The University Of Sheffield.

Enhancing growth using carbon dioxide, and improving efficiency of sedimentation using Chitosan, of *Chlorella vulgaris* in a photobioreactor

By

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Summary

Two of the major problems facing mankind are the energy crisis and environmental changes. The burning of fossil fuels to produce energy impacts on the effects of "greenhouse gasses" and global warming. This study looks at how carbon utilisation by using enhanced levels of CO_2 in the air provided to grow algae in a photobioreactor may be one a method of reducing the levels of CO_2 entering the atmosphere. In addition the microalgal biomass can produce various forms of energy, thus encouraging the concept of converting waste to energy and the production of "green" energy. The study also looks at how the efficiency of flocculation and sedimentation (as part of harvesting the algae) can be improved by using biopolymers (chitosans) instead of metallic salts.

A strain of *Chlorella vulgaris* (*C.vulgaris* TISTR 8580) isolated in Thailand, and thus likely to be most suitable for industrial scale growth in that country, was used as the main microalgae to study. Following a series of initial experiments to establish the culture parameters, this algae was grown in a purpose designed photobioreactor system. In this photobioreactor growth rates and yields were measured for cultures with the addition of 0, 6, 12, 24 and 50% CO_2 (v/v in air). Growth was measured by turbidity, optical density (OD_{680}), cell count and visual inspection and these measurement techniques were compared and contrasted.

The study found that algae grown in 6-12% CO_2 (v/v) give the highest yield at 0.98-1.25 $gL^{-1}d^{-1}$ with the highest specific growth rate of 1.04-2.21 d^{-1} on Day 2. However, the results are more complex when time to harvest is taken into account as higher CO_2 levels (12 %) give better results when harvested at a shorter time scale. At 6% CO_2, the cumulative turbidity is 2,145.69 NTU. for 4 harvests over 28 days. OD_{680} and cell number is 41.97 and 2.15 $x10^7$ cell/mL respectively. The cumulative dry weight is 18.20 g/L. At 12% CO_2 and harvesting every 4 days over the same 28 day period, measurements of biomass by cumulative turbidity, optical density and cell count are 1,852.34 NTU, 41.82, and 2.80 $x10^7$cell/mL respectively. In contrast, growing algae in air alone, and in high CO_2 concentrations of 24% and 50% (v/v), results in fluctuating or low growth which, in the case of 24% added

CO_2 may appear better, but show problems that would limit biomass production on an industrial scale.

Bicarbonate additions of 0.1, 0.01, and 0.001 M were investigated to see if there was any growth enhancement from this "solid" form of CO_2. The study found that 0.001 M is the maximum amount that could be added because higher levels (0.1, 0.01 M) lead to pH change, which causes growth inhibition. Even at the 0.001 M. level there is no evidence of growth enhancement.

Harvesting is a major challenge to the industrial exploitation of microalgae. A major part of this harvesting is separating the algae from the liquid media, particularly without damaging or contaminating them. There are many methods and aids to the separation/concentration of algae and chemical flocculants are one of the most widely used. However, adding chemicals may compromise the quality of the product and may even create a hazardous waste. In this study a comparison was made between some well known flocculating chemicals and natural biopolymer alternatives; chitosans and crab-shell (the latter as a low cost, unprocessed form of chitosan).

The study found that metallic salts added at 0.6-1.0 g/L have the ability to flocculate and sediment algae with a removal efficiency of more than 90% in 1 to 12 hr. Crab shell and medium molecular weight chitosan were able to achieve 95% removal in 24 hr. Surprisingly, adding high molecular weight chitosan gave no advantage to the settling process. Although metallic salts have a high efficiency of removal in a shorter time compared to biopolymers, the advantages of their inert nature, non toxicity and, in the case of crab-shell, low cost, may be of considerable advantage in an industrial harvesting process.

The thesis reports studies that show that this particular strain of *Chlorella vulgaris* is likely to be a good candidate for commercial exploitation in Thailand. The study estimates that 5.04 $x10^6$ L yr^{-1} algae (*C.vulgaris*) volume in 1 ha would be used 4-6% CO_2 (v/v) around 6.58 $x10^5$ tyr^{-1}- 9.87$x10^5$ t yr^{-1}. The biomass productivity is around 1,000 $t.yr^{-1}$. Total algal oil is about 300 t yr^{-1}. Biodiesel product from algae should be around 150 tyr^{-1}. Crab shell and chitosan would be applied in the harvesting process.

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Nomenclatures

BOD	Biochemical oxygen demand
CO_2	Carbon dioxide concentration
CO_3^{2-}	Carbonate
DO	Dissolved O_2
H_2CO_3	Carbonic acid
HCO_3^-	Bicarbonate
HW chitosan	High molecular weight chitosan
MW chitosan	Medium molecular weight chitosan
NOx	Nitrogen oxides
OD	Optical density
TC	Total carbon
TIC	Total organic carbon
TOC	Total inorganic carbon
Shearing forces	The forces are unaligned force pushing one part of a body in one direction, and another part of the body in the opposite direction. When the force are aligned into each other, the are called compression forces
Shear stress	The stress component paralleled to a given surface, such as a fault plane, that results from force applied to the surface or from remote forces transmitted through the surrounding rock

Units

h^{-1}	per hour
J $d^{-1}m^{-2}$	Joule per day per square meter
g/L	Gramme per litre
$gL^{-1}d^{-1}$	Gramme per litre per day
L/m	Litre per minute
m/L	Milligramme per litre
v/v	Volume by volume
$vv^{-1}m^{-1}$	Volume air per volume liquid per minute
μm	Micrometre
$LL^{-1}m^{-1}$	Litre air per litre culture suspension per minute

Chapter 1 Introduction

1.1 The energy crisis and environmental problems

Globally we are reliant on fossil fuels (coal, oil and gas) for the majority of our energy supply. There is much global concern regarding the "energy crisis" and green house gas (GHGs) emissions. The energy crisis refers to "peak oil", the possibility of oil demand outstripping production leading to scarcity of energy supply and an unstable economic situation. In theory, a lack of supply should lead to a higher oil price and, while this may well become a major problem in the future, it would also be an encouragement to the development of energy from alternative sources. In addition the world must deal with the serious problem of the effects of burning fossil fuels releasing greenhouse gases into the atmosphere and the need to reduce these emissions. These two problems, peak oil and the need to reduce the use of fossil fuels, should be, and are, a considerable motivator in the development of lower-carbon fuels and alternative technologies in many countries. However, the situation is not as simple as this. What peak oil, or the threat of peak oil creates, combined with other economic-political factors, is an unstable and fluctuating fossil fuel market that does not encourage governments and private concerns to invest in alternative energies to the extent that might be expected.

In 2014, global energy use comprised of oil (31.3%), natural gas (21.2%), coal (28.6%), nuclear (4.8%), and renewable energy such as biofuels and waste, hydro, wind, solar and heat (about 14.1%) (Birol, 2016). The production of fossil fuels goes to generate energy. The burning of fossil fuels is the cause of most of the man-made input of greenhouse gases into the atmosphere (e.g. about 92% of carbon monoxide emissions, see **Figure 1-1**). The release of these pollutants gives rise to a number of effects ranging from direct effects on the health of people and the environment (smog and acid rain) to the threat of climate change and uncontrolled global temperature rise due to greenhouse gases.

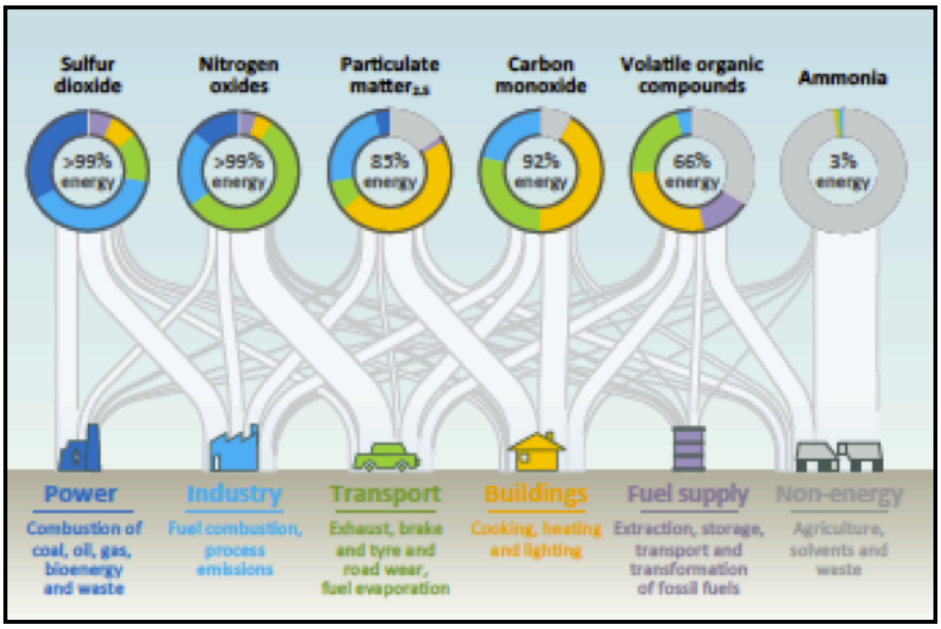


Figure 1-1 Air pollution and their sources in 2015 (Birol, 2016)

The main gases in the atmosphere are N_2 78%, O_2 16%, Ar 0.93 % and CO_2 0.037%. In the Paris agreement, implemented on 4 Nov 2016, The International Energy Agency (IEA) scenarios predicted that the international community will need to slow down the projected rise in energy-related carbon emission from an average 650 million tonnes per year in 2000 to around 150 million tonnes per year in 2040 (IEA, 2016). The broadly accepted goal is to reduce greenhouse gas emissions such that the average global temperature increase due to global warming should not be more than 2^{o}C. It is a challenge that engineers and scientists have the vital task of achieving.

In a new policy scenario, IEA expected that global primary energy demand between 2013 and 2040 to be around 17,900 Mtoe (IEA, 2016). To balance viable energy production with environmental protection, many countries are turning their attention to the development of new, "clean", carbon-neutral and sustainable energy sources. Among these, biofuel is expected to play a crucial role in the global energy infrastructure of the future.

1.2 Biofuel

Petrol has anhydous chain length $(C_4 - C_{12})$ and diesel $(C_3 - C_{25})$. Carbon content by mass (%) is 85-88% and 84-87% respectively (Boyle, 2003). Therefore, transportation fuel technology has to deal with these components which is challenging. Biofuel produces liquid or gaseous fuels from biological processes. Methane is the main gaseous fuel and is produced by

anaerobic digestion of biological material. Liquid biofuels are either produced by fermentation (ethanol – similar in use to petrol) or by the natural production of oils by plants (similar in use to diesel).

Liquid biofuels were first used as motor fuels in 1900 when early diesel engines were designed in 1890 by Rudolf Diesel for use with biodiesel (peanut oil) in Germany (Soccol *et al.*, 2016). However, these were soon replaced by petroleum and diesel derived from fossil oil. Decades later, in the 1970s, the shortage of fossil fuels due to geopolitical conflicts, and the subsequent rise in the price of crude oil in 2000s, alongside energy security and climate change concerns, once again attracted the attention of governments to the use of biofuels.

1.2.1 Bioethanol

Bioethanol is widely used in many countries such as Brazil, the USA. and Thailand. Ethanol or ethyl alcohol (C_2H_5OH) is a clear colourless liquid. It can be produced by the hydrolysis process and sugar fermentation from energy crops such as corn, maize, wheat, waste straw, and sorghum (Schenk *et al.*, 2008). There are three methods of an extracting sugar from biomass: 1) concentrated acid hydrolysis, 2) dilute acid hydrolysis, and 3) enzymatic hydrolysis.

The hydrolysis process breakdowns the cellulosic part of the biomass into sugar solutions. This can then be fermented into ethanol using yeast (**Figure 1-2**). Yeast converts the sucrose sugar into glucose and fructose as a giving by **Equation 1.1** and then to ethanol by **Equation 1.2.**

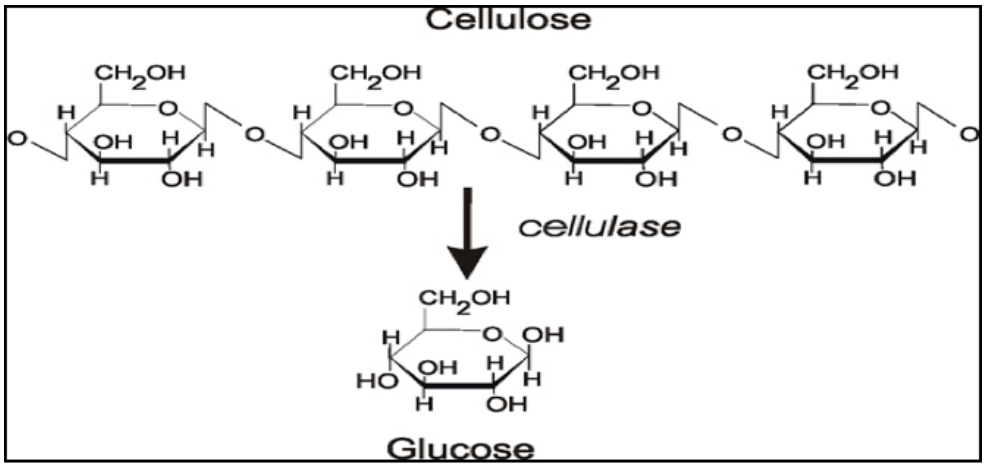


Figure 1-2 Cellulose structure (Held, 2012)

$$C_{12}H_{22}O_{11} + H_2O \xrightarrow[\text{catalyst}]{\text{Invertase}} C_6H_{12}O_6 + C_6H_{12}O_6 \quad (1.1)$$

Sucrose water Fructose Glucose

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 + \text{heat} \quad (1.2)$$

Ethanol can also be derived from ethane (ethylene) by combining water and catalysts at high temperature (**Equation 1.3**).

$$C_2H_4 + H_2O \rightarrow C_2H_5OH \quad (1.3)$$

In fact, fermentation is not a 100% conversion and compounds such as acetic acid and glycol are also formed and have to be removed. The initial ethanol content from biological fermentation is around 15% and the liquor has subsequently to be purified by absorption and distillation techniques. Ethanol reacting with O_2 (an oxidative burn e.g. in an engine) will be converted to CO_2, water, and heat.

