so they can thrive below the surface deep in the epilimnion. Blue-green algae can control their buoyancy via gas vacuoles. Cells exposed to dim light form more gas vacuoles and become positively buoyant, floating upwards to regions of higher light intensity. The resulting increase in photosynthesis, increases production of low molecular-mass sugars, these increase the tugor pressure within the cell, when tugor pressure reaches a critical value, some of the gas vacuoles collapse and the cell begins to sink promoting formation of new gas vacuoles. Buoyancy is then lost near the surface due to increased production of starch grains, this extra ballast make the algae sink. In dense blooms of blue-green algae, there can be large competition for limited $CO_2$ which can depress photosynthesis. The tugor pressure in the cells can no longer rise to the critical value. All the algal cells will then congregate on the surface and become trapped there. These conditions can kill the algae causing them to rot.

In lakes that have been polluted with organic sewage or nutrient salts, e.g. phosphates, certain blue-green algae often form enormous blooms. Lowered oxygen concentration in the water, brought about by heterotrophic bacteria breaking down organic material, stimulates blue-green algal photosynthesis. In water that has become badly polluted with organic sewage, blue-green algae are often almost the only algae that can still survive and grow.

## 2.1.5. Key variables

Algae rely on a number of essential parameters for growth. Light, temperature, P, N, C, trace nutrients and pH. Any of these could be a rate limiting factor for growth. The balance of these parameters is affected by many factors, some linked to the season. Where nutrients are in excess then $CO_2$ or light could easily be the limiting factor, in conditions where fast growth rates are observed, Silicon could be the limiting factor for diatoms. Any number of scenarios can exist that place each variable as the limiting factor. Maintaining the correct balance for a specific species is the most important aspect of creating optimal conditions.

### 2.1.5.1. Light

A minimum intensity of light is required as sunlight (or artificial light) as a source of radiant energy for photosynthesis to occur. Each algal specie has its own optimal light intensity but as a general rule, more is better. Seasonal variations in light intensity affect the growth rates of photosynthesising organisms. Within a water body, the depth of penetration will depend on the concentration of algae or the turbidity of the water. The wavelength and type of light can also affect the penetration and how efficiently algal cells can use it.

## 2.1.5.2. Temperature

Algal cells also have an optimal working temperature. Seasonal variations in temperature are closely associated with that of light although the monthly temperature variation lags behind the illumination.

Temperature has little effect when light is limiting. When light is not limiting, increase in temperature can increase the rate of photosynthesis, growth/doubling rates are consequently increased.

## 2.1.5.3. Nutrients

The major nutrients of concern to algal growth are consequently those which are important for all plants; Carbon, Nitrogen, Phosphorus. Sulphur and Silicon can also be added to the list. Minor nutrients tend to be metals and are only required in trace amounts; most are toxic at larger concentrations. However, the iron content of the medium may have a significant effect on the algae's growth.

P is one of the essential elements for the algae and is found in two types, dissolved inorganic P (DIP) or dissolved organic P (DOP) and particulate P bound into various forms which may vary greatly in biological availability. The majority of P in the open environment comes from municipal waste, urban and agricultural runoff. $PO_4$ is the ubiquitous form of the element, found in both the organisms and in the environment. No oxidation or reduction steps are required for environmental cycling, metabolism and macromolecular synthesis.

When P levels are high, phytoplankton cells are able to accumulate P reserves well in excess of their immediate requirements. This is the phase of 'luxury consumption'. They can then utilise these reserves during periods of low concentration, hence when P in the water is depleted, growth will not necessarily stop immediately. For these reasons, growth rates do not follow exactly the concentration of P as they will tend to uptake whatever is available as

fast as possible. When P is readily available uptake will be quicker. This could be used advantageously for increasing P update in wastewater treatment.

Small cells tend to grow faster and have the highest affinity for P uptake. Small cell size means that the surface area / volume ratio is high, so that the uptake of nutrients from the water is efficient and rapid. Algae may take up sufficient DIP in one hour to last at least two weeks but over a long time period there is a strong link between P concentration and algal growth. Slower growth has the advantage of leading to longer life and lower loss rates.

### 2.1.5.4. Carbon (specifically $CO_2$)

This is also a crucial factor when considering the design of algal bioreactors / HRAPs. It has been noted that $CO_2$ can be deficient in municipal wastewaters in relation to their P and N content; for the greatest biomass production of algae additional $CO_2$ supplementation to that naturally found in the atmosphere could greatly improve performance. Increasing levels of $CO_2$ within the bioreactor will have a toxic effect on bacteria found naturally in sewage effluent that has a negative effect on algal growth and production.

### 2.1.5.5. Bacteria

The presence of bacteria in wastewater however is also considered as a factor stimulating algal growth owing to $CO_2$ production. They are also responsible for the heterotrophic biodegradation of mixed organic load. The presence of bacteria can adversely affect growth of the algae, decreasing rate of divisions and/or yields. Some algal species are more resistant to the presence of bacteria and hence be found to dominate in poor quality water. Microscopic analysis of my culture samples shows that bacteria may be feeding on algal cells and hence reducing productivity.

### 2.1.5.6. pH

pH is an important factor for algal growth, $CO_2$ supplementation can be introduced to improve growth rates and control pH at an optimal value.

## 2.2. Uses of algae

### 2.2. Applications and uses of algae

Algae Biomass has a wide range of different uses which will be important as we move toward a more environmentally sustainable and less damaging urban society. The uses of algae in modern society include food, industry, pharmaceuticals and fuel; the combination of its growth applications and processes make it a highly economical product (see 3.2.3.). Its traditional use involves treating waste streams such as sewage or flue gas. During these applications, the growth process sequesters $CO_2$ and other pollutants.

### 2.2.1. Why are algae so important?

Much of the oil deposits are thought to be derived from sedimentation of algae in the oceans for millions of years. A product that took millions of years to form will be exhausted by humans in less than 200 years. The carbon from $CO_2$ that was trapped within the algal cells and subsequently in fossil fuels over a period of millions of years is being returned to the atmosphere in an instant.

- Algae are the origin of all plant life on the planet, without which animals would not survive.
- Algae are the basis for many food chains.
- Algae are responsible for all the oil and gas that we are now using to power our lives.
- Algae are responsible for perhaps 50-80% of the world's oxygen production; mainly due to the vast quantities found in the oceans.
- Algae are responsible for the original aeration of the planet's oxygen atmosphere (When oxygen producing micro organisms evolved and started releasing oxygen, initially levels in the atmosphere did not rise; all the oxygen produced was used in oxidation reactions that occurred with other elements to produce the oxides that we now use).

By using algal biofuels and avoiding fossil fuels we can attempt to reduce global warming and run our economies on cleaner power that is at least carbon neutral. Biofuels from algae are far superior to traditional biofuels originating from higher plants, crops and trees for a number of reasons:

- Algae are more efficient photosynthesisers than terrestrial plants.
- Algae have much higher growth rates and require a much smaller land area.
- They do not compete for land with food crops or require the use of limited resources such as fresh water.
- Algae are much more suited to growing in conditions that are not suitable for other plants such as hot dessert and in waste or saline water.
- Due to their micro size they can be used in a number of useful applications such as treating wastewater and flue gas from power stations; waste heat is likely to be available in these situations to further increase growth rates.

## 2.2.2. Methods of cultivating algae

Light nutrients and mixing are required to maximise growth, they are cultured in clear tanks or shallow ponds. There are four common systems used for cultivating algae:

- Open photo-bioreactor tanks
- Closed photo-bioreactor tanks
- Shallow raceway tracks or ponds
- Large scale oceans or desert pools

Each system would typically use a certain type of water (fresh, waste or saline), however there is no hard and fast restriction and ultimately it would depend on the application and geographic location. The nature of closed tanks more or less restricts it to fresh water to prevent contamination. Similarly, oceans and to a large extent desert pools, are restricted to saline water (with the possible exception being areas where vast quantities of wastewater are available, such ideal locations exist in the UAE). A great advantage of algae is that many species can grow in saline or poor quality water. This means that no extra demand has to be placed on fresh water.

Any translucent container can be classed as a photo-bioreactor. The closed systems are protected from the outside environment and have two major advantages: Conditions can be more controlled and are much safer from external contamination from unwanted species. The most common use of a closed system is when the requirement is to grow a single species that may be wanted for a specific trait (high oil content), product, food or pharmaceutical. The most common way of producing a monoculture is serial dilution. A sample containing the desired algae is diluted with filtered water. Small amounts from this diluted sample are added to a large number of growing containers. The dilution is performed in such a way using microscopic analysis with the expectation that a number of the containers will contain only cells of the required specie. Successful growing containers are used to start larger cultures. When growing monocultures, the environment must be closely controlled to prevent the culture being contaminated with unwanted organisms. These bioreactors can require large capital investment.

If the tank is not protected from the outside environment it is classed as an open system. These open systems are exposed to the elements so can be contaminated by a whole range of microorganisms, algae or bacteria. Growth factors are difficult to control in open systems. Monocultures can work in open systems when the required specie can grow in some extreme condition and out compete other algae. It can also work if there is a simple inexpensive system of selecting out the desired algae.

Algae can also be cultured in open pond systems like raceway tracks and lakes. Large tanks or ponds can be made as big as required for large scale production. For their size they tend not to be as costly as photo-bioreactors as they can be simple waterproofed lined shallow excavations with simple mixing.

The biggest system for potential large scale cultivation and harvesting of algae is in oceans or huge desert pools. There are still a number of significant issues to be overcome, i.e., providing the resources other than sunlight to make the process work.

There is a limit to how deep (or wide, if a vertical tank is used) a tank or container can be, due to the penetration of sunlight though densely populated algal cultures. The depth can be increased by adding agitation methods to move the algae in and out of the lighted zone or even by using species with lower chlorophyll and light requirements, however this is considered an unrealistic approach when attempting to maximize productivity.

### 2.2.3. Applications of algae growth

The use of algae does not have to be limited to the biomass produced. Its growth phase can also have an important role in modern society and make the production of algae more economically attractive. Instead of focusing on producing algae; it can be the useful by-product of another process, namely important pollution control methods:

- Wastewater treatment
- Flue gas cleaning and $CO_2$ sequestration
- Water pollution limitation, i.e. capture of fertilizer runoff from farms

All methods of algae production have their own advantages, although the use of wastewater as a medium for growing algae has a number of environmental benefits and appears to be a good option initially. However, algal production from wastewater treatment is not seen as having large-scale impacts on replacing global fuel demands; other methods may be needed for this. Careful life-cycle and cost / benefit analysis are required and include rapidly changing data, information

Sewage lagoons provide a method of growing algae and have the benefit of being naturally high in nutrient concentration. The large scale production potential is limited by quantity of resources (wastewater and land). Lagoons are also highly susceptible to seasonal variations and foreign invasion with little control over what species will grow. In terms of biomass production, these changes in species could alter biomass growth rate and nutrient uptake rate. This could potentially be a benefit or a problem; the specie best suited to a specific set of environmental conditions should dominate however their N:P nutrient ratio requirement

may vary widely amongst species, creating unpredictable nutrient removal performance within the system.

The main focus is on the tertiary treatment for removing plant nutrients, P & N. Algae are also capable of removing other nutrients including heavy metals and potentially absorbing persistent organic pollutants (PDPs). Where high quality water standards must be met, less sustainable chemical processes can be replaced by biotechnological processes such as algal reactors that can not only reduce costs but also produce valuable by-products.

Flue gas emissions are a source of $CO_2$. In addition to pumping exhaust gases directly into a pond, or some kind of bioreactor, on which the algae feed, the bioreactor can be installed directly on top of a smokestack. This technology has been pioneered by Massachusetts-based GreenFuelTechnologies.

Water pollution limitation can be provided locally by small scale systems designed to remove nutrients and other elements such as heavy metals. It is also reported that growth is possible at low water qualities.

### 2.2.4. Can we put all the pieces together?

As mentioned in the previous section, when considering algal growth there are a number of factors that influence its efficiency. If all the essential ingredients are in excess (light, temperature, $CO_2$ and nutrients) and other key variables are controlled (bacteria and pH) then there is no reason why excellent algal growth could not be achieved. When considering algal growth on a scale large enough to affect the worlds fuel supply, two more essential ingredients arise that need to be in excess; water and land/space. By definition, one of the six essential ingredients will always be the limiting factor restricting further increases in growth rates and productivity. The challenge is to maximise the availability of all six essential parts.

All algal systems have the potential to utilise waste $CO_2$ from sources such as power stations. To be economically viable (avoiding transportation) the $CO_2$ source must be located close to the bioreactor especially if any waste heat is to be utilised to enhance algal growth. Another benefit of using flue gas is the potential to utilise the waste heat that is normally associated with the flue gas as well as improving growth rates. This may attract additional financial support from the ROC system.

An STW would provide water and nutrients. It is likely that the works will be located close to or have its own AD and/or CHP engine producing, waste heat, $CO_2$ or both. This incorporates four of the six major essential ingredients for significant algal biomass production. The land requirement will always be relative to the water quantities available.

Light is the essential component not fully freely available for WWT. It is dependent on geographical, seasonal and random weather variations. For high value materials, we could place the whole system in a geographic location guaranteed to receive strong sunlight all year round. This however may produce more issues than it solves. Nutrients, water and $CO_2$ all become scarcer as you move towards these light intense geographic locations like deserts (bright areas tend to be hot, this normally also means dry with high evaporation rates, a disadvantage for water-based life forms). Urban centres gather around fresh water resources and are subsequently the major producers, directly or indirectly of nutrients and $CO_2$.

## 2.2.5. Focused algae production

Areas that are bright, hot, wet and spacious do exist; such as tropical rainforest. Even if tropical rainforests are not suitable for large scale algal production, some potential locations do exist. Large hot deserts in coastal regions will have an unlimited supply of warm saline water (or examples like the UAE, large quantities of warm wastewater).

Ensuring the excess availability of all six essential ingredients for algal growth on a large scale is the major difficulty with the mass utilisation of algae. This thesis however suggests

that large scale processing into a manageable operation may not currently be within reach except in these circumstances.

Four locations that have been suggested are:

- Desert regions in mid/south USA – Issues with availability of nutrients and $CO_2$
- Desert coastal regions of Africa – Issues with availability of nutrients and $CO_2$
- Desert regions in the middle-east located close to coastal cities
- Warm tropical oceans close to nutrient discharge – Issues containing algae
- Centralised STW

## 2.2.6. Harvesting and concentrating techniques – Algal separation from water

Harvesting:

- Gravitational settling
- Polymer flocculation and settling
- DAF
- Membrane filtration

Concentrating:

- Further gravitational settling or Centrifuging
- Drying

The main limitation preventing a wider exploitation of sewage algae is the lack of a cheap and effective harvesting technique. Harvesting of algal biomass, whilst essential is also expensive and difficult. Benemann and Oswald (1996) report that these options are available but an optimum solution has not been found or incurs huge costs, deeming it unsustainable.

The most productive algae are the single celled microalgae. These cells have such good buoyancy that it may take up to 48 hours for them to settle even in perfectly still conditions. Using gravitational settling with this type of algae is not an option as shown by this thesis. However further experiments showed that selecting for fast settling algae solved this problem but incurred additional agitation costs.

Polymer flocculants would reduce settling time. However, one of the aims of this thesis is to avoid using additional chemicals. Flocculants may also have a negative effect on re-using the algae.

DAF may be a possible technique to separate buoyant algae however the small size of the cells can still cause significant problems without coagulants.

Another process is cross flow membrane filtration. In cross flow filtration, the feed is passed across the filter membrane (tangentially to the filter membrane) at some pressure difference. Material which is smaller than the membrane pore size passes through the membrane as permeate or filtrate, and everything else is recycled and retained on the feed side of the membrane as retentate.

This mode of operation is used for high solids feeds because of the risk of blinding in dead end filtration. With dead end filtration, solids material can quickly block (blind) the filter surface, causing a cake formation. With cross flow filtration the tangential motion of the bulk of the fluid across the membrane scows the particles from the filter surface and rub them off. This means that a cross flow filter can operate continuously at relatively high solids loads without cake blinding.

The permeate is of very high quality and all the algae are retained in the bioreactor. The major disadvantage to this process is the energy requirements to create a fast enough flow rate and high enough pressure. This is required to keep the membrane clean and produce permeate flow through the membrane.

Once the algae have been separated from the water, concentrating is relatively easy due to the greatly reduced volume. A wet sludge can be produced by further gravitational settling within an hour. A dry sludge can be produced by using the energy intensive method of centrifuging and a dry solid can be produced by allowing the sludge to dry in air over a

period of 24 hours, this however requires additional land area than the previous two options. This is common at STW. The actual time and space required is dependant environmental factors.

## 2.2.7. Use of biomass (Non fuel types)

Algae have a wide range of uses apart from as energy crops as listed and explained below. The use of algae in a number of these applications is not new and has been traditional procedure for many years, e.g. food in Japan, agar gels and glues.

- Human food and nutritional supplements
- Livestock feed
- Colourings
- Fertilizers and soil conditioners
- Pharmaceuticals
- Agar

Algae are of increasing commercial interest, cultivated as a high nutritional value supplement. They can be high in protein and are excellent sources of vitamins A, B1, B2, B6, niacin and C. They are rich in beta-carotene, iodine, potassium, iron, magnesium and calcium. It is a known fact that fish oil contains the omega-3 fatty acids docosahexaenoic acid, commonly known as DHA and eicosapentaenoic acid, or EPA. The Martek Biosciences Corporation discovered that DHA in fish originates from algae (Martek, 2010). They have begun to manufacture it directly from the algal source. A wide range of different species of algae are eaten directly by humans. Algae are also used as a successful livestock feed due to the low cost and high nutritional value; this is especially useful in fish farming.

The natural pigments produced by algae can be used as an alternative to chemical dyes and coloring agents. Many paper products used today are not currently recyclable due to the presence of chemical inks. Paper recyclers have found that inks made from algae are more easily broken down. There is also much interest in the food industry into replacing the coloring agents that are currently used with coloring derived from algal pigments. Algae has

long been used as a fertilizer due to its high nutrient content and can be used to make certain pharmaceuticals; both cosmetic and medicinal. There are well known commercial uses of algae as agar for both food and substrate for other growth.

## 2.2.8. Algae biofuels

Currently the major driving force is for the use of algae in large scale production of biofuels. The idea of using algae as a fuel source has been considered since at least the middle of the last century but is only now becoming more popular due to an increase in cost and damage from fossil fuels. Scientific research, better technologies and processes are improving the economic viability.

There are numerous ways that algae can be used as an energy source. Algae have a huge environmental benefit over fossil fuels but the most important factor is making the production of biofuel more economically viable.

Some uses are:
- Oil Extraction from Algae
- SVO and Biodiesel
- Alcohol (Ethanol, Methanol, Propanol and Butanol)
- Methane/Biogas
- Pyrolysis
- Gasification
- Biomass
- Hydrogen

Algal biomass contains three main components, carbohydrate, protein and natural oils. The economics of fuel production from algae (or from any biomass) demand that we utilize all the biomass as efficiently as possible. It depends on the recovery of the hydrocarbon and use of the remaining components. A series of techniques have been used to extract energy as above from algal biomass to generate renewable fuel.

## 2.2.8.1. Algae and oil

Algae can produce vastly superior amounts of vegetable oil, compared to terrestrial crops grown for the same purpose. Microalgae have much faster growth-rates than terrestrial crops. The oil yield per unit area of algae is estimated to be between 5,000 to 20,000 gallons per acre, per year (4.6 to 18.4 l/m$^2$ per year); this is 7 to 30 times greater than the next best crop, Chinese tallow (0.64 l/m$^2$ per year) or Palm Oil (0.6 l/m$^2$ per year). Currently most research into efficient algal-oil production is being done in the private sector, but if predictions from these small scale production experiments bear out then using algae to produce biodiesel, bioethanol and biobutanol may be the only viable method by which to produce enough automotive fuel to replace current world gasoline usage.

The difficulties in efficient oil production from algae lie not in the extraction of the oil. This can be done using methods common to the food-industry such as hexane extraction. There is still a requirement to find an algal strain with a high lipid content, fast growth rate, easy to harvest and a cost-effective cultivation system (i.e., type of photo-bioreactor).

Open-pond methods have largely been abandoned for the cultivation of algae with high-oil content. Many believe that a major flaw of the Aquatic Species Program (Benemann, 1998) was the decision to focus their efforts exclusively on open-ponds. Algae in an open-pond environment are subject to wide swings in temperature and pH, and competition from invasive algae and bacteria. Open systems using a monoculture are also vulnerable to viral infection. The open-pond method makes the entire process dependent upon the hardiness of the strain chosen, requiring it to be resilient compared to a closed system in order to withstand these environmental variables. For a given amount of photosynthetic energy, an algae strain producing relatively high levels of oil will produce relatively less protein and/or carbohydrate, usually resulting in the species being less hardy, or having a slower growth rate. Algal species with lower oil content, not having to divert their energies away from growth, have an easier time in the harsher conditions of an open system.

Research into algae for the mass-production of oil is mainly focused on microalgae; organisms capable of photosynthesis that are less than 2 mm in diameter, including the diatoms and cyanobacteria; as opposed to macroalgae, e.g. seaweed. This preference towards microalgae is due largely to its less complex structure, fast growth rate, and high oil content (for some species).

The aquatic species program selected around 300 species of algae that could be potentially useful as a source of oil. These were mainly found to be green algae and diatoms (Aquatic Species Program: close out report).

It is known that under certain growth conditions, algal cells produce more oil. Conditions which increase growth rates reduce their oil content. Nutrient starving such as limiting nitrogen with green algae increases the oil content. This was studied extensively by the Aquatic Species Program. There was consensus among the studies showing increased oil production under stress and linked to cessation of cell division. While the rate of production of all cell components is lower under nutrient starvation, oil production seems to remain higher, leading to an accumulation of oil in the cells. The increased oil content of the algae however does not to lead to increased oil overall. In fact, oil production per unit reactor is lower during periods of nutrient deficiency. Higher levels of oil in the cells are more than offset by lower rates of cell growth.

In diatoms, with the activity of ACCase, a lack of silicon also leads to an augmentation in lipid synthesis.

### 2.2.8.2. Oil extraction from algae

Algal oils have a number of commercial and industrial uses as previously discussed and are extracted through a wide variety of scientific methods.

Expression/Expeller press: The simplest method is mechanical crushing. When algae are dried it retains its oil content, which then can be "pressed" out with an oil press. Since

different strains of algae vary widely in their physical attributes, various press configurations (screw, expeller, piston, etc) work better for specific algae types. Many commercial manufacturers of vegetable oil use a combination of mechanical pressing and chemical solvents in extracting oil. Estimates of the cost to extract oil from microalgae vary, but are likely to be around $1.80/kg (compared to $0.50/kg for palm oil).

Soxhlet extraction: An extraction method that uses chemical solvents. Oils from the algae are extracted through repeated washing, or percolation, with an organic solvent such as hexane, benzene or petroleum ether, under reflux in safety protected equipment. This process is widely used in the food industry and is relatively inexpensive. There are problems for example, Benzene is classified as a carcinogen and most chemical solvents are a flame and explosion hazard.

Osmotic shock: Osmotic shock is a sudden reduction in osmotic pressure; this can cause cells in a solution to rupture. Osmotic shock is sometimes used to release cellular components, such as oil.

Ultrasonic-assisted extraction: Ultrasonic extraction, a branch of sonochemistry, can greatly accelerate extraction processes. Using an ultrasonic probe and reactor, ultrasonic waves can be used to create cavitation bubbles and pressure / temperature waves in a solvent material. When these bubbles collapse near the cell walls, shock waves and liquid jets are created that cause the cell walls to break down and release their contents.

Enzymatic extraction: Enzymatic extraction can be used to degrade the cell walls and release the oil. The costs of this extraction process are estimated to be much greater than hexane extraction but may have fewer risks for food and pharmaceutical grade oils. The enzymatic extraction can be supported by ultrasonication. The combination "sonoenzymatic treatment" allows for faster extraction and higher oil yields.

Supercritical fluid: In supercritical fluid/$CO_2$ extraction, $CO_2$ is liquefied under pressure and heated to the point where it has the properties of both a liquid and a gas. This liquefied fluid then acts as a benign solvent for extracting the oil.

Other methods are still being developed, including ones to extract specific types of oils, such as those with a high production of long-chain highly unsaturated fatty acids.

### 2.2.8.3. SVO and biodiesel

The algal-oil feedstock that is used to produce biodiesel can also be used for fuel directly as SVO. Whilst using the oil in this manner does not require the additional chemical and energy needed for transesterification, (processing the oil with an alcohol and a catalyst to produce biodiesel), the use of SVO does require modifications to a normal diesel engine, whereas biodiesel can be run in any modern diesel engine, unmodified. SVO can be used in an unmodified diesel engine providing the viscosity is reduced with white spirit, petrol or similar (as a dual fuel mixture).

SVO, extracted from oil can be refined for traditional transport fuels to produce gasoline, diesel, propane, or kerosene.

Biodiesel is alkyl esters made from the transesterification of vegetable oils. Algal-oil can be processed into biodiesel as easily as oil derived from land-based crops. Biodiesel is biodegradable and non-toxic, and produces significantly fewer emissions than petroleum-based diesel when burned. Biodiesel is a better solvent than standard diesel and so causes less engine wear. Currently, only some vehicle manufacturers state that their diesel engines can be run with 100% biodiesel.

### 2.2.8.4. Alcohol

Biologically produced alcohols, most commonly ethanol and methanol, and less commonly propanol and butanol are produced by the action of microbes and enzymes through

fermentation. This is a potential use of the algal biomass, specifically the remaining fraction after initial processing, i.e. oil extraction.

### 2.2.8.5. Biogas

Biogas produced and recovered by the process of anaerobic digestion of organic material is the most widely used biofuel at the moment and has the advantage that most large UK sewage works already have anaerobic digesters.

### 2.2.8.6. Pyrolysis

Pyrolysis is the chemical decomposition of organic materials by heating in the absence of oxygen or any other reagents. The resulting carbonization produces various grades of hydrocarbon residue.

### 2.2.8.7. Gasification

Synthesis Gas (Syngas) is generated during the pyrolysis and gasification of a carbon containing fuel to a gaseous product. The product will contain varying amounts of carbon monoxide, $CO_2$ and hydrogen. Syngas can be used as a fuel or as an intermediate for the production of other chemicals.

### 2.2.8.8. Solid biomass

Algae can be grown to produce biomass, which can then be harvested, dried and combusted in the same manner as wood, to produce heat and electricity.

### 2.2.8.9. Hydrogen

Algae can be used as a biological source for the production of hydrogen. In 1939 Hans Gaffron observed that the algae, *Chlamydomonas reinhardtii* (a green alga), would sometimes switch from the production of oxygen to the production of hydrogen. Gaffron

was unable to explain this change but in 1998, professor Anastasios Melis discovered that depriving the algae of sulfur was the switch from the production of oxygen (normal photosynthesis), to the production of hydrogen. He found that the enzyme responsible for this reaction was hydrogenase, but the hydrogenase did not work in the presence of oxygen, suggesting a role for sulphur oxidation. Melis found that oxygen transport was dependant on a sulphur enzyme, diverting metabolism via hydrogenase activity. C*hlamydomonas moeweesi* was found to be a good strain for the production of hydrogen.

In 2001 Melis started his own company to try to commercialize hydrogen production, the company failed however research continues at the Photobiological Hydrogen Production Program, started in 2004. Ely and Chaplen are also researching this field, hoping to develop oxygen-tolerant strains of cyanobacteria that can produce hydrogen continuously.

## 2.3. The basics of wastewater treatment, why phosphorus removal is important and a guide to eutrophication

### 2.3.1. Background of wastewater treatment

With the growth of the human population, people began to live in larger and more densely populated groups; the removal of human waste became a serious issue. Where small populations exist and land is abundant, small low energy treatment plants to deal with the sewage to remove carbon, ammoniacal, nitrogen and pathogens were normal.  In modern times, centralisation of facilities is more popular as land has become less accessible and a broader range of pollutants have been consented. When an area becomes densely populated, it is much more difficult to install individual systems and becomes more efficient to deal with the problem by installing more pipes and pumps that carry the waste away to a well controlled centralised outlet. European wide regulations (WFD2003) are now in place which aspires to ensure that wastewater streams are of natural quality and not exerting an influence on biodiversity.

Sewage consists of water-borne domestic and industrial waste which is enriched with dissolved and suspended organic and inorganic constituents. It also contains faecal and other potentially pathogenic bacteria. Currently the sewage has to be treated before its disposal for three main reasons. Firstly, the anaerobic digestion of sewage produces stinking and offensive odours, secondly the contamination of potable water with raw sewage is potentially a health risk and thirdly the waste stream is a sustainable reusable source of inorganic nutrients such as nitrogen, phosphorus, potassium and sulphur that left untreated can have detrimental effects on the environment. Recycled sewage effluent is now also an important component in water supply (5-10%).

## 2.3.2. Overview of the main wastewater treatment processes

A typical wastewater treatment plant operates four main processes; the processes within these stages consist of physical, biological and chemical processes. The actual standard required for the effluent discharge depends on the flow rate of receiving water.

### 2.3.2.1. Preliminary treatment

The first stage consists of two main physical processes. Screening through a series of screens decreasing in size removes the coarse, medium and fine solids. This is mainly for protection of the subsequent treatment units. The second stage removes sand and grit via settlement in a grit chamber. This dense material settles much faster than the organic matter which stays in suspension for treatment in downstream units.

### 2.3.2.2. Primary treatment

The main aim of primary treatment is to remove a large part of the suspended solids and hence reduce the BOD (typically by 30%) directed to the secondary treatment. This is done by gravitational settlement tanks; this remaining mass of solids is the raw primary sludge. Material that has a lower density than water such as detergents will rise to the surface of the sedimentation tanks where it is collected and removed for subsequent treatment. The

process can be enhanced with the use of phosphate precipitating chemicals such as Fe and Al.


### 2.3.2.3. Secondary treatment

The main aim of the secondary treatment is to remove the dissolved organic matter and any remaining fine suspended organic matter. The processes are controlled accelerated natural decomposition mechanisms in bioreactors. The controlled conditions ensure short time scales for treatment. The removal of organic matter in the secondary stage is carried out by adapted soil and river bacteria which include ammonia oxidisers. The microorganisms, particularly the nitrifiers, need to be kept in contact with and have a good supply of oxygen as it is an aerobic process. This is the basic process however there is a wide variety of secondary treatment processes, the main ones are:


- Algal stabilisation ponds
- Land disposal systems
- Anaerobic reactors
- Activated sludge systems
- Aerobic biofilters


Going into detail about each process is beyond the scope of this thesis however details of them can be found for example in the CIWEM British Practice Manual.


### 2.3.2.4. Tertiary treatment

The main aims for tertiary treatment are:


- Achieving low solids values
- The removal of pathogenic organisms
- The removal of heavy metals
- The removal of nutrients, mainly N and P

Tertiary treatment is mainly achieved using sand filters whose backwashing requires large amounts of power which can be costly. To remove or kill pathogenic organisms, sometimes UV lamps are used. Chemical precipitation processes are used to remove nutrients into a sludge. Biological processes using P accumulating organisms may not be able to achieve the consistency of chemical treatment.

### 2.3.3. The importance of nutrient removal, specifically P removal

Most stages in the treatment process work well and easily meet the required standard. The later tertiary stage of nutrient removal is not so successful, can be costly and involve the use of large amounts of chemicals. This is especially true for the removal of Phosphorus.

A standard of 1mg/L $PO_4$ needs to be met 95% of the time to meet the UWWTD or WFD. This is difficult and costly with traditional chemical methods. Meeting N and P standards are important for preventing / controlling Eutrophication. P is often regarded as the main problem in cases of eutrophication in natural waters subjected to point source pollution from sewage since the normal background concentration is less than 0.5mg/L. The concentration of algae and the eutrophic state of natural waters correspond well to phosphorus level. Ecosystems receiving more nitrogen than the plants require are called nitrogen-saturated. Phosphorus is much less soluble in water than nitrogen so consequently, phosphorus is a much more important growth limiting nutrient in aquatic systems.

### 2.3.4. Eutrophication

Growth of algae in water; reservoirs, rivers and other water supplies have a number of undesirable effects. Waters that contain an abundance of dissolved nutrients are capable of supporting a dense crop of algae. N & P are essential to growth and if these are limited in quantity, little growth occurs. Water which is fertile i.e. rich in dissolved nutrients, is termed eutrophic. In eutrophic situations, the deeper waters, which are often cool and dark, usually become totally depleted of oxygen, particularly during summer.

The most general problem is a lack of oxygen in the water needed for animals to survive. Algae also cause discolouration and bad taste in the water with some species producing toxins harmful to higher forms of life involved in that food chain. Algae can also cause additional problems during water treatment and reduce the aesthetical and recreational value of natural waters.

The oxygen depleted water results from the microbial breakdown of sedimenting algal and animal wastes / remains that build up during active periods like the summer, especially in eutrophic environments where nutrients are in excess. In autumn, as the surface water (epilimnon) cools to a temperature lower than the deep hypolimnon a switch or mixing process occurs in the stratified layers. The now less dense, murky oxygen depleted water from the bottom comes to the surface replacing the clear cooling water. As a result, fish and other organisms, which need oxygen, can sometimes be killed through suffocation by this upwelling. The water may produce bad odours as a result of the decomposition. Some waters are naturally eutrophic, but many lakes and rivers also become so, through the effects of human activity. P in detergents is prohibited in Finland / Switzerland to protect the predominately lake surface waters.

### 2.3.5. P & N removal techniques from wastewater

Phosphate removal from wastewater involves the incorporation of phosphate into a particulate form which is then removed as suspended solids. The phosphate can be incorporated as either micro-organisms or metal phosphate precipitates.

Growing biomass to treat sewage is a technique that has been used for many years. Duckweed covered sewage lagoons and reed beds are the most common non-bacterial options. The growth of biomass requires a certain amount of nutrient to be present; sewage is an ideal location. A fast growing water based plant is favourable.

A reed bed is an artificially created wetland planted with specially selected species of reed. They are mainly used in rural areas that do not have nearby sewage treatment facilities. A reed bed is capable of fully treating sewage and relies on biological processes. They are efficient and cheap to run, produces reeds which can be harvested for compost and avoids the need for chemical treatment. (See "Reed Beds for the Treatment of Domestic Wastewater, Grant and Griggs")

A sewage lagoon has the more specific purpose of removing P and N. They are usually one of the final processes in a water treatment system and consist of a large tank / pond growing some form of plant life with good nutrient removal properties such as duckweed. The nutrient absorption is proportional to plant biomass growth. A study by Al-Nozaily showed that the P and N uptake rates and duckweed growth rate are inhibited by high levels of ammonia, N removal is not affected by the depth of tank and P removal is lower than that of algae-based ponds (Arceivala, 1981). It is noted that duckweed is considerably easier to harvest than algae.

Nitrification is the biological oxidation of ammonia to nitrate. The micro organisms, Nitrosomonas and Nitrobacter carry out the reaction but acid produced by the process lowers the pH so alkalinity may have to be added. The nitrate is then biologically converted to gaseous nitrogen by denitrification.

Biological treatment is generally a more attractive option although physical/chemical processes exist. Breakpoint chlorination is accomplished by the addition of chlorine to the waste stream to oxidise ammonia-nitrogen to gaseous nitrogen. Air stripping of ammonia consists of raising the pH of the wastewater to pH11.5; this is induced artificially however it occurs naturally during plant growth when the $CO_2$ concentration is reduced. At high pH the solution will contain mostly ammonia as a dissolved gas. With enough air-water contact, the ammonia gas will be stripped from solution. Selective ion exchange is accomplished by passing the wastewater through an ion-exchanger bed which exhibits a high selectivity for

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the ammonium ion. Filtration of the wastewater is usually required before the ion-exchanger to prevent fouling of the natural zeolite used in this process.

## 2.3.6. The use of controlled eutrophication through algal bioreactors and HRAPs to remove P and other pollutants

A solution to meeting P standards and preventing the release of nutrient rich sewage effluent may exist by performing a wastewater treatment stage of controlled eutrophication.

Algae adapted specifically for growing in wastewater and consuming P and other pollutants can be grown in a controlled environment. They can be used to remove these pollutants before being discharged to natural waters, avoiding downstream growth. The algae would not be discharged with the treated effluent (free of nutrients and suspended solids) but instead used to treat the continuous flow of wastewater and be regularly harvested to maintain the algae concentration at an optimum value. The collected biomass rich in carbon and nutrients can be used to produce higher value products such as fertiliser or sustainable fuel source.

During the algae's growth process they fix large amounts of $CO_2$ from the atmosphere. Additional $CO_2$ can be used to promote more intensive growth and maintain unfavourable conditions for bacteria. The presence of algae in water, even in small quantities may give it an odour or taste. However the majority of freshwater algae tend towards the purification of water due to their capacity to absorb and utilise many diverse kinds of organic substances. It is unlikely that wastewater would be used directly for drinking water treatment without first being discharged into a natural water course for further natural purification or involve treatment processes such as activated carbon to remove dissolved organics.

# 3. Literature Review

## 3.1. Summary of literature review

The progress in this field has been very fast over the past 5 years and the population of literature is on-going, this has meant that the literature review had to be updated regularly. The initial search for literature in 2006 turned up very little data. Therefore, whilst in the experimental planning phase a vast majority of this literature did not exist. Re-planning a similar project with the combined accumulation of knowledge over the past 5 years would give a very different approach. There is a detailed description provided of the experiments that still need to be examined based on the culmination of the project findings, the literature and recent data up to early 2011.

A comprehensive search for literature has been carried out that covers the range of topics related to this project. Where possible, the literature is from modern sources, avoiding older papers that are either out of date or referenced by more modern work. The range of experimental work carried out is fairly small however the nature of this topic provides so many variable parameters that many combinations of these can be tested. In general, there is much agreement about the mechanisms and processes involved in this field. Most experimental data provide similar results and suggested theory.

The main disagreement is not that the process can work but instead, if nutrient removal rates and biomass production can be sustained in unfavourable conditions. The disagreement within the field is related to the economic viability of producing large quantities in the open environment to match the production of energy from fossil fuels. Most people accept however that when the main focus is wastewater treatment, the economics are no longer a barrier; instead any by-products such as algal biomass and improvements in sustainability become benefits.

The main lack of work so far has been implementing all the individual successful procedures into a single unit based at a wastewater treatment works. This is a difficult step to take however due to minimal work having been carried out using natural wastewater in uncontrolled environments which are realistic to real life. Previous work has either tested for individual parameters or optimised laboratory setups, mainly using artificial wastewater.

The focus is to test real life conditions, wild algae and variable sewage under low light conditions to determine if the process is possible in the UK and how this could be combined with the industrial processes shown in the literature. A scale up design should make best use of the most effective pond procedures and mechanisms whilst overcoming the issues and problems other people have experienced across the range of variables and environmental parameters.

Throughout the literature I have then examined the numerous tools available for controlling these parameters to optimise efficiency. The work leads on to describe a suggested design on a treatment facility based on my experimental data and the findings from literature.

Table 1 – Summary of papers that were read and not reviewed, in favour of using other papers

| Title | Author | Year | Reason |
|-------|--------|------|--------|
| Chemical Processes for Phosphate Removal | Jenkins et al. | 1971 | Due to the age of the paper and the evaluation that chemical treatment is not the way forward |
| Modelling of Phosphorus precipitation in wastewater treatment plants with enhanced biological phosphorus removal | Maurer & Boiler | 1999 | Having read through other more suitable papers, I felt this did not contribute anything for my research |
| The Removal of Phosphorus During Wastewater Treatment: A Review | Yeoman et al. | 1988 | Focusing on a review of chemical P removal from 1988; the paper did not provide any useful information |
| Microalgae as a Source of Liquid Fuels | Benemann et al. | 1982 | With the amount and quality of recent papers, it was unnecessary to include this |

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|---|---|---|---|
| The relative importance of Lemna gibba L., bacteria and algae for the nitrogen and phosphorus removal in duckweed-covered domestic wastewater | Korner & Vermaat | 1998 | Taking into consideration the amount of papers I have on this topic, the age of the paper and the specific relevance. Having looked over this paper I felt it was not beneficial for inclusion |
| Sewage nutrient removal by a shallow algal stream | Hemens & Mason | 1968 | Taking into consideration the amount of papers I have on this topic, the age of the paper and the specific relevance. Having looked over this paper I felt it was not beneficial for inclusion |
| Ecological Water Treatment System for Removal of Phosphorus and Nitrogen from Polluted Water | Drenner et al. | 1997 | Taking into consideration the amount of papers I have on this topic, the age of the paper and the specific relevance. Having looked over this paper I felt it was not beneficial for inclusion |
| Nutrient Removal by Thermophilic Fischerella (Mastigocladus laminosus) in a Simulated Algaculture Process. | Radway et al. | 1994 | Taking into consideration the amount of papers I have on this topic, the age of the paper and the specific relevance. Having looked over this paper I felt it was not beneficial for inclusion |
| Nitrogen Removal in Pond Systems With Different Configurations and Geometries | Silva et al. | 1995 | Taking into consideration the amount of papers I have on this topic, the age of the paper and the specific relevance. Having looked over this paper I felt it was not beneficial for inclusion |
| Wastewater Nutrient Removal by Marine Microalgae Grown on a Corrugated Raceway. | Craggs et al. | 1997 | Taking into consideration the amount of papers I have on this topic, the age of the paper and the specific relevance. Having looked over this paper I felt it was not beneficial for inclusion |
| Microalgae and Wastewater Treatment. | Hammouda et al. | 1995 | Taking into consideration the amount of papers I have on this topic, the age of the paper and the specific relevance. Having looked over this paper I felt it was not beneficial for inclusion |
| Where is biological | Barnard & | 2006 | The paper did not include any |

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|---|---|---|---|
| nutrient removal going now? | Steichen | | information about the use of algae for wastewater nutrient removal |
| Nitrification in bulk water and biofilms of algae wastewater stabilization ponds | Babu et al. | 2007 | Taking into account the content, age and relevance of this paper compared with the numerous other papers I have on a similar area of research, I have decided not to include it in the literature review |
| Process performance assessment of algae-based and duckweed-based wastewater treatment systems | Zimmo et al. | 2002 | Taking into account the content, age and relevance of this paper compared with the numerous other papers I have on a similar area of research, I have decided not to include it in the literature review |
| Selection of natural treatment processes for algae removal from stabilisation ponds effluents in Brasilia, using multicriterion methods | Neder et al. | 2002 | Taking into account the content, age and relevance of this paper compared with the numerous other papers I have on a similar area of research, I have decided not to include it in the literature review |
| Comparison between algae-based and duckweed-based wastewater treatment: Differences in environmental conditions and nitrogen transformations | Zimmo et al. | 2000 | Taking into account the content, age and relevance of this paper compared with the numerous other papers I have on a similar area of research, I have decided not to include it in the literature review |
| The role of algae in a deep wastewater self-regeneration pond | Arauzo et al. | 2000 | Taking into account the content, age and relevance of this paper compared with the numerous other papers I have on a similar area of research, I have decided not to include it in the literature review |
| An integrated duckweed and algae pond system for nitrogen removal and renovation | Steen et al. | 2000 | Taking into account the content, age and relevance of this paper compared with the numerous other papers I have on a similar area of research, I have decided not to include it in the literature review |
| Performance of a pilot-scale high rate algal pond system treating abattoir | Evans et al. | 2005 | Taking into account the content, age and relevance of this paper compared with the numerous other |

| wastewater in rural South Australia: nitrification and denitrification | | | papers I have on a similar area of research, I have decided not to include it in the literature review |
|---|---|---|---|
| Nitrogen Transformations and Removal Mechanisms in Algal and Duckweed Waste Stabilisation Ponds | Zimmo | 2003 | Taking into account the content, age and relevance of this paper compared with the numerous other papers I have on a similar area of research, I have decided not to include it in the literature review |
| Design and Analysis of Microalgal Open Pond Systems for the Purpose of Producing Fuels. | Welssman & Goebel | 1987 | Taking into account the content, age and relevance of this paper compared with the numerous other papers I have on a similar area of research, I have decided not to include it in the literature review |
| Systems and Economic Analysis of Microalgae Ponds for Conversion of $CO_2$ to Biomass. | Benemann & Oswald | 1996 | Taking into account the content, age and relevance of this paper compared with the numerous other papers I have on a similar area of research, I have decided not to include it in the literature review |
| Waste Stabilization Ponds: Effluent Quality Requirements and Implications for Process Design | Mara | 1996 | Taking into account the content, age and relevance of this paper compared with the numerous other papers I have on a similar area of research, I have decided not to include it in the literature review |
| Biogas Production from Algae Biomass Harvested at Wastewater Treatment Ponds | Salerno et al. | 2009 | The topic of the paper would be useful but it is so badly presented with important information missing, it is impossible to make sense of how they carried out the experiments. From what I can tell they have serious floors in the control of the experiments they carried out. |

## 3.2. General Findings

G. Shelef (1980) studied algal species in wastewater under varying parameters; season, temperature, pH, organic loading, retention time, depth and agitation methods. It was

found that the algal population in wastewater was made up of six major algae species. Euglena gracilis, Scenedesmus dimorphus, Chlorella vulgaris, Ankistrodesmus falcatus, Actinastrum gracillimum and Micractinium pusillum. Although they all have optimal temperatures around 25°C, Scenedesmus and Euglena are more tolerable to low temperatures and tend to dominate at around 15°C.

A resource availability analysis by the aquatic species program indicated that significant potential land, water and $CO_2$ resources exist to support the algal biomass technology.

"The first work done in earnest by DOE on demonstration of algae technology for energy production predates the Aquatic Species Program. In 1976, the Energy Research and Development Administration (before it folded into DOE) funded a project at the University of California Berkeley's Richmond Field Station to evaluate a combined wastewater treatment/fuel production system based on microalgae. Over the course of several years, the Richmond Field Station demonstrated techniques for algae harvesting and for control of species growing in open ponds. By the time the Aquatic Species Program took on microalgae research, emphasis had already moved from wastewater treatment based systems to dedicated algae farm operations. From 1980 to 1987, the program funded two parallel efforts to develop large scale mass culture systems for microalgae. One effort was at the University of California, and it was based on a so-called "High Rate Pond" (HRP) design. The other effort was carried out at the University of Hawaii, where a patented "Algae Raceway Production System" (ARPS). Both designs utilized open raceway designs. The HRP design was based on a shallow, mixed raceway concept developed at Berkeley in 1963 and successfully applied in wastewater treatment operations in California. The ARPS was really a variation on the same concept. Both efforts carried out their test work in ponds of 100 square meters or less. They studied a variety of fundamental operational issues, such as the effects of fluid flow patterns, light intensity, dissolved oxygen levels, pH and algae harvesting methods. At the conclusion of the smaller scale tests conducted in California and Hawaii, the program engaged in a competitive bidding process to select a system design for scale-up of algae mass culture. The HRP design evaluated at UC Berkeley was selected for

scale-up. The "Outdoor Test Facility" (OTF) was designed and built at the site of an abandoned water treatment plant in Roswell, New Mexico. From 1988 to 1990, 1,000 square meter ponds were successfully operated at Roswell. This project demonstrated how to achieve very efficient (>90%) utilization of $CO_2$ in large ponds. The best results were obtained using native species of algae that naturally took over in the ponds (as opposed to using laboratory cultures)." *Aquatic species program*

Finding algal strains to grow is not very difficult. Cultivating specific strains of algae for biodiesel could, however, be a bit more difficult, as they can require high maintenance and could get easily contaminated by undesirable species. The algal ponds are open systems and hence vulnerable to being contaminated by other algal species and bacteria. The real challenge with open ponds is that the species of algae that have the highest oil content are not necessarily the quickest to reproduce. This creates a problem where other species take over the pond.

In an open pond there is much less control over water temperature, $CO_2$ and lighting conditions. Ponds in which algae are normally cultivated are called 'raceway' ponds, whereby the water, algae and nutrients are moved around a circuit. The ponds are kept shallow and the algae are kept in suspension to aid photosynthesis. The ponds operate in a continuous manner with a steady production of algae.

Photo-bioreactors can be used for a more controlled environment; this comes with extra cost and energy usage. The efficiency and oil yields would be significantly higher and could work out more beneficial in the long run.

In work carried out by the aquatic species program, they found an inability to maintain laboratory organisms in the field. Algal species that looked very promising when tested in the laboratory were not robust under conditions encountered in the field. In fact, the best approach for successful cultivation of a consistent species of algae was to allow a

contaminant native to the area to take over the ponds. This evidence almost suggests that testing with specific species for use in open field environments is pointless.

A problem with the HRTs in bioreactors and tanks is not necessarily due to nutrient removal rates but can be limited by the reproduction rates of algal cells. This can be a common problem and has been stated by Abeliovich (1976). "Whenever we tried to reduce the retention time of the wastewater in the high rate oxidation ponds to less than four days, the algal culture was washed out of the pond, and we were left with an anaerobic pond practically free of algae."

Abeliovich (1976) also found that high ammonia concentrations inhibit photosynthesis and growth of algae at pH values over 8.1.

The most common set up for algae cultivation is long shallow 'raceway track' tanks with the use of rotary paddles. Another popular choice is small diameter tall circular Perspex tanks; these provide the best surface area to volume ratios for maximum sunlight. A disadvantage to this type of tank is the small amount of air contact surface; gives rise to a reduced $CO_2$ concentration in the tank.

A popular method of agitation is the use of paddle wheels that move the fluid around the tank and help keep algae in suspension. It may be possible for these to be wind powered.

## 3.3. Nutrient removal technologies

### 3.3.1. Phosphorus removal

**Chemical techniques**

Morse et al. (1998) produced a comprehensive review of technologies that remove and recover P from wastewater. These technologies include chemical precipitation, BPR, crystallisation, novel chemical precipitation approaches and a number of wastewater and

sludge-based methods. They provide a very useful and informative paper about the existing technologies. However they do not mention, algal ponds, lagoons or any kind of algal based treatment. They provide a full 2 page summary of all the other technologies. The main details are as follows:

Removal was initially achieved by chemical precipitation, which remains the leading technology today (1998). Chemical precipitation is in essence a physico-chemical process, comprising the addition of a divalent or trivalent metal salt to wastewater, causing precipitation of an insoluble metal phosphate that is settled out by sedimentation. The most suitable metals are iron and aluminium, added as chlorides or sulphates. Lime may also be used to precipitate calcium phosphate. Anionic polymers may be used to assist solid separation. Chemical precipitation is a very flexible approach to P removal and can be applied at primary, secondary or tertiary stages during WWT.

Strickland (1998) looked into the development and application of P removal from wastewater using biological and metal precipitation techniques. They look at the program of technology development in response to the wastewater treatment directive, biological treatment, iron salt dosing and biological filters.