Ethanol can be blended with petroleum up to 10% ethanol (v/v), with no need to modify vehicle engines, but up to 85% (v/v) can be used in specifically designed engines such as in flex-fuel vehicles (FFV) (Antoni *et al.*, 2007).

1.2.2 Biodiesel

Biodiesel is commonly obtained from vegetable oils which may compete with edible vegetable oil for agricultural land, waste frying oils from restaurants etc. and from animal fats, oils or fats having fatty acid methyl (or ethyl) esters (FAMEs) which can be separated (and, in the case of some oils and fats, converted to liquid) by using transesterification (**Figure 1-3** and **Figure 1-4)** The process can be catalysed by bases, acids, or enzymes. It can take place either at low or high temperature. The by-product is glycerol.

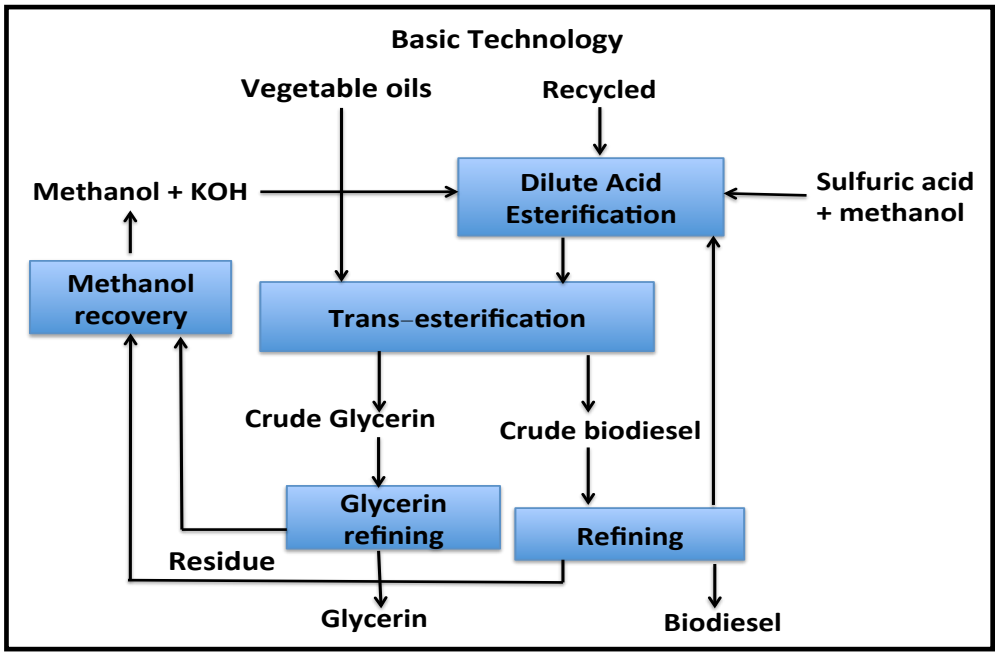


Figure 1-3 Basic biodiesel technology (Marchetti *et al.*, 2007)

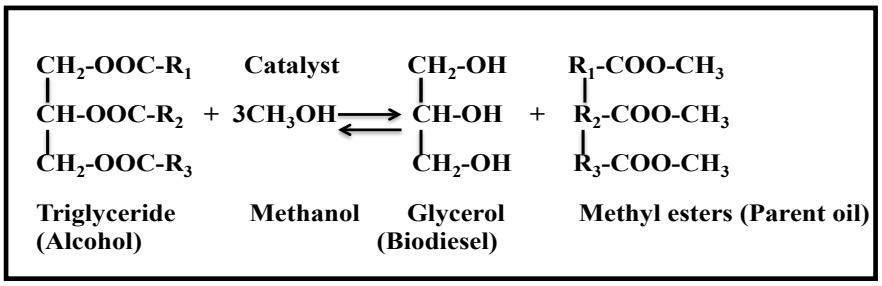


Figure 1-4 Biodiesel production where R_1, R_2, R_3 are the long chains containing C and H atom (fatty acid chain) (Chisti, 2007).

1.2.3 Biofuel from Algae

Microalgae – single celled microscopic plants – utilise sunlight energy to fix CO_2 from the atmosphere and have a high potential to produce biofuel when compared with terrestrial crops. They can be cultivated on non-arable land and have the potential to fix CO_2 from industrial flue gases thus removing a greenhouse gas from a man-made source. Under suitable conditions, some species can accumulate 20-50% of oil/lipid (dry weight) (Blinová *et al.*, 2015; Chen *et al.*, 2011; Chen *et al.*, 2011; Juneja *et al.*, 2013). These can be extracted, dewatered and converted to biodiesel. The remaining biomass can also be used – e.g. in anaerobic digestion to produce more fuel (Blinová *et al.*, 2015).

1) Cultivation: Microalgae can be cultured by photoautotrophic methods (algae require light to grow and create new biomass) in open or closed ponds, or by heterotrophic methods (algae are grown without light, and are fed a carbon source such as sugars, to generate new biomass) (Blinová *et al.*, 2015).

However it is the photoautrotrophic method that is of interest as it both uses "free" energy and nutrient sources and has the potential to fix unwanted CO_2. Designing a cultivation system depends on the algal species and strain, temperature, pH, light, nutrient, salinity and aeration or mixing (CO_2 flow rate). These are the major factors influencing the photosynthetic activity and the growth rate of the algae. Choosing an appropriate cultivation system is key to the affordability, scalability, and sustainability of the algal biomass production.

2) Harvesting and dewatering processes: After algae are grown they have to be concentrated prior to extracting the oils or other components. This step is energy intensive and should be carried out with as little chemical additions as possible (to avoid contamination of the oils and of the remaining biomass).

3) Extraction: There are three major components which can be extracted from algal biomass: 1) lipids (including triglycerides and fatty acids), 2) carbohydrates, and 3) proteins. Lipids and carbohydrates are precursors to fuels e.g. bioethanol, biodiesel, and bio-jet fuel. Proteins can be utilised for by-products (i.e. animal or fish feeds).

4) Conversion: Technologies comprising chemical, biochemical, and thermochemical processes, and/or a combination of these are used to obtain various desired products. The remaining biomass can be used as e.g. a feedstock for anaerobic digestion to produce methane or as a soil conditioner/fertiliser.

1.3 Research problems

Since the beginning of research to develop algal biofuels in the 1970s and particularly over the past decade, a number of problems and challenges have been investigated, for example;

1) Screening algal strains for a high growth rate and high oil yield,

2) Improving algal strains (genetic engineering) under diverse environmental conditions (i.e. CO_2, medium, temperature control, and light quality),

3) Scaling up open pond or photobioreactor designs,

4) Optimising systems for high yield biomass and developing efficient algal harvesting techniques,

5) Extracting algal oil to convert to biofuel.

Most individual studies only focus on improving specific areas rather than holistic studies. These may not reflect the real whole problem of developing an efficient and productive algal system. Studies to improve the overall system tend to be commercial-in-confidence.

The choice of a good system is crucial. The efficiency of the entire process depends on high algal mass, low capital investment, low operation costs, (e.g. energy consumption), and low contamination. CO_2 from various sources such as electricity production, industry and households is emitted to the atmosphere as a waste product which is a cause of air pollution. It can be fixed by algae and changed to a useful product such as a biofuel. This is of significant potential benefit in the development of renewable energy and environmental protection in a country such Thailand. However, the proportion of CO_2 (percentage CO_2 (v/v) in the supplied air) suitable for algal cultivation needs to be understood by further research. The study described in this thesis examines algal growth in air which is assumed to contain close to zero CO_2 and in concentrations of 6%, 12%, 24% and 50% CO_2 (v/v).

Separating (harvesting) algae after growth is an important technical and commercial challenge. In general, a particle in liquid, when left without stirring, will tend to settle to the bottom of a vessel. However, as a method of separation this is only suitable for relatively heavy particles, and not suitable for microscopic algae whose density is close to that of water, due to the time any such settlement would take. Addition of a coagulant or flocculant under appropriate conditions can assist in algal harvesting by settlement.

Common flocculating agents used to form lumps or aggregates are of two types: 1) inorganic flocculants, and 2) organic polymer or electrolyte

flocculants. Metallic chemicals are often used e.g. alum (hydrated potassium aluminium sulphate ($Al(SO_4)_2.x\,H_2O$) and ferric chloride ($FeCl_3$). But the main disadvantage of this method is toxicity. Algae may be contaminated with metallic salts and die before oil extraction. In addition, it is difficult to dispose of chemicals remaining in the system, the biomass or recovered from the supernatant. Otherwise useful biomass may become a waste and, in the worst case, a toxic or controlled waste, considerably increasing disposal costs. This is environmentally unsound, inefficient and uneconomical for commercial use. Indirect costs through its impact on other operations for instance; disposal costs, wastewater operation and maintenance costs may limit biofuel development. Harvesting processes make up around 20-30% of the total biofuel production cost (Grima *et al.*, 2003; Mata *et al.*, 2010; Wu *et al.*, 2012) and it is therefore important to develop low cost, environmentally-friendly harvesting systems.

Harvesting processes using natural biopolymer flocculants such as chitosan and crab shell would cause less problems and are less expensive with lower energy consumption than centrifugation or filtration. A comparison of these is described in this thesis and this approach may lead to an efficient low cost sedimentation process while avoiding contamination.

1.4 Aims of this study

In relation to a specific strain of microalgae *Chlorella vulgaris* TISTR 8580 isolated by the Thailand Institute of Scientific and Technology Research (as this is the country where the results will be applied), this study looks to clarify and understand:

1) What are the best conditions in which to grow the algae?

2) How to design a photobioreactor and optimise the system?

3) How will these algae grow under different CO_2 concentrations?

4) To what extent do metallic salts and biopolymers (chitosans and crab-shell) enhance the sedimentation process?

5) How may other additions affect algal growth?

6) How will organic and inorganic carbon levels change when adding chitosan?

1.5. Objectives

The objectives of this project are:

1) To enhance the concept of waste to energy by using CO_2 for algal cultivation

2) To design a simple photobioreactor system growing a specific strain of algae on a laboratory scale and to optimise the system, measuring growth rates and biomass production.

3) To improve the efficiency of a sedimentation process (as the first stage in harvesting the biomass) by avoiding contaminating chemicals. This will be done by using biopolymers (chitosans and crab shell) and comparing these with metallic salts.

In addition, both organic and inorganic carbon changes will be investigated by measuring TC, TIC and TOC when adding chitosan.

1.6 Scope and methodology of the study

The study begins by reviewing the literature on growing algae, engineering designs, optimal photobioreactor systems and enhanced CO_2 supply. Experimental work includes algal selection (comparison of strains and their environmental requirements) and developing a growth system for the Thai strain of *C. vulgaris* and studying its requirements of, for instance; nutrient levels, light variation, degrees and type of aeration.

After the initial experiments a photobioreactor system will be designed, commissioned, and tested and then used to grow the selected strain. Subsequently, algae will be transferred to the photobioreactor system with controlled environmental conditions. The microalgae will be grown in 4-2L columns (1 control, 3 working volume) on a seven day batch basis to study the effects of various levels of CO_2 (v/v) and bicarbonate ($NaHCO_3$) by measuring the growth rates.

Flocculation and sedimentation will be studied by comparison of the effects of adding metallic salts (aluminium sulphate anhydrate, ferric chloride, aluminium chloride) and biopolymers (Medium molecular weight chitosan, high molecular weight chitosan, and crab shell). Mature algae and dead cells will be used to perform flocculation tests. Experiments will vary coagulant dosages, speed of mixing and measure the degree of settling over time. Various

mixing times (min) and speeds (rpm) will be tested and sedimentation measured by turbidity, optical density (OD_{680}) and cell count. The study will also measure carbon changes (Total Carbon, TC, Total Inorganic Carbon, TIC, and Total Organic Carbon, TOC) (**Figure 1-5)**.