He states that for surface water reservoirs, the concentration of P needs to be reduced to below 0.01mg/L to reliably control algal growth. *Based on results from the literature review, I would state that the figure is closer to 0.04mg/L, never-the-less it is still very low.*

The results of the large scale BNR at Northampton STW show that the effluent was reduced to 1.7mg/L on average throughout the year. Cambridge could only reduce P to 2-3mg/L due to the weak sewage at 120mg/L BOD.

When Strickland (1998) was using an established chemical process, two trials were run, one at Letchworth and one at Northampton. At Letchworth STW they tested both ferric and ferrous sulphate. When using a higher Fe:P ratio, the % P removal was greater. It was shown

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that the use of ferrous sulphate was more effective than ferric sulphate (and also lower in cost) and could remove 97% of P in the trial, achieving 0.29mg/L. Northampton STW trials confirmed ferrous sulphate could achieve <1mg/L P with a 2.4:1 Fe:P dosing ratio. Louth STW switched from ferric sulphate to ferrous sulphate and saw an average annual improvement, reducing effluent P from 2.6mg/L – 2mg/L.

The paper highlights the difficulties in reducing P to low concentrations using these methods.

Takacs et al. (2006) provide a case study from the Washington, DC area showing that chemical P removal can achieve extremely low levels. The Chesapeake Bay area of Washington, DC has very stringent discharge P requirements, essentially down to the detection limit (0.01mg/L). They examine two plants that use different chemical P removal technologies.

Using Ferric, extremely low orthophosphate levels (<0.02mg/L) can be achieved over a wide range of pH values (5.7-8), this is shown for a large data set plotting concentration vs. pH. When simultaneous precipitation is used, a 3.9:1 Fe:P ratio is required however when tertiary clarification is used, it only requires a ratio of 2.5:1.

Takacs et al. (2006) also show that chemical P removal can consistently reduce P to <0.2mg/L, often to below the detection limit of 0.01mg/L in the pH range of 6.2-7.

Valve et al. (2002) looked at enhancing BPR from municipal wastewater with partial simultaneous precipitation. They carried out pilot-scale tests to enhance P removal using ferrous sulphate in a biological P and N removal process.

Previous studies in Finland have shown that with biological P removal (BPR) very consistent results with effluent P levels of less than 0.5mg/L can be achieved even under winter conditions as long as N removal or nitrification is not needed. When N removal was

combined with BPR to treat normal municipal wastewater, wide variations were encountered: effluent soluble P varied between 0.2-2.5mg/L.

During the start up period, P removal was effective with an effluent P of less than 0.3mg/L. This was well in accordance with earlier observations that BPR is effective when nitrification is incomplete. The results indicated that Fe(II) competes with the PAOs and will inhibit the BPR process completely at doses exceeding $9g/m^3$ of Fe(II). The goal of an effluent P level of 0.5mg/L can be attained with $5g/m^3$ of Fe(II). A consistent effluent concentration of 0.3mg/L could not be achieved with this method.

It can be concluded that the Fe very effectively binds the free P thus preventing the PAOs from using it for poly-P build up in the cell which is essential for the functioning of BPR.

This review has shown that there are issues with using simultaneous P and N removal during this process. Simultaneous P and N removal is an integral part of algal treatment without the need for additional chemicals.

Morse et al. (1998) talk about the development of crystallisation technology that started in the 1970s, in response to more stringent P removal requirements combined with the desire to produce a more marketable end-product. The crystallisation process is based on the crystallisation of calcium phosphate on a seeding grain, typically sand, within a fluidised reactor. Process conditions are adjusted to promote calcium phosphate crystallisation by adding either caustic soda or milk of lime. The high rate of crystallisation allows a short retention time and therefore a small reactor. Pellets are periodically removed and replaced by smaller diameter seed grains. This allows continuous operation and ensures good fluidisation.

Ion exchange, magnetic attraction and adsorbents have also been investigated for their potential to remove P from wastewater. In the RIM-NUT ion exchange-precipitation process, ammonia and phosphate ions are removed from tertiary wastewater to produce struvite.

The process uses a cationic resin to remove the ammonium ions and a basic resin to remove phosphate ions. Regeneration releases the ammonium and phosphate which are then precipitated as struvite.

From the preceding literature review, it can be seen that see that today, nobody wants to use chemical treatment due to the high running costs, corrosive and unsustainable nature. It prevents easy recycling of the sludge to agriculture and inhibits anaerobic digestion. It is mainly used at the tertiary stage to focus on P removal. In the following sections we review the biological techniques.

**Biological techniques**

Morse et al. (1998) also state that more recently however, the most popular method has become BPR and is now firmly established. The development of BPR was based on research in the late 1950s, which found that, under certain conditions, activated sludge could take up P in considerable excess to that required for normal biomass growth. Based on this phenomenon, known as 'luxury uptake', a number of applications and processes have been developed and the technology is now firmly established, particularly for new or redeveloped works serving large populations. The technology has the advantage of avoiding the use of chemicals and excess sludge production. However, it requires more complex plant configurations and operating regimes. Recovered P is in general biologically bound and can be released into solution under anaerobic conditions. Careful sludge management is therefore essential.

Craggs et al. (1996) studied P removal from wastewater using an algal turf scrubber. The algal turf scrubber consisted of an inclined floway strip with attached periphyton, micro algae and bacteria. The strip was 6.5m wide and 1012m$^2$ total surface area. Essentially influent would flow down the strip and P removal would occur. The P concentration in the influent was around 3mg/L Biomass was mechanically harvested every 1-2 weeks. The algal species composition varied throughout the year.

The hydraulic loading rate was varied over different times of the year. This had a large effect on the P removal rate, as you increase the volume, the growth rate and P removal rate / L decrease. During the winter growth rate dropped to 4.45mg/L/d. During the summer, the highest growth rates were achieved at 68.6mg/L/d.

When the lowest hydraulic loading rate was used, P concentration was reduced to 0.4mg/L, when it was doubled, P concentration was only reduced to 0.9mg/L. The influent pH of between 7.5-8 would rise during the treatment to around 9.5-10 depending on the loading rate and subsequent algal activity / L. This correlates well with other literature and my own work.

Achieving growth rates of up to 68.6mg/L is good for achieving a sub 1mg/L P target. If loading rate was reduced the growth rate / volume would increase however the total weight would still remain fairly constant. In this case it is unfair to compare against other growth rate experiments.

Toms et al. (1975) wrote an extensive early paper regarding the details of polishing lagoons and the affects of algal growth on performance. They show that lagooning buffers effluent between the treatment works and river, changing the BOD, reduces the existing SS however may increase SS due to algal growth which plays a big role in nutrient stripping, especially P.

They pose questions relating to design and performance that are very similar to the questions asked about the process suggested in this thesis: What is the required HRT? Seasonal variations? Operating conditions? Are nutrients reduced and what are the removal mechanisms? What should the depth of the lagoon be? What effect will algal growth have when discharged to the river? They investigate the factors that affect the growth of algae in the lagoons.

They note that different operating conditions still produce similar dominant algal genera and relative proportions; the population being dominated by green and green-yellow algae

with blue-green algae only occurring occasionally. *Scenedesmus, Ankistrodesmus, Actinastrum, Dictyosphaerium, Chlorella, Coelastrum, Kirchneriella, Chlamydomonas, Heteromastix, Cryptomonas* and *Rhodomonas.*

Algae require and complex combination of environmental factors, particularly light, $CO_2$, N, P and trace elements. They require sufficient time to grow and divide. Due to high P and N loading, algal growth is unlikely to be limited by lack of these elements. They state that in the early stages $CO_2$ is unlikely to be limited but given sufficient time and light to grow, inorganic carbon would become the limiting factor. Algal growth are largely controlled by physical factors, seasonal light and temperature, HRT, turbidity and biological grazing.

It was seen that growth should only occur if the HRT exceeds 2 days and the algal population declines with depth of lagoon. The model they created indicated that for a given HRT, if it is desired to produce the maximum quantity of algae for nutrient stripping and / or harvesting the lagoons should be as shallow as possible. HRTs would be longer in the autumn and spring than in the summer and they state it is unlikely that exploitable quantities of algae could be grown in the winter in the UK. When the lagoons were operated as a series, extensive growth of filamentous algae was observed. They do not offer an explanation for this.

The growth of algae in the lagoons produces 15x more oxygen than they use for respiration, aiding to improved BOD removal. The paper reports no recorded instance of algae harvesting from a lagoon system in the UK but propose flocculation and lime addition as possible methods. They state that the control and growth of algae may provide a commercially exploitable product from the lagoons.

With increasing concern about eutrophication, considerable interest has been attached to the removal of P from sewage effluents. They show a strong correlation between growth of algae and significant removals of P. Where algae did grow they also show seasonal variations between high summer populations (May - August) and high P removal (80%

reduction) compared to the reduced P removal (20% reduction) when populations were low in the winter.

Interestingly they show that the P content of the algae was 3.5%, considerably higher than the 0.5-1% normally assumed. They show that additional P reduction was from precipitation of hydroxyapatite due to high pH values caused by photosynthetic uptake of $CO_2$ by algae. There was 4x more P as sediment in the mud in lagoons with high algal growth than those without algal growth.

They produced a very interesting graph plotting soluble P vs. pH showing that P is 100x more soluble at pH8 than it is at pH10. This could be a factor associated with the importance of keeping pH low to aid P uptake by algae.

For lagoons to remove P, they state that the HRT must be long enough and the depth must be shallow enough, speculating that it is unlikely sufficient algae could be grown in the winter to bring about significant removal of P.

Nitrate removal did not correlate as well with algal growth, suggesting that a mechanism other than uptake by algae may be important such as bacterial denitrification; nitrate to nitrite to nitrogen gas.

One of the principal mechanisms of ammonia loss is diffusion to the atmosphere, this loss depends upon the concentration of free ammonia which in turn depends on the pH. The concentration of free ammonia increases by a factor of about 70% if the pH value is increased from 7-9 at 20°C. With the rise in pH from algal growth, considerable losses of ammonia would be expected. They state lagoon pH could be raised artificially due to lagoons not offering a feasible means of significantly reducing the concentration of ammonia.

Barnard & Oleszkiewicz (2005) write a report into the recent development in biological nutrient removal. They state that the term biological nutrient removal (BNR) designates the simultaneous removal of N and P in a single sludge system. Over the last 30 years BNR has grown from a few plants in sensitive areas to widespread global use.

Recent developments in the technology have been stimulated by the requirement for even higher levels of treatment to protect water bodies from eutrophication. BPR is facilitated by PAOs that require a basic sequence of an un-aerated zone free of oxygen and nitrates, referred to as the anaerobic zone followed by an aerated zone. An anoxic zone may be introduced before and/or after the anaerobic zone where endogenous denitrification takes place.

The PAOs take up P in the aerobic zone and, after recycling with RAS to the anaerobic zone, use that P form of energy to take up and store VFAs that is in turn used to take up and store P during aeration.
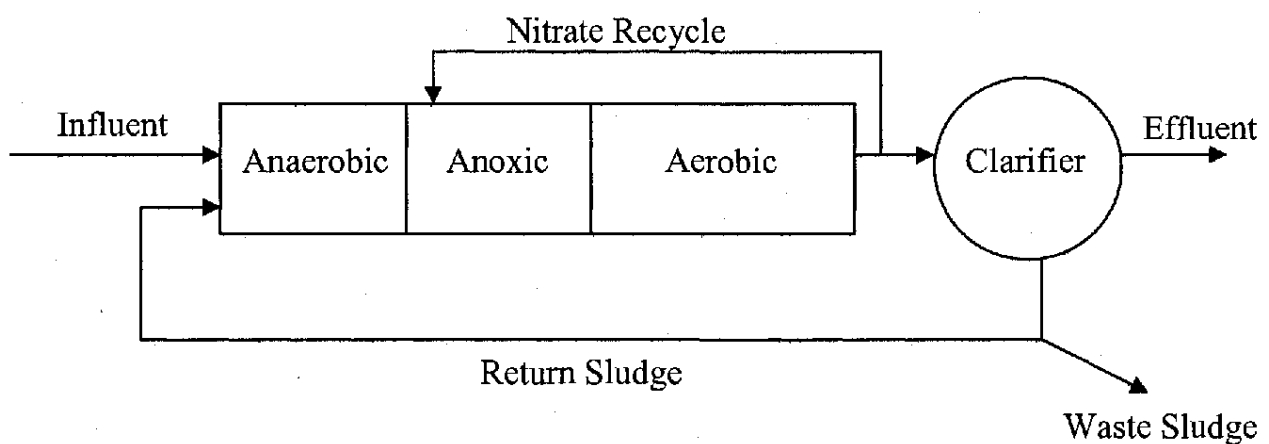


**Figure 2 - Showing the three stage Bardenpho process**

Nitrification is the rate limiting step in the BNR plants and determines the plant size. The growth rate of the nitrifying organisms reduces sharply with a drop in temperature and they may be washed out of the system at low temperatures. Floating plastic media may be

added to the aeration basin for the organisms to grow on. The media is retained by fine screens.

Manyumba et al. (2008) looked into the problem of meeting the P consent with biological nutrient removal under UK winter conditions. They state that P removal can be achieved by biological uptake of P, chemical precipitation or a combination of both of these. However, the biological route is generally considered to be the more sustainable alternative and for larger sites, it may also offer significant savings in capital and operating costs

Although biological P removal is considered to be the most sustainable option for P removal, it has always been problematic for plants that remove both N and P due to the inadequate concentration of organic material during wet periods. P removal systems can either be P limited or carbon limited. P-limited systems achieve a near-complete P removal whereas carbon-limited operations achieve only partial P removal – this is an obvious problem when carbon is limited during wet weather periods.

They looked at two BNR configurations, the Johannesburg (JHB) process and a combined JHB and five-stage Bardenpho process. They were evaluated over a period of 2 years to assess the impact of sewage strength on bio-P removal. The JHB achieved an average effluent total P of 2.4mg/L and the combined JHB and five-stage process averaged 1.4mg/L effluent  total P.

They found acetate dosing proved successful as a source of volatile fatty acids (VFAs) in the anaerobic zone during periods of low-strength sewage. An acetate dosing strategy based on the influent flow rate to the plant was found to be a simple and effective technique that ensured that a consent of <1mg TP/L could be met.

Christen (2007) wrote a short report stating that in the future there will be requirements for much lower levels of P discharge, between 0.01-0.05mg/L. With a growing population, there is a need for better nutrient removal technologies. A Las Vegas STW expects to achieve

annual averages of 0.02mg/L. He states that research now shows a near 100% P removal may be possible and no scientific limit exists. Work should be started now to develop these technologies for the future. He adds that economics is a big factor and other large sources such as urban and agricultural runoff remain virtually unregulated.

Thus, two types of P removal strategies exist, retaining the P within the solids or biomass within the plant or removing it at the end of the process, e.g. by chemical precipitation. The design approach can be black box based on empirical experience or mechanistic using fundamental knowledge. This leads to the aim or research question to investigate if algae can remove P more sustainably or more economically than the alternatives. The biomass and oxygen production are the potential advantages compared to other tertiary treatment techniques.

### 3.3.2. Algal nutrient removal from wastewater

Aslan & Kapdan (2006) looked into batch kinetics of N and P removal from synthetic wastewater by algae. Batch experiments were carried out to investigate the effect of the initial N and P concentrations on nutrient removal performance of microalgae *Chlorella vulgaris.*

The N concentration was varied between 13.2–410mg/L while P concentration was between 7.7–199mg/L, keeping N:P ratio around 2:1 in the synthetic wastewater.

The experiments were performed maintaining the pH at 6.5-7.0 and at room temperature (20±2°C) with artificial illumination (39.8W/m$^2$) provided continuously from one side of the flasks by using 36 W/54 fluorescent lamps. The flasks were aerated to provide $CO_2$ and for mixing via an air pump. Each experiment lasted 10 days. The experiments were conducted in batch by using 1000ml flasks. At the beginning of each series of experiments, 800 ml of culture medium was inoculated to flasks with a suspension of pre-cultured cells.

From what I can tell from the crowded graphs, is a P removal of 5mg/L in 10days, 0.5mg/L/d or 0.02mg/L/h. The N removal was about 25mg/L in 10 days, 2.5mg/L/d or 0.1mg/L/h. The graphs show so much data, all the lines appear straight. If I had wrote this paper, I would have looked at the graph and considered that this shows nothing, then split it up into smaller ranges.

They carried out a good experiment but struggled to convey any decent useable results, they do not state a growth rate and their main findings are: Experimental results indicated that effluent water quality decreases with increasing nutrient concentrations. Algae culture can remove N more effectively compared to P.

The paper is badly written: They state that experimental results indicated that *C. vulgaris* can completely remove up to 21.2mg/L ammonia- N concentration. This is useless data because is this in 1 day, 50 days, ever?

The author goes on to state the efficiency of removal as a %. How is this useful, if the initial concentration is 1000mg/L P, the algae are still only going to remove the same amount leaving the efficiency as a tiny figure, and over how much time? This could be % removal with a HRT of a year; it is as if the author has not thought through the data they are presenting. What is the point of doing an experiment and writing a paper if you are going to provide results in useless units that cannot be compared to any other work. I can take from this an example of how not to present data.

Garcia et al. (2000) studied HRAPs operating strategies for urban wastewater N removal in Spain. N removal efficiencies under different HRTs were compared. This was to allow a comparison of N removal efficiency as a sole function of HRT.

They used two experimental HRAPs (1.5m$^2$, 570L per unit), each with a secondary clarifier for algal biomass separation (0.025m$^2$, without recirculation), were fed with urban

wastewater for a one-year period using two peristaltic pumps connected to a storage tank. The HRAPs were agitated using a paddle wheel mixer.

Monthly average total solar radiation varied between 150cal/cm$^2$/d in November to 630cal/cm$^2$/d in June. Monthly average temperature varied between 11°C in January and 25°C in July. The nutrient concentrations were 51mg/L N and 8.5mg/L P – Similar to Loughborough before RAS installation. pH was measured but no results were shown.

HRT was the same as the cellular retention time, because there was no biomass recycling from the clarifiers. HRAP A was always operated at a higher HRT than HRAP B. Both HRAPs were subjected to the same environmental conditions of solar radiation, air temperature and influent water quality. Grab samples of influent, effluent of the HRAP (mixed liquor) and final effluent from the clarifiers were taken once a week. The annual average N removal was 73% for HRAP A, and 57% for HRAP B.

The concentration of N and P was only slightly affected by sedimentation in the storage tank, as compared to raw wastewater, because these nutrients were mainly present as dissolved inorganic compounds. This is an important point as I will be storing the effluent for my experiments in a similar way.

The conclusion of this study is that HRT determines both the N removal efficiency and the distribution of N forms in the effluent of a HRAP. The N removal level can be controlled through suitable HRT operating strategies. By operating at a HRT of 4 days in spring and summer, and 10 days in autumn and winter, N concentration in the effluent of a HRAP system can be reduced to less than 15mg/L N.

Camargo & Mara (2007) and Camargo & Mara (2010) produced two papers regarding ammonia volatilisation in waste stabilisation ponds. The 2007 paper reports findings of experimental data. They collected ammonia gas coming out from WSPs to determine the importance of ammonia volatilisation as a mechanism of nitrogen / ammonia removal,

showing that it accounted for just 3% of the total N removal in their experiments. They concluded that Ammonia volatilization was not the most important mechanism involved in either total nitrogen or ammonia removal processes. Ammonia and total nitrogen were mainly removed by biological algal uptake under summer conditions and Ammonia removal in maturation ponds cannot be predicted with models based on mass transfer equations for ammonia volatilization as this is only a minor pathway for ammonia removal in maturation ponds.

The 2010 paper continues the topic in more detail and concludes that ammonia removal by volatilisation makes little or no contribution to nitrogen removal by WSP either in summer or winter. High pH and water temperature values should not necessarily favour ammonia volatilisation over alternative mechanisms like algal uptake. An increasing pond water temperature increases phytoplanktonic activity and consequently, in-pond algal biomass would take up and remove ammonium to a faster rate than expected via ammonia stripping. The increment of pH in WSP is a consequence of algal activity and it makes a small contribution to ammonia volatilisation process as ammonia concentration drops due to algal uptake.

The findings reported here are important for algal bioreactor and HRAP design when considering the importance of nutrient removal mechanisms and the optimal pH and temperature parameters to ensure an efficient performance. Taking into account these findings, it may not be worth factoring in ammonia volatilisation as an N removal mechanism. This would be dependent on the N removal rate of the algal species and how much gas sparging affects ammonia volatilisation.

Woertz et al. (2009) produced a study investigating lipid productivity and simultaneous nutrient removal by green algae grown during treatment of municipal wastewaters supplemented with $CO_2$ for the production for biofuel feedstock. I have focused on the municipal wastewater experiments.

Algal growth in wastewater treatment ponds contributes to treatment mainly through dissolved oxygen production and nutrient assimilation. However, the C:N and C:P ratios in domestic sewage are 3.5:1 and 20:1 respectively. These are low compared to typical ratios in rapidly growing algae biomass of C:N - 6:1 and C:P - 48:1. This lack of carbon leads to limitations in algae production and incomplete assimilation of wastewater nutrients by algae. The experiments described in the present research overcame the carbon limitation of the wastewaters by addition of $CO_2$ to the cultures.

The municipal wastewater experiment was run under semi-continuous operation for 18 days to study the effects of $CO_2$ levels and HRTs on algal growth and nutrient removal. Control cultures with addition of air only were used to simulate the carbon limitation typical of wastewater ponds and to differentiate the effect of $CO_2$ addition on productivity.

Municipal wastewater was treated in semi-continuous indoor cultures with 2–4 day HRTs. It was illuminated from two sides by a total of four 40W full spectrum fluorescent bulbs operated on a 16:8 hour L:D cycle. When on, the bulbs provided an average luminance that totalled 41.7W/m$^2$ at the two faces of each bottle. The cultures were agitated by magnetic stirrers and the temperature ranged from 23 to 25°C.

Primary clarifier effluent was collected at the San Luis Obispo, California, municipal wastewater treatment facility. The wastewater had a starting pH of 7.2, 51mg/L N and 2.1mg/L P. The $CO_2$ concentration in the blend was set to maintain pH between 7.0 and 8.0. Results were collected using 4, 3 and 2 day HRTs.

The municipal wastewater cultures were dominated by algae in the *Chlorella*, *Micractinium*, and *Actinastrum* genera. The lipid contents of the algae from the municipal wastewater experiments ranged from 4.9–11.3% of the biomass by weight. Ammonium was the main form of N in the influent wastewater, and after algal growth, organic N was predominant. Ammonia volatilization was minor, the greatest amount was from the air-sparged treatment,

which accounts for 7% of the influent total N. Since this treatment developed the highest pH at 10.3 due to lack of $CO_2$ sparging, it was the most prone to ammonia volatilization.

3 and 4 day HRTs provided similar results, increasing from 100-800mg/L algae concentration in 18 days. Whilst the 2 day HRT only achieved 300mg/L. Sparging with $CO_2$ more than doubled the biomass concentration compared to sparging with air.

Maximum lipid productivity for the municipal wastewater was 24mg/day/L, observed in the 3-day HRT cultures. Over 99% removal of N and P was achieved. The lipid content of the volatile solids peaked at Day 6, during exponential growth, and declined thereafter. Peak lipid content ranged from 14–29%. The lipid productivity was 17mg/day/L which is equivalent to 11,000L/ha/year if sustained year round.

The results suggest that $CO_2$-supplemented algae cultures can simultaneously remove dissolved N and P to low levels while generating a feedstock potentially useful for liquid biofuels production.

Ruiz-Marin et al. (2010) looked at the growth and nutrient removal in free and immobilized green algae in batch and semi-continuous cultures treating real wastewater.

One of the limitations for the development of wastewater treatment systems based on microalgae is the harvest of the biomass at the end of the treatment process. However, the immobilization of cells can represent an alternative for solving the problem as well as providing advantages such as an increase in the cell retention time within bioreactors and higher metabolic activity.

Two species of microalgae were used: S. obliquus, isolated from a hypereutrophic soil and C. vulgaris, isolated from agricultural soil. They were grown as immobilized and free-cells and were compared to test its ability to remove N and P in batch cultures of urban wastewater.

For initial experiments, artificial wastewater was only used for the acclimatization of cells and for direct comparison with real urban wastewater. For all other experiments, real secondary effluent was used. Due to its nature, there was large variation in the concentration of N in the wastewater used for the experiments; 34–48mg/L (this is not a comparatively large variation) so percentages of removal were used to determine removal efficiency. Why? That is the worst thing to do, this creates a bigger problem than it solves; if the algae are removing N at a specific rate, percentage removals are going to cause widely different results depending on the initial concentration whilst having no value for comparison. Just be sensible and measure it in concentration reduction per day. This statement does however highlight an important point about the difficulties of using natural wastewater for experimentation.

Stock suspensions of C. vulgaris and S. obliquus were cultivated in 3L bioreactors containing 2.5L of artificial wastewater at 25 ± 1°C and light intensity of 27W/m$^2$. The bioreactors were aerated to keep free-cells and immobilized beads in suspension and in completely mixed conditions.

The best microalgae-growth configuration for urban wastewater treatment was established and chosen to be used in bioreactors running under the same conditions as batch cultures but in semi-continuous mode. Moreover, photosynthesis–irradiance curves suggested an optimum light intensity of 40W/m$^2$ to be used.

Scenedesmus obliquus showed a higher N and P uptake rate in urban wastewater than Chlorella vulgaris. When tested in semi-continuous mode, S. obliquus was more effective in removing N and P for longer periods (181h) than batch cultures. The beads require more effort to keep in suspension.

They state that immobilized systems could facilitate the separation of the biomass from the treated wastewater although in terms of nutritional value of the biomass, immobilized systems do not represent an advantage over free-cell systems.

The results showing a pH of 10.1 as standard for the experiments. This is a normal value to be recorded in experiments without $CO_2$ addition. The control, without algal activity had a pH of 8.5.

The experiment is good but without using biomass productivity to determine growth rate it is impossible to compare with other experiments.

Jimenez-Perez et al. (2004) performed laboratory experiments to study growth and P and N uptake in two species of free and immobilized planktonic green algae, *Scenedesmus intermedius* and *Nannochloris* sp.

They carried out practically the same experiment as the previous paper. They did not measure growth rate in mg/L for comparison either. It is understandable why researchers do not want to carry out absolute growth rate studies, They are difficult, invasive, time consuming and inaccurate however they are the only way to produce results that can be directly compared to other work. My results are taken from experiments carried out in this way to determine absolute biomass quantities.

Both culture types (free and immobilized) were maintained under standard conditions in an incubation chamber with a 14:10 L:D cycle at $24W/m^2$ supplied by cool-white fluorescent tubes and kept at a constant temperature of 20 ± 2°C. Culture pH ranged between 8 and 9 but it does not state how it was maintained. No method of agitation is given.

P and N uptake rates for *S. intermedius* were 0.014 and 0.012mg/h P and 0.022 and 0.009mg/h N for free and immobilized cells respectively. Rates for *Nannochloris* sp. were 0.006 and 0.009mg/h P and 0.011 and 0.006mg/h N. Per what quantity? Per $m^3$? Without supplying the unit of volume it means nothing.

They state that P and N removal rates obtained in the present study, which used species isolated from wastewater, are markedly higher than rates described in experiments that used commercial species, probably because the former species are better adapted to high nutrient concentrations. I would agree with that.

Their analysis of the results is ludicrous, measuring random sized cell growth, and P and N uptakes per arbitrary culture size. This paper is a waste of time, no recordable numerical data can be taken from it.

Hameed (2007) performed laboratory experiments to study N and P uptake by the unicellular green microalga *Chlorella vulgaris* immobilized in calcium alginate beads. He studied the effect of algal density in bead, bead size and bead concentrations on wastewater nutrient removal.

The cultures were kept at pH 6.5 - 7.2 under aseptic conditions, aerated by filtered air and maintained at temperature of 25 ± 2°C, with light intensity of 36W/m$^2$ using a 16:8 L/D cycle.

Increasing bead concentrations caused significant reductions in the treatment efficiency. Dense beads would reduce the amount of light penetrating through the reactor, and enhance the self-shading effects, which limit the metabolic activities of algal cells. In the reactors with high beads concentrations, large numbers of beads settled at the bottom of the reactors due to the heavy weight of the large numbers of beads and the insufficient supplied air to completely suspend all the beads in these reactors.

P removal was 0.25mg/L/h and N removal was 0.97mg/L/h. These were only achieved using a medium bead density. The high bead density did not perform as well. They mention air-striping of ammonia is a possible mechanism for N removal in an intensely aerated micro-algal system with alkaline pH. See my other comments regarding this.

The N and P uptake rates were initially high for the first 12 hours followed by a period of decline for 12hours until uptake almost stops. This could be due to a lack of C as none was added. They state that the pH in all the reactors was alkaline and ranged between 7.9 usually recorded in the control reactors in the different experiments and 9.3 recorded in different reactors with different types of beads. This verifies my theory. They produce a similar pH curve as my experiments show.

Lau et al (1995) carried out laboratory scale batch experiments to examine the effect of initial inoculum sizes of algae on the reduction of nutrients from primary settled municipal sewage. The microalgae, Chlorella vulgaris was used with four initial inoculum sizes.

Shatin Sewage Treatment Works is a major secondary sewage treatment plant in Hong Kong. Such wastewater had average values of 84.3mg/L N, 4.29mg/L P and pH of 7.10. 500ml conical flasks containing 300ml settled sewage were used containing four different initial inoculum sizes: Low, medium, concentrated and super-concentrated. The algae were cultured at 24 ± 1°C, with illumination of 41.7 ± 3W/m$^2$ from cool white fluorescent tubes with L:D cycles of 16:8 for 10 days. The flasks were aerated with filtered air.

The initial concentration size did not affect the growth rate, lower initial concentrations had greater capacity for growth before reaching a maximum concentration. The drop in growth rate in the 2 more concentrated batches towards the end of the experiment was put down to depletion in nutrients, creating an unfavourable N:P ratio. Higher concentrations provide a faster removal rate. The results showed that 33mg/L N was removed in 8-10days (0.15mg/L/h).

The graph of pH shows a sharp rise from pH7 to around pH8.5 which then increases more slowly, reaching a pH of around pH9.5. This correlates with all other experiments that do not use additional $CO_2$ to control pH.

P removal was shown to be around 3.5mg/L in 10 days (0.015mg/L). The fast initial uptake followed by a period of slower uptake correlates with the inhibitory increase in pH. If pH control was used through $CO_2$ addition, I would expect their uptake rates to be more linear.

During the experimental period, even in the super-concentrated cultures, the self-shading problem was not found probably due to good mixing.

These results indicated that the efficiency of reducing wastewater-borne nutrients by an algal system was directly related to the physiological activity and growth of the Chlorella cells which in turn were affected by the initial inoculum size. The super-concentrated culture seemed to be more beneficial as this treatment achieved satisfactory nutrient removal within 7 days instead of 10.

Chen et al. (2003) looked at nutrient removal by the integrated use of HRAPs and macrophyte systems in China. They operated at pilot scale to investigate the performance of a HRAP under the temperate climate conditions of Shanghai, China. The results indicated that the HRAP gave good efficiency for nutrient removal, especially during summer. With a HRT of 4 or 8d according to the season.

The HRAP system was continuously fed from a settling tank with raw domestic wastewater from the university. The HRAP was 0.3m (except in August 0.45m). A paddle wheel operated continuously to maintain the water flow at a constant velocity of 0.15m/s.

Preliminary assays were conducted during the first 5 months in order to optimize the HRT. The HRT was changed to follow the seasonal variations in climatic conditions – This has been typical for all similar experiments in the literature.

The temperature ranged between 10-30°C and the solar radiation ranged between 5-20MJ/m$^2$

The influent was at pH7.5 which increased to 8.6 and 9.1 in the effluent for winter and summer respectively. This is a sign of carbon limitation, the extent of which was dependant on the productivity. Summer N removal was 5.15mg/L/d which dropped to 2.86mg/L/d in the winter. Summer P removal was 0.25mg/L/d which dropped to 0.2mg/L/d in the winter.

Cromar et al. (1996) looked at the influence of environmental parameters on biomass production and nutrient removal in a HRAP. This is a very relevant paper which reinforces certain literature data from other papers however they do not represent nutrient removal as an absolute value. They use a $3.1m^3$ raceway pond with synthetic media, producing a mean growth rate of 76mg/L/d. The only pH data they give is a total range measured over the whole 7 month period of pH7.4-10.8 which is expected for a setup not using additional $CO_2$.

They show that the HRAP algal concentration is a function of day length and produce a 3D graph of algal concentration vs. temperature vs. irradiance. This is a great graph and shows the importance of temperature and irradiance in combination. **A low temperature or irradiance will be inhibitory regardless of the level of the other parameter**.

Camargo et al. (2010) studied N removal in maturation waste stabilisation ponds via biological uptake and sedimentation of dead biomass and reported findings from work carried out between 2004 and 2007. Using an experimental pilot scale WSP system at Esholt Wastewater Treatment Works. They collected samples of settled organic nitrogen from the maturation ponds in order to estimate seasonal sedimentation rates of organic nitrogen.

They found algal N uptake to be the major mechanism for ammonium removal under favourable environmental and operational conditions for phytoplanktonic activity in maturation WSP. In summer, algal N uptake was found responsible for the majority of the ammonium removed and along with sedimentation of dead algal biomass, it constitutes the dominant N removal mechanism in maturation ponds. However, it is important to highlight

that once dead algal biomass reaches the bottom of the pond, anaerobic digestion of pond sediments partially recycles ammonium N to the water column.

They go on to state that solids removal units would complement the highly efficient algal N uptake. I would totally agree with this important comment and highlight regular biomass removal as an important part of the process of nutrient removal and subsequent production of biofuel.

Griffiths (2009) produced a detailed paper containing very relevant information about parameters involved in algal P removal from wastewater.

The Logan City Environmental Department operates a facility that consists of 460 acres of fairly shallow lagoons (~ 5'deep) for biological wastewater treatment that meets targets for primary and secondary treatments (solids, biological oxygen demand (BOD), and pathogen removal). Significant natural algal growth occurs in these lagoons, which improves BOD removal through oxygenation and also facilitates N removal through volatilization as ammonia under high pH conditions created by algal growth. P, however, is non-volatile and stays in the water and likely cycles in and out of algal cells as they grow and die in the lagoons. In the near future, the regulatory limits on P released from the Logan wastewater treatment facility are likely to become significantly lower to counter potential downstream eutrophication. One way to potentially lower P levels in the wastewater effluent is through management of algal growth in the lagoons. As mentioned above, algal growth naturally occurs in the treatment lagoons and if the algal biomass is harvested when growth yields are highest, the P contained in the cells could be removed to obtain P-free water. The algal biomass could then be used for production of biofuels. This research focuses on laboratory and pilot assessments to show the ability of algae indigenous to the Logan lagoons to uptake P and produce biomass that can be used for biofuel production.

Because temperature changes significantly from summer to winter at the Logan wastewater facility, algae were grown at 4, 10, 20, and 30°C to determine the effect of temperature on algae growth.

Logan lagoons effluent was incubated in 250ml Erlenmeyer flasks in the laboratory to stimulate growth of native algae populations. The flasks were placed on a shaker table to keep the samples well mixed and to prevent the algae from settling. 'Plant growth and natural sunshine' fluorescent lights were placed around the shaker table to promote photosynthesis.

Alkaline conditions lead to the volatilization of ammonia ($NH_3$) since its solubility in water is a strong function of pH. Higher temperatures also facilitate volatilization. Increased algal growth raises the pH due to the use of $CO_2$ by algae during photosynthesis. pH > 10 can easily be reached in algal cultures in the absence of a significant buffer or $CO_2$.

P uptake rates were observed to be the greatest at higher temperatures. During the first 5 days of growth, P uptake rate at 30°C is much faster than at colder temperatures. When the light intensity is held constant, the results indicate that the maximum growth rate can be described as a function of temperature.

They then carried out a $CO_2$ addition experiment: The lab scale raceway reactors were made of ¼" acrylic sheeting.  The reactors have two channels and a length to width ratio of 2:1. Mixing was accomplished by a paddlewheel set-up. An electric motor was used to rotate paddle wheels at approximately 10rpm.

The transparent acrylic was used to allow light penetration for photosynthesis to occur in a laboratory environment. Plant growth fluorescent bulbs were used for the light source. The bulbs provided the red light necessary for photosynthesis around 680nm. Four plant growth fluorescent bulbs were mounted on the bottom and two on each 2ft side for a total of eight bulbs around each reactor. The eight bulbs provide an average light intensity of 764 ±

$20.8W/m^2$ in an empty reactor using a L:D cycle of 14:10. Temperature was held at $21 \pm 3°C$. The variation in temperature came from the lights warming the reactors followed by the reactors cooling after the lights were turned off.

$CO_2$ addition was achieved through pumping pure $CO_2$ through spargers mounted to the bottom of the reactors. The amount of $CO_2$ added to the reactor depended on the pH level. A gas solenoid was placed in between the $CO_2$ source and reactor. The solenoid was controlled by a program written in Lab View based on pH level. pH8 was chosen because the optimal pH level has been reported to be in the range 7.5-8.5. In this experiment, if the pH level went above pH8, the solenoid would open allowing $CO_2$ to flow into the reactor until the pH level dropped below pH8 again.

Whilst most other papers report the addition of $CO_2$ to double productivity, this experiment showed that the productivity only slightly increased. An important finding was shown that the oil content of the algal cells was 3-4 times higher when $CO_2$ addition was used.

Adding my own thoughts to this paper based on a collaboration between this and other reviewed papers; N:P ratio can be controlled by pH which can in turn be controlled by $CO_2$ addition. If the N:P ratio is too high, N can be reduced through ammonia volatilisation by raising the pH which also slows P uptake due to lack of $CO_2$. In contrast, P uptake can be increased relative to N reduction by keeping the pH low (high $CO_2$ content), reducing ammonia volatilisation and improving algal productivity.

Larsdotter (2006) produced a paper on microalgae for P removal from wastewater in a Nordic climate as part of a research project aiming to develop and evaluate a hydroponic system for wastewater treatment in Sweden. They carried out testing on nutrient removal by microalgae.

The P assimilation was dependent on algal growth which is directly associated with inorganic carbon assimilation by the algae which causes a pH increase. Results confirm the rise in pH, 7.6-8.7 for a reduction in P of 1.5mg/L/d.

He states, results from an intensive study during summer showed that culture depths of 17cm gave higher removal efficiencies (78% - 92%) than cultures of 33cm (66% - 88%). On the other hand, the removal rate per area was higher in the deeper cultures, which implies that these may be preferred if area is of concern (regardless of the available land factor, one would always use the most efficient system which in this case is deeper tanks so this point is invalid). This is a perfect example of why you cannot measure in % removed, for real comparison you must use absolute values. In this case, a greater depth is going to remove a greater quantity of nutrient for the same biomass concentration in absolute terms however due to the more limited availability of light, the removal rate per volume is obviously going to be lower. An optimum depth must be chosen to minimise the HRT for a specific reduction in nutrient concentration.

He does state an important point regarding an advantage of shallower tanks; the temperature is slightly increased due to the energy input being greater per volume. This is still irrelevant if a deeper tank is still more efficient.

Over the year, there were large fluctuations in algal growth and removal efficiency as a result of the seasonal variations in light and temperature. During winter, P removal efficiencies lowered. Additional illumination during winter improved the P removal in the shallow cultures but did not have a significant effect on the deep cultures.

Longer HRT gave higher algal biomass concentration and higher P removal efficiency. Hence, the residual P concentrations were lower with longer HRT. – Obviously but what about when measured as uptake per day? Is not the aim to maximise the P uptake rate to minimise the HRT? If you leave the effluent in for an extended period, it will obviously drop to 0mg/L.

The results of his experiment show that there is higher P precipitation at higher pH. All literature states that optimum performance for algal productivity is at lower pH, Using increased pH for P removal by precipitation in a system like this is not a viable mechanism.

Gronlund (2002) looked at using microalgae for wastewater treatment in a cold climate. The paper does not provide any experimental data but makes the following statements.

He concludes that the production of micro-algae, given proper conditions may be high enough to improve wastewater treatment in ponds in cold climate, from a treatment perspective as well as a sustainability perspective.

Microalgae may perform tertiary treatment due to their ability to incorporate inorganic N and P at growth, and also have the capacity to remove heavy metals as well as some toxic organic compounds.

The laboratory experiments showed that micro-algae collected in mid-Sweden regions can grow readily in wastewater between 5-10°C.

In my opinion, based on results from literature, the issue is not whether the process is possible in a cold climate, it is the greatly reduced productivity compared with 30°C that reduces its feasibility when the temperature is not artificially raised. It is promising that algae will grow at 5°C as wastewater temperature is fairly consistent and typically above 5°C year round unless a region experiences a long period of very cold weather. For the process to be in a temperate region, waste heat will be required.

### 3.3.3. Algal ponds

Silva et al. (2008) looked at integrating filamentous 'green tide' algae into tropical pond-based aquaculture. 'Green tide' algae with fast growth rates and efficient nutrient uptake

bloom in eutrophic environments. These same characteristics are sought after for algae in integrated aquaculture systems.

They used three filamentous 'green tide' algae, Cladophora coelothrix, Chaetomorpha indica and Ulva sp. First testing survival and growth were across the extremes of salinity in ponds (0 to 45%). Subsequently, the interactive effects of salinity (15, 36 and 45%) and a broad range of N concentrations were examined (0, 0.1, 0.5, 1, 10mg/L).

They showed that the species tested have a broad tolerance to salinity (ranging from 5 to 45‰) with each having a different optimum for growth (C. coelothrix 30%, C. indica 20% and Ulva sp. 15%).

They do not provide much data on the effect of N concentration however the concentrations they used are in an order of magnitude lower than normally found in wastewater, so the results are not useful. The interesting point in terms of literature data is their analysis and findings that these specific species and no doubt many others are tolerant to a wide range of salinities which could be useful in large scale algal biofuel projects in the future.

Zimmo et al (2005) studied the effect of organic surface load on process performance of pilot-scale algae and duckweed-based waste stabilization ponds.

Removal efficiencies in pilot scale algae-based ponds, ABPs and duckweed-based ponds, DBPs were assessed during two periods of 4 months each. During Periods 1 and 2, the effect of low and high organic loading was studied.

The pilot plant experimental pond system set-up was at the new campus of Birzeit University, Jerusalem. The pilot plant was built with reinforced concrete walls to ensure water tightness. Each system consisted of four equally sized ponds in series 3m length, 1m width, and 0.9m depth and was fed with a constant flow rate of $0.86m^3$/d. A 10cm sand

layer was brought into each pond to simulate a real pond bottom. The theoretical retention time in each pond was 7 days. Baffles at the outlet of each pond were constructed to avoid short-circuiting and transfer of floating materials to the consecutive ponds. The ABPs and DBPs were fed with three categories of influents during three successive periods. From December to August, treatment systems received wastewater from the Birzeit University of low organic strength. From September to February wastewater from Al-Bireh was used which contained high N concentrations (100mg/L) - This resulted in complete decay of duckweed and the ponds were operated temporarily as algae-based ponds. From February to August treatment systems received strong wastewater from the Faculty of Commerce. Two periods of 4 months each were monitored in order to compare the removal efficiency of the two systems at low and high organic loading conditions.

During the course of the two experimental periods, water samples were collected from the influent and effluents of each pond. Removal of BOD, ammonia, total N and total P were investigated.

The duckweed *Lemna gibba* biomass in DBPs was maintained at a wet weight density of 600g/m$^2$ by harvesting the surplus every 5 days. This density was selected to prevent overcrowding and to maintain sufficient cover to minimize the development of algae in duckweed ponds.

No mixing was mentioned and this is reflected in the results that show a variation in parameter measurements over the depth of the pond. Higher pH was measured in the ABPs compared to the DBPs. The pH was slightly higher near the surface of the pond, which is expected in an unmixed pond with greater productivity occurring in the more heavily lighted regions.

N removal rates in ABPs were linearly correlated with BOD surface loading rates while in DBPs, N removal rates were almost constant irrespective of BOD. Overall N removal rate in

the algae system was significantly higher than in the duckweed system. Higher N removal rates would be expected in the pond with the highest pH which was the case.

Organic loading had no effect on total P removal efficiency in both systems. Higher P removal efficiency was achieved in the duckweed system than in the algae system. In ABPs, P removal is due to sedimentation of incorporated P in particulate and decayed algae. The P removal rate in the algal pond was only 0.004mg/L/h which is expected without biomass harvesting. None of the nutrients contained in the algae can be removed from the system. Duckweed uptake of P in the DBPs and subsequent harvesting accounted for higher removal compared to ABPs.

The development of a slime layer on duckweed roots in Ponds 1 and 2 during the period of high organic loading was observed which probably inhibited plant nutrient uptake.

The duckweed growth rate was around 11mg/L/d which is on the low end of the spectrum. Average growth rates for algae are recorded at 10-15x this rate.

During low loading, the well-maintained duckweed cover prevented elevation of pH through algae growth due to the shading provided by the duckweed cover. Therefore the unionized fraction of ammonia was kept below the level of toxicity.

Yan & Jameson (2004) looked into the application of the Jameson Cell technology for algae and P removal from maturation ponds.

This paper describes a cost effective induced air flotation process utilising the Jameson Cell technology to simultaneously remove algae and P from wastewater maturation ponds. The Jameson Cell is a patented technology. It uses a plunging jet to generate air bubbles and results in a more effective flotation process. It has major advantages over conventional flotation technologies, including a more compact design and lower capital cost, a minimum amount of maintenance and the ability to operate at elevated temperatures.

Optimum conditions for algal flocculation were identified using jar tests. Pilot plant trials were then carried out to examine the robustness of the chemical regimes found and to optimise the Jameson Cell operating parameters.

This new treatment process has been shown to be capable of achieving algal removal efficiencies of over 98% and residual levels of total P to below 0.3mg/l in the treated effluent.

The process requires chemical dosing to achieve flocculation which we are trying to avoid in this project. It is however an interesting technology with possible applications pending further testing without using chemical coagulants. They state that the chemicals are added to precipitate dissolved P. If a chemical coagulant is not required for algal flocculation then it could be an effective method of harvesting. This is the only paper that report on the use of this technology in this way so further research would be needed.

Zimmo et al. (2004) assessed N removal processes and N mass balances across pilot-scale algae and duckweed-based WSPs during three periods of 4 months, each under different operational conditions.

During periods 1 and 2, the effect of cold and warm temperature was studied. During periods 2 and 3, the effect of low- and high-system OLR was studied in warm seasons operation. The pilot-scale system consisted of two parallel treatment streams, each with four similar ponds in series fed with domestic sewage with HRT of 7 days in each pond. Each pond had a depth of 0.9m and a surface area of 3m$^2$. Inflow was kept at 0.38m$^3$/d in both ABPs and DBPs.

Period 1 was from December to April during cold season (average water temperature=10 ± 3°C) and the influent wastewater was of low/medium strength (BOD=149 ± 20mg/L). Period 2 was from April to August during warm season (average water temperature=20 ± 4°C)

using the same wastewater source as in period 1 (BOD=167 ± 15mg/L). Period 3 was from April to August the following year during warm season again (average water temperature=21 ± 4°C) and the wastewater used was of high organic loading (BOD=375 ± 32mg/L).

In ABPs sedimentation and de-nitrification were the two main fluxes responsible for removing N from the wastewater. Depending on temperature and OLR, ABPs showed higher N removal via sedimentation (46–245% higher) compared to DBPs. The difference increased with higher temperature and lower OLR. Higher increase in sedimentation in ABPs over DBPs was observed during period 2. De-nitrification and volatilisation rates were higher in ABPs than DBPs by 7–37% and 7–51%, respectively.

The contribution of algae to sedimentation was similar during periods 2 and 3. They state that sedimentation was the main removal pathway. This is an important point and can be utilised providing a method for regular sediment removal is employed otherwise the nutrients stay in the system.

Overall N removal was higher during warm temperature in both ABPs and DBPs, but similar during periods 2 and 3. N removal in DBPs was 20%, 10% and 8% less than removal in ABPs during the three experimental periods, respectively. In DBPs, the duckweed cover prevents sunlight penetration and consequently algae will not develop as in algae ponds. Therefore, sedimentation was limited. They found that Ammonia volatilisation in both systems was not a large contributor to N removal during the entire experimental period.

N recovery via duckweed harvesting represented an important N-removal component. Harvesting algae will have the same effect, if not greater as identified by their higher production rates. Annoyingly in their conclusions they do not state whether they think the use of duckweed is a good idea. From the results they present I would say that incorporating duckweed has a number of disadvantages:

- Prevention of algal growth through shading

- A single surface layer of duckweed vs. algae dispersed throughout the volume

- Reduced gas exchange at the surface of the pond

- Less productive than algae

Zimmo et al. (2003) provides another paper using a similar experimental setup as his previous papers, they focus this study on the comparison of the ammonia volatilisation rate in ABPs and DBPs treating domestic wastewater. They conclude that ammonia volatilisation is the least important method for N removal.

I agree that the effectiveness of this mechanism is directly related to high pH. The high pH required for any considerable ammonia volatilisation is something we are aiming to avoid and therefore further analysis in this area is not required.

Kim et al. (2002) operated HRAPs to evaluate the design parameters and characteristics of filamentous algae matrix (FAM) as the predominant species for treating polluted rural stream water. The porous and gelatinous FAM was formed like a sponge, which functions to prevent excessive loss of the algae in the effluent and can easily be retrieved from the ponds.

A pilot-scale plant was built at the Agriculture and Rural Development Corporation. A steel storage tank was $8m^3$ and the HRAP size was $6m^3$. Each basin was baffled, separated into five compartments; length:2m , width:1m and height:0.6m. This was used to maintain plug flow. The dominant algae were *Spirogyra* species adhering at a low water depth and seeded in the HRAP to form the FAM.

The experiment was performed during the period of June to October. The water depth of HRAP was 0.3m and HRT was 4 days. N concentration was 6.59 to 15.01mg/L and P concentration was 0.88 to 1.89mg/L. The concentrations were artificially controlled by added nutrients.

The organic fraction of harvested FAM was about 88%, which is suitable for use as fertilizer. The HRAP system using FAM was found to be an effective nutrient removal process not requiring any artificial carbon sources for nitrification. At HRT 4 days, the N and P removal efficiencies were 85.9% and 65.8% (from what initial concentration? Not stated), respectively. When the pH and water temperature were maintained above 9 and 15°C, HRT required for achieving a 70% N removal efficiency could be reduced by about 3 days (or to 3days? It is not clear). The oxygen production rate by FAM was calculated as 1.45mg/L/m$^2$ O$_2$. The design surface area of HRAP needed per rural inhabitant was about 2.72m$^2$.

The English is quite bad so it is difficult to understand what they mean. I have done my best to translate but it is not helped by the bad use of data analysis. I do not know why they are working in removal efficiencies. In this paper they state the concentration range in the effluent and then mix about % values and HRTs for different experiments. I cannot use this data for comparison. All they need to state is the environmental conditions and the corresponding nutrient uptake rate. A % is irrelevant if you are required to meet a specific absolute standard, i.e.1mg/L. I have used this paper as an example of poor data available within this subject area and to show why using % removal is not a fair measurement for comparison.

If we take an absolute value of 40mg/L/d and our influent has a concentration of 60mg/L. We can instantly show that a HRT of 1.5days is required for treatment. Stating a % removal means we also require data for the initial concentration and HRT used for each specific experiment which often is missing.

Ramanan et al. (2010) Looked into enhanced algal CO$_2$ sequestration through calcite deposition by Chlorella sp. and Spirulina platensis in a mini-raceway pond. Biological CO$_2$ sequestration using algal bioreactors is one of the most promising and environmentally benign technologies to sequester CO$_2$. This research study was taken up to alleviate certain

limitations associated with the technology such as low $CO_2$ sequestration efficiency and low biomass yields.

Industrial revolution in the 20th century has pumped in huge volumes of $CO_2$ into earth's atmosphere. Concentration of $CO_2$ in the atmosphere is still increasing in the current century and the outcome of this increase has already witnessed a profound effect on global environment. Recently, it has been widely accepted that global warming is because of greenhouse gas emissions from anthropogenic activities. Thus actions are being taken to mitigate the greenhouse gas emissions from anthropogenic activities, especially in the case of large point source emissions, through various mechanisms and protocols. $CO_2$ capture and storage technologies form an integral part of these measures considering their potential for greenhouse gas emissions abatement.

Photosynthesis has long been recognized as a means to sequester anthropogenic $CO_2$ and algae have also been identified as fast growing species whose carbon fixing rates are much higher than those of terrestrial plants. The continuous cultivation of algae would not only help in bio-fixation of $CO_2$ but also yield value added products from biomass such as proteins, fatty acids, vitamin A, minerals, pigments, dietary supplements for human, animals and aquaculture and other bio-compounds. On the other hand, processes in algal photo-bioreactors are still under scrutiny with problems of low biomass yield and inefficient $CO_2$ utilization.

Carbon concentrating mechanism (CCM) plays a major role in photosynthesis and has evolved in algae and green plants. However, CCM acts as an enhancer for higher growth rates in algae and hence have been preferred for sequestration studies. Cyanobacteria and microalgae have been recently subjected to numerous investigations for their ability to utilize DIC. It is proven that these photosynthetic organisms are able to change their relative affinities for DIC ($HCO_3^-$ and $CO_2$), depending on the concentration in the external medium, and there is some active accumulation mechanism which allows $CO_2$ to be concentrated inside the cell. However, there has been no general agreement on the roles of $CO_2$,

bicarbonate ion and carbonic anhydrase (CA) in algal photosynthesis at high external $CO_2$ concentrations. Some interesting phenomenon such as calcite formation is associated to DIC mechanism and may serve as an additional route to sequester carbon.

Preliminary flask experiments were carried out to screen for high $CO_2$ fixing organism among 10 different algal strains. The $CO_2$ fixation inside the mini-raceway pond depended on variables including pH and calcium chloride concentration for calcite production and was therefore optimized in flask scale studies prior to actual experimental run (mini-raceway pond). About 10 algal species were screened for their ability to exhibit high growth rate at 10% $CO_2$ concentration in flask scale experiments. S. platensis and Chlorella sp. showed higher growth rates among the 10 species of algae in flask scale experiments. Both these species exhibited higher intake of $CO_2$ for growth as evidenced from the reducing $CO_2$ concentration on an hourly basis.

Pure cultures of Spirulina platensis (Cyanobacteria, Oscillatoriales) and Chlorella sp. (Chrolophyta, Chlorophyceae) were used. S. platensis was grown in modified Zarrouk medium devoid of bicarbonate ion (carbon free media) whereas Chlorella sp. was grown in Bold Basal Media. Both cultures were acclimatized with 2% $CO_2$ enriched air prior to inoculation in the mini-raceway pond. The mini-raceway pond was maintained under sterile conditions to avoid contamination. It was constructed consisting of a shallow tank with a working volume of 8L and a headspace of 156L which was enclosed to avoid $CO_2$ leakage and injected with a range of $CO_2$ concentrations (1%, 5%, and 10%). The system was maintained at 30°C under 12:12 L:D illumination cycles of 6W/m$^2$ during light cycle supplied by artificial lighting. The mini-raceway pond was inoculated with a constant inoculum (0.15g/L) and one experimental set was allowed to run for 25 days. Three experimental sets were run in each $CO_2$ concentration.

Growth, alkalinity and pH measurements were made in triplicate every 24h. The growth was measured by monitoring the optical density at 670nm. The dry weight was also calculated

to determine the biomass concentration. The pH of the mini-raceway pond was maintained at 10.0 during the $CO_2$ sequestration experiment runs.

The $CO_2$ concentration in the mini-raceway pond was maintained by injecting the $CO_2$ into the headspace of the system. Both the algal species were subjected to a range of $CO_2$ concentration (1%, 5% and 10%). The $CO_2$ fixation efficiency was calculated daily in terms of $CO_2$ consumed by the organisms in the mini-raceway pond. After the measurements, the system was again maintained at the initial $CO_2$ concentration of the experimental run. The calcite production inside the photo-bioreactor was carried out by the addition of 0.1M of calcium chloride for S. platensis and 0.8 M of calcium chloride for Chlorella sp. in the culture medium and the resultant calcite formed was removed daily from the photo-bioreactor. A blank experimental run was also performed without the inoculation of cyanobacteria.

The study demonstrates an increase in $CO_2$ sequestration efficiency by manoeuvring chemically aided biological sequestration of $CO_2$. Chlorella sp. and Spirulina platensis showed 46% and 39% mean fixation efficiency, respectively, at input $CO_2$ concentration of 10%. The effect of acetazolamide, a potent carbonic anhydrase inhibitor, on $CO_2$ sequestration efficiency was studied to demonstrate the role of carbonic anhydrase in calcite deposition. Calcite formed by both species was characterized by scanning electron microscopy coupled electron dispersive spectroscopy and X-ray diffraction.

In the actual experimental run, Chlorella sp. showed remarkable sequestration efficiency in the order of 59%, 51% and 46% for input $CO_2$ concentrations of 1%, 5% and 10%, respectively. This strain showed higher growth and subsequently higher fixation efficiency within 10 days of experimental run, however, mean fixation efficiency declined after 15 days. In contrast, S. platensis was a relatively slower growing organism with lesser mean fixation efficiency of 53%, 41% and 39% for input $CO_2$ concentrations of 1%, 5% and 10%, respectively. S. platensis had sustained mean fixation efficiency until 25 days of experimental run. Growth of both the species was enhanced in presence of $CO_2$. The biomass growth rate of S. platensis was 110.4mg/L/d at input $CO_2$ concentration of 10%

whereas biomass growth rate of Chlorella sp. was 84mg/L/d at the same input concentration. The growth rate of both the species seemed to increase with increasing $CO_2$ concentration.

The organisms did not have a lag phase at concentration higher than the atmospheric concentration which implies photosynthetic capability of these organisms is limited by atmospheric $CO_2$ concentration. Higher $CO_2$ concentration would therefore aid in higher photosynthetic activity, nevertheless, ability to fix $CO_2$ gradually decreased. A host of photosynthetic organisms have shown a similar trend where higher growth has been observed with increased $CO_2$ concentration suggesting the possibility of $CO_2$ being a limiting factor for growth. Conversely, concentrations higher than 15–20% have been shown to be inhibiting the growth of algae.

Chlorella sp. is known to be a high $CO_2$ fixing organism which has been validated in our study. The overall scheme of calcite deposition coupled $CO_2$ fixation with commercially utilizable biomass as a product seems a viable option in the efforts to sequester increasing $CO_2$ emissions.

The calcite deposition coupled $CO_2$ fixation approach undertaken in this study is a novel advance in $CO_2$ sequestration research. The characterization of material obtained after precipitation has demonstrated that the material is calcite formed from biologically aided chemical conversion of bicarbonates. The $CO_2$ fixation efficiency and growth rate of algae were substantially higher when coupled with calcite deposition even in mini-raceway pond system. Both the species were efficient sequesters of $CO_2$ with Chlorella sp. showing better sequestration efficiency compared to S. platensis. However, S. platensis had much higher calcite deposition rate and calorific value, 4303kcal/kg biomass as compared to 2298kcal/kg biomass calorific value obtained for Chlorella sp.

They made an interesting and important finding: The influence of pH, a critical parameter for growth rate of algae was monitored and a study on pH at which maximum growth rate

was achieved was conducted prior to actual experimental run. Both the organisms were subjected to a pH range of 6–13 at constant input $CO_2$ concentration of 10%. A pH of 10.0 was found to be the optimum for both the organisms with highest growth rate of 140mg/L/d and 80mg/L/d observed for S. platensis and Chlorella sp., respectively. The media pH was found to play a vital role in growth of the organism with slightly acidic pH of 6.0 showing the least growth rates for both the organisms. The total alkalinity profile of the experimental run was also monitored to ascertain the role of alkalinity on $CO_2$ fixation. It revealed that the total alkalinity levels inside the mini-raceway pond increased with time and this may be because of the formation of bicarbonates which was subsequently converted into calcite keeping the pH of the media high even after high input $CO_2$ concentrations.

This is an important finding and the only one of its type reported. Up until now, high pH has been considered detrimental to productivity because they were associated with carbon limitation. This shows that when $CO_2$ is in excess, the pH can be artificially raised and may produce a benefit. We obviously cannot take 1 experimental data set as fact however pH is a very important parameter in optimising growth and therefore more research needs to be done in this specific area. This 2010 paper is excellent and a great example of how fast new research in this area is evolving.

Park & Craggs (2010) produced a report on wastewater treatment and algal production in HRAPs with $CO_2$ addition. HRAPs provide improved wastewater treatment over conventional wastewater stabilisation ponds; however, algal production and recovery of wastewater nutrients as algal biomass is limited by the low C:N ratio of wastewater.

The C:N ratio of domestic wastewater (typically 7:1) is low compared to that of algal biomass (typically 15:1); therefore algal production and recovery of wastewater nutrients as algal biomass in HRAP could be enhanced by $CO_2$ addition. Controlling pond water pH to below 8 with $CO_2$ addition may also enhance algal production by preventing ammonia inhibition of algal growth.

For example, when the pH rises to 9.5 (20–25°C) total free ammonia concentration of only 34 and 51g/m$^3$ has been found to cause 50 and 90% reductions in photosynthesis, respectively, of the freshwater alga Scenedesmus obliquus , which often grows in HRAP. Moreover, aerobic heterotrophic bacteria which use photo-synthetically derived oxygen to break down dissolved organic compounds (BOD removal) and release $CO_2$ and nutrients (N and P) have an optimum pH of 8.3, above which bacterial activity is increasingly inhibited. However, controlling HRAP pH to below 8 will reduce nutrient removal by physico-chemical processes such as ammonia volatilisation and phosphate precipitation occurring at pH9, which could reduce overall nutrient removal unless offset by increased algal assimilation.

They state that there is little information in the literature on $CO_2$ addition to wastewater treatment HRAPs and that Laboratory scale research on $CO_2$ addition to wastewater grown algae cultures has demonstrated higher algal photosynthetic efficiencies and productivities compared to controls without $CO_2$ addition. $CO_2$ addition to pilot-scale nutrient media HRAPs has been shown to more than double algal production.

They investigate the influence of $CO_2$ on wastewater treatment performance and algal production in two pilot-scale HRAPs operated with different HRTs (4 and 8 days) over a New Zealand Summer (November–March). Weekly measurements were made of influent and effluent flow rate and water qualities, algal and bacterial biomass production, and the percentage of algae biomass harvested in gravity settling units.

Experiments were conducted using two identical pilot-scale HRAPs (West and East), which were a part of an Advanced Pond System (APS) treating domestic wastewater in New. Each HRAP was a single-loop raceway (surface area: 31.8m$^2$, depth: 0.3m, volume: 8m$^3$) with semi-circular end-walls; lined with HDPE plastic; and with a HDPE dividing wall separating the two raceway channels. A free standing, 1m wide, galvanised steel paddlewheel circulated the pond water around the HRAP raceway to give mean surface velocity of 0.15m/s. The HRAPs each received anaerobic digester effluent (1m$^3$/d) which was added at

the pond bottom downstream of the paddlewheel. The influent to the West HRAP was diluted with $1m^3$/d of tap water (simulating recycle of treated effluent after complete algae and nutrient removal) to give HRTs of 4 and 8 days respectively for the West and East HRAPs. Effluent from the HRAPs was taken from the pond bottom upstream of the paddlewheel to ensure complete mixing within the HRAP.

The maximum pH of the HRAPs was maintained below 8 through pH controlled addition of $CO_2$. The $CO_2$ addition system consisted of a $CO_2$ gas cylinder, a $CO_2$ gas regulator, a gas flow meter (0–12 L/min range), a solenoid valve and gas diffusers. Pond water pH was measured every five seconds with a pH probe and, when the pH exceeded the pH8 set point, the controller opened the solenoid valve and bubbled $CO_2$ into the ponds (2L/min) through two $CO_2$ gas diffusers placed on the pond bottom in turbulent zones (one just before the paddlewheel and the other before the downstream pond corner). When the pond water pH reduced to pH 7.8 the controller closed the solenoid valve halting $CO_2$ addition. The pH probes were calibrated 1–2 times a week with standard pH solutions (pH 7 and 10). The temperature range of the experiment was 18.5 to 23.5°C. The dominant algal species present in both HRAPs during this 5 month study included the colonial algae Scenedesmus sp, Microactinium sp, Pediastrum sp, and the single cell alga Ankistrodesmus sp.

250L gravity algal settling cones (ASCs) were used to harvest the algal biomass grown in the HRAPs. Effluent from each HRAP was divided between two ASCs and introduced horizontally into each cone at mid-depth.

The maximum DO was consistently about 100% saturation during the day with high photosynthetic activity. The DO dropped at night to the minimum values which never reached 0% showing that sufficient algal photosynthesis occurred. During day and night cycles the pH varied between 8 and 6.5 respectively.

Their results showed high dissolved organic compound removal rates which may indicate that $CO_2$ addition to HRAPs enhances aerobic heterotrophic bacterial degradation of organic matter by preventing high pH (>9) inhibition and ammonia toxicity.

This research shows that the wastewater treatment HRAPs with $CO_2$ addition achieved a mean and maximum algal productivity of 55.7mg/L/d and 82.3mg/L/d respectively for the HRAP at 4d HRT and 30.0mg/L/d and 50.7mg/L/d for the 8d HRT HRAP. They state that the reduced algal productivity in the 8d HRAP may have been due to increased light shading by the higher bacterial solids concentration in this pond.

The aim is to reduce the HRT, which in cost terms means, less space required to treat the same volume of wastewater. Reducing HRT is expected to increase biomass production due to the greater availability of required nutrients. i.e., on a daily basis, the 4d HRAP received twice as much nutrient than the 8d. If all the nutrients were used, you could expect twice the growth rate. So I disagree with them with regard to their reasoning behind reduced 8d productivity as it looks like they may have overlooked this important factor.

Algae biomass produced in the HRAPs was efficiently harvested by simple gravity ASCs (mean harvested algal productivity: 38.3mg/L/d for the 4d HRAP and 25mg/L/d for the 8d HRAP respectively). Higher bacterial composition and the larger size of algal/bacterial flocs in the 8d HRAP increased harvest ability (83%) compared to that of 4d HRAP (69%). They state that the algae could be efficiently harvested in simple gravity settlers with a retention time of 3h or less.

This is a well writing paper with good presentation of data and a good source of information.

Heubeck et al. (2007) investigated the influence of $CO_2$ scrubbing from biogas on the treatment performance of a HRAP. However, from the paper I could not work out if any of the experiments involved any biogas, it seemed like only $CO_2$ was added. They state: The

experiments presented in this paper were conducted to examine the effects of $CO_2$ addition (and reduced culture pH) on algal growth and wastewater treatment in batch cultures.

Biogas produced by anaerobic treatment of wastewater can be collected and used for power
generation. However, the biogas may require scrubbing to prevent corrosion by $H_2S$ and to improve engine efficiency by reducing the $CO_2$ content. HRAP can be used to scrub biogas during the daytime when they are carbon-limited and have high pH. This study investigates the influence of the $CO_2$ addition from biogas scrubbing on high rate algal pond wastewater treatment performance and algal production.

Firstly $CO_2$, $H_2S$ and most other trace gases would be scrubbed from the biogas, leaving almost pure methane which is more easily handled and utilised. Secondly the $CO_2$ transferred into the HRAP water may overcome carbon limitation of algal growth (due to $CO_2$ depletion) and increase algal productivity and nutrient assimilation. Thirdly the reduction in pond water pH (often 10) to more neutral levels (optimum: pH 8.3) by $CO_2$ addition may further stimulate aerobic bacterial degradation of the waste and prevent ammonia toxicity of algal growth in wastewater cultures.

Batch culture experiments were conducted in 2L laboratory microcosms and in 20L outside mesocosms.

Inside: The cultures were grown under constant conditions (20 ± 1°C, 12:12 L:D cycle, 1.61MJ/m2 provided by fluorescent light bulbs and constantly mixed with magnetic stirrer bars). One flask had $CO_2$ addition and pH control set to 8 whilst three went without $CO_2$ addition.

Outside: Four batch culture mesocosms were run (20L HDPE buckets, surface area: 750cm$^2$ and depth:27cm) with continuous mixing from magnetic stirrer bars. They were filled with 19L of HRAP water. Cultures were dominated by colonial algae *Microctinium* sp. and

*Scenedesmus* sp. that are typical for HRAP. The temperature varied between 20.9–22.2°C. Two had $CO_2$ addition and pH control set to 8 whilst two went without $CO_2$ addition.

The 2L laboratory experiments had algal growth rates around 54mg/L/d for experiments with $CO_2$ addition and 44mg/L/d without. Non pH regulated experiments reached pH10.8 and did not increase further – showing a strong upper limit. N reduction was 7mg/L/d (0.3mg/L/h) reaching 0mg/L. $CO_2$ addition gave the lowest P concentrations of near 0mg/L, the average rate of removal was about 1mg/L/d.

The results of the outside experiment were only carried out over 3 days and were not very conclusive This is not a long enough time period to make any real judgements however they did achieve a biomass growth rate of 33mg/L/d and N reduction of 4mg/L/d. The pH in non regulated experiments again reached 10.8. I do not know why the experiments were only carried out for three days.

Results indicate that $CO_2$ addition and reduced culture pH increased algal production and nutrient assimilation, decreased high pH mediated nutrient removal processes (phosphate precipitation and ammonia volatilisation), but had little influence on the ability of the culture to remove filtered BOD. Disinfection, as indicated by E.coli removal; was reduced, however, further research on virus removal, which is not affected by culture pH, is required.

They conclude: These experiments demonstrate the potential to scrub $CO_2$ from biogas using HRAP
water without decreasing the effectiveness of wastewater treatment and enabling increased recovery of wastewater nutrients as algal biomass. I would say they demonstrate the potential to add $CO_2$ into HRAPs for improved productivity and treatment efficiencies but experiments would have to be done with biogas to confirm this claim.

It would be interesting to investigate if this process could be incorporated with $CO_2$ addition and whether or not it had negative effects on the quality of scrubbed biogas. Consensus in

the literature is that this process can improve the biogas quality and greatly reduce the $CO_2$ content.

Al-Nozaily et al. (2000) studied the performance of duckweed covered sewage lagoons, N and P balance and plant productivity.

Laboratory scale experiments were performed in a non-continuous batch reactor system with

0.8 to 41.2L with domestic sewage exposed to constant light intensity, temperature and humidity. The contribution of duckweed (L. gibba) to N and P removal in duckweed-covered sewage lagoons was studied at N concentration of 25±96mg/L with 10, 30, 70 and 95cm deep reactors, and liquid mixing intensity of 0, 0.3, 1.0, 2.3 and 34.1W/m$^3$. Duration of each experiment was 20d with biomass harvesting every 5d.

I wanted to use this paper to get results of P and N removal rates and biomass generated using duckweed however the paper is so badly written it is difficult to pull any data from it. From what I gather the maximum removal rates were 2.2mg/L/d N and 0.1mg/L/d P. I could not find a figure for duckweed growth rate.

Shilton et al. (2008) wrote a paper on the advantages of WSPs with regard to solar aeration and disinfection, co-digestion, bio-$CO_2$ scrubbing and biofuel production. They also considered the performance and benefits of HRAPs making this statement:

Daytime algal photosynthesis in WSP assimilates $CO_2$ from the pond water at a rate that is faster than both diffusion of $CO_2$ from the atmosphere and $CO_2$ release from bacterial degradation of the organic waste. The growth of 1 tonne of algae biomass in an HRAP assimilates approximately 1.6 tonnes of $CO_2$ (assuming a carbon content of, 46% of the algae dry weight) (Benemann 2003). As a result, the pond water becomes $CO_2$ depleted and can be used to scrub $CO_2$, and impurities such as hydrogen sulphide, from biogas or exhaust gases that are sparged through it (Heubeck et al, 2007). Scrubbing $CO_2$ using WSP also

boosts algal production by overcoming carbon limitation. $CO_2$ addition to HRAP has been shown to more than double algal productivity.

They also recognise the potential of algae as a biofuel, having higher productivity than most land plants and containing oils that can be converted to biodiesel. The paper is a good account of sustainable water treatment technology and echoes the findings in other reports.

### 3.3.4. Algal Bioreactors

Ai et al. (2008) carried out research to develop an algal bioreactor for use in space to provide protein and oxygen for future astronauts and remove $CO_2$ as part of a controlled ecological life support system. They evaluated the performance of the bioreactor.

The experiment was highly controlled and detailed as follows: Spirulina platensis, a type of filamentous Cyanobacterium was grown in optimal artificial medium, sterilized by supplying ozone. Light was provided by two electromagnetic inductive lamps (80W) at $60W/m^2$, 24h/d (well in excess of any other literature data). The temperature was maintained at 30°C +/- 2.0°C. The pH was constantly monitored and maintained between pH8-10 which they state to be optimal. This was achieved by providing the required amount of $CO_2$. The dissolved Oxygen was maintained between 6-10mg/L. The salinity and electric conductivity were also continuously measured. The bioreactor was agitated by mechanical rotation at 40-60rpm, enough to meet the demand of mixing. The PVC reactor was 45cm in length and 30cm in internal diameter, making it a total of 3.18L.

The demonstration results were successful, meeting the design requirements. They report that the density of algae in the photo-bioreactor increased from 0.174g/L to 4.064g/L after 7 days growth. This is almost double the maximum concentration I took my bioreactors to and equates to a growth rate of 560mg/L/d which is vastly in excess of any other literature values. Benemann, who has carried out extensive research into the subject produced conservative estimates of 33mg/L/day. Bioreactors, under controlled conditions will

produce growth rates significantly higher. The maximum growth rate I observed was just over 150mg/L/d, I estimate that I could have achieved in excess of 300mg/L/d with complete optimisation. Consistent growth rates over 7 days of 555mg/L/d may be possible with the experimental set up they demonstrate. The design of this particular bioreactor was highly complex and highly optimised; growth rates in excess of 300mg/L would be expected.

The experiment was really well structured; all the variables were controlled and constantly measured providing excellent data. All of the parameters were kept at optimum levels; this provides a good idea of maximum growth. I feel the experiment could be extended by a) repeating with the same conditions and b) repeating with various strains of algae. The paper provides quality details, diagrams and pictures of the experiment.

Hu et al. (2008) carried out a study into producing estimates of biomass growth with variable light and temperature conditions using a mathematical model for computer simulation. They focus on the accuracy of the mathematic model so the details about their experimental data are a bit thin.

They state that experiments show that the utilization of red lights of 620nm wave length, light intensity within the range of 10-60W/m$^2$ can significantly affect the growth of microalgae, so red LEDs were used as the light source in their experiments.

They grow a type of green microalgae, *Spirulina platensis* in artificial medium in a bioreactor of unspecified dimensions. Details about agitation, $CO_2$ and pH conditions are omitted.

Experiments with an unspecified constant temperature and varying light intensities were carried out. At 20W/m$^2$ the initial growth rate was around 144mg/L/d but only reached a maximum concentration of 0.8mg/L. At 40W/m$^2$ the initial growth rate was around 126mg/L/d but reached a higher maximum concentration of 1.2mg/L. These growth rate results are within a standard reported range found in the literature and correlate with the

theory of faster initial growth at lower light intensities with inhibition as the concentration becomes denser and the penetration depth of the light reduces.

A second experiment was carried out maintaining a constant unspecified light intensity and varying the temperature between 28-34°C. At 28°C the growth rate was 108mg/L/d, this increased to 168mg/L/d at 30°C and then fell to 127mg/L/d and 120mg/L/d at 32°C and 34°C respectively. These values do correlate with other temperature data that shows that each strain of algae have a working temperature range where growth rate increases until a point where it becomes inhibitory. Exact temperatures vary with specie however these results match other literature data.

Despite lack of detail regarding the experimental methods and parameters, the results are useful. The temperature results would have been more beneficial if the experiment had been carried out over a wider range, 20, 25, 30 and 35°C for example.

Nakajim & Takahash (1991) looked into the use of a photo clarifier, a type of partially shaded bioreactor for algae-liquid separation. They aim to maintain a high density of algae whilst separating them from water, maximising the efficiency minimising the cost. They identify that solids-liquid separation remains a difficult problem. They state, considering that the removal efficiency may be higher as the contact area between the organisms and the treated wastewater increases, and that the surface-to-volume ratio increases with a decrease in the size of organisms, it is advantageous to use unicellular microalgae, which grow dispersively. Thus, dispersing the cells in order to increase reaction efficiency while at the same time separating the cells from the treated wastewater becomes an important problem for the development of a photo-bioreactor using photosynthesis.

They ran an experiment to compare the difference between using a photo clarifier for separation and a control without a photo clarifier. The photo clarifier runs on a continuous loop (whilst lit), medium from the main bioreactor flows into the photo clarifier and flows back to the main bioreactor from a small illuminated area, where the algal cells gather.

Effluent is pumped out of the photo clarifier from a shaded region at the same rate the main bioreactor is continuously fed to maintain equilibrium.

The algae used are a unicellular green algae, *Euglena gracilis* grown in artificial medium. The temperature was maintained at 22.5 +/- 1.5°C. Light was irradiated in a 12-h cycle at 38.8W/m$^2$, all pumps stopped during dark. The bioreactor was aerated, no pH data is given.

Unfortunately they give their growth results in cells/ml which I cannot compare to other literature, however the growth curve is in the standard form of slow initial growth when the density is low followed by a period of fast growth rate until a limit is reached (normally based on the light intensity) and the algal concentration plateaus.

The main finding is that the use of a photo clarifier produced an algal concentration 4x higher than the control after 31 days growth.

Guo et al. (2008) investigated improving hydrogen photo-production regulated by carbonylcyanide m-chlorophenylhrazone from marine green algae *Platymonas subcordiformis* grown in a $CO_2$-supplemented air bubble column bioreactor. They wanted to develop an integrated process of $CO_2$-fixation and $H_2$ photo-production.

$H_2$ photo-production by microalgae has been documented among the genus Chlorococcales and Volvocales (Chlorophyceae) (Happe et al. 2002), with some algae tolerant to extremely high $CO_2$ concentration (850%) and even in exhaust flue gas. The freshwater green alga, Chlamydomonas reinhardtii, is a well-established model strain that produces $H_2$ photo-biologically under sulphur deprivation in a two-stage system. To further improve $H_2$ production, this paper investigates two new process strategies of $CO_2$-supplementation and the use of an air bubble column photo-bioreactor to increase both cell growth and starch accumulation of P. subcordiformis, eventually leading to improved $H_2$ production.

P. subcordiformis, wild-type, was grown photo-autotrophically in a 600ml bubble column bioreactor system (50mm diam, 400mm ht) at 25°C in an autoclaved natural seawater medium, supplemented with micronutrients with initial pH8.

Compressed air and $CO_2$ were mixed and metered through calibrated flow meters, and sterilized using 0.22µm membrane filters before entering the system. The bottom of the bioreactor was filled with a porous quartz sieve (10mm diam), which dispersed the airstream. The cultures were illuminated from two sides with cool white fluorescent lamps which provided an average light intensity of 10W/m$^2$ under 14h:10h L:D cycle. $CO_2$ was added up to 15% in air; normal air, 1%, 3%, 5%, 10%, and 15% $CO_2$. The $CO_2$ was supplemented only during illumination. For $H_2$ production, 7-day old cells from the bioreactor were harvested. They were prepared for photo-biological $H_2$ production under continuous illumination by cool-white fluorescent light (16W/m$^2$).

The maximum cell concentration was attained when 3% $CO_2$ was used. This clearly indicated that air (containing 0.03% $CO_2$) is deficient for optimal growth. 1% and 5% $CO_2$ also performed better than air whilst 10% and 15% performed worse.

The pH in the air-grown culture increased from an initial pH8 to a maximum pH9.6. The initial increase in pH is due to the photosynthetic consumption of dissolved $CO_2$ and then the pH value remained constant. When the algal cells were grown under $CO_2$-supplemented air, the pH continually decreased. This decrease in pH was expected because $CO_2$ supplementation buffered the medium as a result of the $CO_2/HCO_3^-$ balance. The decrease in pH had a positive correlation with the supplementation of $CO_2$ concentrations.

During the $H_2$ production stage, $H_2$ was detected soon after the beginning of illumination and its yield was higher in $CO_2$-grown cells when compared to that of air-grown cells. $H_2$ photo-production was enhanced by 60% for 3% $CO_2$-grown cells when compared to air-grown cells, with the highest rate of $H_2$ production of 3.2 ± 0.12µmol $H_2$/mg Chl /h. Improved $H_2$ production correlated well with the increase in starch accumulation. The

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results were in agreement with Yoon et al. (2002) who reported that $CO_2$ injection in the cell growth phase increased not only the cell concentration but also the $H_2$ production per g of *Anabaena variabilis*.

Rodolfi et al. (2008) carried out an experiment with similar design parameters to my experiment however it is not so relevant as they looked into nutrient deprivation. Their experiment was extremely well done and reported and the results are very useful with regard to my project.

They looked at strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photo-bioreactor for microalgae oil production. They screened thirty micro algal strains in a laboratory for their biomass productivity and lipid content. Four strains (two marine and two freshwater) were selected because they were robust, highly productive and had a relatively high lipid content. They were cultivated under N deprivation in 0.6L bubbled tubes.

Of these four strains, only the two marine microalgae accumulated lipid under such conditions. One of them, the eustigmatophyte Nannochloropsis sp. F&M-M24 attained 60% lipid content after N starvation. This was then grown in a 20L Flat Alveolar Panel photo-bioreactor to study the influence of irradiance and nutrient (N or P) deprivation on fatty acid accumulation.

For strain selection, cultures were grown in an orbital incubator flushed with air/ $CO_2$ (5%) at a temperature of 25°C, under continuous illumination (100µmol PAR photons/m$^2$/s) provided by daylight fluorescent tubes. The second stage of strain selection used 0.5L of culture, bubbled with a sterile air/$CO_2$ mixture (3%) to support growth and maintain pH within the desired range (7.5–8.1). The tubes were immersed in a water bath thermo-regulated at 25°C. Continuous artificial illumination (about 200µmol PAR photons/m$^2$/s) was provided by daylight fluorescent tubes on both sides of the water bath. The 20L bioreactor

was kept at 25°C, the optimal temperature for Nannochloropsis sp. $CO_2$ was continuously added to the air stream (3%) to maintain pH7.5 +/- 0.2.

The outdoor scale-up experiment used modules that were 1m high, 2.5m long and, on average, 4.5cm thick, with a culture volume of 110L. For mixing, compressed air was bubbled at the bottom of the reactor through a perforated plastic tube. $CO_2$ was injected into the culture through a gas diffuser placed in an un-aerated zone, as carbon source and for pH regulation. A control unit provided temperature regulation. $CO_2$ was injected during daylight hours to maintain pH in the range 7.5–8.0. The cooling system prevented the culture temperature to exceed the value of 30°C. The natural solar irradiance was between 14-17MJ/$m^2$/day (a normal day is around 20MJ/$m^2$/day).

An N-sufficient control was measured against the deprivation experiments, nutrient deficiency was attained by replacing the daily harvested culture volume with nutrient (N, P or both N and P) depleted medium. In the N-sufficient culture, N was added in amounts equal to 10% of productivity. N-limited cultures were obtained by adding N in amounts equal to 5%, 2.5%, or 1.25% of their productivity. The experiment started with an N-sufficient culture which was distributed in four equal volumes and diluted with fresh medium containing the different N levels.

With N-deprivation it took a few days to decrease productivity and about 2 weeks to halt growth completely. Lipid content increased after about 5 days of N-deprivation and reached 50% of the dry biomass by the end of the experiment (21 days).

I note that after 15 days, the lipid content had reached 42%. Following this period, no extra growth occurred. So if the algae were harvested at just 42%, in the long run you would gain 17.6% more oil by saving the extra 6 days to increase the lipid content to 50%. Lack of P elicited a similar response, but it took longer to decrease productivity (about 1 week) and halt growth (20 days). Lipid productivity increased from 117mg/L/d in nutrient sufficient media (with an average biomass productivity of 360mg/L/day and 32% lipid content) to

204mg/L/day (with an average biomass productivity of 300mg/L/day and more than 60% final lipid content) in N deprived media.

In a two-phase cultivation process (a nutrient sufficient phase to produce the inoculum followed by a N deprived phase to boost lipid synthesis) the oil production potential could be projected to be more than 90kg/ha/d. The experiments showed that this marine eustigmatophyte has the potential for an annual production of 20 tons of lipid per hectare in the Mediterranean climate and of more than 30 tons of lipid per hectare in sunny tropical areas.

This experiment is an example of near perfect conditions for algal growth when in N-sufficient conditions. It was also a result of strain selection to identify the most productive micro algae. In their main experiment they report growth rates of 360mg/L/d and up to 1450mg/L/d during their light intensity experiment. 300-600mg/L/d seem to be a reasonable range for fully optimised bioreactors, 1450mg/L/d is nearly 10x higher than my maximum recorded growth rate.

### 3.3.5. Carbon fixation

Higher plants take $CO_2$ from the air but for algae, $CO_2$ is always a limiting factor because it must be dissolved in the water medium in which they grow. However, this drives a reaction in Eq. (4) to produce more $OH^-$, creating the alkaline conditions found in algal ponds as explained in the equations here:

$CO_2 + H_2O <\text{->} H_2CO_3$ (1)
$H_2CO_3 <\text{->} H^+ + HCO_3^-$ (2)
$HCO_3^- <\text{->} H^+ + CO_3^{2-}$ (3)
$CO_3^{2-} + H_2O <\text{->} HCO_3^- + OH^-$ (4)
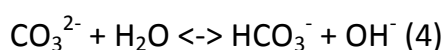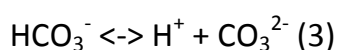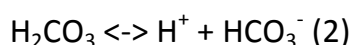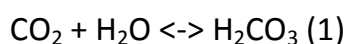
Yun et al. (1997) carried out a study into $CO_2$ fixation by algae, Chlorella vulgaris in wastewater discharged from a steelmaking plant with the aim of developing an economically feasible system to remove ammonia from wastewater and $CO_2$ from flue gas simultaneously. Recently, research on $CO_2$ fixation using actual flue gas from a boiler and a power plant was carried out in a small raceway pond equipped with paddle wheels for mixing and showed that direct blowing of flue gas into algal culture did not inhibit the algal growth.

Since no P compounds existed in the wastewater, external P was added, the optimal concentration required to balance the ammonia was found to be 46mg/L. They made some interesting discoveries; when P was not added to cultures, the initial rate of ammonia removal was very slow because of P limitation, however when P was not limiting the ammonia removal rate was independent of P concentration, reaching its maximum value of 0.86 mg/L/hr. The P content in harvested biomass increased proportionally to the amount of P added showing the algae's large capacity for P uptake and storage. C. vulgaris, utilized ammonia in preference to nitrate as a nitrogen source. Nitrate was not consumed until the ammonia in wastewater was exhausted. The pH value of the culture decreased according to the uptake of ammonia and increased as nitrate was assimilated after the depletion of ammonia. However, P was continuously removed throughout the duration of growth.

All experiments were performed at constant temperature of 27°C in a light incubator in which the average light intensity was maintained at 22W/m$^2$ with 12 x 20 watt 'warmwhite' fluorescent tubes. The media was buffered, controlled at pH8; the algal growth in buffered wastewater was better than that in unbuffered raw wastewater when supplying air or air containing 5% $CO_2$. However, when 15% $CO_2$ was supplied, the algal growth in the raw wastewater was better than that in buffered wastewater. An explanation was not provided. However, I would theorise that the larger quantities of buffering solution required to maintain pH when 15% $CO_2$ is added affected cell metabolism more than the fall in pH in the unbuffered experiments.

When the inoculum was prepared by air bubbling, the best growth was achieved in 5% $CO_2$ The growth was somewhat inhibited in 15% $CO_2$ When the inoculum was adapted to 5% $CO_2$, 5% $CO_2$ still gave the best growth, but the cell growth in 15% $CO_2$ was enhanced compared with the culture grown by supplying air. $CO_2$ removal rates were estimated at 26.0 mg/L/hr $CO_2$. Since the typical concentration of $CO_2$ of flue gas is around 15% $CO_2$, adaptation of C. vulgaris to high $CO_2$ concentration would be necessary for the direct use of flue gas. In a separate experiment, it was found that a gradual increase of $CO_2$ concentration gave even better growth in high $CO_2$ concentrations (up to 30%). This provides a very good case for the fast and effective adaption of algae to suit their environment.

Kaladharan et al. (2009) looked at $CO_2$ sequestration using varying concentrations of $CO_2$ at 0, 5, 10, 15 and 25 mg/L over 2 hour periods. They used 5 types of marine and micro algae but do not state the light or temperature conditions. The results are inconclusive however they show that certain strains of marine algae sequester 100% of the $CO_2$ up to and including concentrations of 15mg/L. At 25mg/L $CO_2$ the sequestration drops to 60% showing this specific strain, *Ulva lactuca* can sequester 180mg/L/day $CO_2$ at a concentration of 1.67g/L algal biomass which I have assumed is wet weight as this has not been made clear.

The paper does not specify enough details about the experiment to provide useful analysis however it does provide some data that can be used as a comparison to other experiments.

Benemann (2003) prepared a report into the biofixation of $CO_2$ and greenhouse gas abatement with microalgae as a technology roadmap. The report provides in depth detail about the technologies and gives some general figures however does not provide specific details about individual experiments. They state that microalgae contain roughly 45% C which equates to 1.64 kg $CO_2$ / kg biomass.

Benemann et al. (2003) produced another report on the controlled eutrophication process: using microalgae for $CO_2$ utilization and agricultural fertilizer recycling. Carrying out a study into the Salton sea, a specific area with high eutrophication problems, they state a figure of

33mg/L/day biomass growth at a cost of $100/mt. The majority of this cost is from the transfer of flue gas from the power plants into the ponds.

From the literature we can expect somewhere between 10-20mg/L/hr $CO_2$ sequestration during photosynthesis which should roughly equate to a growth rate of 6-12mg/L/hr (72-144mg/L/d). Benemann quote a conservative 33mg/L/d which is fair considering the enhanced difficulties with larger production volumes.

Godosa et al. (2010) investigated the influence of real flue gas sparging/scrubbing (7% $CO_2$) on the performance of two 465L HRAPs treating diluted swine manure at 10 days HRT under continental climatic conditions in Spain.

The experimental set-up consisted of two identical raceway HRAPs constructed in white flexible PVC with a total volume of 465L and a surface of $1.54m^2$ (2.3m long, 0.70m wide and 0.3m deep) . The experimental systems were operated from April to July.

The main experiment was operated in three periods, acclimatisation without $CO_2$, 2.2L/min and 5.5L/min gas sparging. The control was operated without additional $CO_2$ for all three periods.

The natural solar irradiation was between 6000-7000 $Wh/m^2/d$. The temperature ranged between 10 and 20°C. A paddle wheel was driven by a motor engine for agitation. Effluent sedimentation was carried out in 7L settlers located at the outlet of each photo-bioreactor.

The plot of pH vs. time shows the pH to be a direct function of the rate of $CO_2$ addition.

N and P removal efficiencies were not significantly affected by flue gas input which suggests that $CO_2$ sparging does not compromise wastewater treatment in HRAPs.

98±1% N removal within the 10d HRT was consistently maintained, regardless of the environmental and operational conditions.

$CO_2$ sparging resulted in lower pH values (≈2 units lower) and an enhanced nitrification compared to the system operated in the absence of flue gas supply.

Biomass concentration was only higher (30% than in the control HRAP) when flue gases were supplied at 5.5L/min, probably due to the fact that the higher irradiances and temperatures prevailing within this experimental period resulted in an inorganic carbon-limited scenario in the control HRAP.

### 3.3.6. Effect of pH

Mostafa and Helling (2001) studied the metabolism of isoproturon by two soil and freshwater microorganisms, green alga *Chlorella kesslerei* and cyanobacterium *Anabaena inaequalis*, as a function of pH.

Isoproturon degradation by *Anabaena* after 10 days at pH 5.5 was 3– 4 times greater than corresponding values at pH 7.5. Since the reduction in biomass due to high concentrations of the herbicide was similar at both pH levels, faster degradation at pH 5.5 seems to result from enhanced metabolic activity of the algal cells under the more acidic media conditions. The isoproturon accumulation by *Chlorella kesslerei* was 5x greater at pH5.5 than it was at pH8.

Beuf et al. (2000) studied the effect of external pH on inorganic carbon assimilation in unicellular marine green algae. Three algae were used, C. littoral, S. bacillary and C. saccharophila. They were isolated in the marine Biotechnology Institute. The algae were grown at 25°C in seawater. The media was bubbled with air for low carbon conditions and air enriched with 5% $CO_2$ for high carbon conditions.

Marine microalgae live in a habitat where the dissolved inorganic carbon concentration is relatively high (about 2µmol/L), but where the dominant form of inorganic carbon is $HCO_3^-$ and the equilibrium concentration of $CO_2$ is only 10µmol/L, Burns and Beardall (1987). Seawater is naturally buffered (usually the pH is between 8.1 and 8.4).

Regardless of the growth conditions, activities related to carbon fixation, that is, photosynthetic oxygen evolution, inorganic carbon uptake and assimilation were enhanced when the measurements were performed at acidic pH. All three algae could grow at pH 4, and grew better at pH 5–6 than at pH 8. This indicates that this marine alga is able to use $CO_2$ more efficiently than $HCO_3^-$.

No evidence could be found for the existence of an alternative inorganic carbon assimilation system specific for low pH and low inorganic carbon conditions. Better growth ability of the algae in acidic conditions could not be explained by an inorganic carbon assimilation mechanism specific to low pH, but is probably explained by a better ability to use $CO_2$ than $HCO_3^-$. However, the results indicated that these algae were able to fix inorganic carbon over a wide range of environmental pH. This stability guarantees the growth potential in surroundings with unstable pH, such as estuarine and coastal waters.

This is an important paper with regards to my research. Not only does it show similar findings to mine with regard to pH response and favoured forms of inorganic carbon. It also shows that even algae whose natural environment is in higher pH respond better in more acidic conditions.

Serralta et al. (2006) studied the effect of pH on biological P uptake. They operated an anaerobic / aerobic laboratory scale SBR to study the effect of pH on enhanced biological P removal. Seven steady states were achieved under different operating conditions. In all of them, a slight variation in the pH value was observed during anaerobic phase. However, pH rose significantly during aerobic phase. The increase observed was due to P uptake and $CO_2$

stripping. A constant P uptake rate was observed until pH values exceeded from 8.2–8.25, decreasing as pH continued rising.

They state that the enhanced biological phosphorus removal (EBPR) process is considered as the most suitable method for P removal from wastewater as it avoids chemicals dosage and reduces sludge production. It results from the activity of polyphosphate accumulating organisms which are capable of taking up P beyond the stoichiometric requirements for growth and storing it as polyphosphate.

The paper correlates with other literature and the findings in my experiments that biological metabolism decreases as the pH rises above an inhibitory value of around 8. Studies agree that this is a direct result of a reduction in $CO_2$, the form of inorganic carbon that is more easily assimilated by organisms.

### 3.3.7. Light and photosynthesis

Radiometric measurements of light such as Watts are quantities of radiant energy whereas photometric measurements of light such as Lux and Lumens are the radiant energy in the visible range (380nm to 770nm). PAR, photosynthetically active radiation from wavelengths between 400 and 700nm is measured in microeinsteins, $\mu E$. We can attempt to convert lux and einsteins into a standard unit of $W/m^2$ however these can only be estimates without knowing the full spectral composition of each specific light source used.

Kirk (1994) writes that light is a continual stream of photons travelling at $3 \times 10^8 m/s$. Full summer sunlight receives about $10^{21}$ quanta/$m^2$/s of visible light. The amount of energy in a photon varies with the wavelength (a photon from the red end of the spectrum contains only 57% as much energy as a photon from the blue end of the spectrum). To add to this complication further, each photosynthetic organism responds differently to different wavelengths of light. In general, green plants reflect mainly green light but have absorption peaks at around 450nm and 680nm which provide a much better rate of photosynthesis.

Photosynthesis works in 2 parts, light reactions and dark reactions. Light reactions take place in the thylakoid membrane system where hydrogen is withdrawn from water to create $NADPH_2$ from NADP, releasing oxygen. Inorganic P and ADP is converted to ATP.

Dark reactions take place in the stroma of the chloroplast where $NADPH_2$ is used to reduce CO2 to the level of carbohydrate, the energy is supplied by the breakdown of ATP.

The Lux (lx) is the SI unit of **illuminance**. **Illuminance** is a measure of the **luminous flux** over a given area. It is the amount of lumens per square metre;  $1 \text{ lx} = 1 \text{ lm/m}^2$.

1 lumen is 1 candela . steradian, 1 lm = 1 cd . 1 sr.

1 cd = 1 lm / 1 sr

**Luminous intensity** is measured in cd or lm / sr

A full sphere has a solid angle of $4\pi$ steradians, therefore a 1 candela light source emits a total **luminous flux** of $4\pi$ lumens.

The candela is the **luminous intensity**, in a given direction, of a source that emits monochromatic radiation at a frequency $540 \times 10^{12}$ hertz and that has a **radiant intensity** in that direction of $^1/_{683}$ watt per steradian.

**Irradiance** (normally referred to as **intensity**) is measured in $W/m^2$.

Therefore it is impossible to make an accurate conversion between Lux and Irradiance unless you know the full spectral composition on the light.

The theoretical maximum is 683 lux = 1 W/m$^2$ however this also needs to be adjusted by applying a specific coefficient for each particular light source. A halogen lamp used in the experiments may have a **luminous efficacy of radiation** of 15% meaning 0.15 x 683 lux = 1 W/m$^2$.

This would mean 1 Lux = 1/102.45 W/m$^2$

Because different wavelengths produce different amounts of energy; there is no single or exact conversion between W and µE/s without knowing the full spectral composition of the light. For typical sunlight, a conversion factor is 1W = 5µE/s which will be used as a guide throughout this thesis in order to keep all light units in W/m$^2$.

Wahal & Viamajala (2010) looked into maximizing algal growth in batch reactors using sequential change in light intensity. They state that algal growth requires optimal irradiance, in photo-bioreactors, optimal light requirements change during the growth cycle. At low culture densities, a high incident light intensity can cause photo-inhibition, and in dense algal cultures, light penetration may be limited. Growth rates and biomass concentrations of cultures exposed to constant light were measured and compared with the growth kinetic parameters of cultures grown using sequentially increasing light intensities based on increasing culture densities during batch growth. Changes to higher light levels were made when growth was observed to stop at the lower intensity. At each illumination level, growth stopped after a certain biomass concentration was achieved. Increase in light intensity led to a resumption of growth but the cultures reached stationary phase again. This phenomenon is likely due to inadequate availability of light to sustain growth at higher cell concentrations.

For this study, they used a lipid-producing micro algal strain, *Neochloris oleoabundans*, grown in batch photo-bioreactors. They used artificial media that was not nutrient limited and maintained a constant 20°C for all experiments. The bioreactors were mixed using a combination of built in paddles and the bubbling of an air / CO$_2$ mixture through each

reactor. After the start of the experiments, gas flow rates were manually adjusted using in-line valves to maintain pH7.

For initial experiments, N. oleoabundans cultures were grown at six different levels of irradiance, 70.8 (14.2), 91.2 (18.2), 130.4 (26), 177.8 (35.6), 220.0 (44), and 273.1 (54.6) $\mu mol/m^2/s$ ($W/m^2$) to determine the effect of different light intensities on algal biomass accumulation under constant illumination. Thereafter, effects of sequential increase in incident light intensities on growth were studied using the three light levels specified below. The irradiance was provided by fluorescent light tubes placed around the bioreactors, and different light levels were attained by changing the distance between fluorescent tubes and the bioreactors along with the number of tubes switched on. The illumination was provided by a bank of 12 (six on each side of bioreactor) Ecolux Sunshine 40W fluorescent tubes. The average light intensities with four, eight, or 12 fluorescent tubes switched on were calculated to be 91.2, 177.8, and 273.1$\mu mol/m^2/s$, respectively. Throughout the experiments, the cultures were exposed to a light–dark cycle of 12h that was maintained using a timer connected to the light circuit.

Their results show that reactors operated under conditions of sequential increase in irradiance levels yield up to a 2-fold higher biomass concentration when compared with reactors grown under constant light without negatively impacting growth rates. In addition, this tailored light supply results in less overall photon use per unit mass of generated cells. At fixed light intensities, around 34$W/m^2$ was found to be the optimum. A maximum growth rate for all experiments was found to be 55mg/L/d, this is within the normal range of other literature data.

Wahal & Viamajala (2010) is a very well presented paper and very detailed experiment. The results are good and provide useful data for my project. Ultimately, when growing algae on a large scale, the use of artificial light is not going to be an option, the algae will be subject to what is naturally available. The results here may be useful if agitation could be adapted to control how long algae spend in lighted zone.

Babu et al. (2010) looked at nitrification rates of algal–bacterial biofilms in WSPs under light and dark conditions. They used synthetic wastewater under 3 light intensities; the light condition was at 17-19W/m$^2$. The results were not conclusive and I feel the experiment could have been better designed.

Bartosh & Banks (2007) provide an excellent report detailing their experiments into algal growth response and survival in a range of light and temperature conditions. They used Scenedesmus subspicatus and Chlorella vulgaris as representative species typically found in waste stabilisation ponds. Their experiments were designed to test the ability of the organisms to survive and grow under a range of different temperatures and light intensities that might occur in mid to high latitude regions.

They state that in temperate and continental regions of both the northern and southern hemisphere, WSPs are subject to seasonal variations in light and temperature: in extreme cases this may include ice cover for extended periods in winter. At higher latitudes reduced light intensity and changes in day length may also have a significant effect on pond performance. The current study examined the effect of these parameters on two algal species commonly encountered in WSPs, with a view to determining their influence on winter survival and spring recovery.