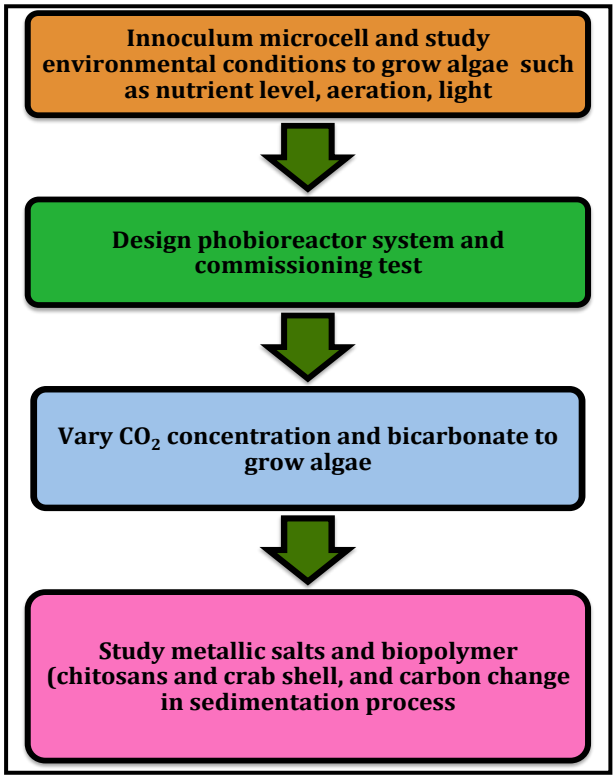


Figure 1-5 The process of the research

1.6.1 Data collection and statistical analysis

1.6.1.1 Data collection

The study will collect useful information from the scientific literature. The literature will be reviewed for algal cultivation, photobioreactor designs, and harvesting process focusing on flocculation and sedimentation.

The laboratory data will be suitably recorded (e.g. in excel spread sheets). Quality control (QC) and quality assurance (QA) are verified by performing blank tests (controls) and taking three replicate readings and using at least three replicates in each experiment. Numerical data will be integrated into Microsoft Excel for Mac OS X EL Capitan version 10.11.5, MacBook Pro (15-inch, version Mid 2012).

1.6.1.2 Statistical analysis

Statistical analysis, for instance mean, regression, standard deviation, correlation, level of confidence, and p-value will be determined using statistics programmes such as Excel workbook Mac pro and Graph pad Prism version 6 for Mac OS X version. Software programmes Auto CAD 2017 for Mac is used to draw engineering works.

1.6.2 The advantages of this research

This study will help to:

1. Promote renewable energy (biofuels), especially in Thailand

2.Target changing waste CO_2 to energy by using CO_2 from flue gas to increase algal biomass in culture

3. Improve the efficiency and environmental safety of initial separation processes.

Chapter 2 Literature Review: Algae cultivation and photobioreactor technology

2.1. Introduction

A review of the literature for biofuel production from algae, the photosynthesis process, environmental factors and photobioreactor designs has been made. The study found that algal biomass yield relates to a number of the environmental factors for example; algal strain, media components, photosynthetic process, pH, light, aeration level, and mixing. Both open pond and closed systems have their limitations in, for example, construction and maintaining the system. Therefore, choosing a system depends on the application, the cost, and the desired operational parameters. The feasibility of any application should be studied in the laboratory before scale-up to a commercial system. PBRs have different designs depending on their use and applications. When grown, the algae and/or their products need to be separated by suitable harvesting techniques to manufacture products such as biodiesel. The literature review will cover:

1) Photosynthetic pigments and algal growth rates;
2) Algal cultivation and types of algal cultivation system;
3) Photobioreactor designs;
4) Harvesting technologies for microalgae;
5) Algal aggregation and flocculation characteristics;
6) Sedimentation characteristics and;
7) Factors influence flocculation and sedimentation processes

2.2 Photosynthetic pigments and growth rates of algae

2.2.1 Photosynthetic pigments

Green plants such as algae absorb light energy via three pigment groups; 1) chlorophylls, 2) carotenoids and 3) phycobilins.

Group 1 (green pigment) and Group 2 (yellow pigment) are lipophilic consisting of chlorophyll-protein complexes. Group 3 is hydrophilic.

1) Chlorophyll

Chlorophyll molecules comprise a tetrapyrrole ring which contains a central magnesium atom, and a long-chain terpenoid alcohol (except for chlorophyll c). The various types of chlorophyll molecules i.e. a, b, and c differ in their side-groups substituted at the ring. The green pigment of chlorophyll has two major absorbent bands: 1) blue or blue-green (450-475 nm., chlorophyll a) and 2) red (600-700 nm., chlorophyll b) (Blinová *et al.*, 2015). The photoreaction takes place in a chloroplast. It is an oval shaped structure. The chloroplast has outer and inner membranes within which are stoma containing stacks (grana) of thylakoids where photosynthesis takes place.

2) Carotenoids

Carotenoids or tetraterpenoids are organic pigments found in chloroplasts and chromoplasts. The structure of carotenoids is a hexacarbon ring linked by 18 carbon atoms with double-bond chains. They are usually either hydrocarbons (carotenes) or oxygenated hydrocarbons (xanthophylls). The chromophore is the part of the molecule responsible for absorption of light and for its colour. The colour arises when a molecule absorbs one wavelength of visible light and transmits or reflects others.

2.2.2 Growth rate

Growth rate can be used to define the phases, in a lifecycle. It can be measured in the form of cell number or total mass. 5 algal growth phases have been described by Fogg and Thake (1987): 1) lag phase, 2) exponential phase, 3) linear phase, 4) stationary phase, and 5) decline or death phase (Mata *et al.*, 2010). The detail is in Chapter 3.

2.3 Algal cultivation

Theoretically, at the stationary growth phase, the maximum algal growth rate should be equal to the maximum rate of photosynthesis. However, it is difficult to optimise and control a cultivation system to this point because a number of factors affect the growth rate; for instance; algal strain, nutrient, light, pH, dissolved O_2, mixing, air and gas supply and inorganic carbon.

2.3.1 Selection of microalgae

There are thousands of species of microalgae in the environment. Cell strains (both the species and the strain (isolate) of the species) and cell selection are important factors in culturing algae. In selecting algae various desirable factors need to be considered such as; 1) CO_2 fixation rate 2) yield of desired component 3) a high tolerance to harsh conditions (such temperature and pH) 4) ease of collection and concentration during the harvesting process. Therefore, algae should be selected to grow well and be easily harvested in the given conditions.

Chlorella sp. is an interesting and versatile algae. Morphologically, the algae are in a genus of single-celled algae belonging to the phylum *Chlorophyta*. Their size is around 2-10 μm and they may be able to be developed on an industrial scale in Thailand to provide a biofuel (Sawaengsak *et al.*, 2014). They can provide a good yield (high lipid content), grow rapidly and tolerate relatively high temperatures combined with good CO_2 consumption. They contain around 45% protein, 20% fat, 20% carbohydrates, 5% fibre and 10% minerals and vitamins such as B_1 (Barghbani *et al.*, 2012). However, the proportions can be varied by the conditions in which they are grown.

Most algae grow within the temperature range 15-40 ℃ with the best growth found between 20 and 30 ℃ (Barghbani *et al.*, 2012; Blinová *et al.*, 2015; Chinnasamy *et al.*, 2009). In a country such Thailand, bioreactors using natural sunlight have no problems with low temperatures, however, it may be that they would need to be cooled or shaded at certain times to avoid too high temperatures.

Chlorella sp. can grow under high levels of CO_2 (v/v). Blinová *et al.* (2015) found that some species can be cultured with sparging up to 50% CO_2 (v/v) in the air supplied. Chiu *et al.* (2008) found that they can yield lipid between 0.97-0.143 $g.d^{-1}$ when 2-15% CO_2 (v/v) is added.

C.vulgris TISTR 8580 is a model in this study because they give a good yield and tolerate high temperature. They are easy to find it in the natural water resource in Thailand where Thailand Institute of Scientific and Technological Research (TISTR) have identified strains which could be used with power plant derived added CO_2 in Thailand (see **Chapter 3**).

Yun *et al.* (1997) found that *C. vulgaris* could grow in wastewater while adding 5% to 15% CO_2 (v/v). The CO_2 fixation rate was around 26.0 g $CO_2/m^3/h$. *C.vulgaris ARC1* strain also grew well when supplied with air enhanced with CO_2 at 5-6% (v/v) at a temperature 30 ℃ giving a biomass yield of 7.86 μg/mL (Chinnasamy *et al.*, 2009). *C.vulgaris* (IAM C-534) was reviewed in a study by Juneja *et al.* (2013) and by Hirano *et al.* (1997) who found that the organelles comprise 37% (w/w) of dry weight and have a high potential to yield starch. Chiu *et al.* (2008) report that the highest total lipid production for *C. vulgaris* was 17.2 $g.L^{-1}.d^{-1}$ using a CO_2 concentration of 15% with aeration at 0.25 $vv^{-1}m^{-1}$ (volume gas per volume media per min). Chiu *et al.* (2009) reported that *Chlorella sp*. (NCTU-2) gives a CO_2 fixation rate exceeding 3 g CO_2 /L in a 4L photobioreactor when adding 5% CO_2 with a light intensity of 300 $\mu mol.m^{-2}s^{-1}$. Lee *et al.* (2000) found *C.vulgaris KR-1* and *C.vulgaris HA-1* could tolerate 15% CO_2 (v/v). They had a stable growth at a cell concentration more than 2 $g.L^{-1}$ (dry weight) in around 30 hr.

2.3.2 Growth media

The media provides a physical support for the algae and must also include essential elements to support their growth and reproduction (Blinová *et al.*, 2015). Favourable culture media for algae include; TAP, BG-11, and BBM (Bold's Basal medium). However, the choice of medium also relates to the algal species and strain. The dilution (concentrations of nutrients in the final culture medium) is also important. 3 groups of substances need to be considered in the media: 1) macronutrients i.e. CO_2, nitrate (NO_3^{2-}) and phosphate (PO_3^{2-}) 2) trace element such Na_2EDTA salt, and 3) vitamins (such as B_1, B_{12}).

As an alternative to commercial media, a natural media resource, such as pond water, may be used to grow algae and to save cost. Differences in the media may affect algal growth rate and this leads to experimental designs to grow algae in various media to clarify which is the best media to produce a high yield

The Crookes Valley Park is located at Sheffield, S10 1BA. It is an area of the public park land, in The Crookesmoor. It belongs to Sheffield City in South Yorkshire, England. The park is about 2 kilometres to the

west of the City Centre at 53.3834 °N, 1.4929° W and is one of the three "Crookesmoor Parks". The others are being The Western Park and The Ponderosa. The Crookes Valley Park covers an area of approximately 4.8 hectares (11.9 acres).

The pond in Crookes Valley Park used to supply water to Crookes and Upperthrope in the 18th century. After this it has been used for fishing and general recreation. It is fed by a small stream which flows through a culvert into the western corner.

Land use has a key influence on water quality assessment (Yuncong and Kati, 2011). Sheffield extends over 3,600 hectares. It is over 500 meters above sea level. There are the low-lying river valleys in the east which are only 10 meters above sea level at their lowest point. The rivers flow from the northern and the western uplands down to the east/the southeast.

The general area is greatly diverse. There are the various local landscapes which consist of the grit stone edge, woodland, grassland, herbaceous park, farmland, open space, arable land, moorland and bog, gardens, allotments and cliffs. The valley has the main green space structure through the city.

There are manufactures and constructions around the dam. Clay, mudstones, sand and gritstones were used as the raw materials for building and manufacture of grindstones. There are also leachate and/or industrial inputs to the lower areas due to iron works and coal mining.

The regions farming produces manure and animal wastes and fertilizer use, the residues of which enter the local surface water system. Pesticide use, storage, or accidental spills in transportation may also be pollutant sources of surface water.

Therefore, when using natural waters to grow algae, at least the major parameters should be monitored such as pH, nitrate, phosphate, ammonia, potassium, calcium and chlorides.

2.3.3 Light

Light is the energy source for algal growth. However, too high or too low light intensity results in photoinhibition. The effects of too high light intensity is due to disruption of chloroplast lamellae caused by inactivating enzymes in the CO_2 fixation process. The spectrum of photosynthetic active radiation, (PAR) can be measured by units of quanta $m^{-2}.s^{-1}$ or in Wm^{-2} or the number of photons per unit area (photosynthetic photon flux density μmol quanta. $m^{-2}s^{-1}$ or $\mu Em^{-2}.s^{-1}$). The conversion factor is 1 Wm^{-2} equals 4.5 µmol photon $m^{-2}.s^{-1}$. The photobiologist prefers to measure the amount of light energy incident on a surface, i.e. radiant flux energy (using a radiometer). Irradiance can be measured in units of power per area (Wm^{-2} or Jm^{-2} s^{-1}), illumination ($lm.m^{-2}$) or foot-candle (1 lm ft^{-2}, i.e. 1 ft. candle equals 10.76 lux). Pyranometer or solar meters can be used to analyse solar irradiance, whilst a lux meter/light meter measures light level.

Light needs to penetrate the algal culture (or the algal culture must be mixing enough for all the algae to receive sufficient light energy). Overheating due to the illumination should be avoided. Light may be supplied by natural sunlight or, in the laboratory, cool white fluorescent tubes.

Blinová *et al.* (2015) found that blue or red light are the most active parts of the spectrum for photosynthesis in *C.vulgaris*. However, Barghbani *et al.* (2012) looked at 4 different light colours (white, yellow, blue, and red) and found the highest yield for *C.vulgaris* was in yellow light. Thus it is likely that different species and even different algae strains will have optimal photosynthesis at different parts of the spectrum. Lanvens and Sorgeloos (1996) reported in (Blinová *et al.*, 2015) showed that light of 1,000 Lux is suitable to grow algae in Erlenmeyer flasks. 5,000-10,000 Lux is required for larger volumes.

Some algal groups, such *Dinoflgellates,* often need light intensity of 60-100 μmol photon.m^{-2} for laboratory inoculum or small culture volumes (Andersen, 2005). On scale up, increasing light intensity up to 200-400 $\mu Em^{-2}s^{-1}$ gives increased algal activity (Munoz and Guieysse, 2006). Pulz (2001) showed that a flat plate PRB with a layer thickness of over 5 mm. requires light around 1,150 $\mu Em^{-2}s^{-1}$.

Dauta *et al.* (1990) found that *C. vulgaris* grow well at 30 ℃ and a light intensity of less than 200 $\mu Em^{-2}.s^{-1}$. In 2012, Sharma *et al.* studied the effects of culture conditions on *C. vulgaris* and the biochemistry in the cell by comparing the effects of light and temperature under different culture conditions. They found that natural daylight and a light and dark regime: 12:12 (2700 Lux) at 25-30 ℃ resulted in the highest levels of chlorophyll a and b (Sharma *et al.*, 2012). Wong *et al.* (2014) found that the specific growth rate (μ) of *C. vulgaris* was 0.801 day^{-1} at a light irradiance of 4,500 Lux.

Several studies show that growth rates and lipid content change with variation in light/dark regimes and with species and strains under those regimes. Gong *et al.* (2014) studied the effects of light and pH by measuring cell density of *C. vulgaris* (FACHB-1227). The light: dark period was 12:12 hr. and they varied the light intensity at 3,960, 7,920 and 11,920 lux. pH was at 7,8,9 and 10 respectively. The results show that, without pH control, cell density is highest in cultures grown at 3,960 lux. When the cultures were controlled to pH 9 light intensity does not affect the optimal cell density even at the highest irradiation of 7,920 lux. The cell density was found to be highest at pH 10. Chandra *et al.* (2012) growing *Selenastrum sp.* at 2,500-3,500 Lux at D/L: 16:8 found that algae could yield a lipid content of 14% dry weight. Many reports find that algae can be grown well under 12:12 light/dark regimes (Dubinsky and Rotem, 1974; Kalhor *et al.*, 2016).

2.3.4 pH

The pH of the culture medium influences algal growth. In general, algal species are best cultured at pH 7.0-9.0 (Blinová *et al.*, 2015; Wang *et al.*, 2012). Dubinsky and Rotem (1974) studied the relationship between algal growth and pH of BBM media for various algae: *Achanantes misnutissima* Kutz*, Synedra radians Kutz, S.ulna Ehr, S. acus (Kutz.) Grun., Cymbell affinis Kutz, Gomphonema parvulum Kutz*. They found that a pH of 8.2 and a temperature of 30 ℃ provide the highest growth as measured by optical density (OD_{665}).

Mayo (1997) built a growth model of *C. vulgaris* containing heterotrophic bacteria at pH 3.0 and pH 11.5 while varying temperatures between 10 ℃ and 40 ℃. The study found that the optimal pH was 6.31-6.84 and temperature was 32.4℃. In this case, the maximum specific growth rate was

0.5 d^{-1}. Pruder and Bolton (1979) found that *T. pseudonana clone 3H* cells could adapt to pH < 6.5 in f/2 media, but declined in media at pH 8.8. The highest cell no. of 5 $x10^4$ to $1x10^6$ mL was obtained at pH 7.5± 0.1 and a temperature of 24 ± 1 ℃ with air containing 159 mol/L CO_2 at a flow rate 0.12-0.15 L/m. Light intensity was 1,500 $\mu W/cm^2$. Nguyen and Rittmann (2016) found that *Synechocystis sp. PCC6803* gives its highest specific growth rate (μ) of 2.4 d^{-1} in BG 11 media at pH 8.5.

2.3.5 Temperature

Temperature does not only affect the metabolic activities of the algae, but also the efficiency of non-biological processes such as gas-transfer rates and the characteristics of particle settling. Generally, algae can grow under a wide temperature range (20-35℃). Most algal cultivation is between 16 and 27℃ (Lebeau and Robert, 2003). Optimal temperature depends on the algal strain.

Low temperatures may result in: 1) reducing electron transport at a given photon flux rate due to a slower rate of CO_2 fixation 2) protecting photosynthesis II (PS II) 3) inhibiting protein synthesis. Temperatures lower than 16 ℃ cause a gradual reduction in growth rate with declining temperature and a similar effect is shown by increasing the temperature higher than 35℃ (Blinová *et al.*, 2015; Lebeau and Robert, 2003).

Mayo (1997) found that the optimum temperature for growth of *C. vulgaris* was more than 30℃. Similar results were found by Barghbani *et al.* (2012) who found that an optimum temperature of 30 ± 2 ℃ provided the highest yield of 1.4 g/L. Sharma *et al.* (2012) give an optimal range of 25-30 ℃ for *C.vulgaris*. The study found that the algae have a highest growth yield at cell number 440 x 10^4 cell/mL, and dry weight by 30.2 mg/50 mL. Chinnasamy *et al.* (2009) studied the effect of temperature (30,40, and 50 ℃) and found that 30 ℃ gives the highest yield (specific growth rate, μ, 0.222 d^{-1}) when adding 6% CO_2. At around 40^{o}C, the cells become less resistant to acidic conditions. Possibly, the cytoplasm is damaged at these higher temperatures due to hydrogen ions penetrating the chloroplast. Additionally, a fluctuating temperature may also cause photosynthetic inhibition (Mayo, 1997).

2.3.6 Types of algal cultivation system

The main types of algal cultivation systems are open pond or closed systems. The components comprise algae, water, nutrient, fertiliser, vitamins, CO_2, and air supply, valves, flow velocity, mixing equipment, electric system, wiring, constructional system and so forth. The selection of the culture system should consider local environmental conditions, economic and social scenarios (Mata *et al.*, 2010; Pawar, 2016).

1) Open pond

The physical open pond is a shallow pond in which algae are cultivated. Nutrients can be provided through run-off from a water treatment plant (thus enhancing water treatment) or from a natural source. Cell circulation can be improved by mixing. This can typically be accomplished by using, for example, paddle-wheels, to circulate the media (Razzak *et al.*, 2013)

An open pond architecture is shown in **Figure 2-1**. Algal ponds are often "raceways". They circulate the water to keep the algae mixed and exposed to sunlight. The size and depth depend on the amount, and hence penetration, of sunlight.

Figure 2-1 Conventional raceway pond by Seambiotic, Ltd. (Xu *et al.*, 2014)

2) Photobioreactor systems

Closed systems or photobioreactors (PBRs) are cultivation systems which are designed to be highly efficient in the conversion of light energy,

use less land, be easy to scale up, and be commercially viable. There are a number of available shapes and applications, for instance; bubble column, airlift reactor, flat-plate reactor, stirred tank, tubular and vertical columns (Mata *et al.*, 2010; Munoz and Guieysse, 2006; Pawar, 2016).

Advantages and disadvantages of the open pond and closed systems are given in **Table 2-1**.

Table 2-1 Comparative open ponds and closed systems (Ugwu *et al*., 2008)

Open Pond	Closed Pond
Advantages	**Advantages**
- Low cost construction - Simple operation - Low cost maintenance - High biomass yield	- Controlled environment and high productivity - Easy to improve productivity. -Simple to control gas transfer. -Reduction in evaporation of growth medium. -More uniform temperature. -Better protection from the outside contamination. - Space saving and reduce fouling -Less contamination, water use & CO_2 losses -Good light utilisation & mixing
Disadvantages	**Disadvantages**
- Poor light utilisation - Difficult to control light and temperature - Trouble with contamination & evaporation - Difficult to grow algae for a long period - Poor productivity - Large land use - Limited to fewer strains of algae - Shear stress to algal cultures	- Cost/complexity - Thermal management - Oxygen accumulation - Construction requires sophisticated materials - Deterioration of construction material

2.4 Photobioreactor designs

To design a closed algal cultivation system, the following aspects need to be considered.

2.4.1 Liquid–gas mass transfer

The algae require that O_2 and CO_2 are available and dissolved in the liquid. Thus, a means of gas-liquid contact needs to be set up in the system.

The gases should be continuously sparged into the liquid. In the process, transfer of gases takes place in several steps. Firstly, the gas travels through the gas-liquid interface, then passes into the bulk liquid. Finally, it reaches and enters the microorganisms. The entire process is driven by the difference (gradient) between the concentration of a component in the gas, in solution and in the organism. The rate of gas transfer in the culture system is proportional to the difference between the existing concentration and the equilibrium concentration. The main factors affecting hydrodynamics or mass transfer are; 1) O_2 mass transfer coefficient 2) CO_2 3) mixing 4) liquid velocity 5) gas bubble velocity, and 6) gas holdup (Ugwu *et al.*, 2002).

1) Oxygen mass transfer coefficient (k_La): O_2 is an essential constituent of the liquid (water) system. Adding O_2 to the water is a means to maintain a level of dissolved O_2 (DO). The main O_2 source for reactors is from the atmosphere. The amount in solution depends on temperature, salinity, and pressure. Colder water will hold more O_2 than warmer water. Freshwater holds more O_2 than salt water. At night and on very cloudy days, algae will remove net O_2 from the water for respiration. On the other hand, during daylight, they produce more O_2 than they consume.

In an algal cultivation system, O_2 sparging can also accelerate mixing to;

1) prevent sedimentation of the algae;

2) ensure that cell populations are exposed the light and nutrients;

3) avoid thermal stratification (e.g. in outdoor cultures);

4) improve gas exchange between the culture medium and the atmosphere

The study of Rubio *et al.* (1999) reported in (Ugwu *et al.*, 2008) found that a 200 L airlift with 100 m. long tubular horizontal photobioreactors gave a K_La of 0.014 s^{-1}. This produced a biomass concentration of 1.5 $gL^{-1}d^{-1}$.

2) Mixing: The type of mixing system relates to the type of photobioreactor and its size. For example, a stirred tank uses impellers (a propeller, paddle, turbine) to circulate a suspension. While a tubular photobioreactor (TPBR) can be mixed by a sparging system.

Good mixing needs to; 1) prevent algal settling, 2) ensure that the cell population is well exposed to even light, pH, temperature and nutrients, 3)

facilitate heat transfer and avoid thermal stratification and 4) improve gas exchange between the culture medium and the gas phase.

Ugwu *et al.* (2002) noted that increasing the number of static mixers inside the reactor column raises the gas transfer coefficient and gas hold up time to culture *C. sorokiniana* with a productivity of 1.47 g/L per day being 15-70% higher than the productivity in tubes without the mixers. Zhang *et al.* (2002) found that CO_2 supplied at a rate of 0.025-1.00 $vv^{-1}m^{-1}$ is optimal in a flat plate reactor. Qiang and Richmond (1996) used an O_2 rate of 0.6-6.3 $LL^{-1}m^{-1}$ to design a flat plate reactor. The mixing rate also depends on the cultural designs.

3) Liquid velocity: An increase in aeration rate can be achieved by mixing, liquid circulation, and mass transfer between the gas and liquid phase. This depends upon the type of cultivation system and liquid velocity and can be accomplished by pumping or mechanical agitation (e.g. rotation wheels, static mixer) as mentioned above.

However, not all algal species can tolerate a high velocity. Mechanical agitation and bubble break-up can lead to hydrodynamic stress. This results in restriction of algal growth and low metabolic activity.

In small scale culture, a fine sparger can be used to control an increasing gas dispersion in TPBRs. Gas bubble velocity can be measured by a flow meter. The bubbles affiliate during flow to form an interface between the liquid and gas. At the wall of the tube, the contacting area is reduced. This may lead to a poor mass transfer rate. The solution should be adjusted to an appropriate flow to control to the optimal conditions.

4) Gas bubble velocity

Gas bubble velocity and size link to the liquid flow rate (Ugwu *et al.*, 2008). The rate and type of circulation will also be altered when baffles or a static mixer are installed inside the reactor.

5) Gas hold-up

The volume fraction of the gas-phase in the gas-liquid dispersion is known as the gas hold-up which depends on the type of reactor, for example; in a bubble column, the gas hold up can be calculated by a differential pressure between the pressure of 1 bar and the actual pressure. Sierra *et al.*

(2008) studied the fluid-dynamics, mass transfer and mixing in a flat-plate PBR. The maximum gas holdup was 0.018 and mass transfer coefficient was 0.0063 Ls^{-1} with an aeration rate 0.32 $vv^{-1}m^{-1}$.

2.4.2 Yield coefficient

During exponential algal growth (logarithmic growth) new cells are fully grown and the substrate is almost consumed. This can be defined by the yield coefficient which depends on various factors such as: 1) the oxidation of the carbon sources and nutrients 2) the level of polymerisation of the substrate 3) the pathway of metabolism 4) the growth rate and 5) various physical parameters of cultivation (such as: temperature, light, pH) (Metcalf and Eddy, 1997).

2.4.3 Types of photobioreactor and technology

A number of designs of photobioreactor have been developed to solve specific problems such as: light capture and flow distribution (i.e. spectral shifting and internal light capturing photobioreactor), mass transfer (such as membrane PBRs), and construction costs (plastic bag PBRs).

2.4.3.1 Tubular photobioreactors (TPBRs)

TPBRs are suitable for large illuminated areas. The system gives a fairly good biomass productivity and economic cost. TPBRs comprise of a solar array, a harvesting unit, a degassing column for gas exchange, a cooling and heating system, and a circulating pump. The array of transparent tubes can be built in different patterns (e.g. straight, bent, or spiral). Most of them are constructed with either glass or plastic tubes. The tube diameter is important. Too narrow may lead to radiant heat losses or photo-inhibition as well as low cell density. Too great and the algae may not get enough light. The diameter should be 0.1 m or less. TPBRs have different arrangements such as horizontal, vertical or sloped (Wang *et al.*, 2012).