They used artificial non nutrient-limited medium however no details of pH, $CO_2$ addition or agitation method were given. The growth was assessed using optical density and photosynthetic rate for a combination of temperatures of 5, 10, 15 and 20°C at light intensities of 7.8 (1.6), 15.7 (3.1), 31.3 (6.3), 47 (9.4), 62.7 (12.54) and 78.3 (15.7) μmol/m$^2$/s (W/m$^2$).

C. vulgaris had a higher rate of growth and photosynthetic activity than S. subspicatus at low temperatures but had reached its maximum growth rate at 15°C. S. subspicatus showed a higher growth rate than C. vulgaris at higher temperatures, and did not achieve its

maximum growth rate over the range of temperatures studied. For both species light was not limiting to growth above 47µmol/m$^2$/s. The photosynthetic rate at least doubled when the temperature was raised from 10-15°C. The photosynthetic rate fell for both species when raised to 20°C.

Survival of the two species under dark conditions was tested at 4°C and -20°C using direct plating and growth tests. C. vulgaris was able to survive at 4°C for a much longer period than S. subspicatus and a portion of the population was able survive -20°C. The different responses of the two species to dark and cold conditions are indicative of the range that may occur across a wider population, and show why in practice some species may appear earlier and compete more effectively in early spring but then lose advantage as the temperature and light intensity increases into the summer.

Curtis et al. (1994) prepared a paper looking at light penetration in waste stabilization ponds. They state the penetration of light into WSPs was studied because of its importance in pathogen removal and algal productivity. The attenuation of light in ponds was dominated by light absorption by humic substances and algae. Longer wavelengths penetrate much better than short wavelengths. Differences in algal concentrations cause the differences in light attenuation seen between ponds, though they also cause some spectral variation because short wavelengths are more affected by changes in algal biomass than long ones. Measurements were taken on experimental wastewater treatment ponds of various sizes and HRTs, detailed in the paper. All measurements were taken in full sunlight.

Rodolfi et al. (2008) carried out a side experiment from the main one carried out (see algal bioreactors section), this focused on measuring the effects of light intensity. When Nannochloropsis sp. F&M-M24 was grown with one-side illumination, an increase of irradiance from 115 to 230µmol photons/m$^2$/s brought biomass productivity from 610-850mg/L/day and FA from 14.7% to 19.6%. With two-side illumination, productivity and FA increased from 970-1450mg/L/day and 24.3-32.5% when the irradiance was increased from

115-230μmol photons/m$^2$/s. It is worth noting that with two-side illumination at an irradiance of 115μmol photons/m$^2$/s, both the culture productivity and the FA were higher than with one-side illumination at an irradiance of 230μmol photons/m$^2$/s, despite the fact that the same amount of photons impinged on the culture in the two different conditions. This is due to halving the required light penetration depth and producing a more evenly lighted zone.

### 3.3.8. Temperature

Cho et al. (2007) studied the effect of temperature and salinity conditions for growth of green algae *Chlorella ellipsoidea* and *Nannochloris oculata.* Four temperatures (15, 20, 25, and 30°C) & three salinity (10, 20, and 30) conditions were tested in triplicates. Specific growth rate (SGR), maximum density, and duration to reach maximum density were measured.

The cultures were grown in 250ml flasks containing artificial medium. The intensity of illumination as maintained at 31μmol photons/m$^2$/s, 24h/d. The flasks were hand agitated twice a day. No pH conditions were given.

They state that the SGR in *C. ellipsoidea* were fairly similar, but the highest maximum density was achieved in *C. ellipsoidea* at 15°C and 10. The maximum density of *N. oculata* was significantly affected by temperature, but not salinity. The highest maximum density was achieved in *N. oculata* at 25°C and 30, but SGR was significantly lower than that of *N. oculata* at 25°C and 10. Based on these results, the condition of 15°C and salinity 10 seemed to be optimal for maximum density of *C. ellipsoidea*, and the condition of 25°C and 10 and 30 for SGR and maximum density for *N. oculata*, respectively.

This experiment shows us that each species varies greatly in growth rate and optimum temperature, here *N. oculata*, preferred warmer temperatures 25°C and 30°C and have a higher growth rate than *C. ellipsoidea* that preferred 15°C. It shows us that using cell count

to measure growth rate is acceptable for comparison within that specific experiment but is totally useless when comparing species or against anyone else's work. This is because cell size of each species varies and would produce misleading results in terms of growth rate. It shows us that the salinity results are inconclusive, probably because species are either adapted for sea water, fresh water or estuarine conditions. Salinity experiments should probably only be carried out as a final experimental stage to find the optimum condition when all other parameters have been confirmed and are kept constant. There were too many plots on each graph to make any sense of them. The growth rate results follow the standard curve found in other literature, it is a shame they cannot be used to compare against other experiments at those temperature and light conditions.

Butterwick et al. (2005) studied the diversity in the influence of temperature on the growth rates of freshwater algae. They state that most planktonic algae will grow, albeit slowly in some cases, at temperatures between 10 and 20°C. It is in this range, and at higher temperatures, that most systematic measurements of algal growth rates in relation to temperature have been made. However, there are actually few supporting experimental studies in which the comparative performance of representative species has been followed at small intervals over a wide range of temperature.

In this study seven species of common planktonic freshwater algae, from a number of major groups, have been grown in culture over a range of temperature between 2-35°C and their growth rates determined. 13 additional species were used to test growth and viability in the higher temperature range, 20–35°C. There were considerable differences between species for growth at low and high temperature.

Unfortunately they measure growth rate in divisions/day so cannot compare cross specie but do produce good results with regard to the effect of temperature range.

All the tests were carried out in 125mL glass-stoppered bottles using unspecified medium. They were all lit by a bank of 'daylight' fluorescent lamps at $20 \pm 4W/m^2$. They state that

this level lies on or near the light-saturation region of growth for many algal species, but may be supra-optimal for some at low temperature. Details about pH and agitation method are not specified.

The results show a trend that nearly all species increase in productivity up until their optimal temperature value and then sharply deteriorate at a critical temperature.

Differences between species appear mainly in the temperature range 25–35°C. All 21 species tested showed active growth at 25°C. At 27°C *A. formosa* died and *C. furcoides* did not grow. At 30°C the diatoms, flagellates and the xanthophyte *Tribonema* died, except for the diatom *Fragilaria crotonensis* which grew vigorously. Growth was obtained at this temperature with all five green algae (chlorophytes), although that of the cyanophytes, *Planktonema bourrellyi* and *P. mougeotii* was not sustained. Only *Aphanizomenon flos-aquae* survived with moderate growth at 35°C, a lethal temperature for the other species.

They produce an informative chart showing all the species plotted against temperature. They show either sustained growth, non-sustained growth or lack of growth represented by three different symbols. This has little use, if they had instead used a single black circle for each bit of data, varied the diameter of the circle to represent growth rate, they could have plotted all of their results on a very useful and easy to read chart.

Unfortunately the growth rates cannot be compared across species; if this was possible we could use this data to draw conclusions as to whether the highest growth rates occur in species that prefer warmer temperatures. This would be useful in system design. If we found a high growth rate specie at a temperature of 30-35°C, a system could be controlled at this temperature using waste heat. Not only would growth rate be high, the risk of contamination from competing species would be low due to the number of species that can survive at this temperature.

Vona et al. (2004) looked at temperature responses of growth in cryophilic and mesophilic algae. They used *Koliella antarctica* and *Chlorella saccharophila* that they kept routinely maintained at 6°C in 250ml Erlenmeyer flasks containing 100ml artificial nutrient medium under continuous illumination (24W/m$^2$). The atmosphere of the incubator was enriched with 5% $CO_2$ in air. *Chlorella sorokiniana* was grown in batch culture at 35°C under continuous illumination (50W/m$^2$) and flushed with air containing 5% $CO_2$. Cool-white fluorescent lamps (Philips TLD 30W/55) provided the illumination. The flasks were stirred magnetically. No pH is specified.

Growth of *K. Antarctica* and *C. saccharophila* was tested at 5, 10 and 15°C; growth of *C. sorokiniana* was tested at 20, 25, 30 and 35°C. Water-jacketed culture vessels (250ml) were used to maintain the temperatures. Growth rate was measured as increase in optical density, I looked into this possibility as a quick and easy way to measure growth rate but it cannot be compared against any other experiments, only being used to measure change.

The paper essentially showed that the two cryophilic algae that grow in extremely cold conditions showed their best growth around 10°C and the mesophilic algae, *Chlorella sorokiniana* would not grow below 20°C but growth rate peaked at 35°C.

This adds to the bank of evidence that 35°C appears to be an upper limit in which algae can grow and that each specific specie has certain temperature limitations based on their natural environment.

Bouterfas et al. (2002) studied the effects of light and temperature on the growth of three freshwater green algae isolated from an eutrophic lake. They were identified as *Selenastrum minutum, Coelastrum microporum, F. astroidea* and *Cosmarium* subprotumidum.

Experiments were performed to determine the growth rate over a wide range of light intensities (6–91W/m$^2$) and temperatures (15, 20, 25, 30 and 35°C), using a 15:9 L:D photoperiod cycle. 400W Phyto-Claude halogen lamps were used for illumination.

They were studied in batch cultures under non-nutrient limited conditions using artificial medium. Air was bubbled through the bioreactor using compressed and filtered air, pH data is not given.

Due to using a growth rate measurement of divisions/day they cannot cross compare species, fortunately they show their graphs for each specie separately. The data produced is very good, it is just poorly presented. If I had access to the raw data I would reproduce the graphs in a different form. The graphs plot the growth rate vs. light intensity, providing a separate graph for each temperature. It would have been good to see an additional graph showing all the temperatures together to make a direct comparison. The data provides solid evidence for the change in performance under different light and temperature conditions.

As the temperature increases, the performance at higher light intensities increases (At 15°C *Selenastrum minutum* peaks at 26W/m$^2$ however at 35°C it peaks at 84W/m$^2$). Light intensity which is too strong for the corresponding temperature is inhibitory. In general there is a sharp rise in performance with increase in light intensity from 0-40W/m$^2$. Between 40-100W/m$^2$ there is only small gain in performance, if any. The performance more than doubles for all species with an increase from 15-30°C. Performance increases with every increase in temperature, however the improvement between 30-35°C is small.

The maximum growth rates occur at 35°C (marginally higher than 30°C), the corresponding optimum light intensities are 420 for *Selenastrum minutum*, 400 for C*oelastrum microporum* and 400 for *Cosmarium subprotumidum*.

The key points from this paper that correspond with other literature are: Growth is significantly enhanced at higher temperature, however anything above 30°C tends not to

provide any benefit. Too high a light intensity is inhibitory, this is temperature dependent however values above 200 generally do not provide a significant gains in performance. Getting the optimum temperature and light intensity for the specie is important as they have shown it has a large effect on growth rate.

Sterner & Grover. (1998) studied algal growth in warm temperate reservoirs, examining N concentrations and temperature.

Essentially, they collected samples from two lakes during various times of the year, with varying light intensities and temperature. They varied the samples nutrient concentration by dilution. They tested each case for growth rates to produce a 3D plot of growth rate vs. temperature vs. nutrient concentration. The graph is excellent and shows consistent responses to both nutrient concentration and temperature in different ways.

In general they state that algal growth was frequently and strongly nutrient limited, particularly when temperature was >22°C. By itself, N was more often stimulatory than P, though strong additional enhancement of growth by P and trace nutrients was often detected. Maximal growth was an increasing function of temperature.

They show that the dissolved inorganic N is limited below values of 0.25mg/L for all temperature values however, as temperature increased and the corresponding growth rate increased the nutrient demand was higher. On nutrient limited samples, temperature had no effect on growth rate. When nutrients were non-limited above 0.25mg/L and temperature kept constant, there was no increase in growth rate. Between 5-30°C, there was an almost linear increase in growth rate for samples where the nutrient concentration was non-limited. Dissolved inorganic N was only tested up to values of 2mg/L which is low compared to wastewater; there is no evidence here that higher nutrient concentrations are inhibitory. Further test would have to be carried out up to values of 100mg/L to determine if certain species are inhibited by high nutrient concentrations.

They attempt to produce a model for these values but state that even with unusually detailed, site-specific fitting of model parameters, accurately modelling algal growth in natural ecosystems can remain a challenge.

Wilde et al. (1991) looked at the cultivation of algae and nutrient removal in a waste heat utilization process. They focus on the design and economics and do not carry out any experiments themselves to provide data. Therefore this is not useful to my project.

Goldman & Carpenter (1974) aimed to produce a model of the effect of temperature on algal growth using collected data. They plot growth rate vs. temperature for all data points and use this to show the maximum growth rate as a function of temperature. The problem is that they are using growth rates measured in divisions per day and comparing them cross-species which I have shown to be problematic. This problem could have been avoided if they had plotted a curve for the mean growth rate at each temperature rather than using the maximum, the result I feel would have been fairly similar, just with a shallower gradient. The sheer number of data points means this is a useful resource.

The number of data points above 30°C is small compared to that below however it is fair to say that on average, the growth rates are slightly higher in the 30-35°C temperature range. They show the growth rate increase to be fairly linear as temperature increases. Compared to 5°C, 15°C is 2x as productive and 30°C is 4x as productive. This is an important finding as it is averaged over so many data points. Compared to Sterner & Grover (1998) who show 30°C as being 3.5x as productive as 5°C, it is fairly consistent. Bouterfas et al. (2002) did not measure temperatures below 15°C however they show that performance at 30°C is at least 2x greater than 15°C which matched the other findings. Vona et al. (2004) show performance to be 4.25x better at 30°C than at 5°C. Butterwick et al. (2005) show the peak performance to be 4-5x greater than at 5°C.

### 3.3.9. Agitation method

Continuous or timed agitation of the bioreactor is required to keep algae in suspension and maintain levels of dissolved gases and mix nutrients. There are limitations to the speed of agitation due to both the high power consumption and the damage to organisms. Turbulence is an important factor for the growth of microscopic algae. A number of techniques can be used to promote mixing in the tanks: the use of surface wind, paddle mixing and also air-lift pumps.

Agitation is one of the major external parameters which regulate algal growth. The advantages of keeping the algal suspension in movement are numerous. The continuous mixing prevents sedimentation of the algal biomass and keeps the nutrients in active contact with the algal cell surface, stimulating nutrient uptake. It will also prevent thermal stratification and avoid photo-inhibition. In tall vertical transparent plastic tubes, good mixing can be obtained by bubbling $CO_2$ from the bottom. In flat tanks, paddle wheels or jet pumps are used to circulate the whole body of water. A study by Persoone showed that in shallow tanks simple air-lift pumps are very efficient in keeping algal suspension in continuous movement. It was found that agitation significantly improves growth rates (about 30% increase) when compared to a non-agitated control. The $CO_2$ in the air being pumped through stabilizes the pH which otherwise is increased by the consumption and net reduction of $CO_2$. Pumping $CO_2$ through the tank not only provides agitation but also increases the amount of $CO_2$ available for photosynthesis.

Any energy expenditure for mixing is going to make up a substantial percentage of the running costs; these need to be assessed for economic viability.

Algae can be grown in a photo-bioreactor, essentially a bioreactor with a light source. As algae grow and multiply, they become so dense that light cannot penetrate very far into the reactor. Algae only need about 1/10$^{th}$ of the light they receive from direct sunlight. A mixing device can be used to move the algal cells about, ensuring they all get time in the lighted zone.

### 3.3.10. Algal growth rate

The work I carried out looks at algal growth rates in mg/L/day to give an indication to the quantity of biomass that could be produced when scaling up the project. The literature varies in the way it specifies growth rate. The good papers tend to state their growth rates in mg/L/day or similar units which can easily be converted. This gives a quantitative approach for cross-experimental comparison. Many papers in the literature state growth rate as cell divisions per day, this trends towards papers that are not so well written or thought out. This may be done for a number of reasons:

- The author(s) have not given any thought to the analytical comparison of data
- The author(s) have chosen not to provide absolute data due to the invasive nature of the experimental technique
- The author(s) have chosen not to provide absolute data due to the effort involved in collecting the data in this form

The collection of absolute growth rate data is a difficult task to undertake, requiring more effort and more disruption to the experiment than other methods such as optical measurement. It requires taking regular samples, the larger the sample, the more accurate the results that can be achieved. The samples cannot be replaced and any biomass removed in this manner is lost. This has a direct effect on the state of the experiment. This problem can be overcome somewhat by accounting for loses however it is still an invasive process

Accurate results tend to be difficult to achieve because of the difficulties involved in collecting a uniform, representative sample. Non-invasive measurement processes are difficult to verify because they are not a physical representation of mass.

Division rate measurements tend to be more accurate and less invasive than absolute mass measurements therefore using cell division rates is a great technique for comparing results

from the same experiment with the same specie whilst repeating the experiment for different environmental parameters. As soon as you want to compare the division rate data with any experiment using a different species, the data becomes useless. The cell size for each algal species varies; therefore division rate is meaningless in terms of mass gain (the most important factor for both performance and comparison).

Findings show that in general cell division rate decreases as cell size increases. This correlates with well known scientific theory that larger cells reproduce slower. Larger cells may divide more slowly but each division is a greater mass increase than it is for smaller cells. It cannot be claimed that a fast division rate represents a high growth rate. Therefore it may be concluded that the fundamental trend in growth rates can be measured as dry matter rather than division rate which must be adjusted for cell size. Many papers lack in reporting a growth rate however most, where appropriate will report a division rate.

Nielsen (2006) carried out an experiment to investigate the relationship of cell size and division rate differences between green algae and cyanobacteria of both unicellular and colony-forming variants. The algae were grown in round-bottomed flasks containing artificial media that was frequently replenished to ensure nutrients were not exhausted. They were lit by 40W/m$^2$ continuous light (fluorescent tubes, Philips TLD Aquarelle and Philips TLD 90 Warm White) and kept in 17°C. The algae were kept in suspension by bubbling with atmospheric air that had been passed through 0.3mm glass microfiber filters (Whatman Hepa-Vent). 19 species of green algae (Chlorophyta) and 9 species of cyanobacteria were grown. They comprised unicellular as well as colony-forming species. The maximum division rate was recorded for each species under exponential growth.

Nielsen found that Eukaryotic green algae and prokaryotic cyanobacteria apparently follow the same algometric relationship between size and maximum growth rate. Both unicellular green algae and cyanobacteria showed significant relationships between cell size and maximum growth rate, growth rate decreased as cell size increased. The relationship between cell size and maximum growth rate was weaker for unicellular cyanobacteria than

for unicellular green algae, due to the wider range of growth rates reported as maximum growth rates for picoplanktonic cyanobacteria. When only colony-forming forms were considered, no relationships between colony size and maximum growth rate for either green algae or cyanobacteria were found. A range of 0.1-4 div/day was found.

Tang (1995) also carried out research, to measure cell division rates. These growth rate results were compiled and adjusted to a temperature of 20°C for direct comparison. Results were only taken where the light was 40W/m$^2$ or greater. The algae species considered were (Chlorophyta, Chrysophyta, Pyrrophyta) comprising five classes (Bacillariophyceae, Chlorophyceae, Chrysophyceae, Dinophyceae and Prymnesiophyceae).

Tang found that algae demonstrate a relationship between both cell carbon content with growth rate and cell volume with growth rate. As cell volume and carbon content increase, division rate decreases. The change was much greater for carbon content than volume due to carbon being an important growth controlling factor. A range of 0.19-3.95 div/day was found, i.e. similar to Nielsen.

### 3.3.11. Metals and algae

Azeeza and Banerjeea (1986) studied the effect of copper and cadmium on carbon assimilation and uptake of metals by algae. They used two species of blue-green algae, *Spirulina platensis*, a filamentous form *and Anacystis nidulans, a* unicellular form grown in artificial aqueous media which was treated with Cu and Cd in varying concentrations. They studied the uptake of the metals, carbon assimilation and Chl A content.

They state that Cu and Cd inhibit many biochemical activities like photosynthesis, N fixation, nutrient uptake etc. Cd exposure commonly causes a decrease in Chl A content and photosynthesis for many algal species. Their experiments showed that the addition of high concentrations of Cu and Cd, 0.1-10ppm were inhibitory for the production of Chl A and

subsequent assimilation of carbon. On average, a small addition of 0.01ppm of Cu and Cd was beneficial for metabolism.

Vymazal (1984) carried out a study of the uptake rates of heavy metals by algae from enriched natural waters in a continuous flow system. Two types of algae were used, naturally growing periphyton community and periphytic filamentous green algae Cladophora glomerata and Oedogonium rivulare. Uptakes of nine heavy metals - Pb, Cd, Cu, Co, Cr, Ni, Zn, Fe and Mn were tested during batch experiments of four hours exposure. During these experiments, a light intensity of $48W/m^2$ was used (measured on water surface). Two types of bioreactor were used, in the first, agitation of water was from a pump motor, the second was slightly bubbled with air. Water in the model was continuously enriched with N & P compounds to speed up the periphyton growth. It was reported that choosing the level of metal concentration to use is difficult due to large variations in toxicity of different metals to different strains of algae. After a period of control, the water in the model was spiked with a mixture of the nine heavy metals. The metals were introduced as inorganic salts.

Uptake mechanisms of each metal varied slightly between the different types of algae, all metals increased during four hours exposure but not in the same way. Some metals were removed continuously (Ni, Cr, Fe and Mn), other metals were removed more rapidly during the first hour or first two hours of exposure and then only slight removal continued (Cu, Pb, Cd, Co). Uptake of Zn was rather unambiguous. Results of these experiments suggest that the course of uptake for individual metals could be similar for most periphyton algae.

According to the results metal uptake courses can be divided approximately into three groups:  1. rapid uptake during the first hour of exposure and then only slight uptake appears, 2. rapid uptake during first two hours of exposure appears and then only slight uptake takes place and 3. continuous uptake appears during the entire four hour exposure.

Iron removal was very rapid during the entire four hour exposure with periphyton as well as with both filamentous algae. In absolute values, up to 94.5% of iron was removed after the four hour exposure. Zinc was removed to a high extent, up to 97.6% but the courses of uptake were quite heterogeneous. Zinc uptake courses were found to be a combination of all types mentioned above. Uptake of nickel increased slightly during the exposure. The uptake courses were very similar for periphyton community as well as for both filamentous green algae. Nickel and chromium were removed to the lowest extent.

This paper is very useful and provides a good basis for comparison with my experiments.

Boullemant et al. (2009) studied the uptake rate of Cd complexes by three freshwater algae, *Chlamydomonas reinhardtii*, *Chlorella fusca*, and *Pseudokirchneriella subcapitata* at two pH values (7.0 and 5.5). Uptake of the Cd complexes over time was characterized by high initial uptake rates but tending toward a plateau after about 30 min. When the pH was dropped from 7 – 5.5, the initial uptake rate decreased by 2-60x depending on the algal strain and Cd ion. The experiments were run at ambient laboratory temperature of 21°C.

The findings in this paper correlate well with (Vymazal, 1984) and my results, showing initially fast uptake rates of metals followed by a plateau. The papers do not specify a reason for the plateau; my main theory is the bio-unavailability of specific ions of each metal that may be present in solution.

Yu et al. (1999) report that Biosorption of heavy metals is an effective technology for the treatment of industrial wastewaters. The uptake capacities of the biomass of a group of nine marine macro algae for heavy metal ions (cadmium, copper and lead) were evaluated. Equilibrium isotherms for each biomass heavy metal system were obtained from batch adsorption experiments. The maximum uptake capacities of the biomass ranged from around 0.8 to 1.6 mmol/g (dry), which were much higher than those of other types of biomass. The results indicated that the biomass of the marine algae is suitable for the

development of efficient biosorbents for the removal and recovery of heavy metals from wastewater.

This experiment used harvested marine algae that had been pre-dried and ground. The biomass was mixed with the heavy metal solution at 21°C for 24hrs. The uptake rates in this experiment were not a factor of biomass metabolism; this shows that macro (and micro) algae cell structure / permeability are highly suitable for heavy metal removal mechanisms.

In general, the heavy metal uptake capacities varied significantly for different types of biomass studied. For divalent heavy metal ions, the reported values for bacterium biomass typically ranged from 0.05 to 0.2 mmol/g; for fungi and yeast, 0.2 to 0.5 mmol/g; for fresh water algae, 0.5 to 1.0 mmol/g and for marine algae, 1.0 to 1.5 mmol/g. Among these values, the capacities of the biomass of a few species of marine macro algae, commonly known as brown algae, were much higher than those of other types of biomass. The results show the metal removal properties for each of the 9 algal strains are very similar, lead is most effectively removed, then copper, then cadmium.

The literature shows that algae have the capacity to remove commonly found metal ions from aqueous solution. Various uptake mechanism exist however it appears common for a fast uptake rate followed by a plateau. Higher concentrations of certain metals have been shown to be toxic to algae as would be expected. Yu shows algal cells to be the most biosorbent biomass material; this may be contributing to the fast uptake rates in live experiments and may be responsible for causing a plateau in uptake rates when the material becomes saturated. Overall it shows the potential for algae to be used as a method of heavy metal removal, a useful attribute to have for wastewater treatment.

## 3.4. Benefits of algae

### 3.4.1. Algal biofuel

The subject of algal biofuel is fast moving with the regular production of articles and papers from a number of different authors stating similar things.

The following is an overview of the common findings:

The plant-like organisms employ photosynthesis to convert sunlight and $CO_2$ into energy so efficiently that they can double their weight several times a day and have the potential to produce significantly more oil per acre than crops like corn or soybeans. Algae oil content can vary from 4 to 50 percent by weight, depending on the species and growing conditions. Algae thrive on a diet of wastewater and carbon dioxide, a greenhouse gas. Wastewater is attractive because it has the nutrients algae need to eat, especially phosphates and nitrates, but wastewater alone does not provide a balanced diet. Most wastewater has too much N and P and not enough carbon. The algae cannot assimilate all the N and P, $CO_2$ must be added. $CO_2$ is created at many wastewater treatment plants when sludge is incinerated or processed in an anaerobic digester to produce biogas Greer (2009).

Algae grow much faster and produce more oil than terrestrial plants (some as high as 50 percent); they accumulate large amounts of oil when they experience environmental stress, Pienkos et al. (2010).

Algae use their oil the same way animals use body fat, as a source of energy when times are lean. For animals, lean times come between feedings; for algae, this happens every night, when there is not any light to power photosynthesis. Oil production also helps algae overcome the stress of growing in full sunlight, which can be hard on these cells, particularly when they are starved of one or more nutrients. Such deprived algae tend to generate highly reactive chemicals called free radicals, which can cause molecular havoc within. The conversion of $CO_2$ to oil prevents the build up of free radicals, helping the cells avoid internal damage.

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A hectare of soybeans, for example, typically produces only about 500L of oil each year, whereas a hectare of algae growing in a shallow pond can easily generate 9000L of oil, perhaps as much as 47 000L annually. That makes algae many times as productive as oil palms, the most oil-rich source of biodiesel now in use. For making the most oil in the least amount of space, algae win hands down, Pienkos et al. (2010).

Some companies have shown they can produce oil at a rate of 1500 gal/ac/yr, and aim to produce 4000-5000 gal/ac/yr in the near future. Some are reporting that they can produce up to 6000gal/ac/yr from algae, even though they are not yet operating on a large scale. In comparison, palm, canola and soy yields 650, 150 and 50gal/ac/yr respectively, Mascarelli (2009).

Growing the alga Chlorella vulgaris in a raceway – a shallow outdoor pond – can give between 10-50t/ha/year of algae, with an oil content of about 25% dry weight. It is estimated that 10% of the UK's surface area would be required by algae production if this method of producing biofuel was used to fuel all our transport, Houlton (2009).

The yield of oil from algae is over 200 times the yield from the best performing plant/vegetable oils. The author states that high oil species of microalgae cultured in growth optimized conditions of PBRs have the potential to yield 19,000–57,000L of micro-algal oil per acre per year. He goes on to say that oil supply is based on the theoretical claims that 47,000–308,000 L/hectare/ year of oil could be produced using algae. Firstly, these figures do not match up, secondly, he then later states that the per unit area yield of oil from algae is estimated to be from between 20,000 to 80,000L per acre, per year. This third figure does not match either of the first two. Three different statements of oil yield have been made, leaving doubt to the validity of the paper! To date, none of the projected algae and oil yields has been achieved. Producing 100 tons of algal biomass fixes roughly 183 tons of $CO_2$. The calculated cost per barrel would be only $20. Currently, a barrel of oil in the U.S. Market is selling for over $100 per barrel, Demirbas (2010).

Pienkos et al. (2010) wrote that one man's pond scum is another man's gold. This attitude has lead to a hundred or so start-ups in the algal biofuel field. He states that large tracks of desert might be the ideal place to grow algae as long as enough water and the proper nutrients can be secured.

There are about 30000 known species to consider but with new technologies we can now grow thousands of cultures simultaneously at the micro-litre scale using advanced liquid-handling devices and robotics. Instruments can isolate single oil-filled cells from their cultures based on how the cells fluoresce. With our improved understanding of flow dynamics, we can engineer ponds and bioreactors that require the least amount of energy to mix. And new polymers that are both stronger and cheaper can withstand months of punishing sunlight, enabling more affordable photo-bioreactors.

Mascarelli (2009) state that several companies now say that they are close to overcoming the technical hurdles to making algae-derived biofuels competitive on a commercial scale.

Companies can choose from a diverse range of growing techniques, from inexpensive open ponds to carefully controlled enclosed tanks, to coax algae into secreting the desired product, which might be ethanol, biodiesel, or pump-ready gasoline. The algae can be indigenous strains or genetically engineered organisms.

Solazyme, Inc., founded in 2003 and one of the first algae companies to emerge, says that his company has used algae to produce more than 10000gal of oil at a quality that meets existing fuel standards.

One issue is that algae cultures grown in an open pond can easily be contaminated and overtaken by invasive species. NREL researchers also ran into difficulties with contamination by non-native algae species and with the replication of laboratory conditions in the field. So,

some companies have opted to grow their algae in enclosed containers that allow them to precisely control the light, $CO_2$, and water conditions needed by various strains of algae.

Closed systems have shown over time that they have significant yield benefits and merits over open ponds, others maintain that enclosed growth systems, commonly called "photo-bioreactors", are far too costly to make algae competitive with fossil fuels.

Greer (2009) write that Sunrise Ridge is among a number of start-ups and universities designing systems to grow algae in wastewater from municipal and industrial treatment facilities.

Sunrise Ridge takes $CO_2$ from the plant's flue stacks and bubbles it through the plastic greenhouses. The enclosed greenhouses maintain desired $CO_2$ concentrations by preventing the gas from bubbling out into the atmosphere. In initial testing, algae reduced nitrate levels in the wastewater to as low as two part per million. N removal is a primary focus of the research, since local treatment facilities must reduce N in treated effluent flowing into the Chesapeake Bay (as reported by Takacs, 2006).

Current biological N removal techniques using bacteria are very expensive. It costs three to four times more to remove N than our projected costs for algae systems. Cost calculations for an algae system at a combined industrial and municipal wastewater plant in Hopewell, Virginia, which included the land, algae ponds and harvesting and biodiesel conversion technologies, were about $25 million. Costs for a conventional biological N removal system were quoted at $100 million. But despite lower costs, STWs are not sold on the technology; they are sceptical about algae because no one has done this on a large scale.

This study looks at adding $CO_2$ to high-rate algae ponds used at wastewater treatment facilities to achieve complete tertiary nutrient removal. The desired result is to eliminate the $CO_2$ limitations of municipal wastewater. The high-rate ponds are shallower than conventional ponds and designed with continuous raceways. A paddlewheel mixes the

water, pushing it around the raceway; it is simulating a shallow river, an environment that favours rapid algal growth. Algae that work well under laboratory conditions do not always perform well after scale up. This is because the sunlight levels are higher and temperature, $CO_2$ and nutrient levels vary more.

Higher algae levels produce more oxygen, which promotes the growth of bacteria that decomposes organic wastes. The bacteria give off $CO_2$ that is used by the algae. Producing oxygen at a faster rate means you can treat the water with a lower HRT. Aeration is an energy intensive process, accounting for 45 to 75% of a plant's total energy costs. Algae in conjunction with bacteria can reduce this cost.

Pittman et al. (2011) looked at the potential of sustainable algal biofuel production using wastewater resources.

It has been appreciated for some years now that microalgae can be potentially utilised for low-cost and environmentally friendly wastewater treatment compared to other more commonly used treatment processes. Although the application of microalgae in the wastewater industry is still fairly limited, algae are used throughout the world for wastewater treatment albeit on a relatively minor scale. Wastewaters derived from municipal, agricultural and industrial activities potentially provide cost-effective and sustainable means of algal growth for biofuels.

The major problem with most wastewaters is the very high concentrations of nutrients, particularly total N and total P concentration as well as toxic metals, which require costly chemical-based treatments to remove them during wastewater treatment. Total N and P concentrations can be found at values of 10–100mg/L in municipal wastewater and >1000mg/L in agricultural effluent.

The ability of microalgae to effectively grow in nutrient-rich environments and to efficiently accumulate nutrients and metals from the wastewater, make them an extremely attractive

means for sustainable and low cost wastewater treatment. Many species of microalgae are able to effectively grow in wastewater conditions through their ability to utilise abundant organic carbon and inorganic N and P in the wastewater. Microalgae are efficient in removing N, P and toxic metals from wastewater and therefore have potential to play an important remediation role particularly during the final (tertiary) treatment phase of wastewater. Algae-based treatments have been found to be as efficient at removing P from wastewater as compared to chemical treatment.

Most of the research on algal wastewater treatment has come from the analysis of laboratory-based small scale and pilot pond scale cultures, and from experimental high-rate algal ponds. High-rate algal ponds which are shallow raceway-type oxidation ponds with mechanical mixing have been shown to be highly effective for wastewater treatment.

Studies have analysed the growth of microalgae under a variety of wastewater conditions. These studies have principally been focussed on evaluating the potential of algae for removing N and P, and in some instances metals from wastewater. These initial experimental studies, particularly those that have also assessed variables for maximal algal biomass production and methods for harvesting algal biomass from wastewater, will be of significant benefit for the evaluation of wastewater-grown microalgae as a biofuel.

The efficient growth of microalgae in wastewater depends on a number of variables. As with any growth medium, critical variables are the pH and temperature of the growth medium, the concentration of essential nutrients, including N, P and organic carbon (and the ratios of these constituents), and the availability of light, $O_2$ and $CO_2$.

There may be some drawbacks in using artificial wastewater to assess conditions in real wastewater. Direct comparisons of artificial wastewater with municipal wastewater have found that although nutrient removal rates are equivalent, micro-algal growth rates are higher in artificial wastewater. This is likely due to increased toxicity of the real wastewaters, inhibitory or competitive effects of indigenous bacteria and protozoa, and by the different

chemical composition of the wastewaters. Only a handful of the literature data comes from experiments using real wastewater, all others are controlled artificially.

The study produced a table of growth rate results from various sources. The majority range from 25-80mg/L/d, one experiment recorded 127mg/L/d and another recorded 346mg/L/d. These are all within the common range that I have found.

Based on current technologies algal cultivation for biofuel production alone is unlikely to be economically viable or provide a positive energy return. Dual-use microalgae cultivation for wastewater treatment coupled with biofuel generation is therefore an attractive option in terms of reducing the energy cost, GHG emissions, and the nutrient (fertiliser) and freshwater resource costs of biofuel generation from microalgae. The high biomass productivity of wastewater-grown microalgae suggests that this cultivation method offers real potential as a viable means for biofuel generation and is likely to be one of many approaches used for the production of sustainable and renewable energy. If micro-algal biofuel production is to be economically viable and sustainable, further optimization of mass culture conditions are needed.

Park et al. (2011) looked at the wastewater treatment process of high rate algal ponds for biofuel production. They produce a great paper focusing on all the important aspects relating to Nutrient removal by algae for bio fuel production.

While research and development of algal biofuels are currently receiving much interest and funding, they are still not commercially viable at today's fossil fuel prices. However, a niche opportunity may exist where algae are grown as a by-product of HRAPs operated for wastewater treatment. In addition to significantly better economics, algal biofuel production from wastewater treatment HRAPs has a much smaller environmental footprint compared to commercial algal production HRAPs which consume freshwater and fertilisers.

HRAPs are shallow, open raceway-type ponds and have depths of 0.2-1m. Mixing is normally provided by a paddlewheel to give a mean horizontal water velocity of approximately 0.15–0.3m/s. Raceway configuration may be as a single loop or multiple loops around central dividing walls. The pond bottom may be either lined or unlined depending on soil conditions and local regulations, and $CO_2$ can be added into a counter current gas sparging sump creating turbulent flow within the pond. They have been used for treatment of municipal, industrial and agricultural wastewaters. However, the use of wastewater treatment HRAPs for algal production and biofuel conversion has received little attention. Both fundamental and field-scale research is needed to optimise algal production and harvest from wastewater treatment HRAPs while maintaining high effluent water quality.

Algae growing in wastewater treatment HRAPs assimilate nutrients and thus subsequent harvest of the algal biomass recovers the nutrients from the wastewater.

The algal biomass produced and harvested from these wastewater treatment systems could be converted through various pathways to biofuels, for example anaerobic digestion to biogas, trans-esterification of lipids to biodiesel, fermentation of carbohydrate to bio-ethanol and high temperature conversion to bio-crude oil. The simplest and most cost-effective option to convert algal biomass to biofuel would be anaerobic digestion in an ambient temperature covered digester pond to produce methane-rich biogas although more expensive heated and mixed anaerobic digesters could also be used. Biogas production rates from laboratory-scale ambient temperature covered digester ponds have been shown to be similar to those of heated mixed digesters (0.21–0.28$m^3CH_4$/kg algal volatile solids (VS) added).

There are many critical environmental (light and temperature), operational (pH, $CO_2$ and nutrients) and biological (zooplankton grazers and algal pathogens) parameters that affect HRAP wastewater treatment. These need to be researched extensively.

In the absence of nutrient limitation photosynthesis increases with increasing light intensity until the maximum algal growth rate is attained at the light saturation point. Increasing the light intensity beyond this point can lead to photo-oxidation (also known as photo-inhibition), damaging the light receptors of the algae and decreasing the photosynthetic rate and productivity. While the light saturation level is dependent on algal strain and culture density, the growth of most algal species is inhibited at light levels $> 40W/m^2$, which is only about 10–17% of maximum summer and winter solar PAR radiation, 400 and $240W/m^2$ respectively.

As algal concentration increases so does the shading effect this biomass creates. For example, an algal concentration of 300mg/L will absorb almost all of the available light (PAR) within the top 15cm of the HRAP, leaving the rest of the pond depth in the dark. Typically HRAPs are designed with a depth of about 30cm, however, turbulent eddies, resulting from water flow around the pond, and paddlewheel mixing provide a degree of vertical mixing through the pond depth thus ensuring that the algal biomass is intermittently exposed to light.

Algal productivity increases with increasing pond temperature up to an optimum temperature above which increasing algal respiration and photorespiration reduce overall productivity. The optimal temperature measured under conditions of maximum algal growth rate (sufficient nutrient and light conditions) varies between algal species, but is often between 28 and 35°C for many algae. The authors state that optimal temperature varies when nutrient or light conditions are limiting. I would argue it is the other way round, optimal light intensity increases with temperature.

The pH of the pond water affects many of the bio-chemical processes associated with algal growth and metabolism, including the bio-availability of $CO_2$ for photosynthesis and the availability and uptake of nutrient ions. Pond water pH is in turn a function of algal productivity, algal/bacterial respiration, the alkalinity and ionic composition of the culture medium, autotrophic and heterotrophic microbial activity (e.g. nitrification and de-

nitrification) and the efficiency of the $CO_2$ addition system. Algal photosynthesis in HRAP raises pH by consumption of $CO_2$ and $HCO_3$, often exceeding pH > 11.

The elevated pH can act to enhance ammoniacal- N removal from the pond liquid via ammonia volatilization. The equilibrium shift to higher free ammonia concentrations at high pH can significantly inhibit algal growth. For example, free ammonia concentrations of 34 and $51g/m^3$ at pH 9.5 (20–25°C) reduced algal photosynthesis of the freshwater algae, Scenedesmus obliquus, by 50% and 90%, respectively. Moreover, aerobic heterotrophic bacteria that oxidize organic matter in wastewater treatment HRAP have an optimum pH of 8.3, above which bacterial activity is increasingly inhibited. The optimal pH of many freshwater algae is about 8. A pH above or below 8 decreases productivity.

Domestic sewage typically contains insufficient carbon to fully support optimal algal production (3–7 C:N ratio in sewage versus 6–15 C:N in algal biomass). $CO_2$ addition has been shown to enhance algal productivity in experimental scale WWT HRAPs and is indeed standard practice at all commercial algal production HRAP systems. By comparison, $CO_2$ addition is presently not used in wastewater treatment HRAPs except in a few small pilot-scale experimental trials.

Intense photosynthesis in HRAPs can increase pond water daytime dissolved oxygen levels to >200% saturation. High dissolved oxygen levels in excess of normal air saturation are believed to impact on algal productivity.

The ratio of N:P in algal biomass can vary from about to 4:1 to almost 40:1 depending on algal species and nutrient availability in algal culture therefore, high productivity may be achieved even at relatively low N:P ratios in wastewater treatment HRAPs. By definition, attempting to reduce N and P to very low levels will usually result in one of the nutrients becoming limiting.

HRAPs are susceptible to grazing by herbivorous protozoa and zooplankton (e.g. rotifers and cladocerans) which can reduce algal concentration and production to low levels within just a few days. (I personally witnessed this process happening under a microscope). Fungal parasitism and viral infection can also significantly reduce the pond algal population within a few days and trigger changes in algal cell structure, diversity and succession.

Maximum algal production in wastewater treatment HRAPs could be achieved by alleviating rate limiting conditions, overcoming inhibitory parameters and through control of algal grazers and pathogens.

$CO_2$ addition to wastewater treatment HRAPs augments carbon availability for algal growth and also serves to mitigate pH inhibition. This can be simply achieved by controlling the HRAP's maximum pH to <8.0 by $CO_2$ addition. While nutrient removal (which is often an important wastewater treatment objective) by physico-chemical processes such as ammonia volatilization and phosphate precipitation may be reduced by $CO_2$ addition to a wastewater treatment HRAP, it has been shown that this reduction in treatment can be offset by the increased algal production and associated nutrient assimilation into this biomass. $CO_2$ addition has been shown to more than double algal productivity in laboratory studies. Waste gaseous emissions, such as flue gas from fossil fuel burning power plants, could be used as a $CO_2$ source to minimize operational costs in full-scale applications. Alternatively at wastewater treatment facilities, if biogas is produced onsite from anaerobic digestion and burned for electricity/heat production, then this exhaust gas could be used as the $CO_2$ source for the HRAPs.

The reduction in loss of N through ammonia volatilization enables greater N recovery from the wastewater through assimilation into algal/bacterial biomass. Providing a potential for nutrient recycling.

The ideal attributes of algal species for use in wastewater treatment HRAPs are: (1) high growth rate (high productivity) when fed with wastewater nutrients which are

predominantly ammoniacal- N and phosphate-P; (2) tolerance to seasonal and diurnal variation in outdoor growth conditions; (3) form aggregates thereby enabling simple gravity harvest. High levels of valuable algal cell components (e.g. lipid for biodiesel) could also be desirable. Algal species' dominance in HRAP may be determined by many environmental (temperature, light), operational (pH, nutrient composition and concentration, hydraulic retention time) and biological parameters (algal pre-adaptation and seeding, gazers and parasites). However, attempts to grow introduced algal species in HRAP as monocultures for periods greater than 3 months have all failed due to contamination by other native algae and/or zooplankton. Control of grazers, parasite and fungi is difficult, research and knowledge is limited. Using a method of selective recycling of harvested algal biomass could be a useful technique.

It seems apparent that we either use photo bioreactors, allow invasive species to dominate or set up strong environmental conditions suitable for only specific species.

Algae are very difficult to remove due to their small cell size (<20μm), similar density to water (1.08–1.13g/ml) and strong negative charge on the cell surface particularly during exponential growth. They have adapted this way as a means of survival, being able to stay in the lighted region with little to no agitation without sinking to the bottom or being held on the water's surface. Chemical flocculation is expensive, reduces the value of the product and makes algal recycling difficult. Mechanical methods are energy intensive, greatly increasing operational costs. Enhancing natural aggregation/bio-flocculation of algae to encourage simple gravity settling would appear to be the most promising option to achieve both a high quality treated effluent in terms of total suspended solids and economically recovering algal biomass for biofuel use. (This correlates with the results of my experimentation). Many of the algal species (Scenedesmus sp., Micractinium sp., Actinastrum sp., Pediastrum sp., Dictyosphaerium sp., Coelastrum sp.) that dominate wastewater treatment HRAPs often form large colonies (50–200μm).

Both fundamental and field-scale research is urgently needed to optimise algal production and harvest from wastewater treatment HRAPs, however for these systems this must be achieved while maintaining high effluent water quality.

Wastewater treatment HRAPs are presently the only economically viable way to produce algal biomass for conversion to biofuels with minimum environmental impact. Optimising harvestable algal yield requires better understanding of the influence of parameters such as $CO_2$ addition, species control, control of grazers and parasites and natural bio-flocculation, as these parameters are currently poorly researched for wastewater HRAP systems. Further research at both fundamental and field-scale will assist optimisation of harvestable algal yield and thus further improve the economic viability and the full-scale implementation of biofuel production from wastewater HRAPs.

Subhadra & Edwards (2010) reports about an integrated renewable energy park approach for algal biofuel production in United States. Another paper was written, Subhadra (2010) providing details on a similar topic.

They both provide a wider view of integration of a number of renewable energy technologies. The papers are not detailed enough on my specific topic and therefore only provide information about the proposed energy projects. It is however a major scale up similar to the process proposed in this project.

The renewable energy project location is aggregated in a 'corridor' primarily comprising regions of New Mexico, Arizona, and Colorado as well as parts of Utah, Texas and Nevada. Further, substantial saline ground-water resources (15 billion acre-feet of brine water) that cannot be used for traditional agriculture or for drinking water, but that can be used for algal culture, are contained within several huge aquifers in this proposed corridor. Most of these states also possess vast stretches of under-developed semi-arid land suitable for large-scale biomass production from feed stocks such as algae and switch grass. The arid

climatic conditions coupled with plentiful sunlight and saline water in the region can support a diversified and strong algal biofuel industry.

I would suggest that a project at that scale is missing one vital ingredient. That being, where are the nutrients coming from?

Radakovits et al. (2010) studied genetic engineering of algae for enhanced biofuel production.

The interest in a variety of renewable biofuels has been rejuvenated due to the instability of petroleum fuel costs, the reality of peak oil in the near future, a reliance on unstable foreign petroleum resources, and the dangers of increasing atmospheric $CO_2$ levels. Photosynthetic algae, both microalgae and macro-algae (i.e., seaweeds), have been of considerable interest as a possible biofuel resource for decades.

The development of algal biofuels has been held back by lack of research in certain areas. These include developing low-energy methods to harvest micro-algal cells, difficulties in consistently producing biomass at a large scale in highly variable outdoor conditions, the presence of invasive species in large-scale ponds, low light penetration in dense micro-algal cultures and the lack of cost-effective bio-energy carrier extraction techniques. The development and engineering of select micro-algal strains are required to improve the yields of bio-energy carriers.

Current commercial agriculture crops have been cultivated for thousands of years, with desired traits selected over time. It stands to reason that with microalgae, it would be beneficial to use genetic engineering in an attempt to bypass such a lengthy selection process. However, despite the recent advances in biotechnological approaches, the full potential of genetic engineering in some micro-algal species, particularly diploid diatoms, can be fully realized only if conventional breeding methods become firmly established, thereby allowing useful traits or mutations to be easily combined. Since the topic of micro-

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algal sexual breeding is beyond the scope of their review, they focus on genetic engineering approaches that could be utilized in the industry's efforts to improve microalgae as a source of biofuels.

Significant advances in micro-algal genomics have been achieved during the last decade. Expressed sequence tag databases have been established; nuclear, mitochondrial, and chloroplast genomes from several microalgae have been sequenced; and several more are being sequenced.

The report goes into significant detail about the genetic engineering procedures however it is beyond the topic of this thesis. The main focus is on engineering for increased lipid content whereas the focus of this thesis is primarily nutrient removal and the production of algal biomass as a by-product. It is however an interesting and important part of the future research as genetic engineering will be essential to maximising productivity.

Beer et al (2009) also look at engineering algae. They focus on bio-hydrogen and biofuel production. There is currently substantial interest in utilizing eukaryotic algae for the renewable production of several bio-energy carriers, including starches for alcohols, lipids for diesel fuel surrogates, and $H_2$ for fuel cells.

Recently, there has been considerable progress in identifying relevant bio-energy genes and pathways in microalgae, and powerful genetic techniques have been developed to engineer some strains via the targeted disruption of endogenous genes and/or transgene expression.

Collectively, the progress that has been realized in these areas is rapidly advancing our ability to genetically optimize the production of targeted biofuels.

Importantly, genetic manipulation techniques have been developed for some species, and are increasingly being applied to optimize biofuel production in several algal systems. In contrast to traditional nutrient manipulation approaches, metabolic engineering improves

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control over metabolic pathways, increases the diversity of available phenotypes, and results in a more reproducible and predictable system.

Significant advances include: (a), the efficient expression of transgenes; (b), a novel mechanism for gene regulation in algae using riboswitches; (c), inducible nuclear promoters and luciferase reporter genes, and (d) inducible chloroplast gene expression. To date, the generation of stable nuclear transformants in microalgae has relied primarily on random genomic integration, intensive screening, and the subsequent isolation of knockout mutants.

Additional genome sequencing efforts are necessary, and research directed toward generating more universal/general genetic transformation tools and screening methods will facilitate the development of informed strategies to optimize the accumulation of targeted biofuels. Significant breakthroughs in the development of improved tools for genetic manipulation in eukaryotic algae, and the current level of interest in algal-based biofuels and phototroph basic research will undoubtedly provide further advances in the coming years.

The paper goes into detail about the advancements in algal genetic engineering for enhanced performance. The main focus is again on Metabolic engineering toward enhanced lipid bio-synthesis. Again, they go into detail regarding genetic engineering processes that are outside the topic of this thesis. Most see this being the area where the majority of the money will come from. The two papers correlate with the information they provide and agree that genetic engineering is an important step to making algal biofuels economically viable. I have no doubt that with the use of genetic engineering, it is possible to make a strain of algae that can be used in large ponds, with all the right properties for oil production and resistance from invasive species.

Demirbas (2010) reports about the use of algae as biofuel sources. He provides a standard report detailing production techniques, costs, algae to energy conversions. It is one of many reports on a similar topic, but one of the most recent, providing up-to-date data.

Biofuels production costs can vary widely by feedstock, conversion process, scale of production and region. Algae will become the most important biofuel source in the near future. Microalgae appear to be the only source of renewable biodiesel that is capable of meeting the global demand for transport fuels. Microalgae can be converted to bio-oil, bio-ethanol, bio-hydrogen and bio-methane via thermo-chemical and biochemical methods.

Photo-bioreactors have higher efficiency and biomass concentration (2–5g/L), shorter harvest time (2–4 weeks), and higher surface-to-volume ratio (25–125/m) than open ponds. However the most commonly used systems include shallow big ponds, tanks, circular ponds and raceway ponds due to the greatly increased capital and running costs of bioreactors.

Tubular photo-bioreactors consist of transparent tubes that are made of flexible plastic or glass. Tubes can be arranged vertically, horizontally, inclined, helical, or in a horizontal thin-panel design. Tubes are generally placed in parallel to each other or flat above the ground to maximize the illumination surface-to-volume ratio of the reactor. The diameter of tubes is usually small and limited (0.2m diameter or less) to allow light penetration to the centre of the tube where the light coefficient and linear growth rate of culture decrease with increasing unit diameter.

A turbulent flow is maintained in the reactor to ensure distribution of nutrients, improve gas exchange, minimize cell sedimentation, and circulate biomass for equal illumination between the light and dark zones. The highest cost for closed system is the energy cost associated with the mixing mechanism. Regardless of using closed photo bioreactors or open ponds, he highlights harvesting as the most difficult problem to solve and the most energy intensive process for the production.

High nutrient wastewater from domestic or industrial sources, which may already contain N and P, can be added to the algal growth media directly. This allows for algae production to be improved cheaply, while simultaneously treating wastewater. Alternately, salt water can

be used, either from saline aquifer or sea water. This means that competition for valuable water resources will be low.

An important statement made is that controlling feed $CO_2$ in response to signals from pH sensors minimizes loss of $CO_2$ and pH variations.

Li et al. (2008) produces a standard report on biofuels from microalgae, backing up the reports by other researchers and repeating much of the same information already found. They do write an interesting section on the use of wastewater.

It is estimated that the biomass productivity of microalgae could be 50 times more than that of switch grass, which is the fastest growing terrestrial plant. One of the major disadvantages of using microalgae for biofuel production is the relatively low biomass concentration in the micro-algal culture (typically in the range of 1-5g/L) due to the limit of light penetration, which in combination with the small size of algal cells (typically in the range of 2-20μm in diameter) makes the harvest of algal biomasses relatively costly and energy intensive. This is a significant concern needs to be addressed properly. The large water content of harvested algal biomass also means its drying would be an energy-consuming process. These problems are expected to be overcome or minimized by further research and technology development.

Sun drying is probably the cheapest drying method that has been employed for the processing of micro-algal biomass (at high solids concentration , after gravitational settling). However, this method takes a long time, requires large drying surface, and risks the loss of some bio-reactive products. I found this to be by far the cheapest and most effective method, it is very easy, requires basic, inexpensive  equipment, is fast enough and has no associated energy costs. It is important to find the balance between the drying efficiency and cost-effectiveness to maximize the net energy output of the fuels from microalgae strategy.

Micro-algal farming using wastewater is the most promising approach in terms of economics. In addition to the apparent benefit of combining micro-algal biomass, and therefore biofuel, production and wastewater treatment, successful implementation of this strategy would allow the minimizing of the use of freshwater, another precious resource especially for dry or populous countries, for biofuel production. Extensive works have been conducted to explore the feasibility of using microalgae for wastewater treatment, especially for the removal of N and P from effluents, which would otherwise result in eutrophication if dumped into lakes and rivers. Ironically enough, it is algae in the lakes and rivers that cause this problem. It is simply a matter of allowing the consumption of N and P by microalgae in a controlled manner that benefits rather than deteriorates the environment. Levels of several contaminant heavy metals have also been shown to be reduced by the cultivation of microalgae. A major concern associated with using wastewater for microalgae cultivation is contamination. This can be managed by using appropriate pre-treatment technologies to remove sediment and to deactivate (sterilize) the wastewater.

Even though the open pond systems seem to be favoured for commercial cultivation of microalgae at present due to their low capital costs, closed systems offer better control over contamination, mass transfer, and other cultivation conditions. The combination of the closed photo-bioreactor and open pond combines the benefits of the two and has been demonstrated to be effective at a 2-ha scale.

Pokoo-Aikins et al. (2010) looked at the design and analysis of biodiesel production from algae grown through the sequestration of $CO_2$ from the flue gas of a power plant. The proposed system provides an efficient way to the reduction in greenhouse gas emissions and yields algae as a potential alternative to edible oils currently used for biodiesel production.

Photosynthetic growth of algae requires $CO_2$, water and sunlight. Temperature should be in the range of 20–30°C in order to have good growing conditions. Algae also need other

inorganic nutrients like P and N in order to grow. The fact that micro algae grow in aqueous suspensions, allows for more efficient access to $H_2O$, $CO_2$ and other nutrients which explains the potential for the production of more oil per unit area than other crops currently used.

Important favourable factors about the process are:

- No competition for land with crops.

- No competition with the food market.

- Ability to grow in water with high levels of salt so there is no additional demand of fresh water. Also, areas with saline ground water that has no other useful applications can be targeted.

- Overall use less water than oilseeds.

- High oil yield: algae (of the aquatic species) require less land for growth than biodiesel feedstock from terrestrial plants because they are capable of producing more oil per hectare. Furthermore, the oil content in algae (per dry weight) can reach as high as 80%. It is worth noting that the oil from microalgae can be extracted with yields up to 80–90%. Under optimal conditions, microalgae have lipid content between 5 and 20% dry weight while under unfavourable conditions lipid content increases between 20 and 50%.

- Efficient sequestration of $CO_2$: another reason why microalgae are attractive is that $CO_2$ (of about half of the of dry algae weight) is needed for growth. $CO_2$ is a common industrial pollutant, thus microalgae can contribute to reducing atmospheric $CO_2$ by consuming $CO_2$ wastes from industrial sources such as power plants.

The choice of algae species should address specific characteristics that allow the use of flue gas as the $CO_2$ source. Much research has been done on the tolerance of different species to flue gases. Several species were found to be suitable for the growth of algae using flue gas. One of these many species is Chlorella. Chlorella is tolerant to $CO_2$ concentrations of up to 40% by volume and up to 40°C. The oil content of Chlorella typically ranges between 28 and 32% dry weight. To try and save on energy costs for the process, drying should be done using excess flue gas.

This paper, along with so many other papers and reports focus on the economics of the procedure base on theoretical values. They also mainly focus on the algae to biodiesel conversion process. Such a small amount of the research being done is focusing on physically doing experiments on wastewater or flue gas to grow algae. Without more solid results on the growth phase, Producing reports of theoretical oil conversion efficiencies is pointless and should be the final stage in the research chain.

Brune et al. (2003) produced a further study into the Salton Sea area - The controlled eutrophication process; microalgae for biofuels production and fertilizer recycling at the Salton Sea, California. A potential site for a large-scale integrated microalgae $CO_2$ bio-fixation process is the Salton Sea in Southern California. This large ($905km^2$), shallow, inland lake receives about 10,000 tons of N and P per year from agricultural drainage waters and other sources, resulting in massive algal blooms, fish kills and other negative environmental impacts. It is 69m below sea level, has no outlet, and has been accumulating salt and nutrients from storm water, treated sewage, industrial waste discharges, and agricultural drainage for nearly 100 years.

Over geologic time, the Salton Basin and Lake Cahuilla have been periodically flooded due to natural diversions of the Colorado River, but after each diversion ceased, the area became a dry lake bed. More recently, as the region developed and large scale agricultural irrigation and municipal and industrial waste streams from the U.S. and Mexico were created, the Sea has become a permanent body of water, with evaporative losses approximately equal to tributary inflows. The build up of salts and nutrients from agricultural, municipal, and industrial discharges from the Imperial and Coachella valley's has led to the development of an inland hyper-eutrophic, hyper-saline lake. As the Sea has increased in salt content, it has seen a transition of ecosystems from freshwater to brackish to salt tolerant organisms.

Reducing the salinity will not resolve one of the most serious water quality problems in the Salton Sea; the high nutrient loads that are causing eutrophication. The large-scale algae blooms, cyclic low oxygen levels, massive fish mortalities, and frequent odour problems that are caused by eutrophication represent major water quality problems that must be addressed.

The need for nutrient management on such a large scale provides an opportunity to utilize and recycle waste nutrients within a private enterprise while providing a public environmental service. The 'Partitioned Aquaculture System', developed at Clemson University, integrates microalgae bio-fixation of $CO_2$ with fish aquaculture promoting a high rate of harvestable algae and fish production.

Nutrient inputs currently eutrophying the Salton Sea may be recovered using 4,000 acres of CEP units producing an algal sludge that may be blended with waste paper to be fermented into methane gas fuelling 20MW of exportable electricity generation capacity, bio-fertilizer production, and 20,000tons of fish biomass, while abating as much as 200,000tons of greenhouse gases, all while generating a cash flow of nearly $20,000,000 per year.

This physical separation of the fish culture ponds from algal production and water purification allows separate optimization of these processes and thus, maximizes overall system performance. In the initial work on the PAS, algal productivities of 6 to 12mg/L/day were sustained throughout the growing season as opposed to 1-3mg/L/day in conventional ponds. These are low values compared to the rest of the literature.

They use a novel harvesting system. A conveyor belt starting at the base of the pond rises out of the water carrying algal biomass, the top of the belt rotates round, and the algae is mechanically scraped off the belt and collected as a thick sludge. Because the water can drain off before the collection point, the biomass concentration harvested is high.

### 3.4.2. Algae for industrial applications

There are many conflicting statements on the potential of micro-algae for high biomass production, but there is a general agreement that the current production systems are not economically viable for biomass production alone. The difficulties include high capital infrastructure costs, problems of contamination through open pond systems and costs associated with harvesting and drying. These costs adversely affect the competitiveness of aquatic biomass production systems, compared to land-based agriculture and forestry. The harvesting technique is highlighted as a key area for research.

Harmelen and Oonk (2006) prepared a report for the international network on biofixation of $CO_2$ and greenhouse gas abatement. The report assesses the practical applications of microalgae in biofuels production, focusing on the global potential of microalgae processes for GHG abatement in conjunction with municipal and agricultural wastewater treatment.

It claimed the most plausible immediate applications are in conjunction with advanced wastewater treatment processes, for the removal and recovery of nitrogen and phosphorus, thus allowing the re-use of these plant nutrients in agriculture. Algal treatment of wastewater is common in rural areas using algal ponds. The next step is to optimise the process and utilise the biomass by using $CO_2$ fertilisation, biomass harvesting and putting the product to beneficial use. They suggest algal ponds need to be fertilized with $CO_2$ (preferably from flue gases) which could double production rates. The advantage of algae is their ability to directly use fossil $CO_2$ from flue gases and similar sources, high productivities over other plants, high nutrient content and their use in environments not suitable for conventional agriculture. Microalgae technologies are helped by their very short generation times and relative simplicity in scalability of their hydraulic production systems allowing faster process development at smaller scales.

The harvesting process was identified as a current technological limitation due to their small cell size along with the relatively high cost of the cultivation systems and undeveloped

nature of this technology. They conclude that harvesting would need to be by flocculation and settling. I agree with these findings.

They predicted 100 tons dry biomass per hectare per year (54.8mg/L/d) stating that the predictions must be demonstrated in practice, requiring considerable R&D. This productivity could reduce the land footprint to as low as $1/10^{th}$ that of conventional biofuels production processes.

Nevertheless, they predicted higher costs of producing biofuel from microalgae than from higher plants and state that the additional costs must be justified in quality of biomass, ease of conversion into desired biofuels, co-production of higher value products, the efficient use of land using small footprint high productivity systems, the use of underutilised land, water and nutrient resources, reduced environmental impact and benefits from growth applications. The combination of these positives could justify long term development of algal bioreactors for multi benefit as well as solely for biofuels production.

In their review, Harmelen and Oonk also state the next 10 year focus of R&D for microalgae biofuels production should be in conjunction with wastewater treatment; basing the economics against the alternative technologies currently employed. In wastewater treatment microalgae substitute fossil energy used in conventional processes for solar energy; reducing $CO_2$ emissions as well as producing renewable biofuels. Fertilisers can be produced from nitrogen-fixing blue-green algae, cyanobacteria, which Harmelen and Oonk predicted could provide 10 million tons in $CO_2$ abatement for every 1 million tons of N fertiliser produced.

They suggest microalgae production processes would be limited to locations with flat land with the availability of nutrients in wastewaters but in favourable sunny climates with average annual temperatures of 15°C. The $CO_2$ required should be provided by flue gases from on site CHP power plants.

Carlsson et al. (2007) also prepared an introductory report on the findings from the EPOBIO project detailing the use of micro and macroalgae for industrial applications.

They also reported the small size of algae (3-30µm) would contribute to the high cost of harvesting (20-30% of the total cost of biomass production). The dilute nature of media (0.5g/L or less) requires large volumes to be handled. They also suggested that attempting to keep cultures to a selected specific species would recommend complex bioreactor design under extreme conditions. They predicted that $1.5km^2$ of algal pond is required to fix the $CO_2$ from a 150MW power plant.

Selection of a suitable production system for the purpose would be important. Bioreactors are not suitable for wastewater treatment because of their costs. Higher value products where additional control is required were more suited to closed bioreactors. Cleaning bioreactors due to wall-growth would also cause abrasion and limit bioreactor life time. Pond biomass concentration could be achieved to between 0.1-0.5g/L compared to 2g/L+ in bioreactors (in fact my bioreactor results correlate, obtaining an algal concentration in excess of 2g/L was easy).

Wastewater applications - Macro- and micro-algae can be applied to sequester, remove or transform pollutants such as excess nutrients, xenobiotics, and heavy metals from wastewater, or $CO_2$ from exhausts. These applications are known as phycoremediation. The treatment processes yield an output in the form of algal biomass that can be used to produce chemicals, biofuels or biogas as by-products (Munoz and Guieysse, 2006).

Micro-algae are often applied in the tertiary treatment of domestic wastewater in maturation ponds, or in small-to-middle scale municipal wastewater treatment systems. The most common designs include facultative ponds, which are relatively deep and support surface growth of micro-algae, and high-rate algal ponds (HRAPs), which are shallow and depend on mechanical mixing to maximize algal production and removal of biological oxygen demand. HRAPs are the most cost effective reactors for liquid waste management

and capture of solar energy; productivities of up to 50t/ha/y (27.4mg/L/day) are feasible. Typically, there is no effort made to control the species composition in wastewater treatment ponds. However, having specific species that sediment, float or flocculate efficiently would greatly facilitate harvesting, which is an expensive step. They estimate production costs of 2 - 4 € / kg algal dry weight. Biofuel production in conjunction with wastewater treatment and fertiliser recycling is seen as a near-term application (5 to 10 years), since the algae are already used in wastewater treatment.

The relative affinity of raw *Sargassum* biomass for various divalent metal cations was determined at environmentally relevant concentrations to be Cu > Ca > Cd > Zn > Fe (Davis et al. 2003). Also micro-algae have been used to remove heavy metals from wastewater (Wilde and Benemann, 1993; Perales-Vela et al. 2006).

## 3.4. Overall summary

It would appear that a major knowledge gap exists in collecting absolute growth rate data to support the large amounts of theoretical oil yields especially when using an open environment.

There has been limited research carried out on using the algae to energy process as a sewage treatment process or more importantly actually using effluent even though one of the initial points raised is that in the short term, this process needs to be combined with wastewater treatment to make it economically viable. Once this is achieved, research can build off this to expand it to more large scale production.

Another knowledge gap is the vessel this process should be carried out in. Bioreactors are deemed uneconomical yet lagoons and HRAPs may not produce the right environment to maximise algal growth through lack of light and agitation issues.

Another area is determining performance using algae; how low can P concentrations be reduced to and at what HRTs? Is it worth fighting to use a specific algal strain or should the most dominant just be prevalent?

Having identified the knowledge gaps, this research project will concentrate on using algae to reduce P to extremely low levels and to <1mg/L within the shortest HRT. Whilst meeting this P standard I will investigate the effects on other quality standards, N concentration and SS. I aim to identify the best vessel and process to maximise the P removal efficiency and biomass growth rate. The work has identified the need to study naturally occurring algal strains that grow in real sewage effluent. Part of the study will look at the feasibility of this process in low light conditions during the UK winter.

# 4. Aims and Objectives

## 4.1. Aim

The main aim of the project is to provide an in-depth review of current research on using algae to remove nutrients, primarily P and operate a 'sewage to algae to energy' process. This existing knowledge should be extended through investigation, using experimental techniques to design and optimise a sustainable method for this process whilst minimising costs (capital investment, operational and maintenance costs and other costs derived from economic and environmental restrictions). The primary focus is to achieve acceptable EU WFD standards for the treated effluent with a secondary focus on maximising the algal yield.

The literature shows us that good technologies for P-removal exist. The purpose of this project will be to show that the tertiary algal treatment method can be more efficient, more sustainable, cheaper (at least in terms of offsetting the by-product / energy generation), more reliable and also reduce P to lower levels or meet the current 1mg/L standard with a shorter HRT.

## 4.2. Key objectives

Compile information and prepare a full assessment of previous work regarding key areas of the research; P and N removal, sewage treatment, algal growth and biofuel. Use this information to carry out detailed, well controlled laboratory experiments using a series of experimental rigs to provide results from which I can derive conclusions and new knowledge.

The experimental methods include studying the growth rates of algae as a by-product of the removal/fixation of N, P and C from wastewater. Measure how the growth rates were affected by changes in light, temperature, $CO_2$ concentration, algal concentration and nutrient concentration to determine an optimal process.

The objectives in 11 key areas have been identified below

### 4.2.1. P and N removal

Reduce P and N concentration in wastewater to meet EU discharge standards of a maximum of 1mg/L P and 5mg/L N (95%) using algae as a sustainable method of cutting costs and minimising energy use, improving sustainability.

Study the uptake rates of P, the total quantities being removed from a real works effluent and what levels of P removal are achievable. Is this method and removal rates competitive with existing processes such as chemical treatment and bacterial processes (acintobacter), BPR, and would it be economically viable?

Study the uptake rates of P, N & C from real sewage effluent and what levels of P & N removal are achievable. To design an algal bioreactor / HRAP to meet P & N concentration standards for discharge and at fast enough rates to be economically viable. The method will be compared for synergy with the standard de-nitrification process and biological nutrient removal processes. Can P & N removal be successfully combined in a single process?

### 4.2.2. Carbon sequestration

Determine the amount of C fixation during this process. Analyse how much C is taken up from the effluent and see if this is a viable C sequestration process. Is the fixation of additional waste C possible, e.g. combustion exhaust? Compare this data with previous work.

### 4.2.3. Algal growth rates & concentration

To create conditions promoting high growth rates of algae and hence high uptake rates of nutrients. Quantify these growth rates and perform mass balance analysis with nutrient

uptake. Compare the algal growth rates achieved with data from literature for similar processes and also with existing methods of algal biomass production specifically for biofuel.

Is the growth rate consistent and can it be controlled? Are the algal growth rates and production high enough to consider them a valuable by-product?

Study the effects of bioreactor algal concentration with regard to growth rates, light penetration and competition for nutrients. Therefore provide suggestions on optimal algal concentration within the bioreactor.

### 4.2.4. Trace nutrients and quality standards

Assess whether or not the growth of algae has other positive or negative effects on the treatment of sewage other than the removal of P &N.

### 4.2.5. Algae / effluent separation

Provide a technique for the separation of algal solids from the discharged effluent. The technique should be fast and have low energy requirements whist being effective at achieving the highest concentration (>6% for direct feeding to AD). The discharge should meet EU standards of a maximum of 35mg/L suspended solids (95%).

### 4.2.6. Seasonal variation

Compare results from artificial light with those from natural light. Establish whether the UK can sustain year round algal growth with the natural light intensity available, providing quantitative data on seasonal variations to allow scale up to open, natural conditions at a STW.

Are the light intensities high enough and daylight hours long enough in the winter to sustain algal growth to meet P removal requirements?

### 4.2.7. Use of algae

Assess the potential uses of the algal biomass by-product as a sustainable fertilizer or fuel source, investigating the options available, AD, liquid fuels and combustion.

### 4.2.8. HRT, bioreactor design and optimisation

Optimise the HRT throughout the whole system to reduce both capital and running costs.

Determine the best design based on literature and experimental data. What type of bioreactor / HRAP design will work best? Use this data to indicate optimal dimensions and values for all parameters involved in the system.

### 4.2.9. Bioreactor design scale up

Devise experiments that determine the suitability, barriers, advantages and disadvantages of this process for use in the UK.

Investigate how these experimental techniques can be scaled up to full scale level. Produce an optimal performance pilot plant design for further studies at the sewage works based on the successful laboratory rig.

To consider the possible use of this process as an industrial technique for value added by-products and provide new information regarding the best designs for an industrial scale operation.

## 4.2.10. Integration

Investigate the prospects for integration with waste heat from the CHP / AD. Assess the effects of temperature on algal growth rate and metabolism. Consider whether heating the effluent would be a barrier to discharge due to watercourse temperature requirements.

Can the same industrial units provide additional $CO_2$.

## 4.2.11. Further work

Using the findings from this project, make new conclusions about the prospects and suggestions for further research in this area to develop the method and improve its effectiveness.

# 5. Methodology

## 5.1. Bioreactor designs

The experimental period consisted of initial testing followed by 3 major phases of bioreactor development, each including numerous experimental stages.

Initial testing was carried out to ensure the algae could be grown in a laboratory bioreactor under artificial lights as a simple batch feed process. A mixture of final effluent was collected from Loughborough, Long Whatton tertiary treatment lagoon and various holding ponds around the university. The mixture was selected to ensure a broad inoculant of natural species best suited to growing in the UK climate in the nutrient rich mixture found in sewage effluent. They could then be refined by selective pressure from reactor design; it was fed on Loughborough final effluent.

Using a 4.5L beaker under artificial lamps (spec in section 5.1.1.) a algal culture began to grow, reaching a concentration of more than 500mg/L, high enough to allow experimentation on a continuous flow process to begin (stage 1). The initial batch testing demonstrated reductions in nutrient concentration of both N and P to below their required EU standard of 5 and 1mg/L respectively. It was noted that the growth of duckweed on the surface of the bioreactor obscured light penetration into the vessel and all duckweed was removed prior to starting stage 1 continuous experimentation.

The feed effluent for all experiments was obtained from Loughborough sewage treatments works and therefore had a large variation in properties such as nutrient concentration. This work was to use real sewage effluent was chosen over artificial media in order to account for the wide variation of environmental conditions likely to occur naturally. It produced less controlled experiments due to the varying P concentration and varying algae concentration / specie mix. This however better represented real life and provided more valuable results that could be reproduced in field experiments.

### 5.1.1. Phase 1 - Continuous Flow Bioreactor – Single Vessel System

The first bioreactor used was a single continuous flow Perspex tube with a conical base. It had a circular cross sectional area of 154cm$^2$ (14cm in diameter) with a usable height of 65cm providing a 10L capacity. The conical base was 12cm in height and led to a flat bottom with an outlet tube on the side, used for sludge removal. The bioreactor also had built in pipes on the side for inflow and outflow (see fig. 3). The inflow of final effluent was continuously pumped into the bioreactor, controlled by a peristaltic pump (Watson Marlow, model 50SS, fig. 4). The pump was variable speed (minimum 0.25rpm) and the flow rate could be adjusted to control the HRT of the bioreactor. The HRT was defined as the time required for the flow throughput to equal the volume of the vessel. The outflow was unrestricted to maintain constant volume in the bioreactor.



**Figure 3 - The bioreactor with light and mechanical stirrer**

**Figure 4 - The Watson Marlow pump**

The feed flowed bottom to top, here the effluent flowed out via the outlet pipe into a settling tank (Fig. 5). The treated effluent spilled over as discharge whilst the algae were collected at the bottom to be recycled back to the main bioreactor (see fig. 5). The algae and effluent in the main bioreactor were mixed by a mechanical stirrer to keep the algae cells moving and in suspension. The stirrer was kept the same throughout all experiments. The algal cells were buoyant enough to stay dispersed in suspension for an hour, therefore the mechanical stirrer was controlled by a timer to come on for 1 minute every 8 hours. The bioreactor had a sludge outlet pipe at the base equipped with a manual valve, to remove dead algal cells or clumps that were too big to remain in suspension would typically collect as a sludge in or around the outlet tube. This meant it was possible to manually remove the algal sludge from the base of the bioreactor. The removal prevented re-mineralization of nutrients from decaying cells.

## System Flow Diagram
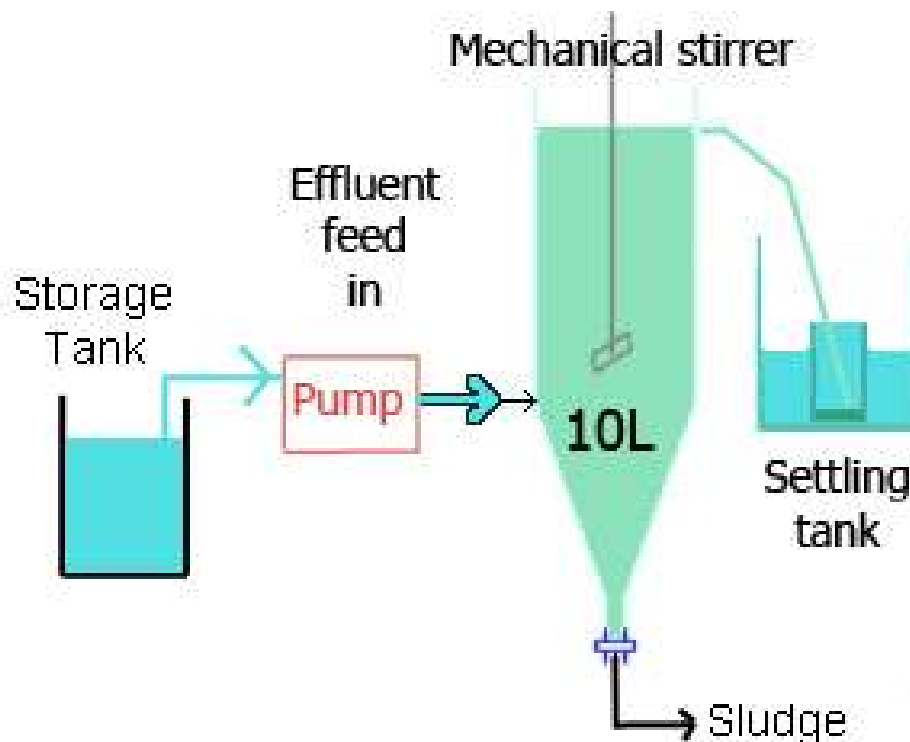


**Figure 5 - System flow diagram of the phase 1 continuous flow process**

Samples were taken daily to measure the P & N levels at the inflow and outflow, monitoring the performance of the bioreactor.

The main problem with this first design of continuous flow bioreactor was that it was completely mixed and without baffling. The nutrient concentration in the feed, introduced into the bottom of the bioreactor, was rapidly dispersed and diluted throughout, therefore the nutrient discharge concentration was equal to the nutrient concentration within the bioreactor. To achieve the required EU standard, the small dilution ratio limited how fast effluent could be pumped in and limited the overall minimum concentration that could be achieved. The maximum uptake rate of nutrients by the algae was restricted (see discussion in section 6.).  A longer HRT could produce lower nutrient concentrations in the treated discharge and low HRTs were used to meet the EU requirements (1mg/L P).

The bioreactor was artificially illuminated from fixed intensity halogen lamps around the tank to provide light for the algae to optimise photosynthesis. The lamps produced a luminosity of 121W/m$^2$ at a distance of 1cm however this declined to 40W/m$^2$ at 8cms from the lamp, less than 20% of the minimum expected from natural daylight (see section 6.). The light energy produced was adequate to perform the experiments in a laboratory however would not be economically feasible for scale up. The lamps could not provide a luminosity to match natural light, see discussion about this in the results. The lamps were kept the same throughout all the experiments however the light intensity was varied (due t the inverse square law) by changing the distance between the lamp and the bioreactor.

These initial experiments were a success, showing P could be reduced to below the required EU WFD concentration level however the bioreactor setup needed re-designing.

## 5.1.2. Phase 2 - Continuous Flow Bioreactor – Second Generation Larger Scale Multi Vessel System

This initial bioreactor designed for the project using a single stage photo-bioreactor design showed promising results. The main issue was the dilution ratio into the single bioreactor when final effluent of high nutrient concentration was added. A high dilution ratio was required to keep the concentration below the standard of 1mg/L, thus limiting the HRT.

The second experimental phase was an evolution of the first in that the process was expanded to allow multi stage flow. Two additional bioreactor vessels were constructed and had a design based on the original bioreactor but using larger dimensions, given below (see Fig. 6).

**Figure 6 - Example of the 2$^{nd}$ generation bioreactors at full capacity**

The two new bioreactors were identical. They had a cross sectional area of 314cm$^2$ (being 20cm in diameter) with a usable height of 95cm providing a 30L total capacity but with an operating capacity of 15L. The design of the base of the bioreactor was improved by providing a steeper conical shape with the outlet tube connected to the bottom, this made the removal of the sedimented decaying algal sludge a much more efficient process and allowed the tanks to be emptied more quickly enabling an easy method of cleaning. The base section was 12cm in height (See Fig. 7). The new bioreactors were equipped with the same inflow, outflow and sludge discharge tube arrangement as the first design.

**Figure 7 - Detail of the conical base**

The original 10L bioreactor was kept in place with the additional two (second generation design) 15L bioreactors added. The three bioreactor vessels were combined in series to solve the short circuit baffling problem from the first experimental phase. Designed to give plug flow and a strong concentration gradient; they reduced the potential for short circuiting (see fig. 8). Basic theory (Levenspiel, 1992) suggests three tanks in series will reduce short circuiting by 50%. By adding a $2^{nd}$ tank that feeds into the final tank, the process of reducing 20mg/L to 1mg/L was split into 20 to 5 and 5 to 1. This makes a more efficient P removal process as the overall concentration in the first tank will be higher, therefore giving faster P removal and faster growth. In theory adding the third tank can split the process further, 20 to 10, 10 to 3 and 3 to 1.

The overall aim of this new design was to achieve lower final nutrient concentrations for the same HRT that are compliant with the EU UWWTD and WFD. At full scale, It may be possible to switch bioreactors in / out of service depending on the P concentrations.

**Figure 8 - Showing overall setup with all 6 tanks**

Final effluent from Loughborough sewage works was pumped into the bottom of the first vessel at a controlled rate. The effluent flows from the top of vessel one to the bottom of the next until the final settling tank. All the bioreactor vessels were lit with fixed intensity halogen lamps. The mechanical stirring was controlled by timers as in the first experimental phase (where stirring in the tanks was found to be required at most for 1 minute every 8 hours) to avoid algal precipitation and decay.

Less stirring reduced the algal concentration in the very top layer of each tank and hence the transfer of cells from one tank to the next, those cells that are transferred to the next tank (were smaller, younger, more buoyant) are thought to be the most productive and

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continue to improve the quality of the process in the later stages, however these are the cells discharged at the end of the process and also the hardest to separate. Occasional stirring is necessary to keep enough algae in suspension throughout the tank without losing them to the sludge at the base, whilst the rest periods allowed sedimentation of unwanted biomass (clumps or dead algal cells). The algae collected in the final settling tank was returned to the first vessel to replenish the algal concentration, whilst sedimented biomass collected from the base of the three bioreactors was collected and stored for digestion.
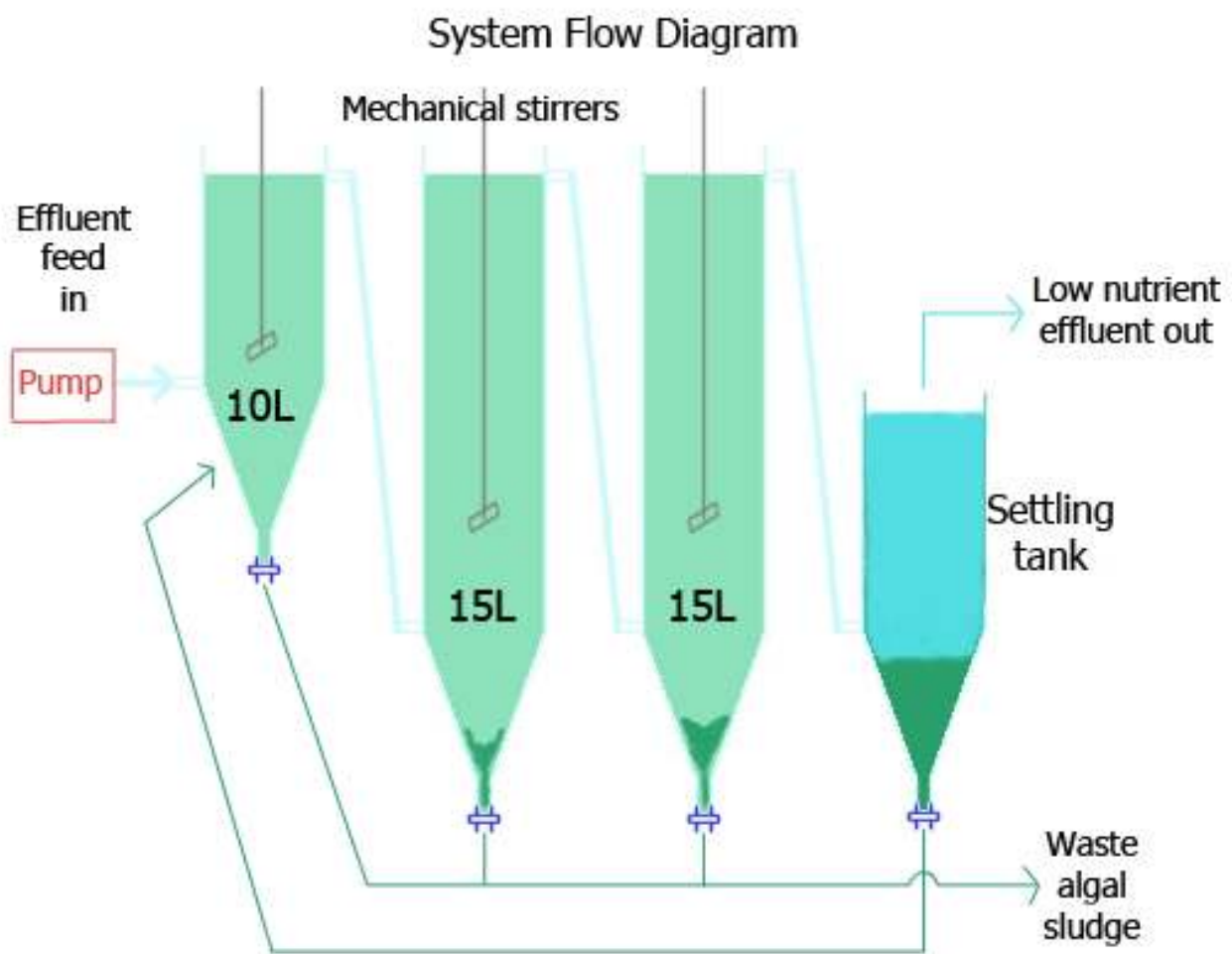


**Figure 9 - Diagram showing the system flow for phase 2**

Algae became attached to the connecting pipes, through physical resistance and to the bioreactor walls where light intensity was greatest. The growth on the walls reduced the illumination within the whole reactor. Rotation of lighting position, regular scrubbing of

bioreactor walls and cleaning the connecting pipes was necessary. All cleaning was carried out between experimental runs.

Tall clear tanks were chosen to allow light penetration to all areas of the fluid whilst land area usage is minimised. Energy costs are reduced because the flow through the system is generated by a small gravitational gradient and agitation of the tanks is barely required. The final stage requires a method of separating the algae from the discharge. The algae settlement rate needed to be predictable because of their rapid deterioration if stored too long or losses if the flow rate was too high.

Throughout the experiments, the HRT was varied. P reduction to meet EU requirements could be achieved at a flow rate of 16L / day (48 hour HRT) however there was poor separation of the fine algal cells from the discharge. The maximum achieved by gravity was 4L / day (8 day HRT).

An increase in bioreactor volume requires an increase in flow rate to maintain the original HRT. This increase in flow meant a faster rate of transfer of algal cells from one bioreactor vessel to the next and subsequently out of the system.

Because dilution was not now a critical factor due to the multi stage setup, it meant the HRT could be reduced by a factor of 2-3. This meant that a 4x increase in total bioreactor volume could increase the flow rate by up to 12x.

The overall concentration of suspended algae in each tank was affected by the algal growth rate, flow rate of the system and return rate back into the 1st tank. The algal growth rate was slower than the transfer rate and as the flow rate was increased, more algae were lost out the end of the system. The separation of algae could not keep up with HRTs of less than 8 days. This meant the return of algae to the first tank could not keep up with the reduction of algae from flow transfer. The effect of this was a wash out of the algae. A maximum HRT of 24 hours would be required when considering feasible capital costs at full scale. As a

consequence, experimental trials were carried out with membrane separation using cross flow filtration.

The artificial light intensity remained constant throughout the experiment at 121W/m$^2$ (see 5.2.2.1.).

The temperature of the experiment was recorded and allowed to vary with the temperature of the laboratory and was in the range of 7-22°C; this better represented field experiments that have a wide range of naturally occurring temperatures.

The key design issue became finding a separation process for removing very fine, buoyant algal cells from water that was quick enough and avoided damaging the algae for re-introduction into the system.

A cross flow filtration membrane system was added to the end of the process as shown in fig. 10.

Effluent In

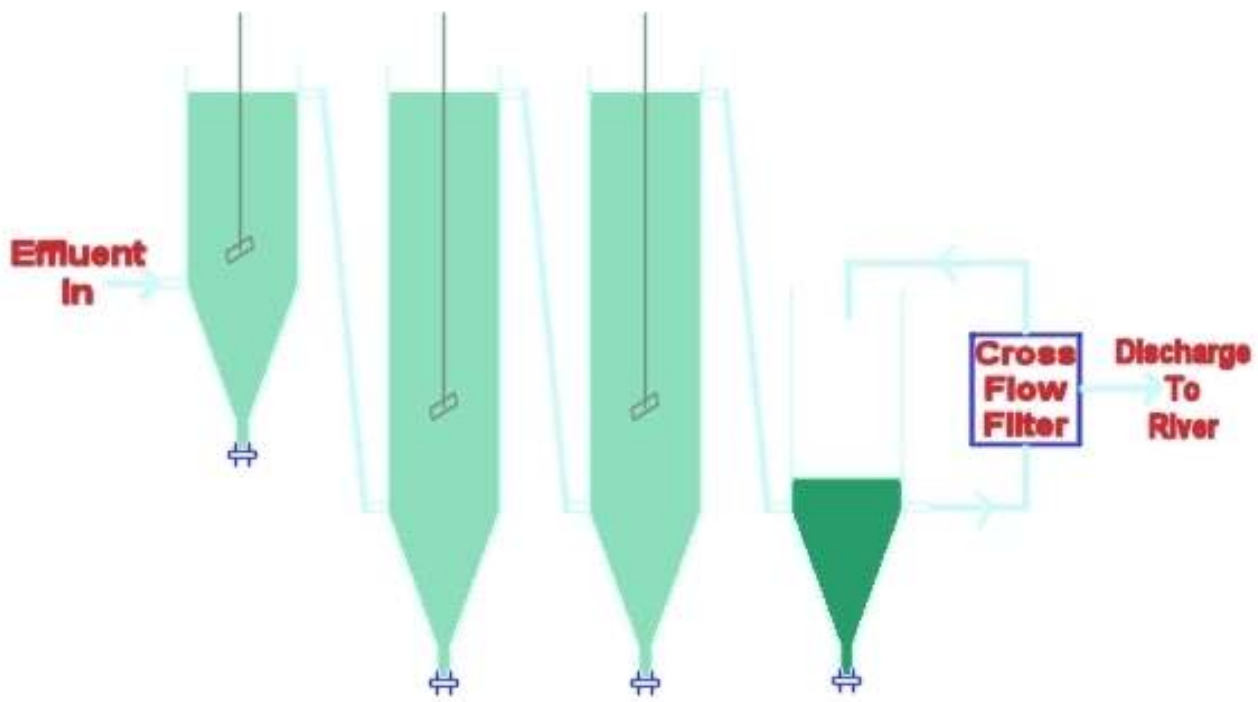Cross Flow Filter — Discharge To River

**Figure 10 - Showing the cross flow filter attachment to the final settling tank**

The cross flow filter was energy intensive, requiring a high flow rate to keep the membrane clean and high pressure to drive the effluent through the membrane.

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- Initial testing showed that a fast enough flow through the membrane could not be achieved.
- There was serious concern that the velocities and pressures may have been stressing the algae causing a release of stored P.
- The system was hard to work with in the laboratory and would be difficult to scale, requiring large numbers of units.
- The project is looking into pushing for more sustainable methods.
- The wastewater treatment industry is familiar with settlement and less familiar with membrane filtration.

For these reasons, further development of the system was not considered.

### 5.1.3. Phase 3 – Sequencing Batch Feed Bioreactor

Retaining algae in the system is very important for this process as it is the algae that are fully responsible for the mechanics. Following the issues maintaining the algal concentration in the continuous flow bioreactor system and poor performance of the cross flow filtration membrane; phase 3 was introduced as a sequencing batch feed process where biomass were not removed and cannot be lost from the system, except during sampling.

After each batch the contents of the bioreactor were left to settle, enabling the separation of the algae from the treated effluent. Every time the effluent was removed, it left behind the fastest settling algal cells and flocs. Using selective pressure by repeating this technique of fast gravitational settlement, a fast settling algal culture developed following the forced removal of the more buoyant algal cells. The separation process had reduced to 5-10mins allowing the HRT to be reduced to whatever was required to obtain final nutrient concentrations of either 0mg/L or the EU standards. Constant agitation, keeping the algae in suspension was a trade-off for fast gravitational settling.

This batch feed bioreactor design was used for the majority of the experiments. The process was much simpler, easier to operate and proved to be able to better achieve lower P

concentrations overall, often 0mg/L than the continuous flow process as the results in section 6.3.2.2. show. The batch feed bioreactor consists of a clear vessel, it was stirred continuously at 80rpm by the same mechanical mixer as used in the continuous and lit by fixed intensity halogen lamps (see fig. 11).

The cross sectional area of the bioreactor was 254cm$^2$ (18cm in diameter) with a usable height of 18cm providing a volume of 4.5L. As a sequencing batch bioreactor, feed and discharge tubes were not required.
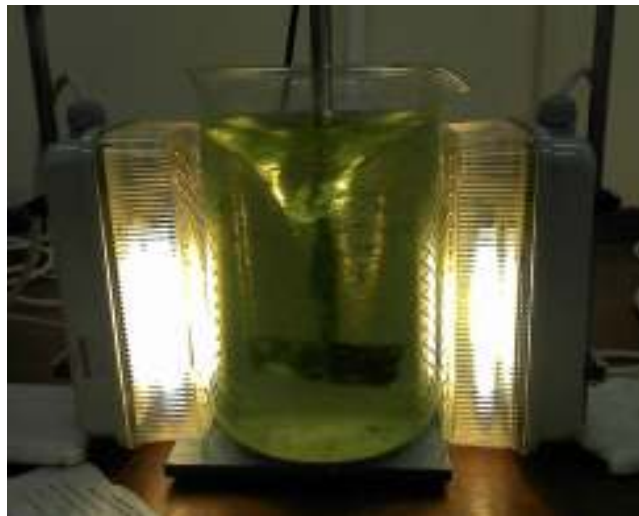


**Figure 11 - Showing the 4.5L batch feed bioreactor soon after an initial inoculation**

The individual batches are not affected by flow rate and do not experience algal losses. Each batch could be designed as a completely separate experiment allowing adjustment of the starting P and biomass concentrations; it also allows detailed control over the HRT. Every batch feed fully replenished the nutrients in the bioreactor. This follows the previous batch achieving the required standard and being drained of the treated effluent. Before feeding, the mixing device is switched off to allow the separation of algae from the treated effluent. 10 minutes settling was sufficient for decanting effluent that met the suspended solids WFD regulation of 35mg/L. The effluent was used for analysis, leaving behind the algae to be used in the next feed. The batch time varied between 24 and 72 hours dependant on the nature of the experiment being carried out. Strictly controlled environmental parameter experiments were carried out over a 24hr period. Longer experimental runs over periods of 8-30 days were batched and sampled at varying intervals of between 1 and 3 days to build

up a picture of productivity vs batch time. An operational process could be expected to be 12 hours for complete P removal, or less to meet the 1mg/L standard. In terms of P removal per time; these results were much better than those from phase 2.

With every 4L feed the algal concentration increased due to growth. Typically an experiment was started with an initial inoculation of about 500mg/L while it was allowed to increase to around 2000mg/L over about 10 days. When the algal concentration became too great it began to affect the light penetration and was harvested for digestion. A proportion was retained as an inoculant to achieve the 500mg/L starting concentration for the next 10 day run for example. The results had shown a further increase in biomass concentration would not be beneficial for algal growth or final nutrient concentration (see section 6.).
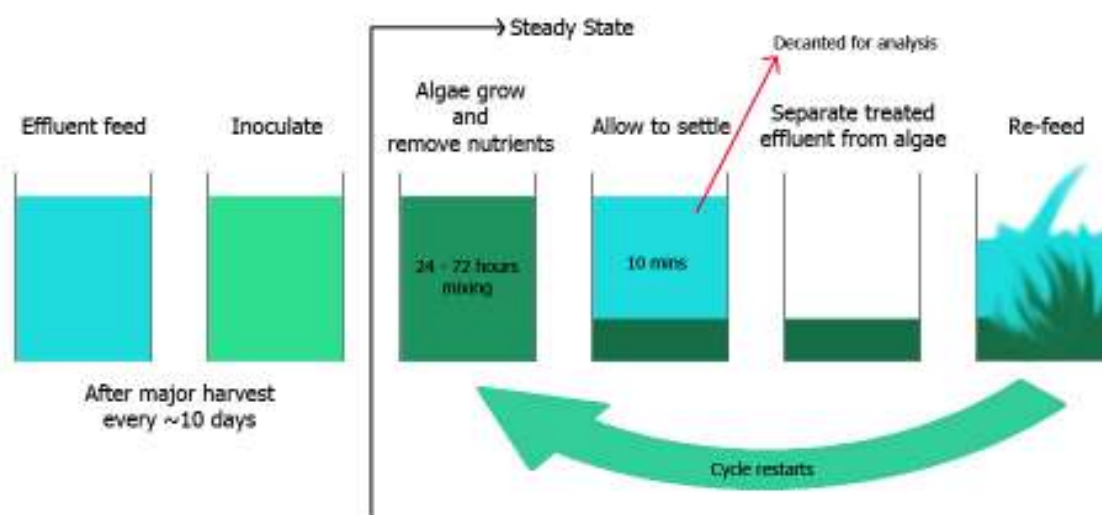


**Figure 12 - Diagram showing the process flow of the batch feeding**

Another major benefit of this process configuration was the ease of operation, maintenance, measurement / analysis and control to a fixed P standard. Specific variables can be better controlled and measured after a set time from feeding. The single controlled tank also makes it easier to monitor growth rates. The experiments can be repeated identically many times to allow improved statistical analysis of feeding frequency and to study when nutrients such as carbon become limiting.

The process was shown to be reducing P concentration to 0mg/L within 24 hours without $CO_2$ addition. A comprehensive set of results were obtained (section 6.3.2.) for the full range of water quality indicators under different lighting and temperature conditions.

## 5.2. Methods of Analysis

### 5.2.1. Standard Methods

These were all carried out in accordance with international standard methods (APHA 2005). The variation in sample size and equipment used are described. The sampling except for the suspended solids analysis did not require the removal of algae. Therefore, during the collection of samples for analysis the mechanical stirrer was switched off for 2 or 3 minutes allowing the majority of the algae to settle. This provided a sample virtually free of suspended solids. The samples were pre-filtered using a Whatman No.1 10µm filter paper to remove any remaining particles that may affect the chromatograph. This type of sample was neither too intrusive nor affected any of the results by removing algae from the system. The suspended solids samples were taken on mixed bioreactor contents and carried out to measure the biomass concentration. These samples did cause significant losses which were taken into account when preparing the mass balance analysis.

#### 5.2.1.1. Phosphorus and Nitrogen

The Phosphorus and Nitrogen measurements used a 20ml pre-filtered sample as noted above. The sample had to be filtered to remove particles that could clog the chromatographic column, no other pre-treatment was needed. Phosphorus and Nitrogen analysis were carried out by means of the Dionex ICS -1000 ion-chromatographic method calibrated against $NO_3$ and $PO_4$ internal standards (APHA, 2005). It produced results in mg/L $NO_3$ and mg/L P. Each test on the machine used an injection volume of 1ml.

The early measurements used the Palin colorimetric test technique however whilst providing scoping data it became apparent that the accuracy of the tests and margin for

error were outside the tolerance level for the project (a range >5% over 3 identical samples). None of these results were used in the data analysis in section 6.

### 5.2.1.2. Suspended Solids

The total mass of suspended solids was determined by filtration of a water sample using the standard 1.2 μm grade GFC, 7 cm discs under the application of a vacuum pump. The disks were weighed (before and after filtration) dry at room temperature (after 5 minutes of cooling in a desiccator). Drying was usually for 2 hours at 105°C. 50ml samples were usually used (1/20 L) therefore the total weight difference was multiplied by 20 to express the mass of SS found in mg/L (APHA, 2005). 100-150ml samples of the treated effluent were used when available.

The suspended solids measurements were used for two reasons, to determine whether the treated effluent was within EU standards of 35mg/L and to determine the concentration of algae in the bioreactor, primarily to measure growth rate. Measuring algal concentration was intrusive. The most accurate result was provided by using the biggest sample from the bioreactor, 150ml, even up to 300ml. Often It was not feasible to remove this much from the bioreactor, especially at the start of the SBR experiment or when repeating the measurements as the algal losses were shown to have a dramatic effect on the performance of the experiment. Often 3 x 50ml samples were used as a compromise between achieving an accurate result and not removing too much algae from the bioreactor.

When measuring biomass concentration, the samples were extracted from the middle of the bioreactor to get the best representation and maintain a consistent standard procedure.

The measurements of suspended solids in the final discharge effluent after the algal had been removed by gravitational settling were carried out initially to ensure the EU standard of 35mg/L was being met. A culture of fast settling, self flocculating algae had been developed meaning very low suspended solids content was retained in the solution. The

results showed that the concentration was always less than 5mg/L (often close to 0) with no increase in inorganic matter from the initial influent. Based on these negligible results, further analysis was not carried out.