1) Horizontal tubular photobioreactors (HTPBRs)

HTPBRs have a higher volume than the vertical photobioreactors. The diameter can be reduced without concern for structural integrity, and angled for incident light (**Figures 2-2 and Figure 2-3**). The systems have a problem because, depending on the country, large amounts of heat input or

removal requires an expensive temperature controller. A heat exchanger may be incorporated in the design to maintain the optimum temperature.

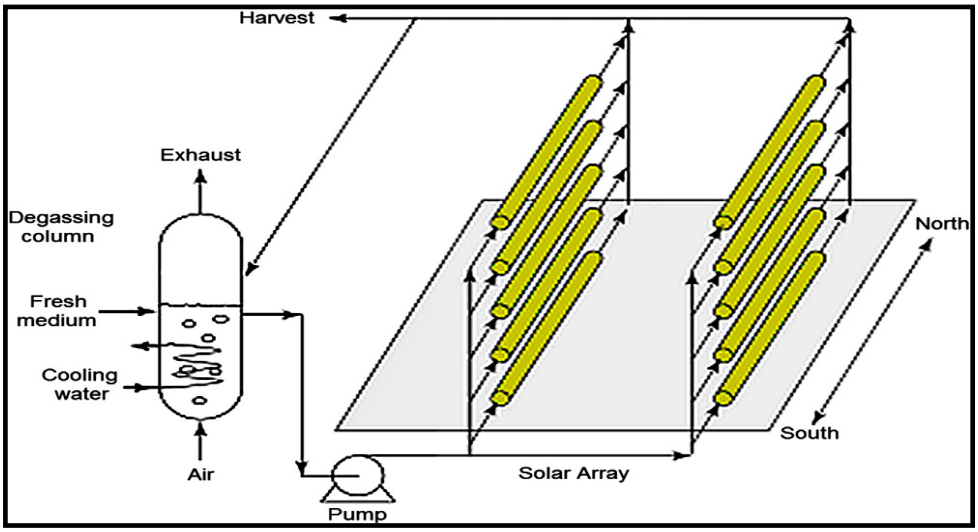


Figure 2-2 Schematic diagram of an HTPBR (Wang *et al.*, 2012)

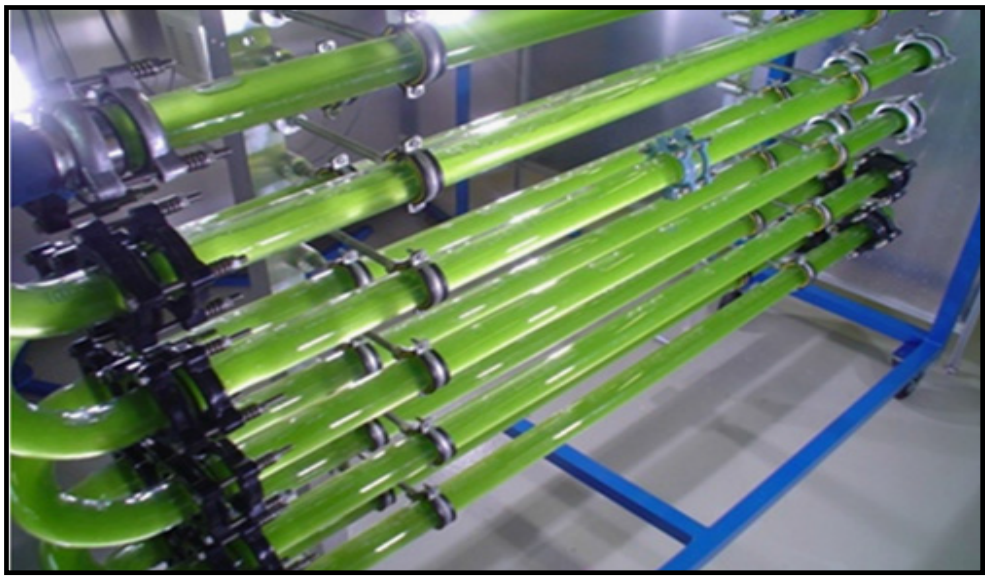


Figure 2-3 HTPBRs (Bitog *et al.*, 2011)

A newer design consists of a series of thin tubes connected at the bottom by a manifold which supplies a compressed gas. The top has a degasser. The tubes are placed on a framework to maintain the desired angle. The inclined tubes increase bubble velocity, gas hold-up, and gas transfer coefficient (The ratio of gas transfer divided by air volume). Ugwu *et al.* (2002) suggest that an angle of 45° is the best to operate the column (1.47 $gL^{-1}d^{-1}$) (see **Figures 2-4**).

Figure 2-4 A near 45 degree TPBR (Bitog *et al.*, 2011)

2) Helical tubular photobioreactors

Helical TPBRs or biocoil are a hybrid between a horizontal and vertical. They comprise several sections of PVC tubes wound around a cylindrical wire frame. The illumination is either sunlight or fluorescent lamps (**Figures 2-5 and Figure 2-6**). Hall *et al.* (2003) used a 75 L helical reactor with a circular frame 1.2 m. in diameter. The reactor was 106 m. long with plastic tubing of 0.03 m diameter. Travieso *et al.* (2001) cultured *Spirulina platensis SP-G* using biocoil cylindrical reactor. The reactor was cylindrical, 0.9 m height with a 0.25 m^2 basal area. The maximum productivity yield was 0.40 $g.L^{-1}.d^{-1}$.

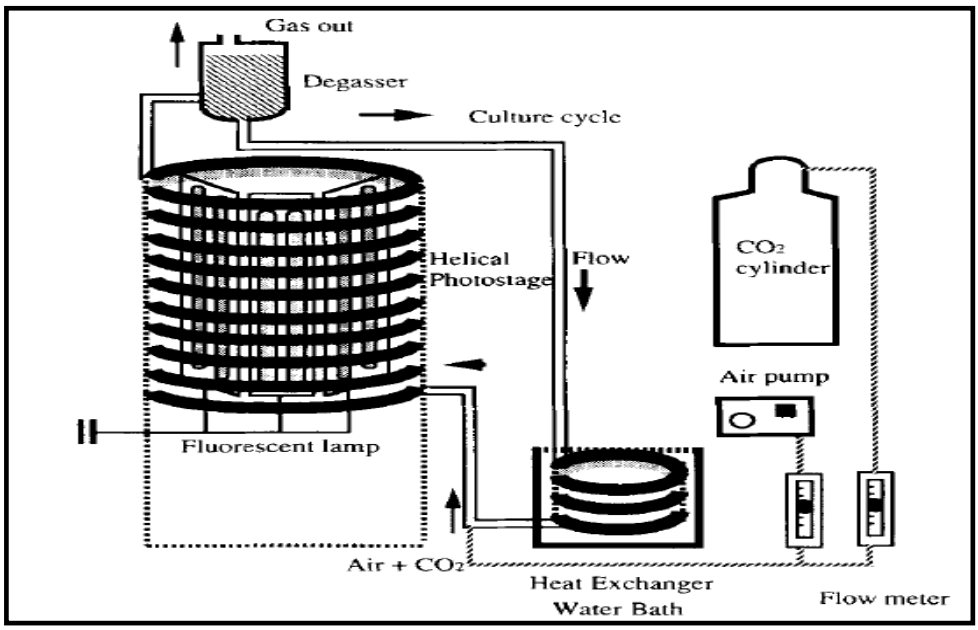


Figure 2-5 Schematic diagram of a biocoil (Watanabe *et al.*, 1995)

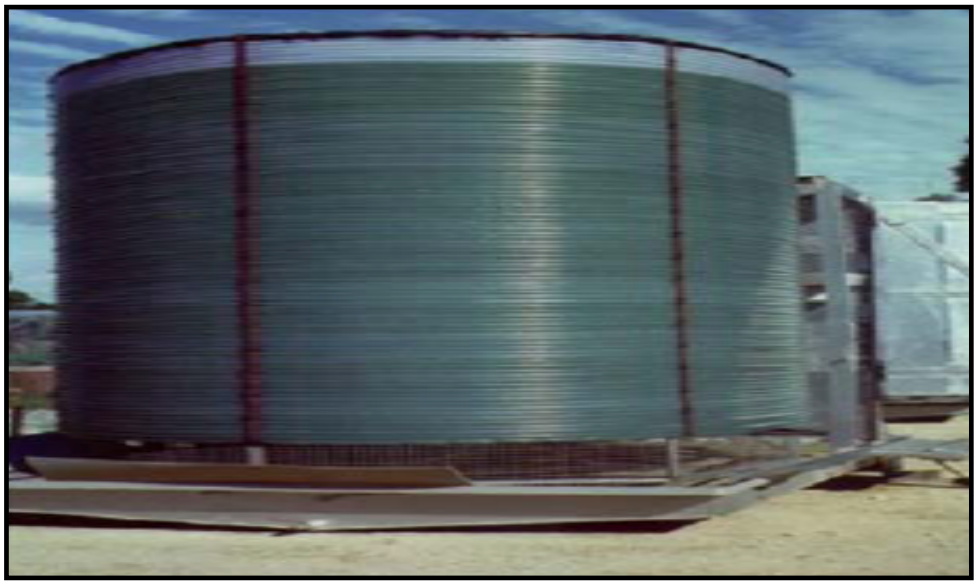


Figure 2-6 A 1,000 L helical tubular photobioreactor (Biocoil) at Murdoch University, Australia. Courtesy of Professor Michel Borowizka (Chisti, 2007).

3) Vertical column PBRs (VPBRs)

Advantages of this system are compactness with low-cost and high volumetric gas transfer. The structural engineering design has to: 1) reduce mutual shading in multi-column facilities, 2) increase the surface area/volume ratio. They should also be concerned about: 1) the height restriction and gas transfer limitation 2) the strength of the transparent materials to construct the columns and 3) the length of the column in relation to the residence time of O_2.

VPBRs are cylinders, the diameter should not exceed 0.2 m. The height is usually less than 4 m (Bitog *et al.*, 2011; Mirón *et al.*, 2004; Wang *et al.*, 2012). The flow velocity should be between 0.125-1.25 $vv^{-1}m^{-1}$ (Ugwu *et al.*, 2002). The air sparger is located at the bottom. The space at the top of the PBR is used for gas or liquid separation. Mixing can be provided by air bubbles. Perforated plates may be installed inside the column to break up bubbles, and to increase turbulence.

2.4.3.2 Airlift PBR

Airlift PBRs are modified bubble column reactors. They are engineered for both good circulation of the medium and good oxygen dissolution. The thickness of the plate determines the surface area and the length of light path. A small thickness allows for better diffusion and light

distribution. **Figure 2-7** includes an internal loop airlift, split column and external loop airlift with an air sparger.

A schematic of an airlift is shown in **Figure 2-7 A**. The advantages of this are simultaneously mixing and good light absorbability, despite the large diameter (which should not exceed 0.2 m (Bitog *et al.*, 2011)). Other benefits are simplicity and cleanability.

Split column airlift PBRs have a flat plate to separate the diameter of the column into two parts: 1) the riser region and 2) the lower region. Gas sequestration occurs at the top of the column, while the degassed liquid falls downward (**Figure 2-7 B**).

An external loop airlift PBR is shown in **Figure 2-7 C**. The gas bubble occurs in the internal region. The degassed liquid is moved through an external circulation column.

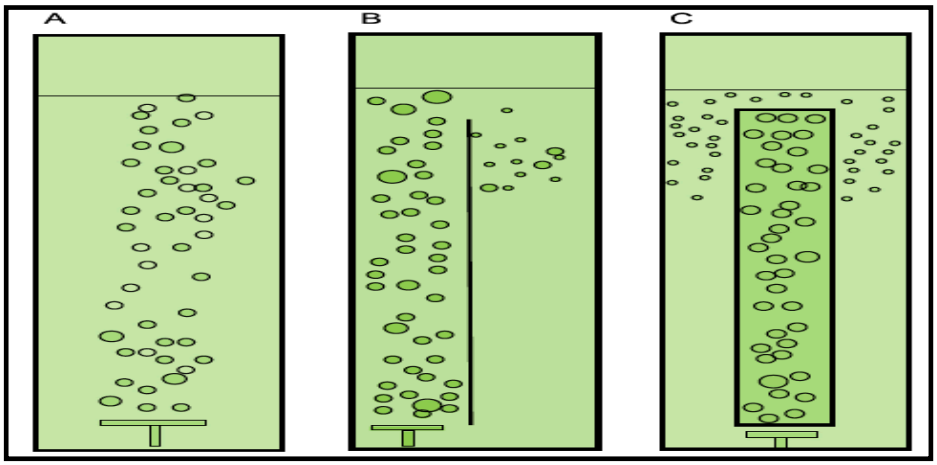


Figure 2-7 Example of airlifts (A) internal loop airlift (B) split column airlift (C) external loop airlift (Pawar, 2016).

2.4.3.3 Flat-plate PBRs (FP-PBRs)

Flat-plate reactors were first proposed by Milner (1953) (as cited in Ugwu *et al.* (2008)). Samson and Leduy (1985) developed a flat reactor equipped with fluorescence lamps to culture *Spirulina maxima*. A year later, De Ortegas and Roux (1986) developed an outdoor flat panel reactor by using thick transparent PVC material (as thin walled PBRs are more costly to build, difficult to clean and result in more fluctuating temperatures). Extensive work since has come up with numerous designs (such as shown

by Tredici and Materassi (1992), Qiang and Richmond (1996), Zhang *et al.* (2002) and Sierra *et al.* (2008).

The optimal tilt providing the maximal incident light can be changed, with the position following the sunlight through a day and/or year. Wang *et al.* (2012) found that in the summer when the sun is high, smaller tilt angles of 10° and 30° resulted in a higher biomass. In the winter, when the sun is lower, tilt angles of 60° were needed. They noted that the optimal angle depended upon on the latitude of the location.

FP-PBRs have received more attention due to their large surface area for maximum solar utilisation. Conventional designs allow immobilisation of algae associated with a suitable light path to achieve high photosynthetic efficiency. Dissolved O_2 (DO) is lower than in HTPBRs. FP-PBRs consist of two categories: 1) airlift FP-PBR and 2) pump FP-PBR (**Figure 2-8**). Flow control can be difficult. The engineering problems are in the construction of cost-effective panels.

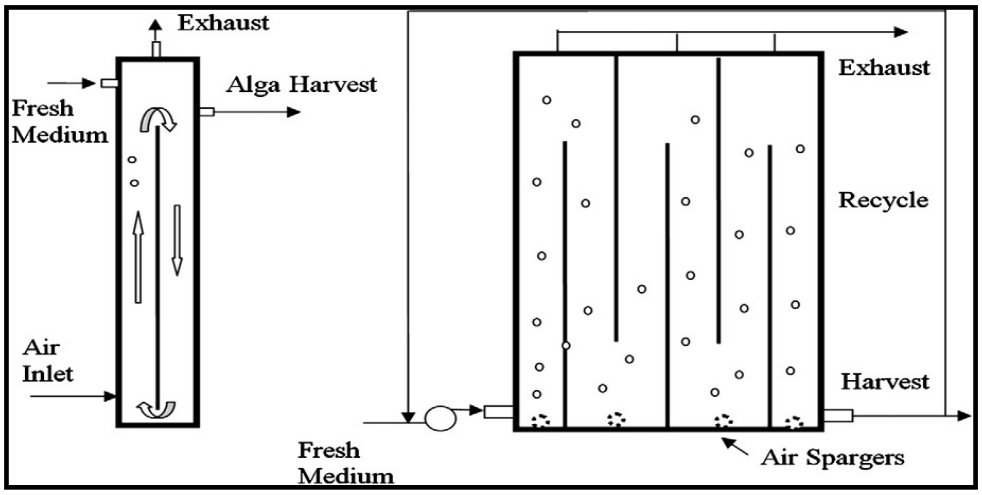


Figure 2-8 Typical flat plate photobioreactors; (A) airlift FP-PBR, (B) pump-driven FP-PBR (Wang *et al.*, 2012)

A pumped FP-PBRs design has,as it's name implies, the liquid circulated by a pump (**Figure 2-9**). A compressed air supply can also provide mixing. Cheng-Wu *et al.* (2001) engineered a flat plate design to produce *Nannochloropsis sp*. The temperature was controlled at 27 ± 2 ℃ with air enriched with 1.5% CO_2 (v/v). The flow rate was 1 L/min. The flat plates in each subunit were 110 cm. x 200 cm. (W x L) and 10 mm. glass plate. Biomass yield was between 5 and 6 x 10^8 cell/mL.

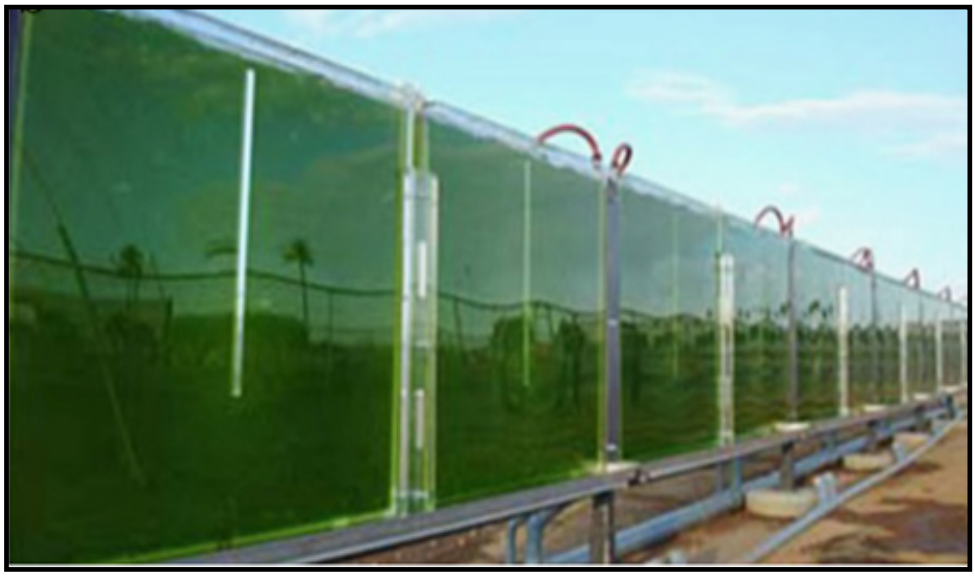


Figure 2-9 Air pump-Flat plate (Bitog *et al.*, 2011)

2.4.3.4 Plastic bag PBRs

Plastic bag PBRs are attractive as commercial scale bioreactors because they are low cost, show good sterility and control of temperature. The problem is the disposal of used plastic bags. In addition, plastic bags may suffer from inadequate mixing causing cell crushing and fragility thus scale-up may not lead to increased productivity (Schoepp *et al.*, 2014).

Trotta (1981) used 50 L-polyethylene bags (thickness 0.3 mm., width 30 cm., length 180 cm.) with mixing aeration enhanced with 1% CO_2 (v/v) at a flow rate of 8-10 L/m to grow *Tetraselmis suecica.* This resulted in a biomass yield of 20-30 $g.d^{-1}$ (wet weight). Moheimani (2013) designed a 6 L- bag photobioreactor (50 cm. x 30 cm. x 40 cm., L x W x H) to culture *Tetraselmis suecica CS-187* in f/2 media and *Chlorella sp.* in 3N BBM media. It was found that *Tetraselmis suecica CS*-187 gave a biomass yield of 0.051 $gL^{-1}d^{-1}$, whilst *Chlorella sp.* gave 0.060 $g.L^{-1}.d^{-1}$ in a 5 L working volume.

2.4.3.5 Biomass productivity

A number of photobioreactors have been designed to increase algal yield. Olaizola (2000) developed a 25,000 L outdoor photobioreactor for producing astaxanthin from *Haematococcus pluvialis*. The study found a biomass yield of 0.2 $g.L^{-1}$ in January 1999, then 0.36 $g.L^{-1}$ in September the same year. Lopez *et al.* (2006) designed a tubular and bubble column to grow *Haematococcus pluvialis*. They found that the tubular column gave a yield

of 0.41 $g.L^{-1}d^{-1}$, whilst the bubble column gave a yield of 0.06 $g.L^{-1}.d^{-1}$ (Lopez *et al.*, 2006). Biomass productivity data are shown in **Table 2-2**.

Table 2-2 Productivity of PBRs design (Ugwu *et al.*, 2008)

Productivity	Volume (L)	Algal strain	Productivity ($g.L^{-1}d^{-1}$)	Ref.
Airlift tubular	200	*Porphyridium cruentum UTEX 161*	1.50	(Rubio *et al.*, 1999)
Airlift driven external loop tubular	200	*Phaeodactylum tricorntum UTEX 640*	1.20	(Fernández *et al.*, 2001)
Airlift driven tubular	200	*Phaeodactylum tricorntum UTEX 640*	1.90	(Molina *et al.*, 2001)
Inclined tubular	6	*Chlorella sorokiniana IAM 212*	1.47	(Ugwu *et al.*, 2002)
Tubular Undulating row photobioreactor	11	*Cyanobacteria (Arthrospira platensis)*	2.70	(Carlozzi, 2003)
Outdoor helical tubular	75	*Phaeodactylum tricornturn UTEX 640*	1.38	(Hall *et al.*, 2003)

2.4.3.6 Advantages and disadvantages of photobioreactors

There are both advantages and disadvantages to any PBR design which have to be improved for the future. Reviews compare the various PBRs (column, flat plate PBRs and plastic bag PBR) which are described in **Appendix A** (Brennan and Owende, 2010; Kunjapur and Eldridge, 2010; Ugwu *et al.*, 2008; Yadala and Cremaschi, 2014).

2.5 Harvesting technologies using flocculation and sedimentation processes

The harvesting process represents a major cost of algal production (Wu *et al.*, 2012). An efficient low-cost harvesting method is essential for large-scale microalgal biomass production and should use environmental friendly technology. There are several techniques that can be used to remove algal cells from suspension. These can be divided into two main groups, bulk harvesting and thickening (Chen *et al.*, 2011).

1) Bulk harvesting: reduces the negative surface charge of microalgal cells allowing a large clump to form and sink. This occurs in, for example, flocculation and gravitational sedimentation.

2) Thickening: This process is one of dewatering and includes techniques such as centrifugation, filtration, or sedimentation.

Choosing a harvesting technology depends on the algal species, medium, concentration and investment cost, amongst other factors.

Flocculation and Sedimentation

Flocculation and sedimentation are a means of separating algae by gravity, overcoming the cells buoyancy by encouraging (physically or chemically) the joining together of many algal cells to from a clump (or floc – hence flocculation). The flocs are usually heavier aggregates than the individual cells and thus sediment much more quickly (some flocs float on the surface and can be skimmed off)

Flocculation and sedimentation consist of three mechanisms: 1) aggregation 2) flocculation 3) sedimentation (settling).

1) Aggregation: Before a cell suspension is at all able to form a floc or agglomerate, cells must be attracted to each other. The attraction between cells is by Van der Waal forces.

2) Flocculation (agglomeration): This is the process in which the cells form a floc.

3) Sedimentation (or settling): This is a physical process in which the flocs in suspension settle out of the fluid and come to rest against a barrier (usually the bottom of the vessel or some catching apparatus). The flocs are dense enough to move through the fluid in response to the forces acting on them; gravity or centrifugal acceleration. This process relies on biomass density to operate. It is, however, slow under certain conditions but can be enhanced by bubbling air or adding chemical salts, polymers, or polyelectrolytes.

2.6 Algal aggregation and flocculation characteristics

2.6.1 Algal aggregation

This mechanism refers to the clustering of (in this case) algal cells form a “colloidal” suspension. The rate of aggregation depends on the collision of particles and usually occurs within a short time (seconds to hours). Adding flocculant to solids suspended at high concentrations may change the

rheological properties as algae cells may bond with chemicals and then sediment.

1) Perikinetic aggregation

The IUPAC Compendium of Chemical Terminology (1972) states that "the rate of perikinetic aggregation can be determined by the frequency of collisions, and the probability of cohesion caused by Brownian motion" (McNaught and McNaught, 1997). Algal cells of different sizes can be induced to come together by Brownian motion, this motion is most significant for particles of one micron (μm) or smaller.

2) Orthokinetic aggregation

When collisions are caused by hydrodynamic motion (e.g. convection or mixing) the component of stress coplanar with a material cross section (called the "shear force") can induce orthokinetic aggregation.

The stress is a force acting on a particle in homogenous media under uniform linear stress. In fluid dynamics, a shear stress is over a distance in a thin-wall structure.

Typically, the dominant particle sizes involved in this type of aggregation are in the range of 1-40 μm (Mokhtari, 2007; Wang *et al.*, 2005).

2.6.2 Flocculation characteristics

Flocculation can be described by four mechanisms: 1) charge neutralisation 2) electrostatic patch 3) bridging and 4) sweeping flocculation. Each mechanism depends on the type of interaction forces between particles to form flocs (Metcalf & Eddy, 1997; Peavy H. S., 1985).

1) Charge neutralisation: Van der Waals forces exists between individual atoms and molecules, also between particles and cells. The force is the sum of the attractive or repulsive forces which are composed of 1) a keesom force (force between two permanent dipoles), 2) a Debye force (force between a permanent dipole and a corresponding induced dipole) and 3) a London dispersion force (force between two instantaneously induced dipoles) (Metcalf & Eddy, 1997; Peavy H. S., 1985).

Charge neutralisation occurs when positively charged ions, polymers, or colloids strongly absorb onto the negatively charged surface of the particle, neutralising any further electrostatic repulsion between the particles. The stability can be determined by the interaction between the algae cells, media components, and any added flocculant.

2) Electrostatic patch: (Re-arrangement of adsorbed polymer): In 1996, Yu and Somasundaran explained that this mechanism occurs when a charged polymer binds to the opposite negative charge. New particles connect with each other through patches of opposite charge. The mechanism relates to electrical double layer forces which occur between charged objects across a liquid boundary (Yu and Somasundaran, 1996).

3) Bridging: When polymers or colloids simultaneously bind to the surface of two different particles, they form a bridge between them. These bridges lead to more collisions between particles and, then, the aggregation of flocs. The interaction between such algal flocs in a colloidal system can form a dense network by their bridging polyelectrolyte chains (universal attraction). The binding force depends on the molecular weight and amount of the added chemical which forms the bridge.