### 5.2.1.3. IC and TOC

The IC and TOC were analysed by the high-temperature combustion method using a Total Carbon analyser *Rosemount Dohrmman DC 190*. Measurements required only a small sample of 5ml to be taken from the bioreactor. Each sample was tested three times for accuracy. The actual injection volume was only 2μl which allowed duplicates. The samples were also pre-filtered as noted above (section 5.2.1.).

### 5.2.1.4. pH

The pH of the effluent was measured by using a portable standard electronic pH-EC-TDS meter (*Hanna HI 9812*) in the bioreactor for 5 seconds. The pH meter was buffered using a standard pH7 solution prior to every use because of drift. The same instrument measured electrical conductivity and total dissolved solids. According to the manufacturer, the EC/TDS only required calibration 1-2 times / year. It was checked quarterly with standard isotonic solutions.

### 5.2.1.5. Metals

The measurement of metals used a 20ml sample taken from the bioreactor. The samples had no pre-treatment other than pre-filtering, as noted above (Whatman No.1), to remove suspended solids. Each metal to be tested required about 5ml and the procedure was the same for each metal.

Dissolved metals were then tested from all samples using an Inductively Coupled Plasma (ICP) analyser *(Thermo Jarrel Ash Atom Scan 16)*. This provided results in mg/L of the specific metals, Fe, Ni, and Zn by comparison with calibration standards. Each metal was

analysed in triplicate and an average was taken from the results. The ICP's detection limit for each of the tested elements is dependent on its wavelength and is presented in Table 2.

<p align="center">**Table 2 - ICP's wavelength and detection limit**</p>

| Element | Wavelength/nm | Detection limit mg/L |
|---------|---------------|----------------------|
| Fe | 259.940 | 0.002 |
| Ni | 221.647 | 0.005 |
| Zn | 213.856 | 0.002 |

## 5.2.2. Non-standard Methods

### 5.2.2.1. Light

The light was measured in Lux using a 'Precision Gold N76CC' light meter. This could determine the intensity of light across the bioreactor from the light source. The units in Lux were converted to $W/m^2$ using a conversion factor of 1/103 as derived from the literature review.

### 5.2.2.2. Temperature

The experiments were run in the unheated pilot plant laboratory. The temperature was measured at the time of taking samples for other experimental variables. A thermocouple was used to give a reading to the nearest 0.1°C. Both the temperature of the effluent in the bioreactor and ambient temperature was measured. Due to the high specific heat capacity of water, the temperature of the effluent was not very responsive to the change in air temperature. A drop in temperature was noticed over weekends when the heating was off in the adjacent room and doors were always shut allowing the bioreactor to slowly cool. This was only ever an issue during cold spells during the winter. The effect would be greater outside, however larger volumes, shorter HRT and possibly $CO_2$ injection from CHP would prevent any serious change in temperature from that of the normal range. The results from temperature experiments are shown in section 6.3.2.

Temperature data was also archived from the sewage works thermocouple to compare with the laboratory data.

## 5.3. Experimental Methods and Stages

### 5.3.1. Experiment Protocol

### 5.3.1.1. Collection of effluent from Severn Trent Water

The final effluent feed for all experiments was taken from Severn Trent Water, Loughborough sewage works from the combined final discharge of all the clarifiers. A 25L bucket was filled by lowering it by rope into the discharge channel, once filled, it was hoisted out and capped ready for transportation back to Loughborough University. The weather conditions were noted.

### 5.3.1.2. Phase 1 - Continuous Flow Bioreactor – Single Vessel System

Every new batch of the Loughborough final effluent collected was analysed for N and P. After the algal bioreactor settling tank, the treated discharge was also sampled for N and P. Daily grab samples were taken from the surface of the settling tank. The feed tank was kept topped up for continuous running and as discussed in 5.1.1., recycling the algal concentrate from the settlement tank to the main bioreactor was done every few days. With the known volume of the bioreactor and the flow rates, uptake rates of nutrients could be calculated. This reactor was run for a period of 6 months (results shown in 6.1.).

### 5.3.1.3. Phase 2 - Continuous Flow Bioreactor – Second Generation Larger Scale Multi Vessel System

The multi tank continuous flow bioreactor followed a similar procedure to the single tank continuous flow bioreactor. However in this case the bioreactor could be sampled at different points in the treatment train along the series of tanks. This was used to show the reduction in nutrient concentration along the series of tanks and how well each tank performed in the sequence compared to a single tank using this method of 'baffling'.

Recirculation to the first feed tank from the final settler was intermitted, replenishing algal concentration in the initial bioreactor tank from that collected in the settling tank and sampling were the main operational tasks. Cleaning the algae from the reactor walls was necessary every 7 days to prevent build up, that, over time reduced light penetration. Discharging algal sludge from the base of the tanks and regularly cleaning the connecting tubes from a build-up of algae prevented re-release N and P. The experimental run was over a period of 5 months.

### 5.3.1.4. Phase 3, Exp.1 - Batch feed bioreactor – P uptake rates and loading

The feed was sampled for P and then the required volume was added to bioreactor to give the desired load. Sampling for P was at 2 ,4, 8 and 24 hours after feeding to monitor the change in P concentration. With the simpler batch system, it was possible to run 2 duplicate bioreactors to compare the reproducibility under the same conditions.

Results would also show periods when more frequent samples were required, the sampling times were adjusted based on this. Samples might be taken typically at 0, 0.5, 1, 2, 8.5, 10.88, 12.35 and 14 hours after feeding to produce more detailed uptake results.

### 5.3.1.5. Phase 3, Exp.2 - Batch feed bioreactor – Algal growth rates 1 (Predominately P uptake)

Starting from an algal concentration of 400mg/L, the bioreactor was batch fed 7 times over 8 days. Before each feeding, the algal biomass concentration was measured using suspended solids. This provided data showing the growth rates to estimate doubling times and compare with literature.

**5.3.1.6. Phase 3, Exp.3 - Batch feed bioreactor – Full measurement tropical and temperate conditions**

Measurements for all variables were taken at the standard intervals over a 24 hour period for one reactor at a constant 34°C maintained in a warm room and a second at ambient temperature in the laboratory. This was repeated for a range of algal biomass concentrations. This experiment was repeated with two levels of light, 140W/m$^2$ and 58W/m$^2$.

At the start of the 24 hour period batch feed, the bioreactor would be fed with 4.5L of final effluent. At this point the biomass concentration was recorded along with the nutrient levels and all other variables, IC, pH, metals, temperature, conductivity, TOC. Samples were taken at 2, 4, 8, 12 and 24 hours after the initial feeding. After 24 hours the biomass concentration was recorded to show the growth over that period. At this point the treated effluent was drained and the cycle was started again to record a new data set for the next batch, this time with a different initial biomass concentration. 4 different biomass concentrations were measured for each light and temperature combination (a total of 16 x 24 hour data sets). The tropical experiments were run with one of the bioreactors in a constant temperature room (34°C).

**5.3.1.7. Phase 3, Exp.4 - Batch feed bioreactor – N uptake rates**

After feeding the bioreactor with 4.5L of final effluent, the N concentration was recorded. Every 2-3 days the final N concentration was recorded and the bioreactor fed again with fresh effluent. Knowing the start and finish N concentrations along with the time period between feeding, an uptake rate could be calculated. Taking the samples for measurement was done in exactly the same way as all other experiments as described in the standard methods, 5.2.1.1.

**5.3.1.8. Phase 3, Exp.5 - Batch feed bioreactor – Algal growth rates 2 (Predominately N uptake)**

Algal growth rates 2 was an evolution on the first experiment. Care was taken to account for all algal loses from the system from sampling and loses in effluent volume from sampling and evaporation. This was necessary to provide accurate results for total algal growth as opposed to net change in biomass concentration within the bioreactor. Providing this algal growth data allows for adjustment to nutrient uptake rates per unit of algal growth.

To help algal loss reduction, instead of sampling every day, the biomass concentration was sampled every 3-4 days. The data set was then collected over 24 days instead of 8 as in the previous experiment, Algal growth rates 1.

**5.3.1.9. Phase 3, Exp.6 - Batch feed bioreactor – Outdoor vs. laboratory conditions**

Two identical bioreactors were fed during summer (July – August) and sampled every 3-4 days. One kept in normal laboratory conditions, under artificial light and the other was placed outside under natural light. The same effluent source was used for both. The experiment was run for 37 days including time to acclimatise to the new environment.

## 5.3.2. Summary

**Table 3 - Summary of experiments**

| Model Type and purpose of experimental study | Light | Temperature | Dimensions |
|---|---|---|---|
| | | | |
| *Single tank continuous flow bioreactor* | | | |
| Nutrient Uptake – Varying the HRT to determine how fast the process works and the effects on the algal concentration and | $120W/m^2$ | $17 \pm 4°C$ | $154cm^2$ x 65cm, 10L |

| | | | |
|---|---|---|---|
| separation process. | | | |
| | | | |
| *Multi tank continuous flow bioreactor* | | | |
| Nutrient Uptake – Varying the HRT to determine how fast the continuous flow process can work now the multi stage system has been included. | $120W/m^2$ | $17 \pm 4°C$ | $2 \times 314cm^2 \times$ 95cm, 30L (15L used) + 1 x $154cm^2 \times 65cm$, 10L. Total 40L |
| | | | |
| *Batch feed bioreactor* | | | |
| To determine P uptake rates during batch process. | $120W/m^2$ | $17 \pm 4°C$ | $254cm^2 \times 18cm$, 4.5L |
| Finding the growth rate of unicellular green algae. | $120W/m^2$ | $17 \pm 4°C$ | $254cm^2 \times 18cm$, 4.5L |
| Studying well controlled batch feed experiments varying the biomass concentration. Recording 13 performance indicators under temperate lit conditions. | $120W/m^2$ | $17 \pm 4°C$ | $254cm^2 \times 18cm$, 4.5L |
| Studying well controlled batch feed experiments varying the biomass concentration. Recording 13 performance indicators under tropical lit conditions. | $120W/m^2$ | $34 \pm 0.5°C$ | $254cm^2 \times 18cm$, 4.5L |
| Studying well controlled batch feed experiments varying the | $40W/m^2$ | $17 \pm 4°C$ | $254cm^2 \times 18cm$, 4.5L |

| | | | |
|---|---|---|---|
| biomass concentration. Recording 13 performance indicators under temperate low light conditions. | | | |
| Studying well controlled batch feed experiments varying the biomass concentration. Recording 13 performance indicators under tropical low light conditions. | $40W/m^2$ | $34 \pm 0.5°C$ | $254cm^2$ x 18cm, 4.5L |
| Studying the N uptake rates for filamentous blue-green algae. | $120W/m^2$ | $17 \pm 4°C$ | $254cm^2$ x 18cm, 4.5L |
| Studying the growth rate for filamentous blue-green algae. | $120W/m^2$ | $17 \pm 4°C$ | $254cm^2$ x 18cm, 4.5L |
| Outdoor vs. laboratory conditions comparison experiment. Initial testing of process in a less controlled environment outside. | $120W/m^2$ Laboratory and $200\text{-}1000W/m^2$ outside | $19 \pm 4°C$ Laboratory and $16 \pm 10°C$ outside | Each $254cm^2$ x 18cm, 4.5L |

## Experimental Timeline

| | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **2006** | | | | | | | | | | | Collection of algal samples, initial testing and maturation of innoculum | |
| **2007** | Preparation of continuous flow, single vessel system | Measurements, technique improvements and testing different separation techniques | | | | | | | | Design phase of improved multi vessel continuous flow system | | |
| **2008** | Measurements from multi vessel continuous flow system, testing different configurations and HRTs | | | | | | Conversion to sequencing batch feed reactor | Standalone experiments on P uptake rates and algal growth rates through batch feeding | | Controlled batch experiments varying light and temperature. Each experiment measured 13 performance indicators | | |
| **2009** | N uptake rate experiments through batch feeding | | | | | Outdoor vs laboratory conditions experiment | | | Further algal growth rate experiments | | | |

**Figure 13 - Timeline of experimental procedure**

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# 6. Interpretation of Results

## 6.1. Phase 1 - Continuous Flow – Single Vessel Bioreactor

Fig. 14 below shows the results from the first phase of experimentation using the continuous flow reactor shown in fig. 3. and described in section 5.1.1.

**Continuous Flow Bioreactor - Phase 1**
**Feed vs Discharge, P and N Concentrations**
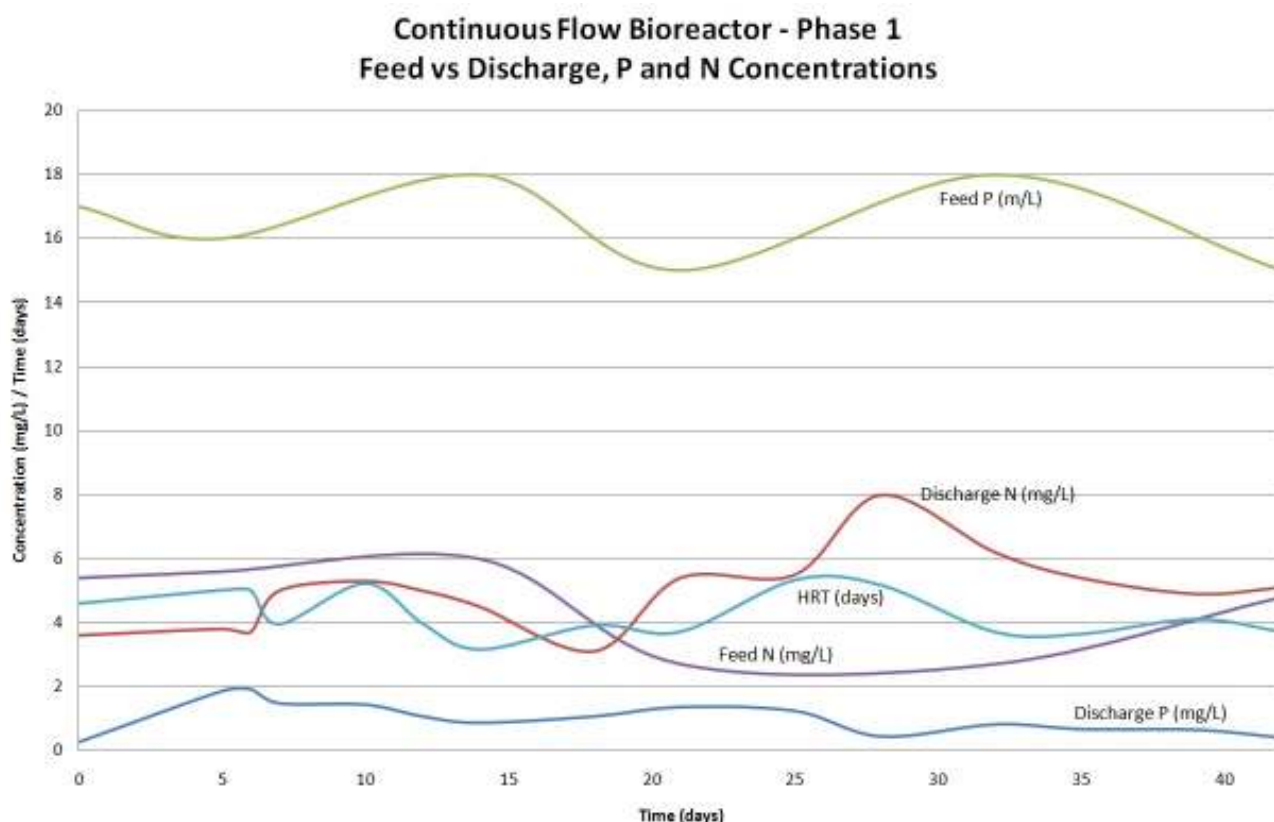
Figure 14 - Comparing the effluent feed P and N concentrations with the discharge P and N concentrations over a period of 6 weeks. The HRT during this period is shown; it varies between 3.5 and 5 days.

Fig. 14 shows the comparison between the feed and discharge nutrient concentrations along with the HRT at the time.

During the 40+ days the experiment ran for, improvements were made iteratively to optimise the performance of the process; this can be seen in the last 16 days where the average discharge P concentration was 0.62 ± 0.21mg/L compared to the first 23 days when it was 1.39 ± 0.52mg/L.

The improvement in P concentration was put down to better control of the flow rate, removal of dead algal cells and more effective return of algae collected from the settling tank.

The performance of the reactor was able to achieve the WFD target and the results showed sub 1mg/L P were possible. The HRT however during this experiment was on average 4.29 days + 1 days settling time (the variation in HRT was caused by adjusting the flow rate to control the biomass concentration). This amount of time is impractical at a sewage works. This HRT was limited by the dilution ratio required to achieve the WFD P concentration in the completely mixed bioreactor, washout of algae from the bioreactor and speed of settling to collect the algae for reuse. To overcome these problems were the objectives of the subsequent designs as detailed in section 5.

The algae species in the system appear to have little to no effect on the N concentration as shown in fig. 14 where the N concentration was actually increased at times. The results show the N concentration of influent and effluent vary without correlation. Integrating the area under each of the curves shows the net change in N is close to 0. The composition of the decaying sludge was measured which suggested only a trace of N indicating a low cell content. Cell growth theory suggests that uptake of N is expected to be at least 5 times as great as for P.

Observations of the solution under 100x magnification showed that the protozoa were eating the algae. It was not clear to what extent this affects the biomass concentration since this was an observation rather than an objective of the thesis, however it was accepted that this will occur across all system designs and is not a factor that can easily be controlled.

## 6.2. Phase 2 - Continuous Flow– Second Generation Larger Scale Multi Vessel System Bioreactor

### 6.2.1. P Uptake
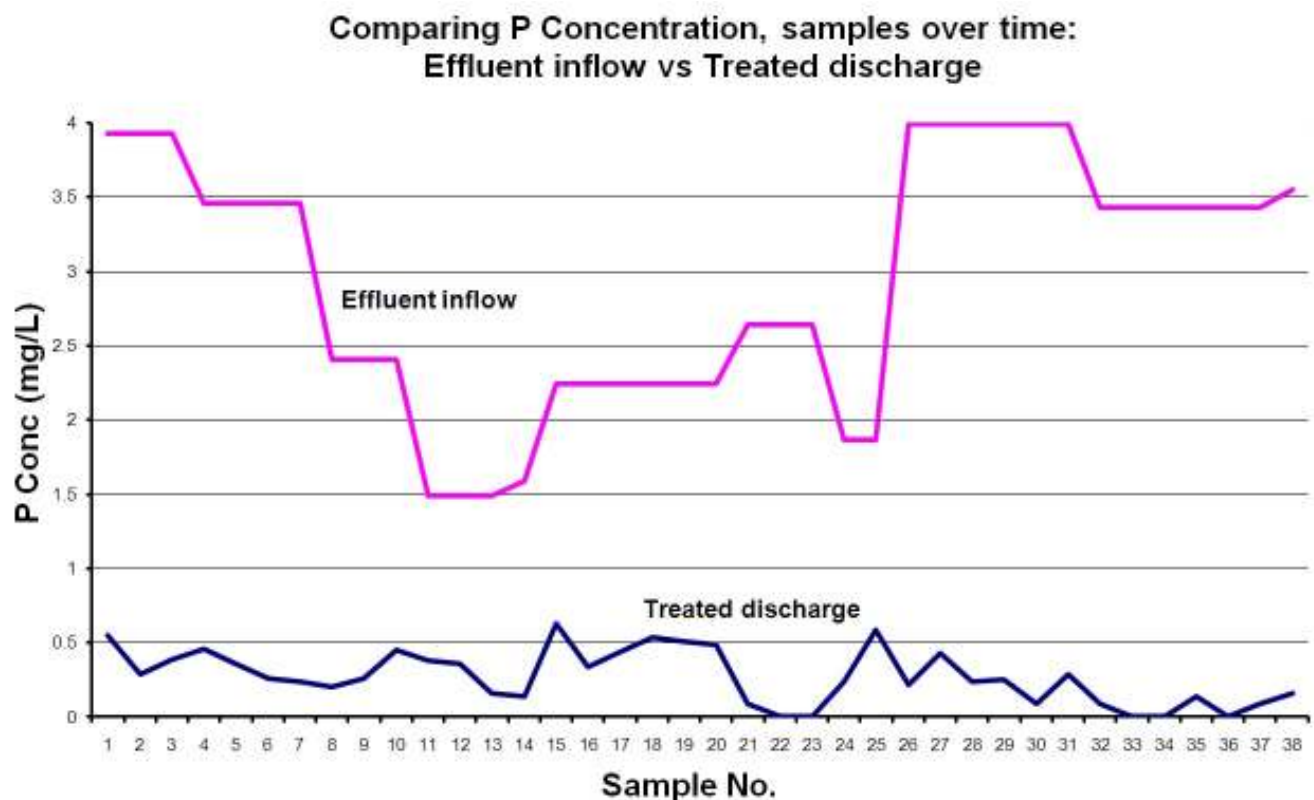


**Figure 15 - Shows the STW effluent feed to the experiment and the discharge P concentrations for the continuous flow multi vessel system at HRT of 48 hours.**

Fig. 15 shows a consistent discharge P concentration of below 0.5mg/L whilst the Loughborough STW final effluent feed varies between 1.5 and 4mg/L. The phase 2 reactor often reduced P to 0mg/L showing complete removal was possible. The results varied between 0-0.63mg/L thus with an average from all samples of 0.27mg/L compared with an average feed P concentration of 2.95mg/L. This was achieved with a HRT of 48 hours, i.e. half that of the phase 1 bioreactor.

The overall process concept was the same as the single vessel continuous flow system but these experiments demonstrate the importance of creating a concentration gradient to ensure the best removal rates. In this way, the HRT can be reduced whilst producing discharge P concentrations of less than half that previously achieved. The feed P

concentrations during this experimental run were less than a third of the original experiment due to the installation of a RAS fermenter at Loughborough STW, see section 6.3.3.

Again however the experiments suggested that the HRT in this experiment was limited by washout of algae from the bioreactor and settling velocity of the algae to collect for reuse.
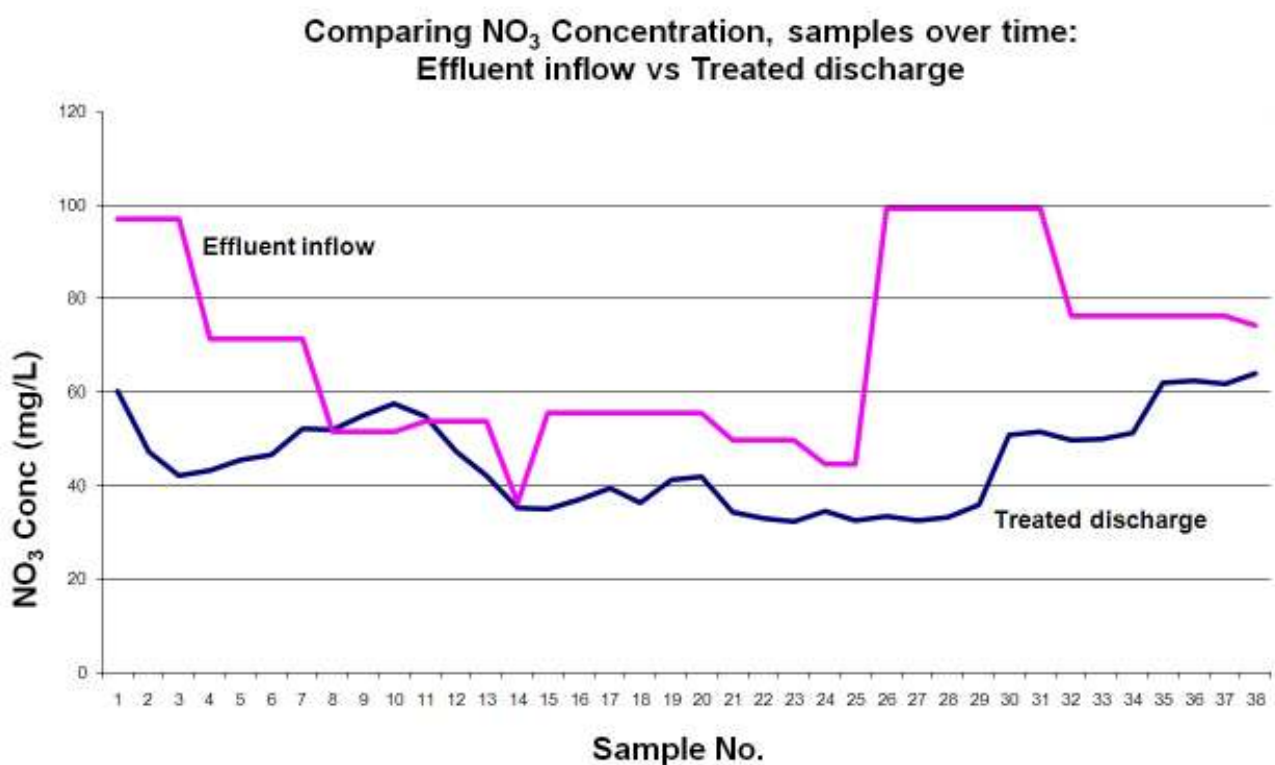
## 6.2.2. N Uptake



Figure 16 - **Shows the feed and discharge N concentrations for the continuous flow multi vessel system.**

Fig. 16 shows some reduction in N concentration however not enough to meet the 5mg/L N standard average residual (22mg/L $NO_3$). The average $NO_3$ concentrations across the experimental run were 45.23mg/L for the discharge from a feed of 69.2mg/L.

When comparing these results to the previous single vessel system where there was no net change in N concentration (Fig. 14), It can be seen that there was a significant improvement in uptake rates. These results from phase 2 suggest greater growth.

There was an average net reduction in $NO_3$ of 23.97mg/L which is 5.41mg/L N. There was an average net reduction in P of 2.68mg/L giving an N:P ratio of 2.02:1. Algal physiology would suggest a larger uptake of N. The excess uptake of P compared to N could be explained by the algae using the P during the energy conversion process of photosynthesis rather than using it in cell biomass.

The suspended solids concentration of the discharge was measured across all the experiments carried out with all results showing a 0 or near 0 result.

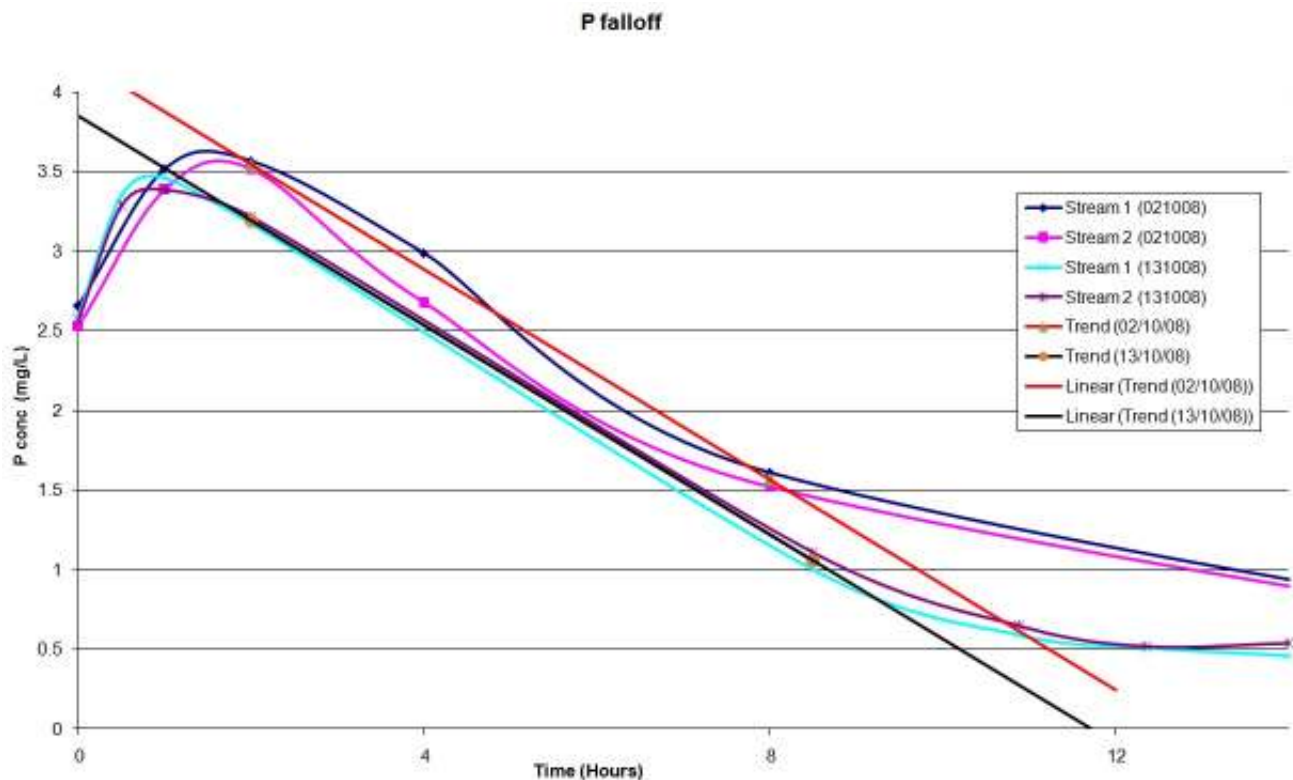## 6.3. Phase 3 – Sequencing Batch Feed Bioreactor

### 6.3.1. P Uptake Rate



**Figure 17 - Shows the change in P concentration after batch feeding.**

Fig 17 is a very important graph, it shows the results from two experiments under the same conditions (using different feed effluent) performed 11 days apart with 2 separated instances run each time. It shows that within each experiment, the separate runs are highly reproducible, producing almost exactly the same results each time. The two experiments differ slightly with regard to their P concentration however the falloff / uptake rate of P is identical at 0.33mg/L/hr.

The trend shows at what point a 0mg/L value of P should occur if the uptake rate was constant. It is expected that the uptake rate would remain constant providing there is available $CO_2$ and soluble P. The raised pH to above 10 could make all the available P insoluble.

If the P becomes insoluble at high pH, it may precipitate out and be retained for the next feed. After feeding the pH is returned to a normal value between 7 and 8 causing the precipitated P to become soluble again; spiking the P concentration to above the initial value of the effluent.

This experiment was repeated many times using varying conditions; the results were highly reproducible and are discussed fully in 6.3.2.2. It was concluded therefore that $CO_2$ is utilised as the preferred source of carbon. The exhausted $CO_2$ and subsequent rise in pH and its effects are attributed to the slowdown in P uptake. This has also been demonstrated by (Ruiz-Marin et al, 2010) who was also researching algal nutrient removal using real wastewater.

## 6.3.2. Comparison between tropical and temperate conditions

Throughout section 6.3.2. I refer to temperate and tropical conditions as stream 1 and stream 2 respectively. Each experiment I carried out was done twice at the same time; in 18°C and 34°C. All the legends used in graphs for this section show 8 lines, 4 from stream 1

and 4 from stream 2. The numbers following the stream indicator on the legend show the start and finish dry biomass weight in mg for that experiment, i.e. the change in biomass over the 24hr experiment. All the experiments were carried out in 4.25L bioreactors.

### 6.3.2.1. pH and Inorganic Carbon

Carbon is required for algal growth in the greatest concentration, according to literature Tezuka (1989), 50-300 times as much by weight than P. The most available source is dissolved $CO_2$ in solution.
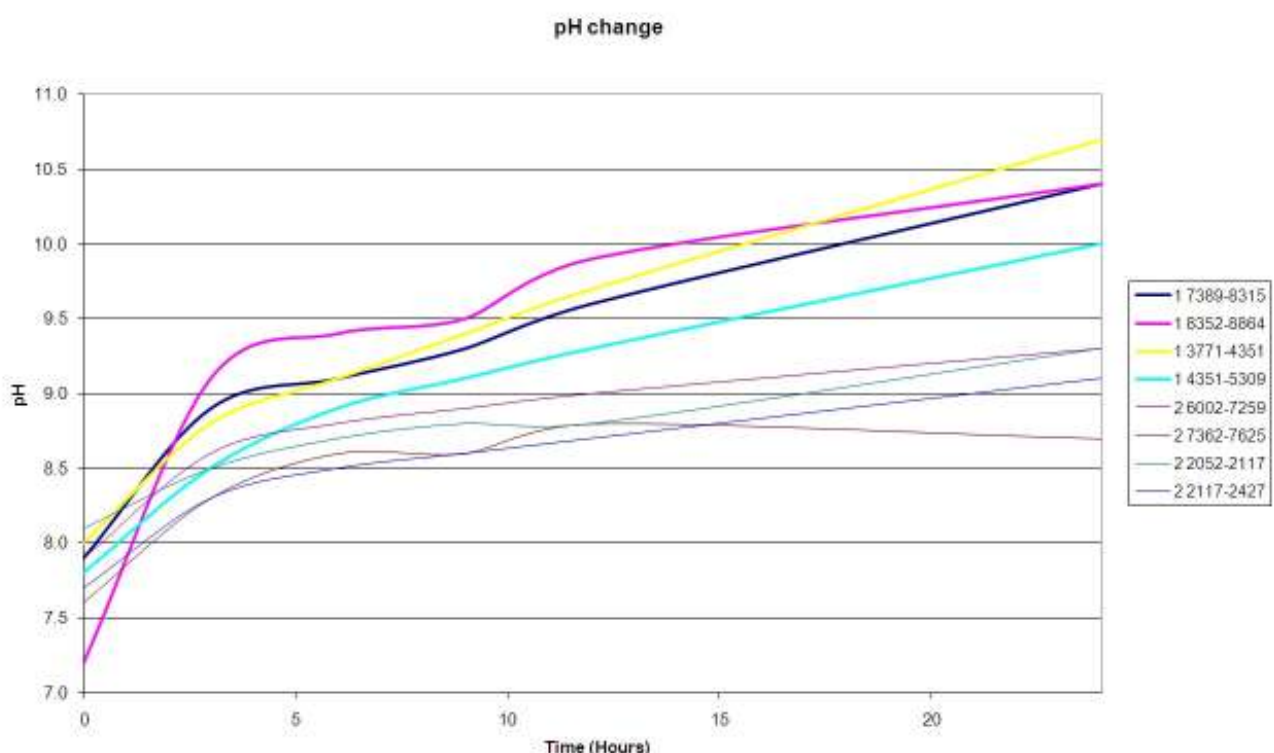


**Figure 18 - Shows the change in pH over time after feeding. The key shows the start and finish total algal biomass (mg) for each repeat of the experiment. The room temperature experimental stream results are highlighted in bold on the graph showing the increased activity.**

If the algae are using dissolved $CO_2$, it would be expected that the solution would become more alkaline as more $CO_2$ via carbonic acid is used faster than it is replaced from the atmosphere. This equilibrium with the atmosphere might be improved by using a shallow turbulent carrousel type reactor that would induce more gas into the solution.

These pH changes are some of the most consistent and reproducible values. The pH is shown to rise from around 7.5 to 10.5 in 24 hours. 50% of the increase in pH happens in the first 4 hours when $CO_2$ is abundant. The rate of increase gradually declines as the available $CO_2$ is reduced.

When $CO_2$ is limited the algae are able to use the carbonates / bicarbonates at a much slower rate, this can be seen in fig. 19 below. This only starts to occur after $CO_2$ has been exhausted at around 10 hours after feeding.
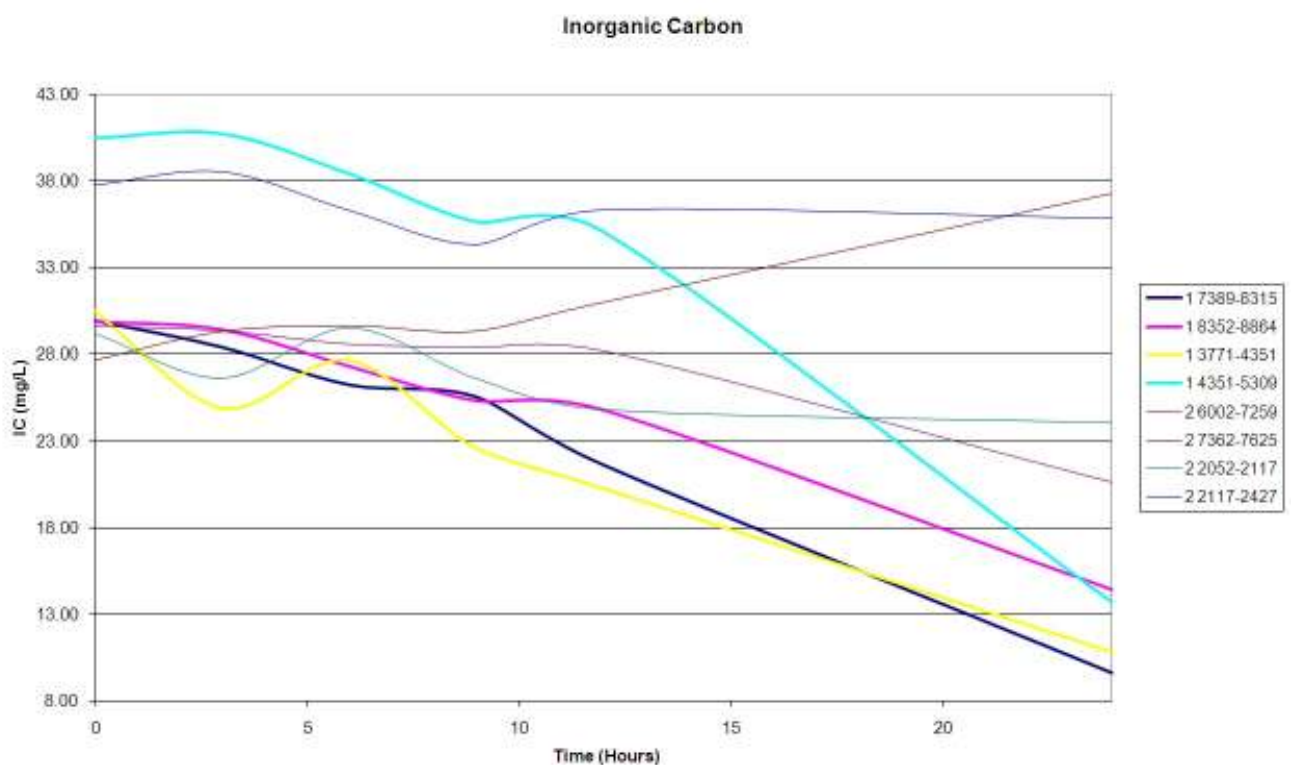


**Figure 19 - Shows the change in IC over time after feeding for different ranges of algal concentration, shown in the key. The room temperature experimental stream results are highlighted in bold on the graph.**

The IC remains constant for the first 10 hours, dropping by about 5mg/L (0.5mg/L/hr) on average in this time. After 10 hours there is a much sharper decline of on average 14mg/L (1mg/L/hr). The data presented in fig. 18 and 19 suggests the $CO_2$ is virtually exhausted within 10 hours of feeding when the carbonates become the source of carbon. The increase rate of IC uptake correlates with the timing of the pH results. As exhaustion of $CO_2$ occurs,

demand for IC increases. The difference between tropical and temperate (normal and highlighted respectively, fig. 19) matches the difference in growth rates.

The lower pH values for the tropical environment compared with the temperate environment suggest $CO_2$ is still available. This correlates with the IC results that show no reduction in IC for the tropical environment. The pH change correlates well with all the other performance indicators, it almost directly links to use of $CO_2$ and hence growth.

When matching this finding with all the other data for the 24 hour batch feed experiments (shown in figs 17-26); the results show a significant reduction in performance after this time, indicating that a lack of $CO_2$ is seriously inhibitory. This is discussed in the following sections.

Growth and nutrient uptake continues but at a much lower rate, suggesting that activity is possible at these high pH values. Work by Ramanan et al showed that pH10 produced the maximum growth rate whilst the pH was artificially controlled and $CO_2$ was kept in excess. This was the only experiment I found in the literature that tested a range of pH values without $CO_2$ being limited.

These results show that algae can use both these common sources of carbon, but showing a preference for dissolved $CO_2$. This would recommend that adding CHP exhaust to the algal bioreactors would improve performance.

$CO_2$ is the least abundant and in greatest demand, and rapidly becomes the limiting factor. The pH can be maintained by replacing the $CO_2$ used, keeping it in excess. This could be achieved by bubbling an additional supply from the base of the bioreactor through the solution. Adding $CO_2$ in surplus would be ideal as long as the pH did not fall to an inhibitory level and could be supplied from the scrubbed biogas or CHP exhaust. The concentration of $CO_2$ addition must be carefully controlled as Yun et al found 5% $CO_2$ to be more effective than 15% $CO_2$. Using a higher $CO_2$ concentration will make it harder to regulate an optimal pH. The literature shows algae typically have a working pH range of between 7 and 12 with

an optimum value of 10. Maintaining optimal pH and surplus $CO_2$ are important and relatively easy variables to manage in the wastewater treatment environment especially with the carbon emission incentives proposed by the regulators.

## 6.3.2.2. P Concentration
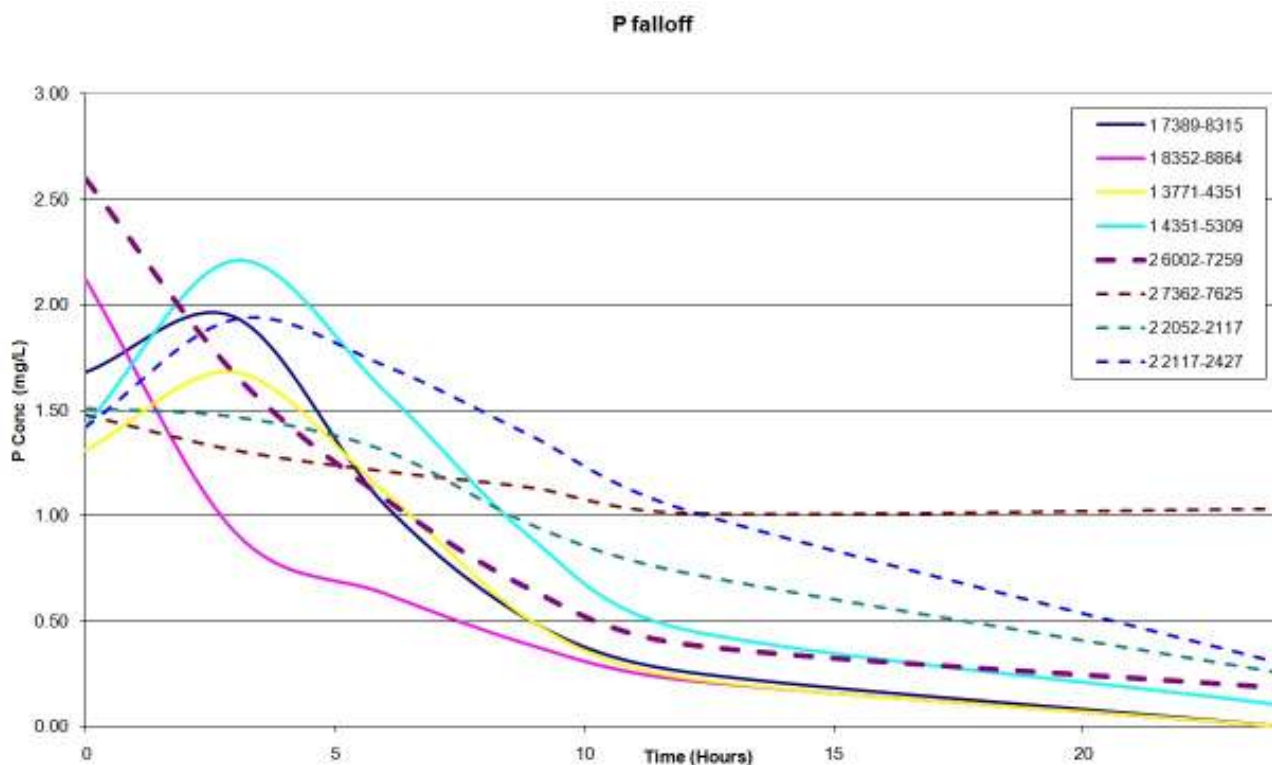


**Figure 20 - Shows the change in P concentration over time after batch feeding for various concentrations of algae. The temperate stream 1 experiments show a better performance over the tropical stream 2 experiments.**

The algal bioreactor at room temperature was consistently reduced the P to 0mg/L concentration. The time taken to reduce the concentration to 0mg/L varies between 12 and 48 hours depending on the availability of essential nutrients and conditions for growth and therefore nutrient uptake. The initial concentration of P was shown to be variable however the results show that the discharge P concentration remains below 0.3mg/L throughout all room temperature experiments. Whilst the P is in demand the uptake rate will increase at higher concentrations. The rapid fall off of nutrient concentration during the first 10 hours could partly be due to an initial excess luxury storage uptake of P which is then held in the

cells for future use when P is exhausted. Algae may buffer the solution by storing easily obtainable nutrients when at high concentration. The uptake is assisted by the concentration gradient. They use their own stores when solution concentration is low. Exhaustion of the $CO_2$ after 10 hours however and subsequent rise in pH could also be affecting the algal metabolism. The trend of reduction for all nutrient uptake and metabolism after around 10 hours is as noted previously across all the data in these results and it is suggested based on this data and previous work that the change in performance are due primarily to available carbon becoming a limiting nutrient.

For fig. 15, 17, 20 there was removal of P down to 0.5mg/L within the first 12 hours after feeding indicating to us that the 1mg/L EU standard could be met within 6 hours (even without further optimisation or $CO_2$ addition). Following this there was a "tail off" of nutrient uptake as might be expected from nutrient limiting conditions. Total removal of P to the detection limit is generally within 24 hours likely to be due to slower metabolism using carbonates as a carbon source. The gradient of P uptake rate shows that maintaining $CO_2$ in excess should result in complete removal of P within 12 hours. This can be seen by the trend line plotted in fig. 17. which is extrapolated and demonstrates total P removal in a 12 hour HRT. This is irrespective of other optimisation factors such as, intensity of light, increase in temperature, selection of specific algal strains, pH control and bioreactor design where the HRT could be further reduced to 6 hours.

In terms of proposed bioreactor design, it is concluded from the data that HRT is one of the most important factors. The HRT is a major influence on cost. The results from these experiments suggest cost-effective HRTs over full life-time analysis.

For a 70,000pe such as Loughborough with a 21ML/day flow, a 5000m$^3$ bioreactor would be required for a 6 hour HRT. At £200/m$^3$ we can expect a capital cost of £1m + other running costs. Some of this cost may be offset by power generation from produced biomass however a full life cycle analysis is recommended to determine full costs and benefits.

According to Severn Trent Water, the costs of chemicals for chemical treatment are rising at 3-4x standard inflation.

An interesting observation on the P concentration patterns was an initial rise in P concentration. This was a consistent observation shown to happen in most of the data collection. Each time the algae are batch fed, the P concentration makes on average a 40% rise for the first 2 hours. It then drops off linearly for the next 6 hours to the $CO_2$ exhaustion point; there is then an inflection and a gradual tail off to 0mg/L at some point within the next 8-16 hours. The times varied according to the initial concentration and HRT as noted.

The phenomenon of an initial rise in P concentration could be explained in a number of ways. Using the theory that algal cells store more nutrient than they can currently use based on the phosphorus accumulating organism model we could hypothesise that when suddenly placed in an environment where P is readily available, the algal cells equilibrium is disturbed causing the release of some of the stored P. This could also be an action in response to the sudden availability of P. This would assume a voluntary release of P.

A second explanation could be an involuntary release of P caused by stress. When the algae are batch fed, the mixing device is switched off and the algal cells allowed to settle to the bottom of the tank. Here they form a concentrated layer enabling the 'treated' effluent to be decanted. The concentrated algal sludge remains in the bottom of the tank, before a new batch of final effluent is added for feeding. This period of 5-10 minutes, in a highly concentrated, non-mixed, dark, low carbon environment could be enough to stress or shock the cells into releasing their stored nutrients. A similar phenomenon is found with bacteria stressed by low redox (BPR). None of the other papers found in the literature went into as much detail in analysing P uptake and release and therefore I cannot determine if anyone else has noted this.

The different batches and experiments with the algae show a very consistent P concentration fall off gradient (0.4mg/L/hr +/- 0.15mg/L/hr) during the first 10 hours when

at steady state. Small variations in it would be caused by: initial P concentration, algal concentration and availability of light. From fig. 20 we have a P uptake of 0.35mg/L/hr along with a matching N uptake of 0.25mg/L/hr, this resulted in an algal growth rate of 30mg/L/hr (360mg/L/day, taking into account the algae really only grow vigorously for 10 hours until the $CO_2$ is exhausted. This equates to 29.4mg/L/hr C giving a C:N:P ratio of around 84:1:1 which is reasonable. A C:N:P ratio of 50-300:6:1 might be expected from the literature (Tezuka, 1989) but the ratio has been shown to vary depending on species as demonstrated from the numerous accounts in Section 4. According to Park et al the ratio of N:P in algal biomass can vary from about to 4:1 to almost 40:1 depending on algal species and nutrient availability in algal culture. All the results gathered with green chlorophyta algal flocs show an N:P ratio of between 2-3.5:1 however the results with the blue-green filamentous algae show more in the region of 5.5:1 which is closer to what is expected for algae in general.

### 6.3.2.3. N Concentration
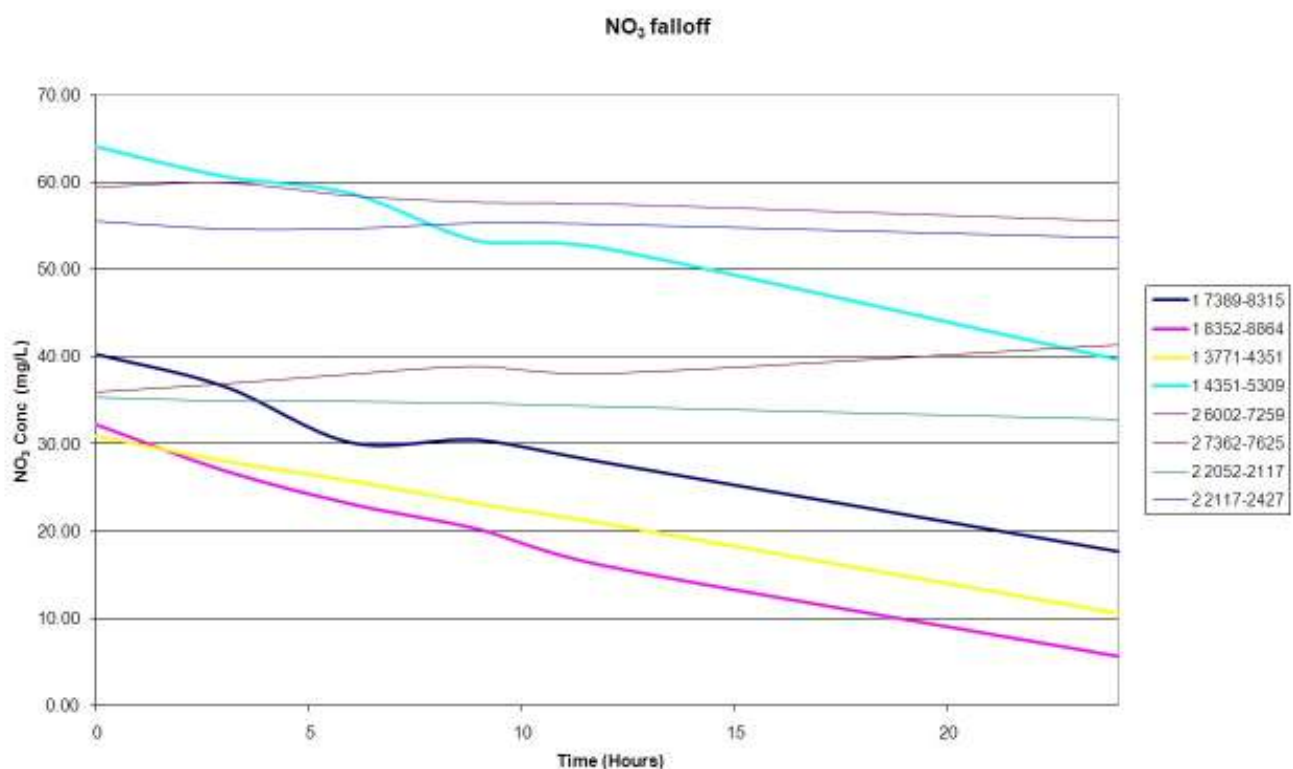


**Figure 21 - Shows the change in N concentration over time after batch feeding. Stream 1 are highlighted bold on the graph.**

Under tropical conditions, the results show there was no uptake of N. Under temperate conditions, the uptake of N was consistent at 0.25mg/L/hr regardless of the initial concentration compared to P as noted before at 0.35mg/L/hr.

Comparing the N uptake with P uptake, over 24 hours there was on average 5.3mg/L/day as N and 1.5mg/L/day as P giving an N:P ratio of 3.53:1. This was higher than the 2.02:1 measured in the continuous flow multi vessel system; in this case the initial P concentration was on average twice as high which could also explain the difference as well as the different reactor design.

### 6.3.2.4. Metals

Originally five elements were selected to test for (Section 5.2.1.5.), 2 of these showed no change in concentration, so the analysis was reduced to 3; Fe, Ni and Zn.

Metal ions will change in solubility with the pH rise caused by the algal growth. I have shown the pH to increase considerably but the rapid drop in metal concentration shown fig. 22 is faster than the rise in pH.
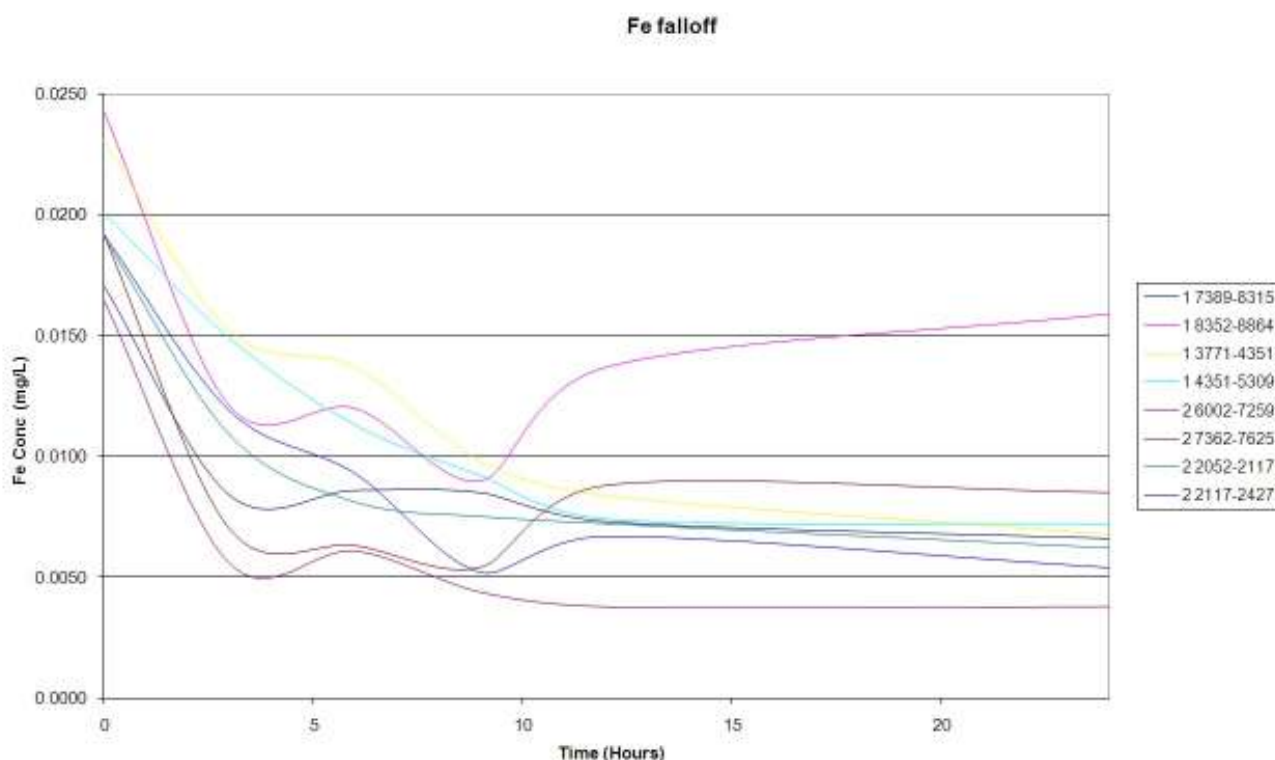
**Figure 22 - Shows the change in Fe concentration over time after feeding.**

There is an obvious drop in FE concentration within the first 2 hours of feeding (0.02-0.0075mg/L average). This could be explained by the ion binding to the algal cells or a metabolic use triggered by the growth rate at the high concentration of carbon. Following the initial 2 hours, the concentration remains constant which could be due to the decrease in solubility of FeOH as the pH rises. Some organic iron would be expected to have a high bioavailability, these may be used quickly resulting in this early drop in concentration. This leaves behind Fe ions which have low bioavailability to the algae, hence showing no change in concentration for the remaining time of the experiment. Iron is also poorly soluble, it could be a trace element that is rate limiting.
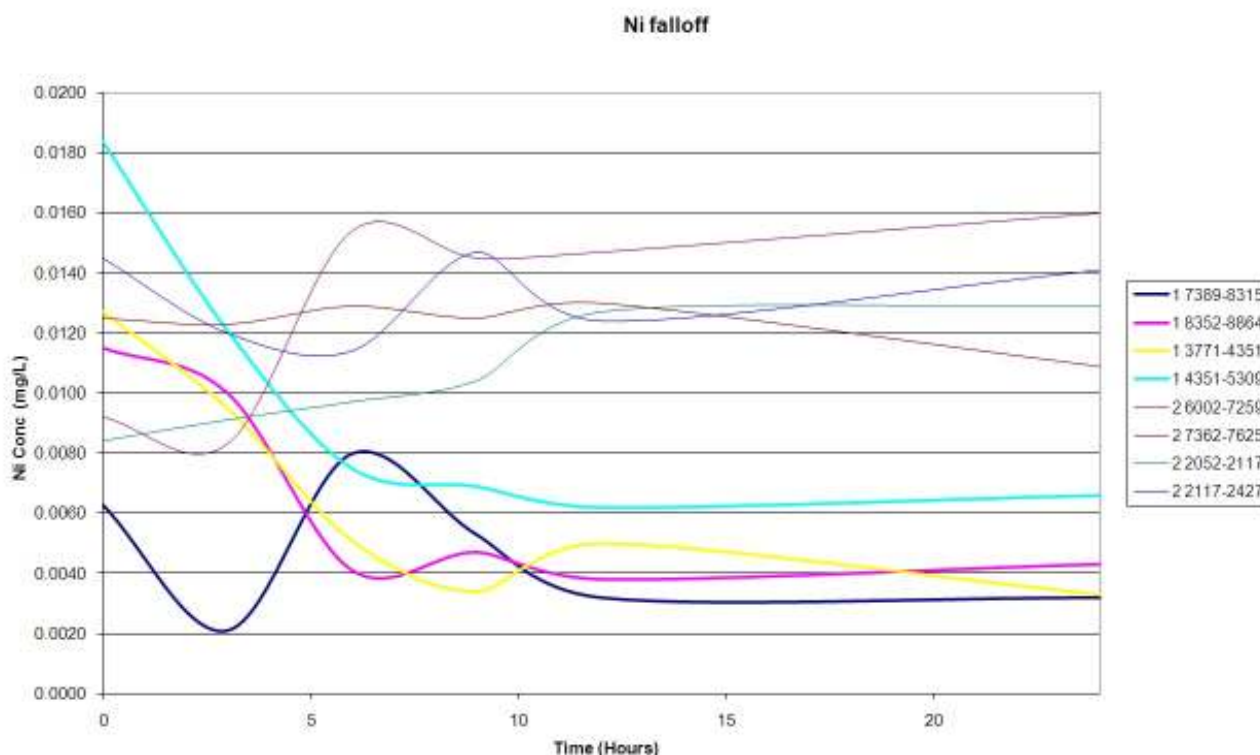
**Figure 23 - Shows the change in Ni concentration over time after feeding. Room temperature experiments are highlighted.**

At temperate conditions, Ni shows very similar properties to Fe, except over a slightly longer time period of about 5-10 hours. In tropical conditions, there is no effect on Ni concentration. Solubility might be expected to increase in a warmer environment but this is not consistent as the results show a slight rise in concentration.

Ni shows similar behaviour to Fe; after the initial 10 hour period, the Ni concentration does not change, this could also be linked to the reduction in metabolism.
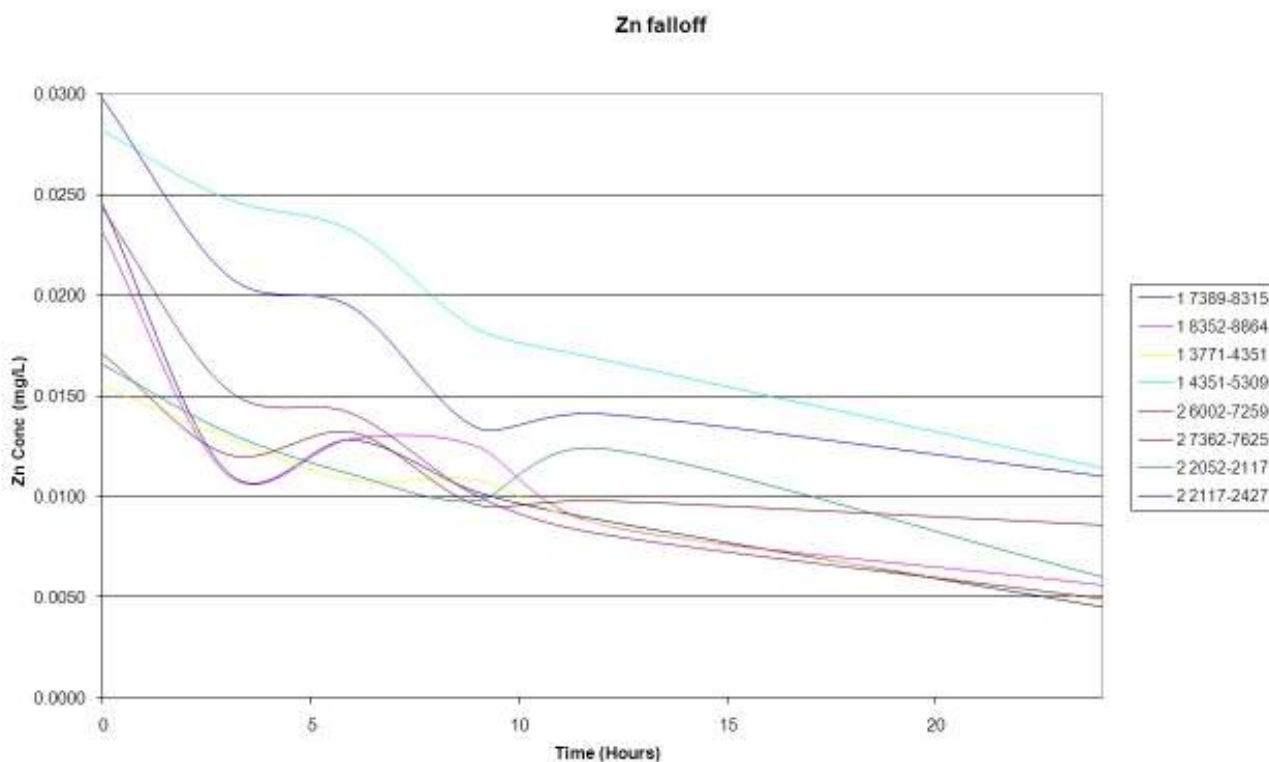
**Figure 24 - Shows the change in Zn concentration over time after feeding.**

The Zinc pattern in Fig. 24 is similar to both Fe and Ni with an uptake followed by a rise. Over both temperature conditions, Zn has a steady fall off over 24 hours (0.025-0.01mg/L average). 0.01mg/L in the first 10 hours and 0.005mg/L in the next 14 hours; a reduced rate after 10 hours as shown with other nutrients.

### 6.3.2.5. Growth rate
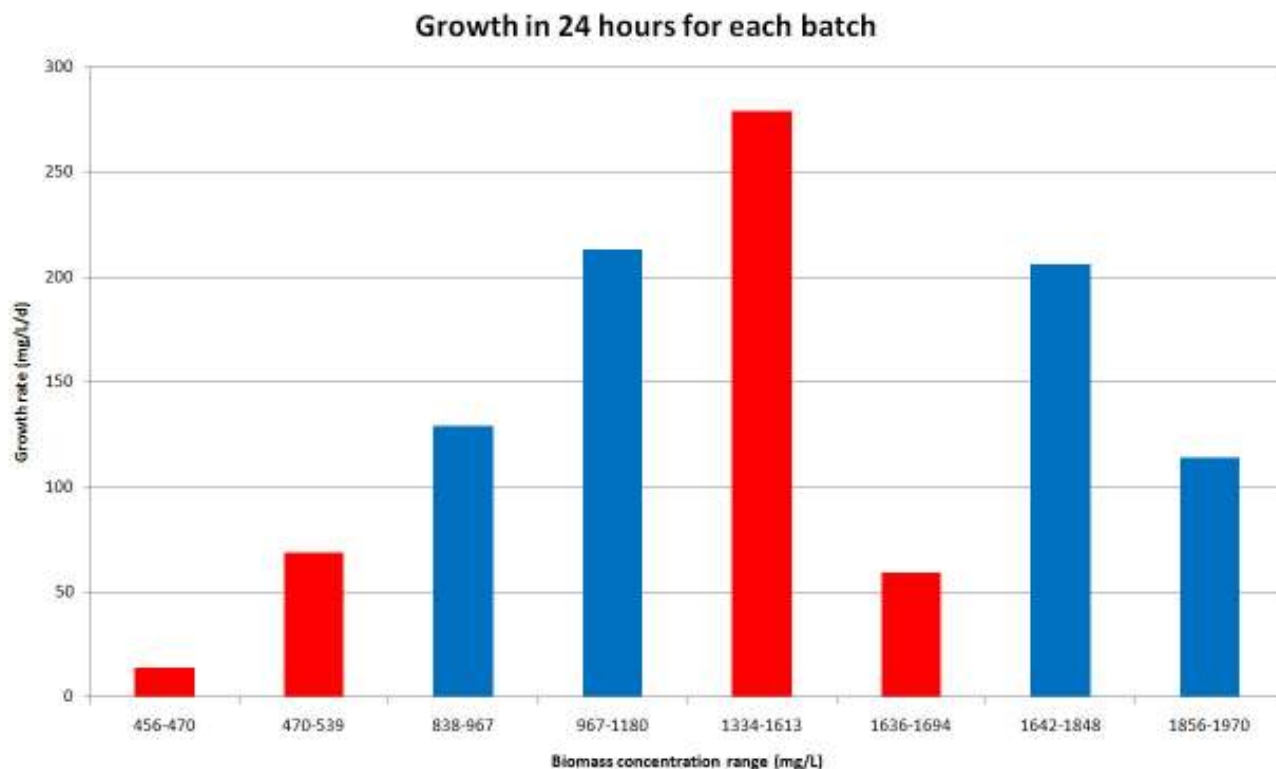


**Figure 25 - Shows the growth rate within the 4.5L bioreactor for a range of biomass concentrations. Temperate conditions shown in blue, tropical in red.**

Fig. 25 indicates that in general, growth rate is a function of biomass concentration, proportional to the original inoculum size up to an optimum of around 1.4g/L. Above this however the reduced growth rates are attributed to competition for light and nutrients. Fig. 25 does show an anomaly at concentration 1636mg/L during the tropical phase of experiments. This anomaly may be due to the unstable nature of the tropical experiments as we have seen from fig. 17 onwards.

### 6.3.2.6. Effects of temperature

Overall the performance indicators it has been shown that the temperate condition experiments noticeably outperformed the bioreactors in the tropical conditions. The exception to this was the tropical experiment between biomass concentration of 1.3-1.6mg/L. This showed the highest growth rate of all experiments and the corresponding P uptake is shown in Fig. 20 which correlates perfectly with this finding. The growth rates for

all the experiments can be seen in fig. 25. They all match up with their respective results for P uptake, heavy metal uptake, pH change and inorganic carbon use showing a strong relationship between biomass concentration -> nutrient use -> growth rate. The best growth in the temperate conditions occurred at an algal concentration of between 1-1.8g/L and between 1.3-1.7g/L in the tropical conditions.

Metabolism of algae that naturally occur in low temperature regions have been shown to perform best and efficiently within their natural temperature range. When the temperature is raised to 34°C+, performance in terms of N, P and C uptake drops significantly. Over a long enough time period, the algae would be expected to adapt to increased temperatures and outperform the cold temperature conditions. At warm temperature, over a number of weeks and months, performance did improve as the culture adapted, a visual change was also noticed in the appearance characteristics of the algae but this was not explored further as the process of natural selection and evolution is beyond the scope of this project. It is recommended that this is an important area for development of the process and should be explored in more depth in further work.

The best achievable performance would in theory be under hot conditions and this is likely to be linked to high light intensity, using an algal strain that has evolved to operate at these tropical temperatures. This has been shown by Cromar et al who demonstrate the importance of light intensity and temperature on growth rate achieved.

Each algal species will have evolved to suit the local climate and conditions, The best suited wild type algae to specific conditions will then dominate at that time. With regard to artificially raising the temperature to tropical conditions in a naturally cold climate, no or few algae species suited to these conditions will be present to form a new culture. Unless a specific culture inoculum is added, but the naturally occurring species will slowly adapt through natural selection enabled by very fast reproduction rates.

In the tropical condition experiment, the P removal was not as adversely affected as the N removal which was completely inhibited (Fig. 21).

### 6.3.2.7. Effects of light

The 8 experimental runs with phase 3 reactor as discussed above were repeated but this time the light sources were moved 10cm away from the bioreactor walls. This reduced the light from 136W/m$^2$ to 39W/m$^2$.
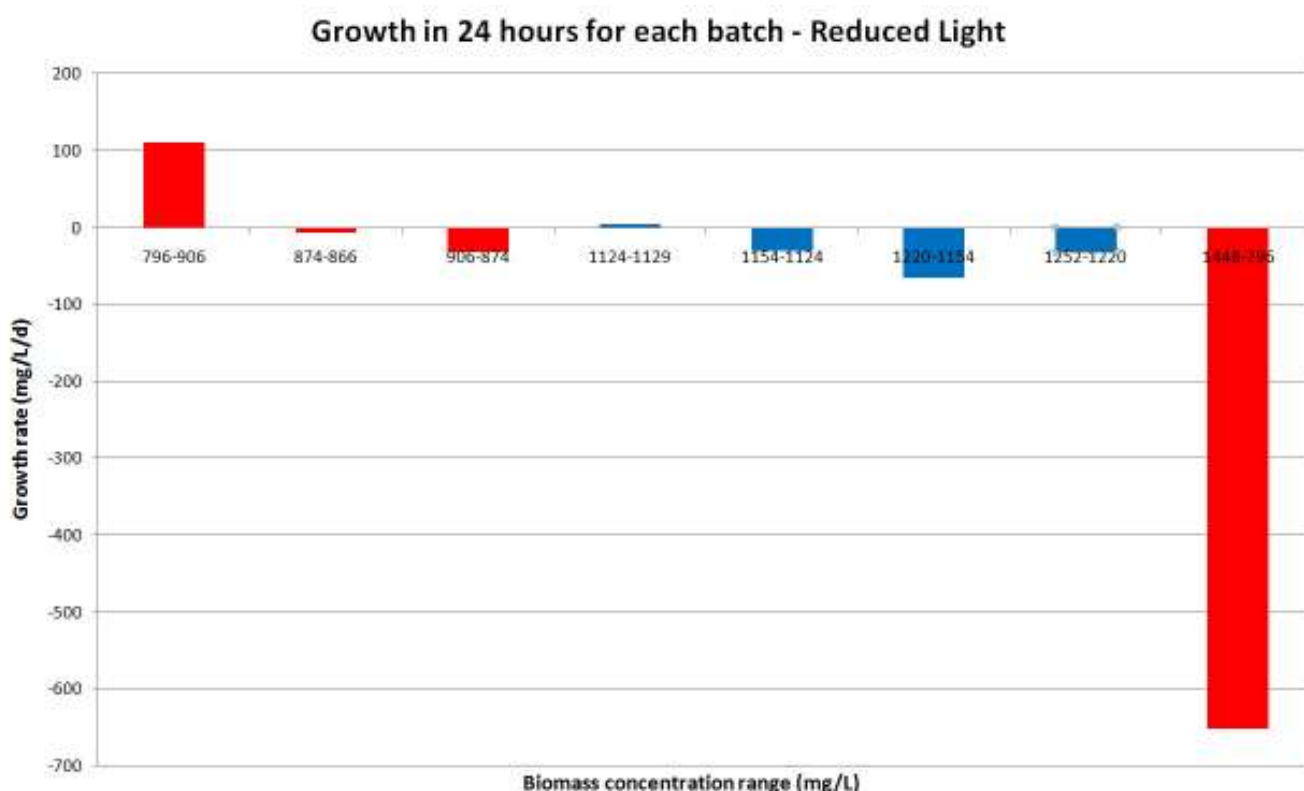


**Figure 26 - Shows the growth rate within the 4.5L bioreactor for the low light experiments at 39W/m$^2$. Temperate conditions shown in blue, tropical in red.**

The experiments shown in fig. 26 show in most cases decay and death of the algae. The growth rates are mostly negative, showing a net loss in biomass. The results for the performance indicators correlated with this and can be found in appendix 1.

It was concluded from these results that the performance of an algal bioreactor system was mainly dependent on the intensity of light. The bioreactor required a minimum of 97-

$136W/m^2$ to be effective. The normal daylight intensity in the UK for the shortest day of the year is $505W/m^2$ in the sun and $78W/m^2$ in fully overcast conditions (as found from the light meter data collected with this project).

### 6.3.3. Change in P concentration over time and the effect on algal species
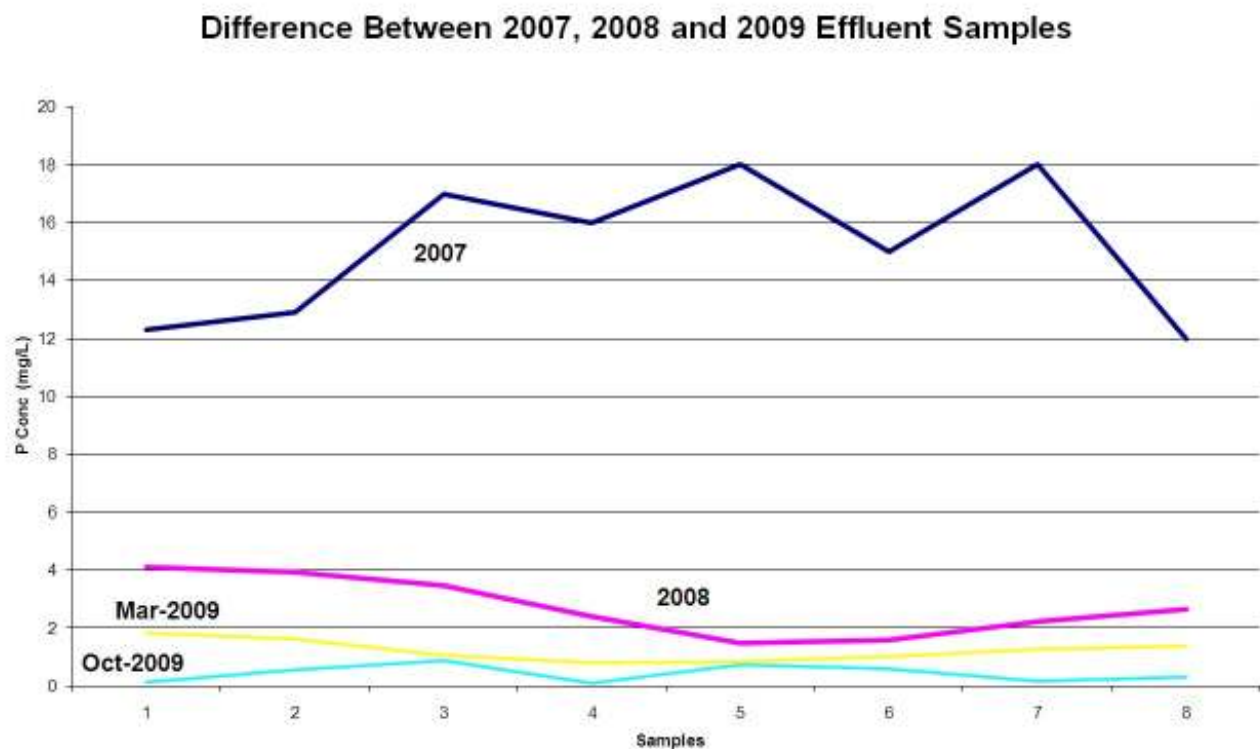


**Figure 27 - Shows the change in Loughborough sewage works final effluent P concentration over the 3 year period of the project. It reflects the installation of a RAS fermenter at the works.**

Over the 3 years of experimentation (Nov, 06 – Nov, 09), final effluent was collected from Loughborough sewage treatment works (LSTW). The P concentration of every sample from LSTW was recorded over this period. The P concentration in the effluent steadily reduced over this period. This reduction is due to the installation of BPR augmented by a new RAS fermenter to provide soluble P removal at the works to meet WFD.

The Severn Trent works in Loughborough choose to use this new technology that extends the Bardenpho process shown by Barnard & Oleszkiewicz (2005) in section 3.3.1. A side stream fermenter is added into the RAS return loop as an extra process as shown in fig. 28.

This side stream takes a small proportion of return sludge and allows it to ferment over several days to release more VFA which then pass back into the anaerobic reactor.
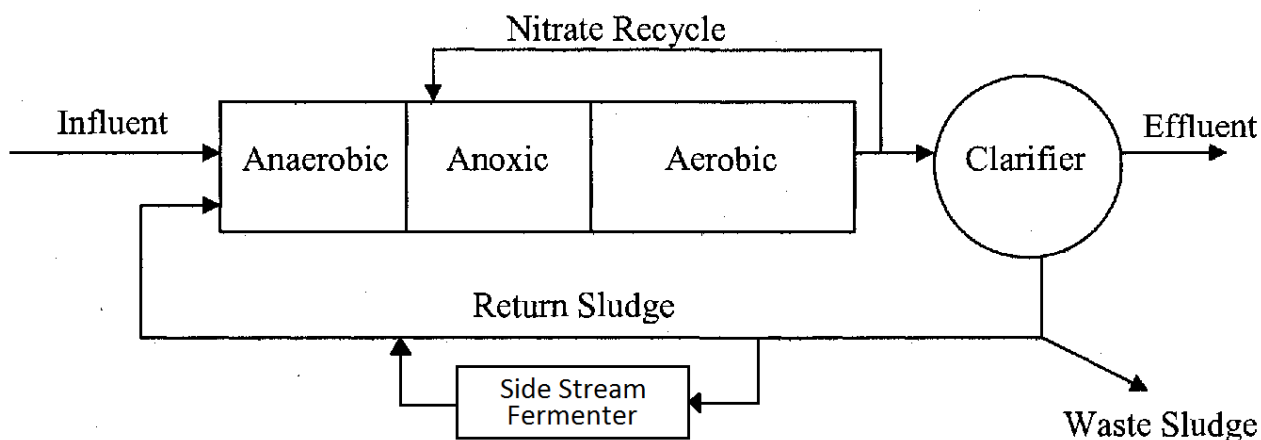


**Figure 28 - Example of a side stream fermenter in the Bardenpho process**

As shown by the graph, the pre-2008 P concentration varied between 12-18mg/L due to rainfall effects. This is many times higher than the required 1mg/L WFD standard for P. With the installation of the new RAS fermenter at LSTW, by 2008 the P concentration is shown to drop suddenly below 4mg/L, ranging between 1.49mg/L and 4.12mg/L through the year.

As this process became established within LSTW, it became increasingly effective, reducing P to between 1-2mg/L by early 2009 and then by late 2009 consistently meeting the WFD standard of 1mg/L P.

LSTW is a great case study for this technology as these independent results show a dramatic improvement in P reduction after its installation, followed by gradual increase in effectiveness to eventually have LSTW able to meet the required EU standard through the installation of the RAS fermenter.

The steady reduction in P caused it to become limited with respect to N, favouring an environment suited to N fixing algae. There was a subsequent change in the algal species from unicellular green algae to a filamentous blue-green algae (known for their N fixing

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capabilities). N removal increased but this had a drastic effect on P removal, producing unfavourable results. This showed that N removal was possible however algal growth rates were significantly lower, less than a 1/3 of that experienced with the mix of P favouring unicellular algae.

Samples from the bioreactor were studied under the microscope on numerous occasions. It was expected that the mix of algal species seen at various times would change. Without in depth analysis it is impossible to say just how the specie concentrations changed. It was noted that in general, the common species remained similar and examples can be seen in the microscope photos taken.



**Figure 29 - Shows the diversity of algal species initially found in the bioreactor samples. A) Scenedesmus denticulatus B) Pediastrum boryanum var. Longicorne C) Micractinium quadrisetum D) Closterium ehrenbergii E) Pediastrum**

boryanum var. Cornutum F) *Scenedesmus dimorphus*  G) *Scenedesmus communis*  H) *Chroococcus turgidus*  I) *Golenkinia paucispina* J) *Closterium moniliferum* K) *Oedogonium obtruncatum* L) *Cylinddrospermum muscicola* M) *Chlorella vulgaris.*
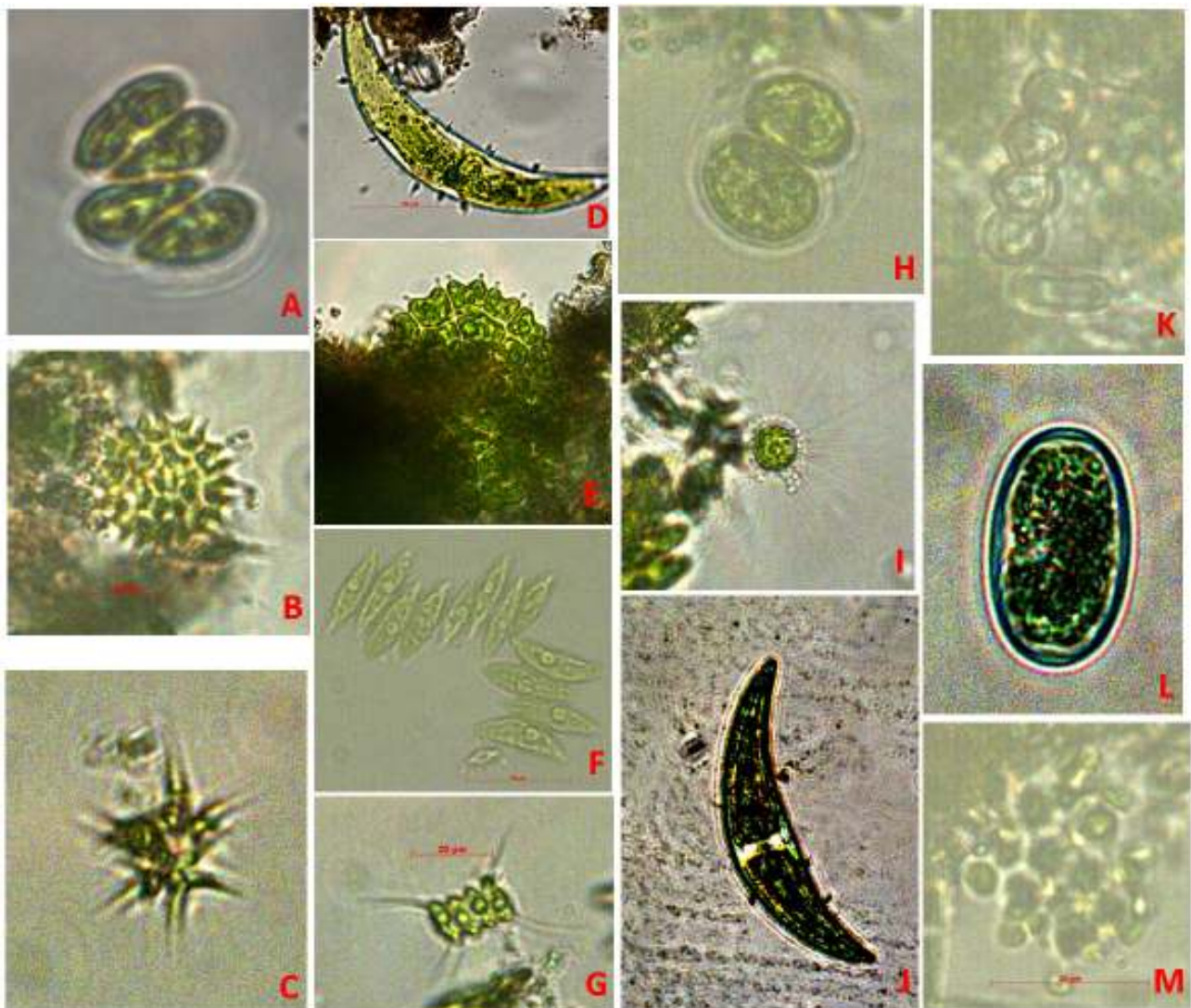
Whilst studying the algal samples under a microscope, many different species of algae were found, far more than the extent of the photos show. Although there are no pictures, members of the *Euglena* class of algae were identified as a common specie.

The microscopic analysis of the samples suggested that the *Scenedesmus* class were the most common species but it is possible they may have been the most easily identifiable algae. A species that tended to clump together such as chlorella were more difficult to identify. The majority of the photos come from cells found alone as most of the algae being viewed were in large groups making it impossible to distinguish individual shapes.

The micrographs taken were identified by comparing them to drawings and images found in the book, "Freshwater Algae: Identification and Use as Bioindicators". The species identified are as follows: *Scenedesmus dimorphus, Scenedesmus denticulatus, Scenedesmus ellipticus, Scenedesmus communis, Oedogonium obtruncatum, Closterium moniliferum, Closterium ehrenbergii, Pediastrum boryanum var. longicorne, Pediastrum boryanum var. cornutum, Golenkinia paucispina, Micractinium quadrisetum, Chlorella vulgaris, Cylinddrospermum muscicola, Chroococcus turgidus. Euglena gracilis* and other species in the *Euglena* class were found although as previously mentioned I did not capture any micrographs of these.

*Scenedesmus denticulatus* and *Scenedesmus dimorphus* were the most commonly identified and are considered an indicator of mildly to moderately nutrient enriched water (John et al, 2003). Sewage oxidation ponds have been shown before to support extensive growth of unicellular algae, e.g., Chlorella, Chlamydomonas, Scenedesmus and Euglena (Kumar and Singh, 1979). When pond temperature is about 15°C *Scenedesmus* and *Euglena* have the advantage (Azov et al). G. Shelef (1980) studied algal species in wastewater under varying parameters; season, temperature, pH, organic loading, retention time, depth and agitation

methods. He found that the algal population in wastewater was made up of six major algae species. *Euglena gracilis, Scenedesmus dimorphus, Chlorella vulgaris, Ankistrodesmus falcatus, Actinastrum gracillimum* and *Micractinium pusillum.* Although they all have optimal temperatures around 25°C, *Scenedesmus* and *Euglena* are more tolerable to low temperatures and tend to dominate at around 15°C.

The experiments I carried out had a normal operating range of between 12-18°C and I also found that *Scenedesmus* and *Euglena* dominated throughout the majority of the experiments until the species switched as detailed below.

The drop in P concentration had a dramatic effect on the algal species and overall growth rate. In 2009 when the average P concentration in the effluent feed dropped to around 1mg/L there was a change in the algal species present in the bioreactor. The algae type changed from flocculating unicellular green algae, *Scenedesmus, Euglena* and *Chlorella* that were predominately P dependant to a uniform long filamentous blue-green algae that formed larger filament strand entangled flocs with a higher affinity for N compared with P. This change in nutrient uptake behaviour was directly paralleled by the drastic change in the type of algae present in these open reactors.

## 6.3.4. N Uptake Rate
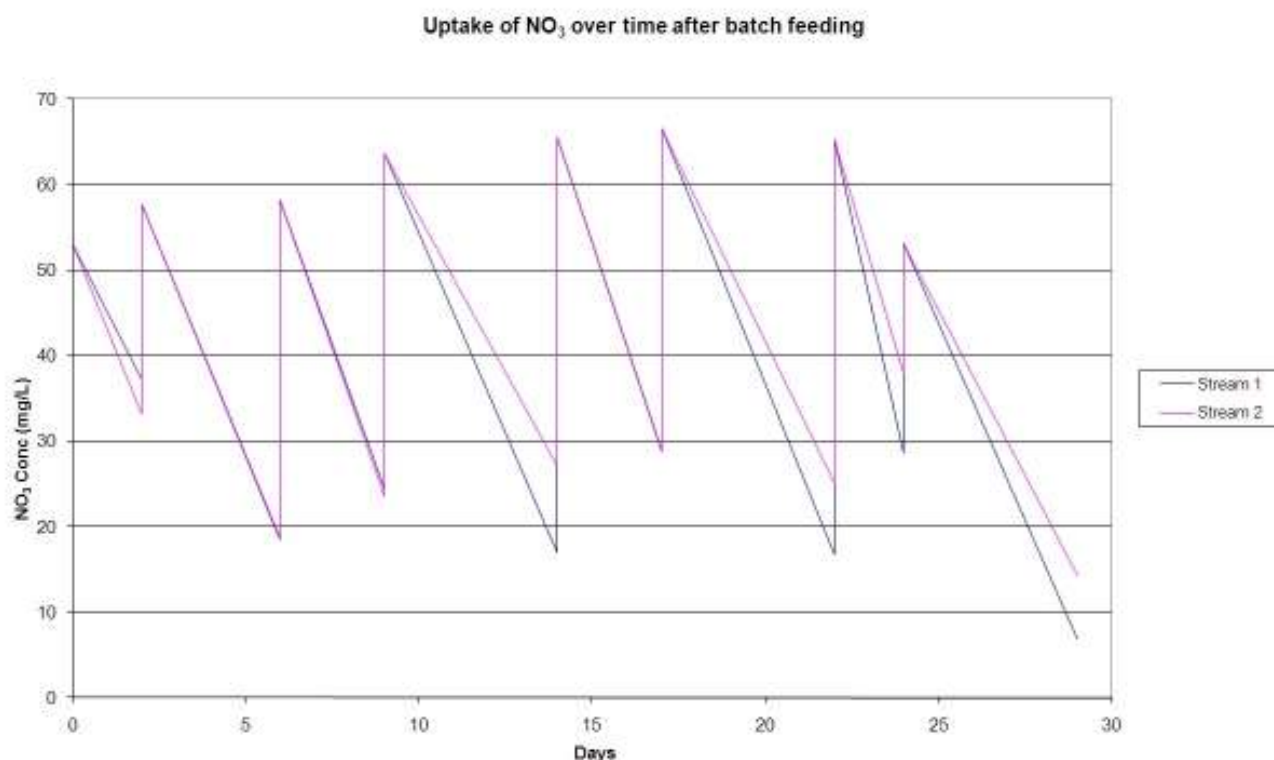


**Figure 30 - Shows the uptake of N over time and the reproducibility of the results between 2 independent streams. The saw tooth effect is caused by the cyclic batch feeding followed by N uptake by the algae.**

The first thing to note from the results shown in fig. 30 is the consistency in values between two independent bioreactors. Using the same effluent feed source, over the same time period, they had an almost identical performance.

The gradients of the lines vary between 7-18mg/L/d $NO_3$ uptake. The change in gradient correlates with the batch process time; the shorter batches have an overall faster uptake rate. This is in-line with the previous results, showing an increased uptake rate immediately after feeding where all nutrients are in excess, compared with the uptake rate after two days where the performance tails off due to the exhaustion of $CO_2$.

Fig. 30 shows that reaching the required standard of 22mg/L $NO_3$ (<5 as N) is possible however with the reduction in productivity after 2 days the required HRT shown here of 4-5 days was not a realistic option without optimisation. The fastest uptake rate from the

results was 0.75mg/L/hr which is slower than the rate achieved with the unicellular green algae in previous experiments of 1mg/L/hr. At 0.75mg/L/hr, a HRT of 2.2 days is required to reduce the concentration of $NO_3$ by 40mg/L.

The N concentration in the effluent feed for this experiment was on average 13.5mg/L N, 18x as high as the P concentration, 0.75mg/L P. Throughout all the experiments, although with this one in particular the N has always been in excess with respect to the theoretical predicted growth requirements based on an N:P growth ratio of 6:1. This would explain why it has been more difficult to achieve an N concentration of <5mg/L as quickly as is possible for P.

Experiments using the green algal flocs (section 6.3.2.3.) were shown to be able to reduce $NO_3$ to less than 22mg/L within 24 hours without optimisation of the bioreactor to enhance growth. This performance in uptake rate and algal growth rate was not be matched by the blue-green filamentous algae used in the low P experiments.

Molecular diffusion mass transfer theory would suggest that nutrient capture rates are greater when concentrations are at their highest. However there are no results in these experiments to back this up because this behaviour seen in results is better accounted for by the predominant effect of $CO_2$ on metabolism. It is recommended that carrying out further experiments keeping $CO_2$ in excess whilst maintaining an optimal pH (8-10) would be better for monitoring of P and N uptake rates. These experiments were limited by $CO_2$.

## 6.3.5. Importance of light and comparison between outdoor and laboratory results
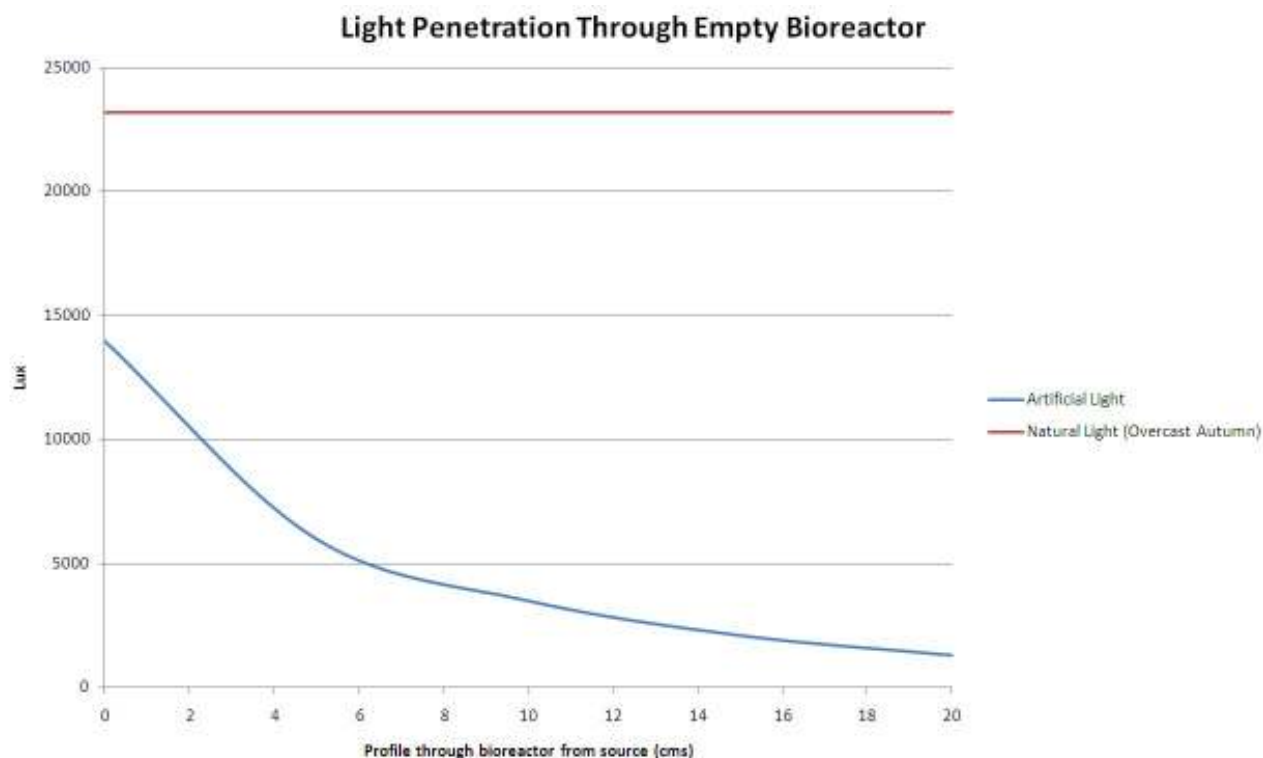


**Figure 31 - Shows the difference in light penetration through an empty bioreactor, comparing natural and artificial light.**

It has been suggested that the most often encountered limit to algal growth rate is the intensity of light (Richmond 2004) and it is the concentration of biomass within the reactor usually limits light penetration and growth. Mixing energy can be used to overcome this limitation by moving algae in and out of the lighted regions.

Fig. 31 shows how low the intensity of light is from an artificial source compared to natural light and how quickly it reduces with distance. For artificial light, the reduction of light intensity with distance through the bioreactor is very high. The light source is adjacent and intensity decreases with distance due to the inverse square law. Natural light intensity does not reduce with distance; the source is of infinite distance away when compared to the range of distances being measured causing the photons to appear parallel in nature. The atmosphere also causes diffraction of the light meaning the photons hit the target from a much wider angle rather than just the point source. Natural light also has a much broader

spectral range. Natural light is far more variable in intensity with large changes between night and day and between cloud and direct sun.

**Table 4 - Shows the results from the light intensity data collected during the course of the experiments. 6 Results were required for each time of year, these were all recorded within 3 days of the date shown and within 30mins of the time shown when the specific conditions were met. Overcast results indicate 8/8 oktas cloud cover.**

| Date | Time of Day | Condition | Light Intensity ($W/m^2$) |
|---|---|---|---:|
| 21st Dec 2009 | 12:00 | Overcast | 76 |
| 21st Dec 2009 | 15:00 | Overcast | 14 |
| 21st Dec 2009 | 12:00 | Direct sunlight | 605 |
| 21st Dec 2009 | 15:00 | Direct sunlight | 256 |
| 21st Jan 2010 | 12:00 | Overcast | 85 |
| 21st Jan 2010 | 15:00 | Overcast | 26 |
| 21st Jan 2010 | 12:00 | Direct sunlight | 700 |
| 21st Jan 2010 | 15:00 | Direct sunlight | 58 |
| 21st Feb 2010 | 12:00 | Overcast | 95 |
| 21st Feb 2010 | 16:00 | Overcast | 41 |
| 21st Feb 2010 | 12:00 | Direct sunlight | 843 |
| 21st Feb 2010 | 16:00 | Direct sunlight | 171 |
| 21st Mar 2010 | 12:00 | Overcast | 125 |
| 21st Mar 2010 | 16:00 | Overcast | 80 |
| 21st Mar 2010 | 12:00 | Direct sunlight | 1103 |
| 21st Mar 2010 | 16:00 | Direct sunlight | 454 |
| 21st Apr 2010 | 13:00 | Overcast | 131 |
| 21st Apr 2010 | 17:00 | Overcast | 107 |
| 21st Apr 2010 | 13:00 | Direct sunlight | 1126 |
| 21st Apr 2010 | 17:00 | Direct sunlight | 707 |
| 21st May 2010 | 13:00 | Overcast | 142 |
| 21st May 2010 | 18:00 | Overcast | 137 |
| 21st May 2010 | 13:00 | Direct sunlight | 1168 |

| | | | |
|---|---|---|---|
| 21st May 2010 | 18:00 | Direct sunlight | 823 |
| 21st Jun 2010 | 13:00 | Overcast | 172 |
| 21st Jun 2010 | 18:00 | Overcast | 150 |
| 21st Jun 2010 | 13:00 | Direct sunlight | 1244 |
| 21st Jun 2010 | 18:00 | Direct sunlight | 994 |
| 21st Jul 2010 | 13:00 | Overcast | 178 |
| 21st Jul 2010 | 18:00 | Overcast | 133 |
| 21st Jul 2010 | 13:00 | Direct sunlight | 1179 |
| 21st Jul 2010 | 18:00 | Direct sunlight | 856 |
| 21st Aug 2010 | 13:00 | Overcast | 141 |
| 21st Aug 2010 | 17:00 | Overcast | 113 |
| 21st Aug 2010 | 13:00 | Direct sunlight | 1142 |
| 21st Aug 2010 | 17:00 | Direct sunlight | 681 |
| 21st Sep 2010 | 13:00 | Overcast | 121 |
| 21st Sep 2010 | 17:00 | Overcast | 76 |
| 21st Sep 2010 | 13:00 | Direct sunlight | 1117 |
| 21st Sep 2010 | 17:00 | Direct sunlight | 497 |
| 21st Oct 2010 | 13:00 | Overcast | 115 |
| 21st Oct 2010 | 17:00 | Overcast | 45 |
| 21st Oct 2010 | 13:00 | Direct sunlight | 865 |
| 21st Oct 2010 | 17:00 | Direct sunlight | 217 |
| 21st Nov 2010 | 12:00 | Overcast | 96 |
| 21st Nov 2010 | 15:00 | Overcast | 30 |
| 21st Nov 2010 | 12:00 | Direct sunlight | 698 |
| 21st Nov 2010 | 15:00 | Direct sunlight | 66 |

**Table 5 - Shows the amount of daylight hours received per day for different months throughout the year in central London, latitude 51° 30' North.**

| Month | Daylight Hours / Day |
|---|---|
| December | 7.5 |
| January, November | 8 |
| February, October | 10 |
| March, September | 12 |
| April, August | 14 |
| May, July | 16 |
| June | 16.5 |

An experiment moving the source 10cm away (in 1cm increments) from the reactor wall, reducing the light intensity from 136-29W/m$^2$ demonstrated a decrease in algal productivity. During the experiments carried out with the light source placed 10cm (29W/m$^2$) away from the reactor wall, algal growth stopped and began to biodegrade. All other performance indicators remained constant showing no algal activity.