4) Sweeping flocculation: In this mechanism particles are entrapped in a large mass. They then break up to form flocs under the influence of an applied shear. The addition of polymers can also create repulsion between groups of cells by a steric force. It occurs when the surfaces are completely covered with a thick layer of polymer. The force depends on the solubility properties of the adsorbed layer (Vandamme *et al.*, 2013).

2.6.3 Types of flocculation

Flocculation can also be divided into 1) auto-flocculation 2) physical flocculation 3) bio-flocculation, and 4) polymer flocculation.

1) Auto-flocculation

Auto-flocculation occurs spontaneously in, for example, cell cultures when the pH increases above 9 and photosynthetic processes and CO_2 uptake are depleted (Vandamme *et al.*, 2013). The formation of flocs rely on

1) ions and interaction of the negative surface charge, 2) inorganic precipitation, 3) adding chemicals to the solution, and 4) pH.

Gravity sedimentation is commonly used as a harvesting technique for algae biomass in wastewater treatment. It is suitable for specific strains such as *Spirulina* whose cell size is greater than 70 μm. (Brennan and Owende, 2010).

2) Physical flocculation

Flocculation can be accomplished by physical forces for example; ultrasound waves, magnetic force or vibration. The flocculation of some algal species can be improved by coating the cells with cationic polymer before providing a force (e.g. adding magnetite (Fe_2O_3) and then using a magnetic force). It is difficult to apply on a large scale and can be expensive (Vandamme *et al.*, 2013).

3) Bio-flocculation

Bio-flocculation involves the use of bacteria or fungi to induce flocs because they have a positive charge to interact with the negative charge of the algal cell surface. However, cultivating bacteria or fungi in combination with microalgae requires a co-culture system needing more skilled techniques. There is less risk of contamination if the bio-flocculant is grown separately and added when required. The use of bacteria or fungi as flocculating agents does avoid chemical contamination. However, there may be a problem in separating bacteria or fungi and concerns over contamination in the recovery of algal components (Vandamme *et al.*, 2013).

4) Polymer flocculation

Polymers are widely used to flocculate particles. There are three main forces responsible for polymer bonding; 1) electrostatic bond, 2) hydrogen bond and 3) covalent bond. The main factors relating to the interaction between algal cells and polymer in solution are polymer and solid characteristics, chemical properties, interaction forces and the charges on the polymer. To prevent contamination of the algal suspension and to reduce hazardous metal contamination of products when using chemical flocculation, there is interest in the use of natural flocculants such as chitosan from shrimp or crab shell.

2.6.4 Flocculants

Most microalgae cells have a negatively charged surface and have a similar density to water. Therefore, the hypothesis is to use positively or neutral charged chemical flocculants to form large algal flocs that are heavy enough for efficient sedimentation.

Most particles in a liquid are unequally charged objects; the surface force may attract them together over a short distance to form flocs. While large algal flocs will sink to the bottom of the vessel naturally this may take a long time because the floc does not have sufficient mass. However, the attractive force can be modified (usually enhanced) by adding an ionised adsorbent polymer or adjusting the pH. This forms a better floc and hence more rapid sinking.

Chemicals such metallic salts (for example; alum, $FeCl_3$, $AlCl_3$) can be used as flocculants to increase mass and hence help settlement (Christenson and Sims, 2011; Granados *et al.*, 2012; Rawat *et al.*, 2013; Velan and Saravanane, 2013). This is because they have a hydrolysable cation (such as Al^{3+}, Fe^{3+}). The positive charges or cations on the flocculant, can bond to and neutralise the negative electrical charge of the algae cell. As more and more cells are bonded together a floc forms and the rising density caused the floc to sediment to the bottom of the vessel. Sukenik *et al.* (1988) and Aitken (2014) found that the sedimentation of algae can be enhanced by ferric sulphate, chitosan and commercial poly electrolytes (Puri-flocs 601 & 602). A number of factors affect flocculation for example; the charge on the algal cell, chemical properties, the density of algae in suspension, molecular mass and dosage of the metallic salts or other flocculants, mixing, ionic strength, and pH in the media. If the floc has been networked with a strong ionic bond the combination of algae and chemicals means that the floc gains enough mass for rapid settlement. In contrast, if the bonds between the algal cell and the flocculant are weak the algal flocs are less dense and may float in the media.

The study described in this thesis investigates six possible flocculants categorised in 2 groups;

1) Metallic salts: aluminium sulphate (Alum, $Al_2(SO_4)_3$), ferric chloride ($FeCl_3$) and aluminium chloride ($AlCl_3$);

2) Bio-flocculants: medium molecular weight chitosan (MW chitosan), high molecular weight chitosan (HW chitosan) and crab shell (an unrefined source of chitosan).

The physiochemical properties, specification and the applications of the chemicals used in this study are given below.

1) Metallic salts

1.1) Aluminium sulphate ($Al_2(SO_4)_3.16H_2O$)

Aluminium sulphate is commonly used as a flocculating agent in the purification of drinking water, wastewater treatment and also in paper manufacturing. Its molecular weight is 342.15 (anhydrous), density is 1.61.

1.2) Iron (III) chloride (ferric chloride) ($FeCl_3$)

Ferric chloride is a fairly strong Lewis acid used as a catalyst in organic synthesis. The MW is 126.20. When dissolved in water, iron (III) and chloride are changed. Chloride bonds with hydrogen to be hydrochloric acid. While, ferric or iron soluted in the water changes to be ferric hydroxide ($(FeO(OH)^-$) in which this negative charge could be bonded with positive charge of algae resulting a heavy floc to drown in suspension.

1.3) Aluminium chloride ($AlCl_3$)

Aluminium chloride is a powerful Lewis acid. In water Cl^- ions are displace with H_2O to form the hexahydrate $[Al(H_2O)_6]Cl_3$. Molecular weight is 133.32 g/mol. Density is 2.4 (25 ℃, g/cm^3). Solubility is 46.6 g/100 mL at 30 ℃.

Grima *et al.* (2003) and Granados *et al.* (2012) found that flocculation agents such aluminium sulphate can aid in the harvest of planktonic algae. However, they found its use not to be economic. Cabirol *et al.* (2003) used aluminium and sulphate at a concentration of 1,000 mg/L of $Al(OH_3)$ and 150 mg/L SO_4^{2-} from K_2SO_4 to form aluminium sulfate as a flocculant. However, the study found that aluminium at 1,000 mg/L adversely affects the activity of methanogenic and acetogenic bacteria during the anaerobic digestion of algal sludge.

Ahmad *et al.* (2006) studied the coagulation of residual oil and suspended solids in palm oil mill effluent (POME) using chitosan, alum,

and polyaluminum chloride (PAC) as the flocculants. POME samples were dosed with chitosan (0.08-0.8 g/L), alum (0.05-5 g/L), and PAC (0.5-5 g/L). The study found that chitosan was able to remove 95% suspended solids and residue oil from an initial volume of 2,000 mg/L when adding 0.5 g/L chitosan with the following conditions: mixing rate of 15-100 (min-rpm), and sedimentation time 20 min at pH 4.0. In contrast for the same 95% removal, alum and PAC had to be at 8.0 and 6.0 g/L with a mixing rate of 30-100 (min-rpm), settling time 50 and 60 min respectively at a pH of 4.5.

In 2012, Granados *et al.* used aluminium sulphate, ferric chloride, ferric sulphate, chitosan and polyelectrolytes as flocculants to settle *Chlorophyceae* microalgae in a wastewater treatment process. The sedimentation process used a rapid mixing rate of 2-150 (min-rpm) followed by a slow rate of 5-20 (min-rpm), flocculatants were dosed at 0,3,5,7,10, and 15 mg/L. The study found that both metallic salts and chitosan are inefficient. In contrast, the polyelectrolytes could recover 2-25 mg/mg of microalgae (Granados *et al.*, 2012).

2) Bioflocculants (Chitosans)

Chitosan is a biopolymer that can be used as a flocculant. The structure is a polycation comprising a linear copolymer of glucosamine and N-acetyl glucosamine β 1 $\longrightarrow$ 4 linkage which is obtained by a partial N-deacetylation of exoskeleton chitin e.g. crab and prawn shell (Rinaudo, 2006) (**Figure 2-10**).

In general, chitosan remains undissolved in water in neutral or alkaline conditions. The polycation becomes soluble in acidic conditions (Dutta *et al.*, 2004). An advantage of its chemical properties is linear polymine which has the reactive amino and hydroxyl groups. Chitosan is a natural polymer, biodegradable and non-toxic. It has been shown to aggregate microalgae cells in a small number of reports (Chen *et al.*, 2016; Divakaran and Pillai, 2002; Dutta *et al.*, 2004; Xu *et al.*, 2013).

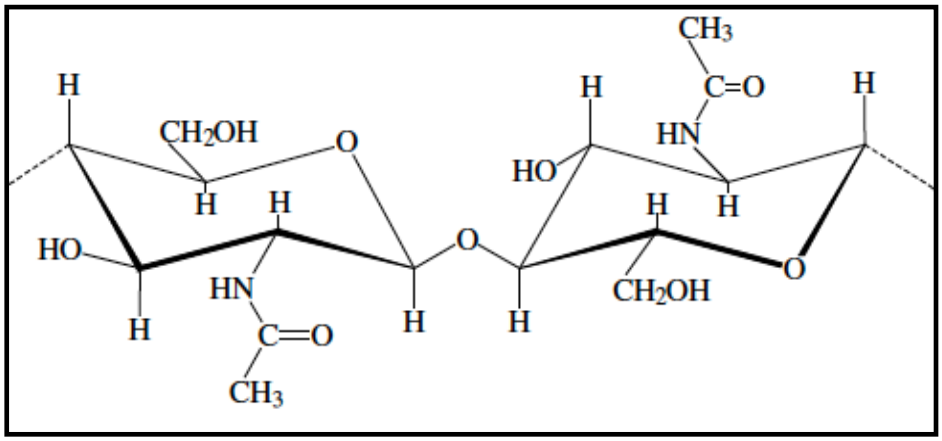


Figure 2-10 Chitin structure (Rinaudo, 2006)

There are many types of chitosan which the average molecular weight of commercially produced chitosan is between 3,800 and 20,000 daltons.

1) Medium molecular weight chitosan (MW-chitosan)

The molecular weight is between 190-310 kDa. The synonym is Deacetylated chitin; Poly (D-glucosamine) $(C_6H_{11}NO_4)_n$ (75-85% deacetyalated). It is an environmentally friendly electrolyte biomaterial. It may be used as a flocculant, in protein precipitation, an encapsulating agent and as an aqueous thickener.

2) High molecular weight chitosan (HW-chitosan)

The molecular weight is 310-375 kDa and it is ≥ 75% deactylated. The benefits are biocompatible, antibacterial and environmentally friendly polyelectrolyte with a variety of applications including water treatment and noval fibers for textites.

3) Crab shell

Crab shell is a unrefined source of chitosan. The composition includes 2-Amino-2-deoxy (1 → 4)– β -D-glucopyranan and Poly-(1,4- β -D-glucopyranosamine). Chitosan is a linear amino polysaccharide composed of approximately 20% β 1,4- linked N-acetyl-D-glucosamine (GlcNAc) and approximately 80% β 1,4- linked D-glucosamine (GlcN) that is prepared by the partial deactylation of chitin in hot alkali. As it contains a very high percentage of chitosan it's use, if it gives good results, would be much less costly than refined chitosans.

2.6.5 Flocculation using chitosans

pH influences the effects of chitosan on settling. The study of Lubián (1989) of 11 strains of marine microalgae (*Phaeodactylum tricornutum, Chaetoceros calcitrans, Chaetoceros gracilis, Dunaliella salina, Nannochloris maculate, Tetraselmis suecica, Nannochloropsis gaditana, Nannochloropsis sp., Rhodomonas baltica, Monochrysis lutheri, Ischrysis aff. Galbana*) found that adding chitosan at between 40-80 mg/L at a pH 8.0 gave an efficiency of flocculation up to 75%. At pH 6.5, chitosan concentration could be lowered to 10 mg/L for the same result. The study showed that other factors, for instance; density and cell characteristics, may also affect flocculation.

Divakaran and Pillai (2002) studied flocculation using chitosan in the range of 80-800 mg/m^{-3} for the fresh water algae (*Oscillatoria, Spirulina, Synecocystis*). Experiments measured turbidity at 10, 20, 30 and 55 NTU. The results found that flocculation is sensitive to pH. A maximum 90% removal rate was found at pH 7. The maximum chitosan used was 15 mg/L. However, settling time was not reported. They report that pH and chitosan dosage are significant factors to sediment, similar to the study of Lubián (1989).

Ahmad *et al.* (2011) conducted flocculation tests using chitosan to flocculate *C.vulgaris*. The microalgae had been cultured in BBM media for 14 days. 10-100 ppm. of chitosan was added and mixed at various speeds between 5 and 250 rpm. with settling times of between 5-100 min. The study found that 10 ppm. of added chitosan can remove more than 99% of algae when the mixing time-speed is at least 20-120 (min-rpm).

In 2014, Hesami *et al.* studied the effectiveness of chitosan as a coagulant aid in the removal of turbidity from water using chitosan combined with $FeCl_3$ as the coagulant agents. The experiment used chitosan doses of 0, 0.5,0.75,1.5,2.0 mg/L mixed with 10 mg/L $FeCl_3$. Mixing was by fast mixing for 1 minute at 100 rpm followed by slow mixing for 20 minutes at 45 rpm and then a 30 minute settling time. They found that 0.5 mg/L of chitosan was the optimal dose to add to the $FeCl_3$ to reduce 50% of initial turbidity. The study also found that the highest removal efficiency is at pH 7.0 (Hesami *et al.*, 2014).