Light intensity through the reactor with respect to depth into the tank is a factor of surface light intensity and concentration of algal biomass. As the concentration of algal biomass increases, light penetration into the bioreactor decreases. Mixing not only helps to keep algae in suspension but also ensures they spend an equal amount of time in adequately lighted regions. The literature data from various papers show a general consensus of a maximum carrousel reactor depth of 30cm.

When comparing full artificial lighting with UK natural light (Fig. 31) it can be seen that the luminosity of natural light is in a much higher order of magnitude than full artificial lighting. Summer natural light – 1165W/m$^2$, winter natural light – 582W/m$^2$, full artificial light – 136W/m$^2$. A typical overcast autumn day will achieve around 194W/m$^2$.

The natural light will contain a spectrum much more suited to biological growth than artificial light due to the evolution of the organism under natural light. The algae being used in this process originally derived from a local wastewater treatment plant will have adapted and evolved to suit this specific spectrum of light. Ignoring luminosity, the growth rates in natural light would be expected to be higher due to the spectrum alone.
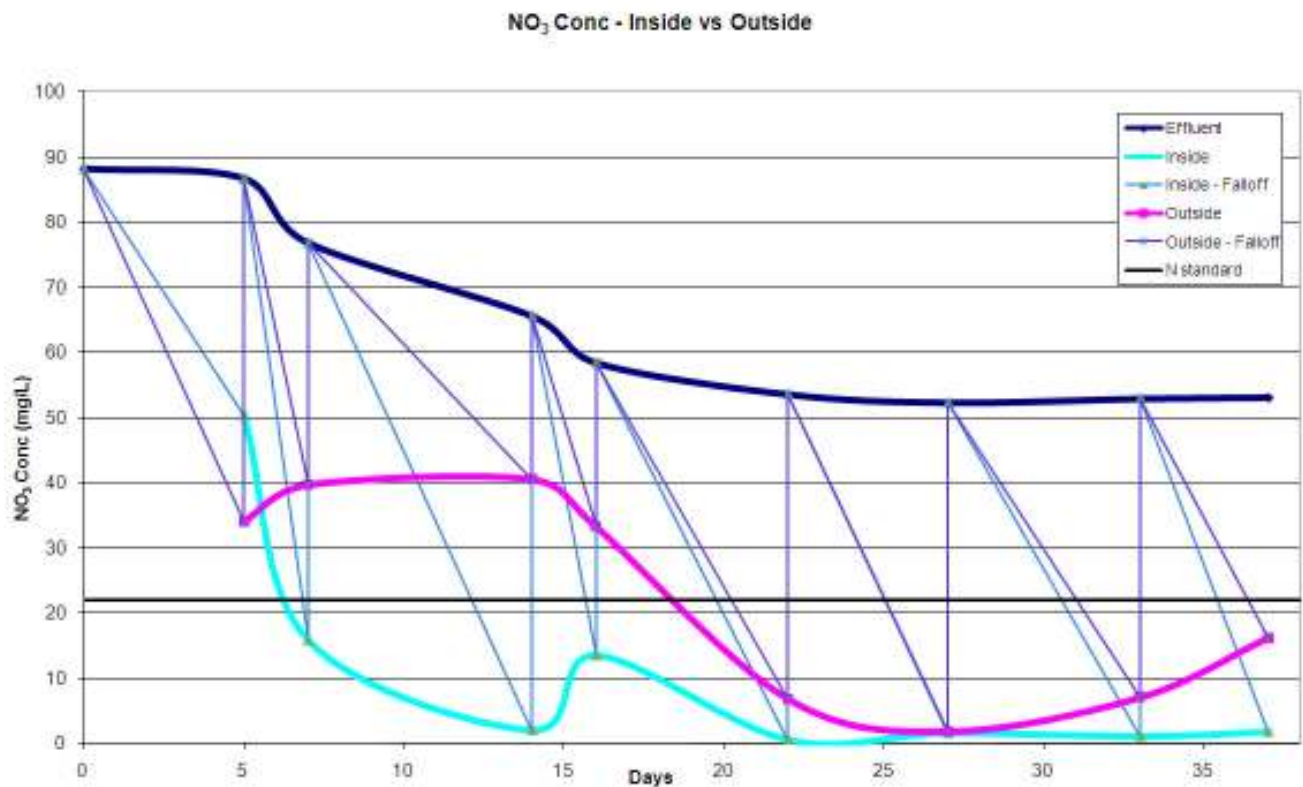


**Figure 32 - Shows the performance of the laboratory bioreactor and the outside bioreactor using the same feeding effluent. The falloff in N concentration over time is shown by the step changed between the 2 curves.**

This experiment was an initial test to see if the results from the laboratory could be matched outside and perform without a constant artificial light source. After a short acclimatisation period, the outside bioreactor stream performed within the EU set standard, as low as 2mg/L $NO_3$.

The results are similar to those shown in section 6.3.4. The uptake rate over the first 2 days after feeding is demonstrated (days 5 and 14 in Fig. 32) to be much faster than the following

days when $CO_2$ has become exhausted. This can be seen where the batch process time is increased and the additional drop in concentration is small.

The first test in external conditions performed almost as well as the laboratory experiments despite being at a disadvantage with diurnal light and large periods of shade, having been placed (for security reasons) in a partly shaded area.

The outdoor experiment was not in an ideal location. To avoid interference, we were limited to this location. The area was secluded but unfortunately also subject to shade for 9 of the 16 hours of daylight experienced in July. As an example of comparison, after 20 days acclimatisation, the outside reactor was consistently reducing $NO_3$ to under 10mg/L whilst the laboratory experiment had reached steady state and reduced $NO_3$ to <1mg/L consistently over the remaining 360 hours of experiment.

It was predicted therefore that an external bioreactor with the correct set up that is with, for example, a shallow optical depth, an established culture kept at a consistent temperature (which effluent usually is) and with carbon kept in excess would perform better than these laboratory results. It was concluded this was due to the increased intensity / wavelength of light and a larger set up that would provide a more consistent and better availability of nutrients.

## 6.3.6. Growth rates
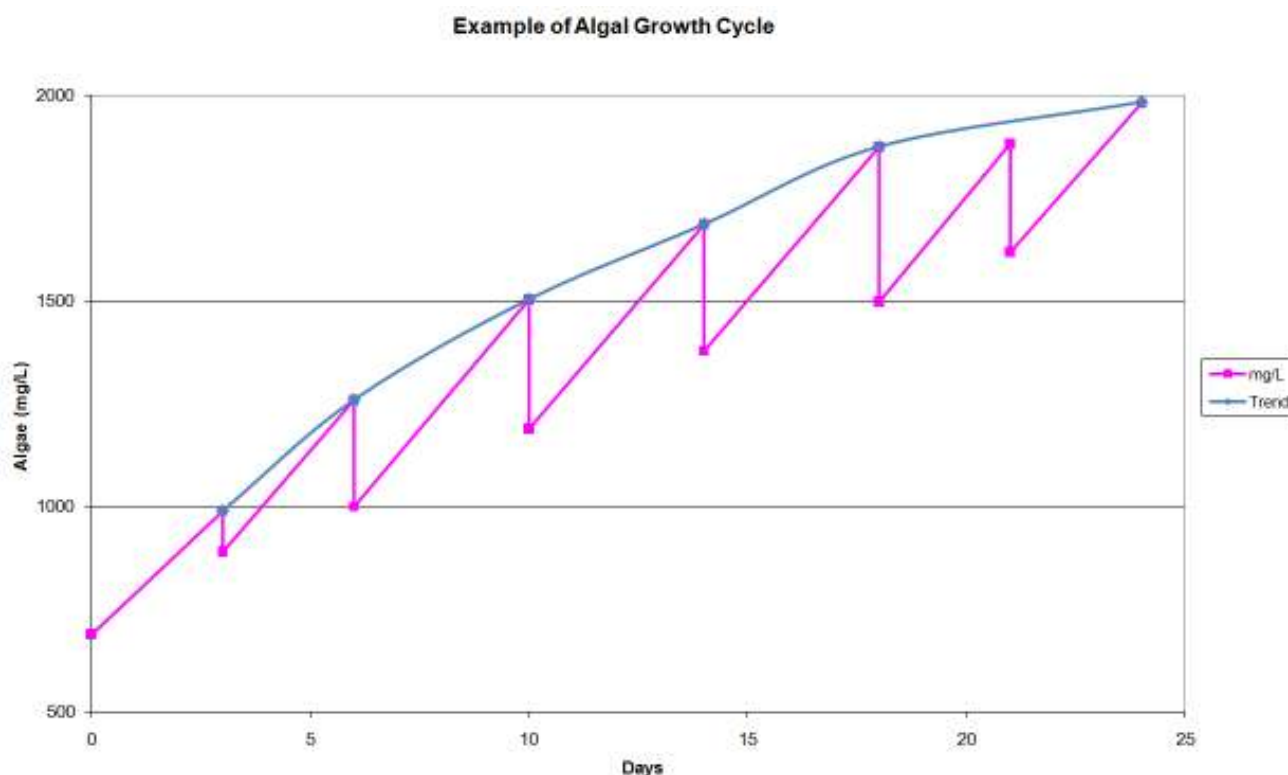


**Figure 33 - Algal growth cycle showing losses at feeding/measuring points. The saw-tooth effect is caused by the reduction of biomass from losses during sampling followed by a re-growth period.**

Algal growth rates for fig. 33 shows a consistent gradient as the total concentration of algae increases at an average rate of 124.49mg/L/day (SD of 2.56, Max of 128.44mg/L/day, Min of 120.89mg/L/day). In the laboratory conditions used, these results were therefore reproducible. Because the concentration of algae increased whilst the growth rate remained constant, this shows the doubling rate of the algae has reduced. At 700mg/L the average doubling rate of all algal cells contained within the system was 7 days compared to 16 days at 2000mg/L. Making the assumption that the algal cell doubling rate is fairly constant we can conclude that at higher concentrations a much larger proportion of the biomass is inactive or dead. The fastest growth rates occurred between 1000-1900mg/L with higher concentrations providing only marginal increases. The growth rate began to drop at concentrations above 1900mg/L and was lower (100mg/L/day) below 1000mg/L. A tail off in growth rate is expected as the biomass increases incrementally. Availability of light diminishes, availability of nutrients diminishes and there is more competition for these

limited resources causing a greater die away. Disease is also more likely to increase. Simple kinetics models would predict an increase in growth rate and biomass concentration until there is a rate limiting nutrient. For algal growth however, nutrient concentrations are low and with a semi-batch operation, the saw effect is shown in Fig. 33 due to batch feeding and sample removal. Growth rate tail off does not appear to happen until algal concentrations of 1900mg/L. We can conclude from this that this rate limiting maximum concentration was not an important factor at these concentrations however it has been shown there is no real advantage in maintaining a concentration of 1800mg/L over 1400mg/L. A healthier bio-reactor would be more beneficial and any advantages of maintaining a high concentration of algae do not compensate for the loss of light. It was concluded that maintaining a concentration of algae in the range of 1000-2000mg/L would be acceptable.
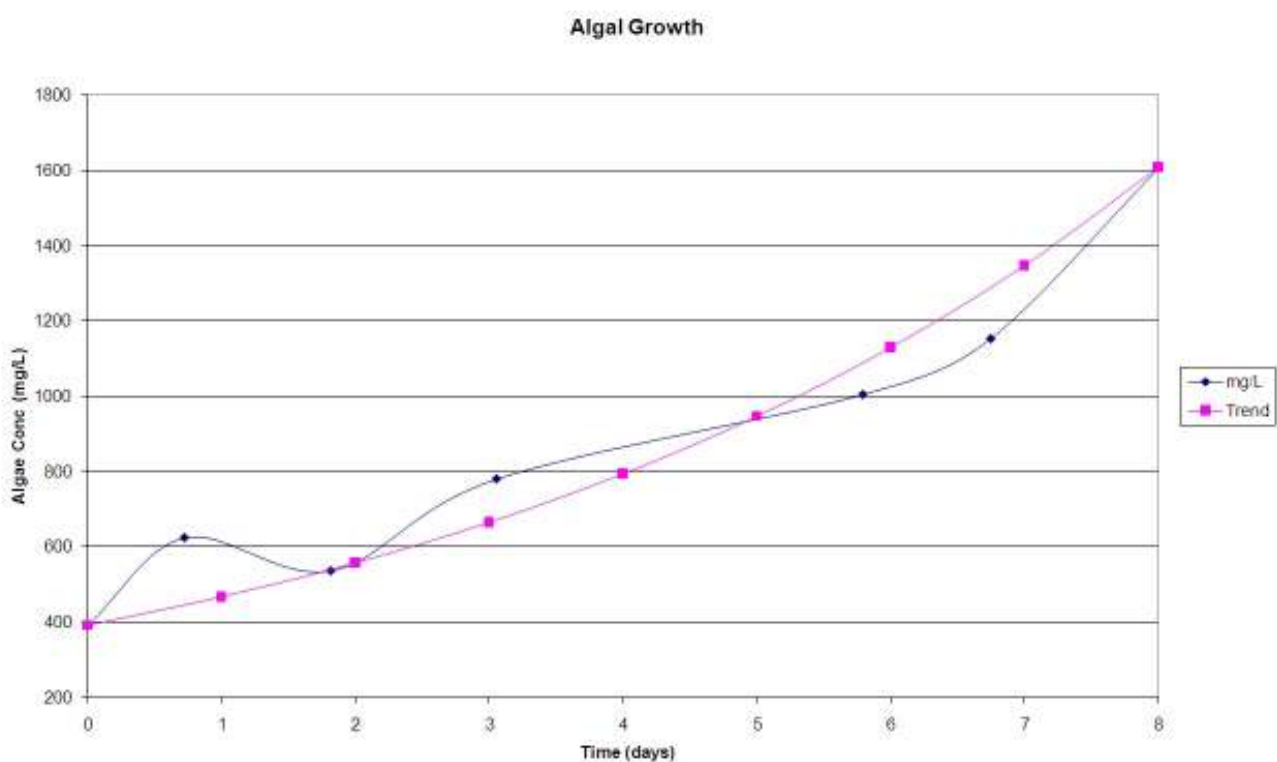


**Figure 34 - An increase in growth rate as the algal concentration increases**

Throughout the project, a range of over 20 growth rate experiments with varying algal species suggested that standard algal growth rates in un-optimised bioreactors vary between 50-200mg/L/day and can be shown to increase with algal concentration. Fig. 34

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shows the trend is not linear but there is a slight increase in growth rate as the concentration increases between 400 and 1600mg/L. It shows a prominent increase in growth rate above 1000mg/L which correlates well with Fig. 33 This behaviour at lower concentrations is expected if a constant doubling rate is assumed from standard microbial growth rate equations. The trend shown in Fig. 34 fits a doubling rate model of 4 days. Initial algal concentration + (doubling factor/day x Death loss factor x days) = final algal concentration. Averaging all the experiments carried out shows an average growth rate of 143.2mg/L/day with SD of 16.2mg/L/day. A maximum of 152mg/L/day was achieved at an algal concentration of between 1200-1600mg/L. One can expect somewhere between 10-20mg/L/hr $CO_2$ sequestration during photosynthesis which should roughly equate to a growth rate of 6-12mg/L/hr (72-144mg/L/d).

Other results collected from the literature show a wide range of values. Wahal & Viamajala looked into maximizing algal growth in batch reactors using sequential change in light intensity. They found a maximum across growth rate across all their experiments of 55mg/L/d. Ai et al reported growth rates of 560mg/L/d consistent growth for 7 days. This is a surprisingly high value compared to the other data found in the literature. It was however carried out is a highly optimised bioreactor and therefore you would also expect the testing to stringent. Comparing with the results I collected, I would suggest this is a feasible growth rate however due to the nature of their experiment, I would also suggest this is close to the upper limit. With complete optimisation of my experiments I would predict the growth rate to be increased 2-4x which is in line with the results they demonstrate. Hu et al found growth rates of 108-168mg/L/d. Rodolfi et al report growth rates of 360mg/L/d. Craggs et al found winter and summer growth rates of 4.45 and 68.6mg/L respectively. Pittman et al produced a table of growth rates they found from literature; the majority range from 25-80mg/L/d, one experiment recorded 127mg/L/d and another recorded 346mg/L/d. Cromar et al produced a mean growth rate of 76mg/L/d. Ramanan et al found growth rates of 84-140mg/L/d. In another experiment, Park & Craggs found growth rates of between 30-80mg/L/d. Heubeck et al found growth rates of between 44-54mg/L/d. Standard experiments appear to show a normal range of between 30-150mg/L/d with highly

optimised bioreactors reaching growth rates up to and possibly in excess of 600mg/L/d. The results from my experiments compared to the literature data indicate good performance for an un-optimised bioreactor, with numerous different configurations producing 120-150mg/L/d. The maximum growth rate achieved in a 24 hour batch experiment was 280mg/L. 80% of this growth occurred in the first 10 hours equating to a growth rate of 540mg/L/d during active periods.

**Table 6 - the summary of different growth rate experiments under varying conditions.**

| | Growth (mg/L/day) | Pinitial (mg/L) | Pfinal (mg/L) | Ninitial (mg/L) | Nfinal (mg/L) | Light | Temp (*C) | Algae Type |
|---|---|---|---|---|---|---|---|---|
| 1 | 152.0 | 1.84 | 0.09 | 44.63 | 23.38 | 100% | Oct (18.5) | Floc |
| 2 | 153.2 | 1.65 | 0.04 | 41.52 | 18.66 | 100% | Nov (18.3) | Floc |
| 3 | 105.4 | 1.91 | 0.39 | 49.30 | 47.68 | 100% | Nov (34.1) | Floc |
| | | | | | | | | |
| 4 | -30.8 | 2.24 | 1.35 | 54.61 | 50.88 | 50% | Jan (13.5) | Floc |
| 5 | -145.3 | 1.88 | 0.81 | 53.12 | 60.89 | 50% | Jan (33.4) | Floc |
| | | | | | | | | |
| 6 | 124.5 | 1.21 | 0.00 | 57.86 | 28.79 | 100% | Mar (16.4) | Floc |
| 7 | 48.4 | 0.37 | 0.42 | 60.33 | 24.18 | 100% | Oct (18.5) | Filaments |

Laboratory experiments using full artificial light produced growth rates of up to 150mg/L/day. As noted this value is similar to other results in the literature and It could be used feasibly to scale up and produce an economic estimate for treatment operation. The laboratory experimental results achieved are un-optimised with respect to; pH, temperature, mixing, nutrient stability, low light intensity used and was not supplemented with $CO_2$. These suggest that growth rates and removal of priority pollutants, P & N could be further improved and optimised by using a pilot/full scale outdoor set up. For example Ai et al reported growth rates of 560mg/L/d in highly optimised photo bioreactors.

The highest growth rates achieved (>150mg/L/day) came from floc algal strains (chlorella sp) that show a high affinity for P. The data shows a direct link to quantity of nutrient removed and growth rate of algae.

When the algal strain changed to filamentous blue-green, the results show the growth rates reduced to less than 33% of the maximum achieved with the unicellular green algae flocs. Nielsen (2006) and Tang (1995) show that the range in growth rates across all the different species is so big that we would expect 2 species chosen at random to have very different growth rates. The focus here is to grow for maximal nutrient removal rates, if the focus was on producing large quantities of biomass, then investigation into the fastest growing species would be more important. I did however find a correlation between growth rate and nutrient uptake rate, with the two expected to be closely linked. The biggest limitation to algal species choice is their ability to thrive in the environmental conditions found in wastewater.

# 7. Summary and Final Discussion

## 7.1. Discussion of results summary

The aim of this project was to determine whether using algae to remove nutrients, primarily P from real wastewater via the production of algal biomass was achievable and sustainable in meeting the WFD standards within a reduced HRT.

The main considerations were:

- Reducing P to very low levels

- Reducing P to 1mg/L within the shortest HRT

- Whilst achieving the P standard, are other quality standards also met, N concentration and SS

- Whilst achieving the P standard what is the growth rate of the biomass produced

- Can this process be sustained year round in the UK

The most conclusive outcome of the results was the reduction of P to below the detection limit. This result was repeatable and clearly showed that algae are capable of removing all available soluble P.

With this knowledge, the question was, under optimal conditions, how fast can the P concentration be reduced to 1mg/L. This of course depends on the starting concentration of P however the uptake rate was fairly constant at about 0.33mg/L/hr whilst $CO_2$ is in excess with the only variation factor being the concentration of algal biomass present. The concentration of algal biomass is an easily controlled variable which can be set at 1500mg/L for optimal uptake rates.

Experiments were not carried out in completely optimal conditions, i.e. set pH, temperature and light intensity whilst nutrients are maintained in excess. Due to this I cannot conclude

exactly what P uptake rates are possible however based on literature findings, it is not unlikely that the rate may be 50-100% higher (producing up to double the algal growth rate).

Using optimal conditions of pH8, 1000W/m$^2$ light intensity, 25°C, 1500mg/L biomass concentration and excess $CO_2$; I would expect a P uptake rate of at least 0.5mg/L/hr and algal growth rate of 250mg/L/d. With an example starting P concentration of 8mg/L, to reach the WFD standard of 1mg/L would require a HRT of 14 hours producing 250mg of algal biomass over the day.

This achievement is realistic during summer months in the UK when daylight hours are in excess of 16 hours. The process would work perfectly with 1 batch feed per day per tank. Gravitational settlement would be completed over night ready for the next feed.

Using all the results gathered it is not clear whether the process would work during the winter months. From the results gathered for varying light conditions matching those experienced during winter; theoretically the algae would grow during light hours. In the above example during winter months with light intensity of 150W/m$^2$ the HRT may be between 2-3 days.

Whilst the P standard of 1mg/L is met, N concentration is reduced and can meet the 5mg/L standard however I can conclude that with using this process alone it is difficult to guarantee that N will always meet the required WFD standard. N removal was not made the primary focus of this research as its removal is less problematic than P, with other sustainable removal mechanisms already existing.

After fast gravitational settling, SS were reduced to below 5mg/L which is well within the WFD standard of 35mg/L. ( see p194 Results and Discussion). Heavy metals, Fe, Ni and Zn were found to also be reduced during this process by 60-80% in the experiments carried out.

## 7.2. Comparison of analysed results with hypothesis

Nutrient removal from wastewater can be achieved more sustainably and more effectively using a biological process of algal growth in bioreactors than it can by using existing methods. - The major energy requirement came from mechanical stirring for agitation. By integrating gaslift $CO_2$ sparging we could reduce the need for mechanical stirring whilst increasing Carbon sequestration. Pumping energy requirements exist regardless of the process being used. The process did not require the use of any chemicals. The shallow tank depth used does require a greater land area though.

Algal growth will sequester Carbon – The project showed that available Carbon was the major limiting factor. For the process to be successful, Carbon would have to be added to increase the level to at least twice of what is naturally available.

The process would improve effluent quality by reducing other pollutants. – Whilst the photosynthesis and eutrophication processes of algae are well understood, their interaction with and use of metals had not been so commonly researched. The hypothesis was based on the theory that algae would require trace amounts of certain metals. This was shown to be the case when results showed a consistent 60% reduction in the concentration of Fe, Ni and Zn.

The biomass by-product can be used as a fuel source. – This project did not carry out experiments using the algal biomass recovered however the growth rates and yields showed that scaled up applications at STWs could produce enough algae to make the process at least carbon-neutral. As demonstrated in the literature, the process of turning algae into fuel is becoming a more commonly researched topic (out-weighing researchers trying to find feasible and economical ways of growing the algae). The focus is on the algae to bio-diesel process. Whilst this has not yet been shown on a large scale level that is competitive with fossil fuel production; commercial organisations are now able to produce bio-diesel on a small scale using more controlled systems.

## 7.3. Contributions

The project tested both the continuous flow process and batch feed process establishing that for operating and performance reasons, the batch feed process was more effective. It is easier to manage, produces more consistent results, has a less complex setup and can reduce P faster and to lower levels, subsequently producing more algae.

The project showed that fast separation of algae from water was possible after switching off the stirrer. To keep the algae in suspension, the system requires continuous agitation. This method of fast gravitational settling could be scaled up to an industrial level.

The project showed that P removal from wastewater using algae is possible to concentrations below the detection limit. Results demonstrated that a maximum of 12 hours HRT would be required for total P removal. Results predict that with an optimised bioreactor working to a standard of 1mg/L P, <6 hours HRT would be required. These would run on an agitated biomass concentration of between 1.4-1.9g/L.

The project showed that N removal from wastewater using algae could be a competitive process however it was more difficult to achieve the required EU standard of 5mg/L due to N being in excess compared to P.

The project showed that significant growth of algal biomass is produced and has vast amounts of potential uses, the simplest and most logical being anaerobic digestion using existing on site equipment.

The project showed that using natural light was better and recommended for all further work. The low artificial light intensity used in experiments showed that the algae could grow for most of the year in natural light. Winter sunlight intensity is 5x greater and has a wider spectrum than the artificial lights used in the experiments but natural winter poor light conditions and short light cycles were not tested.

The project showed that the process requires $CO_2$ in excess of that which can be transferred naturally or available. Bubbling additional $CO_2$ into the base of the reactor was recommended to increase carbon capture, algal growth rates and nutrient removal rates and remove the need for mechanical stirring.

The project also showed that it was important for specific algal strains to operate at their adapted temperatures. Raising the temperature to tropical conditions does not improve metabolism for UK climate algal strains. 34°C was shown to be detrimental.

Literature states that $CO_2$ and light are the most important factors for photosynthesis. The project confirmed they were the key variables with the concentration of P available. This suggests promising results and further work at a larger scale was recommended.

The project provides the fundamentals for a sustainable sewage treatment process showing that high biomass growth rates are achievable, on a large scale this could contribute towards the fight against global warming and in the long term has the potential to be the basis for the production of the worlds fuel supply.

## 7.4. Comparison with closest rivals

Ruiz-Marin et al wrote a 2010 paper entitled "Growth and nutrient removal in free and immobilized green algae in batch and semi-continuous cultures treating real wastewater". Their topic is similar to this thesis. Essentially attempting to remove P & N via the growth of algal biomass in real wastewater. They focused on determining if immobilized algae are better than free algae. They claimed that immobilized systems could facilitate the separation of the biomass from the treated wastewater although in terms of performance, immobilized systems did not represent an advantage over free-cell systems. I only worked with free-cell systems due to enhanced system simplicity and better distribution of nutrients across the cells surface.

They used both *Scenedesmus* and *Chlorella* algae, two of the common algae worked with in my thesis. Their system was also managed without the addition of $CO_2$ and like my thesis, reached a common pH of 10.1 with the non-algal control maintaining pH8.5. They showed that aeration was enough to keep the algal cells in suspension.

Unfortunately the results they obtained could not be contrasted with my experiments. They carried out a decent experiment but measured nutrient uptake as a % rather than an absolute rate whilst not producing growth rate data.

Griffiths produced a 2009 thesis entitled "Removal and utilization of wastewater nutrients for algae biomass and biofuels", a similar title to my thesis. Tasked with the problem of reducing P discharge from the Logan City sewage treatment facility where algae grow naturally in the lagoon, they looked into two aspects, 1) harvesting the naturally occurring algae to remove P from the system and 2) laboratory and pilot assessments on the ability of the indigenous algae to uptake P. Both of these with the intention of using the biomass for biofuel production.

They used plant growth fluorescent bulbs to encourage photosynthesis in a transparent acrylic rig with mechanical stirring for agitation. A similar setup to my experiments. They also experienced the standard pH > 10 whilst not using additional $CO_2$. They also found *Scenedesmus* and *Chlorella* to be the most dominant species observed in the Logan lagoons.

They carried out a $CO_2$ addition experiment where pH was maintained at 8 via automatic $CO_2$ release. They found that oil content within the cells was 3-4 times higher whilst using $CO_2$ addition. Another good finding that backed up other authors was the increased productivity at higher temperatures up to 30°C.

They carried out a smaller number and range of experiments than I undertook in my project, finding similar growth curves however the numbers involved were smaller. P reduction over

days rather than hours under lower algal biomass concentrations producing growth rates of around 15mg/L/d.

# 8. Conclusions

## 8.1. P and N removal

Algal bioreactors were shown to be able to reduce critical nutrient concentrations of N and P to below the detection limit.

The removal rates of P and N were directly related to the strain of algae present in the bioreactor. The flocculent unicellular green algae were predominately found at higher P concentrations whilst the filamentous blue-green algae were predominant at low P, removing mostly N. It is suggested therefore that the specific type of algae present will depend on the concentration of nutrients in the treated effluent as the specie most suited to those conditions will dominate. With low concentrations of P, species that have a high P requirement will not be able to compete with other species with a lower affinity for P will dominate. In the experiments reported, the flocculant green algae were displaced by the filamentous blue-green algae.

The best nutrient removal rates occurred when there was a mixed culture of 10 or more different species of algae present. When a single species completely dominated the performance was inhibited. With uniform filamentous blue-green algae; the growth rates fell to less than 33% and the nutrient uptake rate dropped. A mixture of algae will mean the P:N concentration ratio is less critical for ensuring high growth rates.

When $CO_2$ was freely available after the initial batch feeding, P uptake rates were shown to be up to 8mg/L/d. After 10 hours once the $CO_2$ had become exhausted the P uptake rate dropped to 1.3mg/L/d. There was a strong correlation between dissolved $CO_2$ and P uptake rate. The rise in pH to above 10.5 due to the consumption of $CO_2$ is thought to have been inhibitory however a previous experiment suggested that the optimal pH value may have been around pH10, producing the fastest growth rates; a range of pH6-13 was tested. The majority of previous work however maintain pH8 as an assumed optimum.

The results from my experiments found that $CO_2$ always becomes the limiting factor first. This was shown by experiments maintaining a constant light intensity, constant temperature and availability of P & N; the performance drops in correlation with the consumption of $CO_2$, demonstrated by pH rise. The post 10 hour metabolism reduction was observed across all performance indicators and confirms previous work where pH was measured but $CO_2$ sparging was not used.

In a wastewater treatment system designed for the removal of nutrients; the P and N concentrations should never become rate limiting as the algal bioreactors could be re-fed on demand by monitoring to achieve the EU WFD standards.

P removal is likely to be the major design criteria and the results have shown that this is possible to <1mg/L for the EU WFD and UWWTD within 10 hours in bioreactors with between 1-2g/L algal biomass concentration. The results do not show simultaneously control of the N concentration to <5mg/L as N due to N being in excess compared to P. The results show a steady N uptake of 5.5mg/L/d. This needs further work to optimise.

## 8.2. Carbon sequestration

Effluent pH increase shows a direct correlation with the decrease in IC. The rise in pH is linked to the reduction in dissolved $CO_2$. As the rate of increase in pH level slowed, the rate of decrease in Carbonate increased indicating the switch to Carbonate once the majority of $CO_2$ had been used, clearly shown by the increase in pH. This behaviour was confirmed by all similar experiments found in the literature. It was therefore concluded that $CO_2$ was the preferred source of carbon, only switching to carbonate when $CO_2$ became limited. As noted in section 6.3., this has a dramatic effect on reducing nutrient uptake and algal growth rate.

Maintaining an optimum pH for algal growth is an important design consideration. A rise or fall outside the active pH range becomes a limiting factor. Dissolved $CO_2$ concentration should always be kept in excess so a natural pH8 could be controlled by monitoring the release of additional $CO_2$. If the optimum pH was found to be 10, pH could be artificially raised whilst still ensuring $CO_2$ was in excess.

The need for additional $CO_2$ in this system makes it a perfect candidate for the scrubbing of waste gases rich in $CO_2$. A gaslift stream from the base of the bioreactor is recommended to be incorporated. This gaslift system may be enough to replace the mechanical stirring process for agitation.

## 8.3. Algal growth rates & concentration

Without further optimisation of light, species, temperature or excess $CO_2$, growth rates were found to be between 100-150mg/L/day; this compares well with the literature (findings shown in section 6.3.6.). Light intensity is increased when outside under natural light, direct sunlight was measured to be at least 40x more intense than artificial light when measured at the centre of the bioreactor. Unlike the artificial light from the halogen lamps used, natural light contains the full spectrum of electromagnetic radiation required for optimal plant growth and therefore it is suggested that this may be a further benefit from scale up studies on outside bioreactors. This combined with the cost of power has led to the recommendation that natural light is used for the scale up trials. The lowest intensity of natural light during winter drops below $97W/m^2$ during overcast conditions however sunlight intensity is in excess of $194W/m^2$ and should be enough to maintain a base level of growth. The day length remains unsolved; artificial light may be required to increase the daylight hours during winter.

Algal growth rate and nutrient uptake rate were affected by biomass concentration. Between 500 - 1000mg/L the growth rates increased at a fast rate. A slower increase in growth rate was found as the concentration of algae continued to rise to 1900mg/L. Algal

growth rates began to decline above this limit. No advantage was found in maintaining an algal concentration of 1800mg/L over 1400mg/L. At high concentration, light penetration was reduced and death rate was increased. The results suggest that an optimum bioreactor concentration of algal biomass is around 1500mg/L.

The results show that whilst enough light and carbon are available, P, N and other trace nutrients such as metals will also be removed at a constant rate in proportion to growth rate. Under the conditions of my experiments, maximum growth rates were found to be around 540mg/L/day. This could be increased further with increases in natural light intensity and optimisation of temperature, pH and algal strain; further laboratory work on more intense bioreactors is suggested.

## 8.4. Trace nutrients and quality standards

Trace metals, Fe, Zn and Ni found naturally in the effluent were shown to be reduced by 70% within 10 hours of feeding. The reduction in removal rate after 10 hours correlates with the exhaustion of dissolved $CO_2$ and rise in pH. Metal concentrations may be reduced further if they are not limited by the overriding need to control N & P.

## 8.5. Algae / effluent separation

The selection of a fast settling, self flocculating algal species mix was a simple process. This enabled fast gravitation settling times of less than 10 minutes leaving effluent SS of <5mg/L. The algae free effluent can be separated from the thick settled sludge layer enabling a self seeding process ready for the next batch feed. The gravitational settling worked for both the in situ batch feed process and continuous flow process using a settling tank.

Cross flow filtration and dead end filtration were found to be unsuccessful.

## 8.6. Seasonal variation

### 8.6.1. Light

From the light intensity measurements and the literature data, it was established that natural light has a far greater intensity than is sustainably possible to reproduce with artificial light. The light rays from the sun are parallel, meaning the intensity does not decline over the depth of the empty bioreactor however the origin of the weak artificial light is so close to the bioreactor, the intensity reduces by about 90% over its depth due to the inverse square law. The results showed that nutrient removal could be just as successful outside as it is under controlled laboratory conditions.

On fully overcast days, the range of light intensities experienced during different times of the day and year was small compared to direct sunlight where the angle of the sun was a more significant factor. The light meter data confirms that the light intensity ranges between 78-155W/m$^2$ during mid-winter and mid-summer respectively on a fully overcast day. When the sun is obscured behind 6/8 cloud cover, these values are roughly doubled, for 3/8 cloud cover they roughly tripled to 233-466W/m$^2$. Direct sunlight ranges between 602-1262W/m$^2$ for mid-winter and mid-summer respectively.

It was shown that the minimum light intensity required to achieve the results in this project were between 97-136W/m$^2$. When comparing the required light intensity with those experienced naturally in the UK; it can be seen that for the majority of daylight hours throughout the year, natural light intensity is adequate for algal growth. The question remains whether the days are too short during winter to maintain adequate growth. The bioreactor design would need to be completely open to prevent shading issues due to the low angle of the sun in the winter.

### 8.6.2. Temperature

Algal species being kept at their optimal temperature for growth was shown by results (section 6.3.2.6.) and literature to be important. Raising the temperature for the cold water inoculum algae to 34°C was found to be detrimental however theory suggests that productivity should be higher at warmer temperatures with algae suited to those conditions. From the literature in section 4, I conclude that the majority of algae increase in productivity with an increase in temperature up to about 30°C. By 35°C most algal species cannot function. It is also concluded that the respective light intensity and temperature need to be suitable. One study into the effects of light and temperature on the growth of three freshwater green algae isolated from an eutrophic lake found that as the temperature increases, the performance at higher light intensities increases and light intensity which is too strong for the corresponding temperature is inhibitory. It was also found that the performance increases with every increase in temperature, however the improvement between 30-35°C is small before becoming quickly detrimental to growth. This finding was confirmed by an experiment that looked at the influence of environmental parameters on biomass production. It was found that the HRAP algal concentration is a function of day length. A 3D graph of algal concentration vs. temperature vs. irradiance showed that a low temperature or irradiance will be inhibitory regardless of the level of the other parameter.

Further work on optimal temperature should be carried out once a steady state has been achieved in the pilot plant using constant conditions.

Water has a high specific heat capacity and hence reacts slowly to external temperature changes. Sewage temperature does not vary greatly; temperature data from the treatment works shows a May-September average of 17°C and a November-March average of 9°C.

The temperate algae used will have an optimum operating temperature between 15-25°C, typical UK summer temperatures. The bioreactor will perform better under a constant temperature, maintaining it will not only maximise growth but also provide a more controlled environment to promote steady state. Waste heat from the proposed digester

could be used. A temperature reduction process may be required prior to discharge depending on the effluent discharge standards and results of a seasonal trial. The heated effluent should still be suitable for discharge to the environment as temperatures above 25°C are not envisaged.

### 8.6.3. Comparison: Outdoor vs. laboratory conditions

When comparing performance between laboratory conditions and outside experiments, existing literature suggested that a number of factors need to be considered; temperature variance, availability of light (cloud/shade/night-time/seasons) and contamination from wild species.

The results presented here demonstrate that whilst there is an available supply of $CO_2$, the light intensity was the next most important factor leading to a limitation of growth.

In laboratory conditions using artificial light, light intensity can be maintained constant 24 hours a day however this intensity was low compared to natural light with halogen lamps. The intensity next to the light source is enough for algal growth however this drops exponentially through the depth of the bioreactor. The results showed no growth was possible when the light source was moved 10cms away from the tank corresponding to an 80% reduction in light intensity.

In natural light, the intensity is variable depending on time of day, season, cloud cover and shade. During daylight in winter in overcast conditions, as expected the light intensity at the surface of the bioreactor is poor, less than that produced by the halogen lamp. The light intensity from the halogen lamp diminishes so quickly that by the centre of the bioreactor it was less than that measured in natural winter overcast light.

The results obtained from the external experiments show that performance is almost as effective as laboratory conditions, even with the unfavourable conditions noted. Insufficient

work was carried out in external conditions (specifically during winter season) to draw suitable conclusions and this is discussed more in the further work.

The application of this process externally promises favourable results. This is pending a year round pilot plant experiment located at a sewage works. In tropical countries with less seasonal variation this process looks to be even more attractive.

Contamination in outside conditions is less controllable than in a laboratory due to easier access by wild species but it is anticipated that any working system will have the environmental conditions tuned to a consistent steady state making contamination less likely.

## 8.7. Use of algae

Without further experimentation conclusions cannot be drawn on the most efficient way to use the algal biomass by-product. In terms of fuel it was concluded that making use of the onsite AD equipment would be the obvious choice for turning algae into energy.

## 8.8. HRT, bioreactor design and optimisation

### 8.8.1. Continuous flow vs. batch process

Algae have a better capacity to uptake nutrient from higher concentration environments (at least within the range found in municipal wastewater). The continuous feed process dilutes the high nutrient concentration influent into the bioreactors reducing the overall concentration. This had three significant implications:

1) There is a continuous supply of new nutrient as feed for the algae meaning the nutrient concentration cannot easily be exhausted. However, this continuous dilution does mean that the overall nutrient concentration always remains low which may reduce the algal uptake rate of nutrients based on simple kinetics. A batch process

can be used to provide an initial high concentration of nutrients whilst ensuring the system is re-fed before nutrient exhaustion. If the total amount of nutrient was identical in both cases, simple kinetics shows that nutrient is removed faster at the higher concentration and therefore reducing the time required to meet a specific standard. This was shown to be true in the results.

2) The low overall nutrient concentration can produce a consistent discharge concentration due to the buffering capacity of the system however it could not achieve as consistently low concentrations as is possible in a batch process due to a continuous supply of nutrients into the system. An extensive baffling arrangement or carousel design could be used to solve this problem. Extensive baffling would provide a large distance between the inlet and the outlet, reducing the change of 'short circuiting' the system. This arrangement however sets up another problem, detailed next.

3) The flow of effluent carries the algal cells with it. When the flow rate of wastewater is greater than the growth rate of algae, the biomass concentration is reduced at the start of the system, reducing the nutrient removal capacity and creating wasted volume in the tank. A feedback loop is required to constantly replenish the inlet area.

### 8.8.2. Algal diversity

The algae have evolved to efficiently use the various nutrients available and as shown, can adapt to suit the environmental conditions presented. When an environment is unchanged for a longer period of time, the adaption of algae to this specific environment becomes obvious as the species can become closer to a mono-culture supported in the results by steady state, metabolism factors, nutrient removal, pH change and growth rates. Reduction in nutrient concentrations become greater and more effective when there is a high growth rate. Steady state reactors would avoid changes in algal diversity.

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With regard to cultivating algae on wastewater, there may be potential in experimenting with algal species that are not found naturally in the bioreactor conditions but this requires specific work and is unlikely to be useful due to the dominant nature of more suitable algal species. Trying to put specific species into open systems was reported in the literature review and it failed. Whichever species is best suited to the current conditions will naturally dominate and further adapt to the conditions hence we expect to see the best possible growth developing over a year to compensate for seasonal effects. The efficiency of algal growth and nutrient removal are expected to improve by changes to the physical process design.

If the process incorporated waste heat to raise the temperature to levels that may not be suitable for naturally occurring algal species; investigation into species more tolerant to high temperatures would be worthwhile.

The dominating algal species could vary throughout the year. In the results reported here, they were mostly dependent on the concentration of nutrients available. Temperature and light effects due to the current season could also be important factors. This may give rise to inconsistent yields of algae and varying performance in terms of nutrient removal.

## 8.9. Bioreactor design scale up and industrial integration

See section 9.

## 8.10. Overall Conclusion

The feasibility of implementing a wastewater nutrient removal system combined with the production of algae biomass on a large scale in the UK is good. The use of waste heat would mitigate the problems associated with low winter temperatures experienced in the UK climate. Regardless of this, the process could be successful in warmer areas of the world and is a promising system for developing countries.

This project assessed the feasibility of this system as a nutrient removal process in which it was successful; it also produced a valuable biomass by-product. In terms of using wastewater ponds as a method of producing algal biomass, the scale of the process is limited by the volume of wastewater processed by each specific sewage treatment works and the land available for use. It would be possible to produce a significant amount of biomass but in the grand scheme of things, the contribution from wastewater processes will only scrape the surface of what is required for global energy consumption. To really make a difference by using this technology as a renewable fuel, large areas of desert will need to be filled with specifically designed lagoons for massive-scale algal growth.

The UK is not highlighted as a specific part of the world where algae production rates are likely to be significant, however as a process used primarily as a method of controlled eutrophication for the treatment of wastewater with the algal by-product harvested at low cost for energy recovery then the whole system is likely to be very successful. With the integration of waste heat to control effluent temperature, there are no barriers to success as the results are successful under lighting conditions less than those experienced naturally during UK winter. The effluent would benefit from any increase in temperature up to around 30°C, therefore using any available waste heat. Waste $CO_2$ would also be utilised based on availability controlled automatically by pH monitoring.

Field conditions are different from the lab. Light is only available during certain hours of the day and the temperature falls at night. Seasonal variations will also play a massive role in biomass production, growth and efficiency.

The findings of this project may not indicate that a combined wastewater treatment and biomass production system is the best approach to solving the worlds fuel problems but this research has shown that algae is the answer in some capacity. The process may not currently be working as efficiently as it could but it is close to achieving its potential with

the use of a well regulated temperature and excess $CO_2$; continued research in this area is essential, a mass form of alternative fuel is critically needed.

In terms of wastewater treatment, the use of algae has many benefits to enhance performance. These included P and N removal, heavy metal removal, oxygenation and reduction of BOD. Without focusing on biomass or biofuel production, algae are definitely the answer to future improvements of large scale wastewater treatment.

# 9. Design of an Optimum Performance Pilot Plant

Based on the results from my experiments and the data recorded in more recent literature, I can make a decent proposal for the design of an optimum performance pilot plant to carry out the treatment of wastewater using algae.

Specific details are pending further experimental research as detailed in the further work section of this thesis. However, I believe there is enough data to enable the outline design of this system to provide maximum efficiency.

For this design we will assume the use of real sewage effluent that contains a P concentration of between 2-15mg/L and excess N in relation to P based of an N:P ratio of 5:1. The process will be tertiary treatment and occur as the final stage.

## 9.1. Design specification

The process must ensure that steady state is maintained to keep a healthy consistent mix of algae optimised for efficient nutrient removal. The primary focus is to maximise the nutrient uptake rate to reduce capital cost and land requirements by lowering the HRT. This is achieved by providing optimal environmental conditions for algal growth.

$CO_2$ is to be kept in excess. Natural light should be used; the bioreactor will require an open design to ensure the light intensity and availability is maximised. The pH should be maintained at the optimal value. Temperature should also be kept at the optimal value however this is likely to be as high as is possibly feasible up to a maximum of 30°C.

## 9.2. Physical dimensions and system processes

Transparent tall narrow tubes have the greatest surface area for light penetration along with a smaller land usage however they are not economically viable when compared to the shallow tank carrousel design.

Two types of tank are required, a more complex active tank - a HRAP incorporating the air-$CO_2$ gaslift process where the algae grow and a basic settling tank ensuring the active tank can be used continuously for growth.

The active tank should be shallow (between 30-60cm pending further experiments) to ensure maximum light capture. The gaslift process could operate through a tall baffled tube built into the base of one side of the tank to allow maximum contact with the solution. Combined with a paddle wheel to keep the algae in suspension; this would create a gentle flow across the shallow tank (2-4m wide and 10-30m long) and back on a return loop to the entry point of the gaslift. The $CO_2$ depleted solution would then be refreshed to maintain $CO_2$ concentration in excess of algal requirements.

When the effluent has met the required P & N standards, the agitating flow would be switched off for 10 minutes allowing the majority of the algae to settle and remain in the active tank. The majority of the treated effluent would be drained into the settling tank to enter the settling phase. The active tank would be re-filled with untreated influent and the batch process started again.

The settling tank would be of basic design with dimensions set by the size of the works. It would incorporate a circular cone shaped bottom, funnelling down towards a sludge collector. The clear treated effluent could spill off the top of the settling tank and be discharged. The settled algal sludge would be regularly removed to prevent nutrient re-mineralisation. The active tank would also incorporate a method for collecting the settled algal sludge when necessary. Any collected algal sludge would either be returned to the

active tanks or sent for processing into biofuel. The exact amounts would be set by ensuring the active tanks were maintained at an algal biomass concentration of around 1.5g/L.

A 21,000,000L/d works (requiring 5000-10000m$^3$ tank volume) using an average tank depth of 60cm would require a land area of approximately 100m x 100-200m using a 6 hour HRT. The exact size requirements would depend on the established night time and winter performances.

## 9.3. Environmental parameters

$CO_2$ will be controlled and kept in excess. Light will be maximised. Temperature should be maximised where possible up to the determined optimum value, likely to be 30°C. pH will be maintained at the optimal value as determined by further work; this is likely to be somewhere between pH8-11.

## 9.4. Temperature control

I recommend the addition of heat to raise the temperature to as close as possible to the optimum value. This could be achieved through the gaslift process or through a heat exchanger. This recommendation is based on a waste heat source being used from a local process such as power generation.

The temperature may need to be reduced slightly during winter months as the optimal temperature is a function of light intensity.

# 10. Further Work and Recommendations

## 10.1. CO$_2$

Further work is necessary to study the effect of keeping CO$_2$ in excess, preventing it from becoming a limiting factor. This should help maintain a constant pH level for optimal algal growth. The mechanical stirrer should be replaced by an gaslift system that maintains dissolved CO$_2$ based on the real time monitoring of pH.

Further work on how increased CO$_2$ affects algal growth and the uptake of N and P with the results from this research is needed.

Studying the introduction of excess CO$_2$ is important because of the extra benefits derived from sequestration and its potential to increase biogas and biofuel from waste. The data suggests it is necessary for improved performance.

## 10.2. Additional heat and temperature optimisation

Addition of waste heat e.g. from digestion, to raise and standardise the temperature should be addressed as priority. This should increase metabolism when using an algal strain suited to those specific temperatures. Experiments could be carried out using temperatures between 20-30°C on temperate strains for UK conditions. Tropical inoculums are suggested for temperatures of up to 35°C.

Carrying out temperature optimisation experiments for the range suggested would be an important part of developing the technology. Temperature optimisation varies widely between species and depends on other environmental factors such as light intensity; this may explain the lack of literature data regarding conclusive temperature optimisation results.

## 10.3. pH optimisation

The majority of the literature assumes an optimum of pH8. This is possibly due to the relationship with low carbon concentration, creating high pH and subsequent low productivities. The only experiment in the literature to carry out a fair pH optimisation test whilst maintaining excess carbon concentration found the optimum pH to be 10.

pH optimisation would need to occur whilst keeping the other environmental variables constant and take into consideration the effects from buffering the solution at high pH values. I recommend testing a range between pH8 – 11.

## 10.4. Tank dimensions

A shallow tank, covering a larger surface area allows more light to enter the system and ultimately increase the temperature. The larger surface area would in turn result in greater heat loss if the air temperature was lower than the water temperature.

Whilst a shallow tank at 30cm depth will receive more energy per area, a 60cm tank depth may actually be more efficient as it can treat twice as much volume. Determining the most efficient configuration in terms of light, temperature and absolute removal rate per square metre is recommended for pilot plant design.

## 10.5. Control of algae species

The results have shown that a mixed culture works better than a dominating single strain. Further research is suggested to control the algae species within the system. Controlling of environmental conditions and maintaining optimum conditions for the chosen species is a recommended start to this research.

## 10.6. Alternative organisms

The findings from this project would suggest that using alternative plants such as reeds, duckweed and hyacinth in artificial wetlands are not as effective as the algae with respect to N, P and C uptakes. Whilst these have been shown to work, higher efficiencies are likely to be found by pursuing further research focused on algae.

## 10.7. Pilot plant

Based on the recommendations from this report; a study of the cost benefit of optimal aspect ratios of algal bioreactor design compared to the alternatives is suggested along with performance vs. economic viability.

An outside reactor without artificial lighting was suggested for the extended study of small scale pilot experiments located at sewage works. The main focus should be to compare summer and winter results. The experiments would incorporate agitation by $CO_2$ gaslift.

Following refining of the small scale pilot experiments then a large full scale demonstration plant could be planned after a season of pilot plant data. The design should incorporate the successful techniques established whilst using control where necessary to maintain steady state.

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