Ahmad *et al.* (2006) found that chitosan, aluminium sulphate and polyaluminium chloride (PAC) can be used a coagulant in wastewater treatment using in removing residue oil and suspended solid from palm oil mill effluent (POME). Chitosan was used at 0.5 g/L while aluminium and PAC was dosed at 8.0 and 6.0 g/L.

2.7 Sedimentation characteristics

Once flocs are formed, the sedimentation process (rate) is related to the three main forces acting on a particle moving through a fluid; 1) external force i.e. gravitational or centrifugal force, 2) buoyan force, and 3) drag force. If the external force equals the combined buoyant and drag forces, the particle is neutrally balanced and there is no movement. However, if an external force is more than the buoyancy and drag forces combined, the particle will sink.

Both the shape, size and fluid dynamics of the particle will influence its settling. A low drag coefficient indicates that the object will have less hydrodynamic drag. The drag differs depending on the flow regime and Renolds'(Re) number.

The flow regime, such as laminar, transitional or turbulent flow, results in different drag coefficient values. Laminar flow is low velocity which occurs when a fluid is flowing through a closed channel such as a pipe or between flat plates. Transition flow is a mixture of laminar flow and turbulent flow. Turbulent flow is a flow in which the fluid undergoes irregular fluctuation.

The mechanisms of settling from a suspension can be classified into four regions; 1) discrete particle settling 2) flocculent settling, 3) Hindered or zone settling and 4) compression settling (**Figure 2-11**).

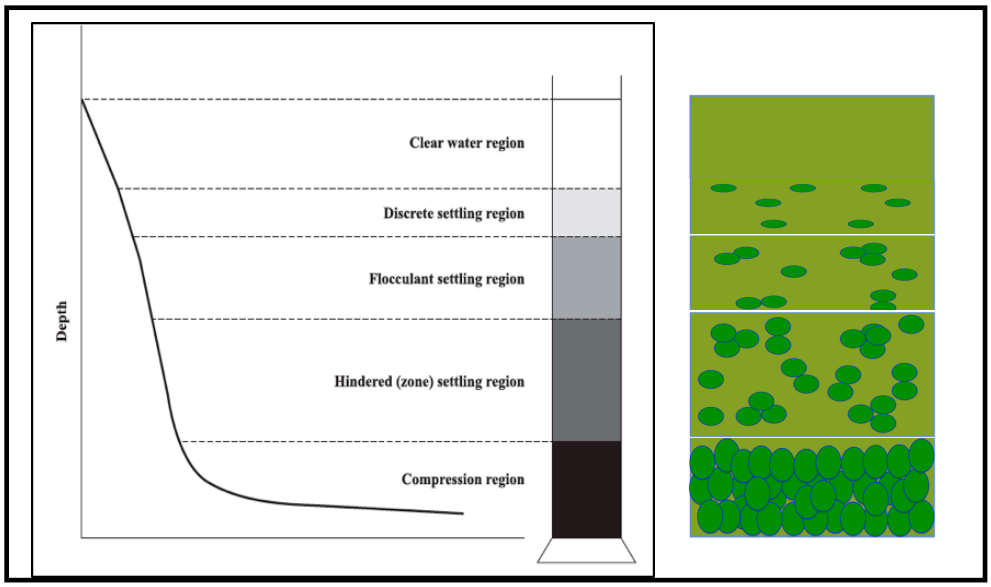


Figure 2-11 Settling regions in suspension. The clear water region is at the top of the vessel. Then various algal densities are in the different settling zones which are; discrete settling, flocculant settling, and zone settling. At the bottom of the vessel, the algal particles are most compressed (Metcalf and Eddy, 1997).

1) Type 1: discrete particle settling

This type of settling occurs at low solid concentrations. The particle will travel at a constant velocity through the liquid. Discrete particles move down the water column by independent settling without interaction with neighboring particles. The constant velocity for spherical particles only depends on gravity force.

The settling velocity of discrete particles (v) can be tested in a columnar vessel. The velocity can be calculated by dividing distance travelled by time (**Equation 2.2**).

$$v = \frac{\text{distance traveled}}{\text{time of travel}} = \frac{d}{t} \tag{2.2}$$

where, d is the distance of travelling (m). t defines a travelling time (s).

2) Mass fraction settling (Flocculent settling)

In a dilute solution, particles may not act as a discrete setting entities but may coalesce during a settlement. This increases the particle (aggregate) mass, which can be enhanced by adding chemicals etc., resulting in a clump (floc). The removal rate can be written as a percentage using **Equation 2.3**.

$$x_{ij} = \left(1 - \frac{C_{ij}}{C_0}\right) \text{ x } 100 \qquad (2.3)$$

where, x_{ij} is the removed fraction in percent. C_{ij} is the mass concentration or a removal velocity at depth (i) and at time (j). C_0 is the initial mass concentration or velocity.

3) Zone settling (Hindered settling)

High levels of suspended solids (more than 500 mg/L) are sufficient to produce a hindered settling. The liquid moves through the contacting particles. The floc particles adhere together resulting in a contacting zone. They tend to fix positions with respect to each other as a unit forms. The settling constant is proportional to the solid concentration. For example, if the density and viscosity of the liquid surrounding the particle increases, the settling velocity tends to decrease (Guibai and Gregory, 1991).

4) Compressive setting

In very dense and high mass solid suspensions, particles settle by compressing in a mass. This consolidation is proportional to depth and settling time (**Equation 2.4**).

$$H_t - H_\alpha = (H_2 - H_\alpha)e^{-i(t-t_2)} \qquad (2.4)$$

where, H_t is an algal height at time t. H_α is sludge depth after a long period such as 24 hr. H_2 is sludge height at time t_2. i is the depth for a given suspension.

The setting characteristics of algal colloids relate to the turbidity, density, the radius of the algae, cell size, and also the sedimentation velocity.

2.8 Factors influence flocculation and sedimentation processes

Parameters influencing flocculation and sedimentation can be categorised in three main variables affecting floc formation:

1) Particle characteristics (i.e. cell density, size, weight);

2) Flocculation and sedimentation types (see **section 2.6-2.7**);

3) Media characteristics – components and physical and chemical attributes such as ionic strength, pH, solid to liquid ratio, viscosity, chemical elements in the media, gas and air flow, viscosity, and gas velocity (Ahmad *et al.*, 2011; Chen *et al.*, 2016).

2.8.1 Particle characteristics

The algal strain, cell surface charge, density, cohesion or adhesion forces, and growth phase influence flocculation behaviour. Growth rates, such as exponential or stationary phase and cell condition such as living or dead cells show different floc formation and settling velocity characteristics. Other factors also affect the process such as;

1) Particle size: for example, smaller size algae require more flocculant dosage than larger size (Vandamme *et al.*, 2013). For very small size algal cells, Brownian motion is dominant which leads to a difference in floc formation and sedimentation characteristics (Hogg, 2000; Russel, 1981).

2) Cell density: Algal settling depends on the density, (mass per unit volume) of the cell, and the force of gravity.

3) Void ratio and porosity: The void ratio (e) is defined as a ratio of volume void-space (V_v), in the water, to volume of solid (V_s) as shown in **Equation 2.5-2.6**.

$$e = \frac{V_v}{V_s} \tag{2.5}$$

$$n = \frac{V_v}{V_T} = \frac{V_v}{(V_v+V_s)} \tag{2.6}$$

where, n is the porosity and V_T is total volume.

4) Cohesion and adhesion: These are forces acting between two substances (two cells or a cell and a coagulant molecule). Cohesion is the force of attraction between molecules of the same substance, whilst, adhesion is the force of attraction between different substances. Both these forces operate over very short distances. An example is the different forces acting on algae in the bulk of the media compared to the forces acting at the wall of the vessel.

2.8.2 Media components and characteristics

The characteristics of the liquid medium, for example hydrodynamics such as currents, turbidity direction of flow, stirring rates, shear stress, together with pH, gas and aeration characteristics and chemical components are significant to algae forming flocs and setting;

1) Flow system and bubble size: gas transfer rate is influenced by increasing velocity and bubble size of the gas supplied. The study of Zimmerman *et al.* (2008) found a dramatic rise in air velocity related to an increasing bubble diameter. A slow velocity improves algal flocculation and bulk settling. Thus, it is important to optimise flow rate. When a gas mixture is introduced into water, the mixture will move towards an equilibrium condition. It is then possible to decrease gasification to provide sufficient O_2 (or other component gas) saturation for oxidation (or some other process) but not to over aerate the system. Gas velocity can be calculated by dividing force by the cross sectional area of the vessel as a giving by **Equation 2.7**.

$$v_g = \frac{F_g}{A} \tag{2.7}$$

where v_g is gas velocity, F_g is the volumetric gas flow rate (m^3/s). A is the cross section area.

2) pH: Rattanakawin and Hogg (2001) found that pH of the media is very important in the flocculation process in relation to floc size. The study looked at pH 7,8.6,10, and 11 and found that the maximum floc size (around 10 μm.) is at pH 8.6. At pH below 5 and above 11, the conditions do not allow alumina and polyacrylamide to form flocs.

2.9 Summary and conclusions

Algae can play a role in alternative energy sources by capturing CO_2 using light energy via the photosynthesis process to produce biomass. While this whole biomass can be used to produce a fuel (for example by anaerobic digestion) the main interest is in the lipid (oil) content of the algae. If the production of lipids can be maximised and economically harvested and extracted, then, due to the potential efficiency of microalgae in converting light energy and CO_2 to biomass, microalgae could contribute significantly to the alternative energy market (Carlsson, 2007; Lam *et al.*, 2012). To achieve the best yield, the correct algal species and strain needs to be selected for a particular environment and environmental conditions. Light, growth medium and mixing need to be optimised in a culture system (a bioreactor or photobioreactor). Each individual design has benefits and limitations.

A number of harvesting techniques can separate algal cells from the growth media. Flocculation (bringing together algal cells into clumps) and sedimentation methods are, potentially, the most efficient per unit cost than other methods. There are three main processes; aggregation (coagulation), flocculation and sedimentation. Particle aggregation can be induced by adding chemicals (coagulants or flocculants). Mechanisms relate to flocculate such as; charge neutralisation, electrostatic patch, bridging and sweeping flocculation. Several factors affect flocculation and sedimentation mechanisms for example; particle characteristics, flocculant, and medium components and characteristics.

Chapter 3 Materials and methods

3.1. Introduction

A number of microbiology laboratory techniques were used in this study. Most of the experiments and laboratory practices were set up by applying the methodology of: 1) Standard methods for the examination of water and wastewater 22nd edition by American Public Health Association (Rice *et al.*, 2012); 2) Algal culture techniques by Andersen (2005); 3) Bioscience laboratory technique by Bonner and Hargreaves (2011), and 4) Recommended practice from the equipment manufacturers. In this chapter, the material and methods are given for:

1) Algae strains and chemicals;

2) Equipment;

3) Experimental set up

3.2 Algae strains and Chemicals

3.2.1 Algal strains

There are 2 algae groups used in this study:

1. *Chlamydomonas reinhardtii* and *Chlorella sp*. received from Dr. Joseph Longworth, The University of Sheffield. *Chlamydomonas reinhardtii* was grown to study light and aeration. While *Chlorella sp*. was used as a model to study effects of the medium.

2. *C.vulgaris* TISTR 8580 obtained from Thailand Institute of Scientific and Technological Research (TISTR), Ministry of Science and Technology, Pathumthani, Thailand. This was the main algal strain used in this study.

3.2.2 Isolation and Identification of microalgae strain

C.vulgaris TISTR 8580

Microalgae may be obtained from a number of natural resources. There are several steps involved in selecting the desired algae and getting it to a pure form. Algae suitable for biofuel production should have a high lipid content and be adapted for growth in the required environment, be fast growing and be easily isolated and sub-cultured.

Purification and using a pure strain helps to eliminate contamination from bacteria, fungi, and zooplankton and to allow all available food resources to be used in producing the desired outcome – the maximum number of algal cells with the desired properties. While this can be done in any laboratory from wild strains, it is more usual to select a suitable strain from a culture collection where it has already been purified and its properties and characteristics noted.

As this study is aimed at the production of algal biomass in Thailand, not only should an algal species and strain suitable for biofuel production be used, but also the strain should be capable of being easily grown in the Thai environment – ideally it should be one isolated from Thailand.

C.vulgaris TISTR 8580 is a Thai strain originally collected from freshwater at Bung Sigan, Amphoe Muang, Nonthaburi, Thailand. The alga was identified, isolated, and purified in the BIOTEC laboratory, Microbiological Resource Centre, Thailand Institute of Science and Technology Research (TISTR), Ministry of Science and Technology (MOST)(http://www.tistr.or.th/tistr_culture/list_en.php?type=a&key=C) where the cultures are maintained in N-8 media at 26 ℃. The sample was purchased on 14 February 2014.

3.2.3 BBM Medium

The main medium used for the cultivation of microalgae was 3 N Bold's basal medium (BBM). Vitamin B_1 (thiamine) and B_{12} (cyanocobalamin) were added at 1 ml/L media liquid. B_1 is antioxidant that helps to protect the plants from environmental stress. It can improve plant resistance against bacterial infection. In addition, it stimulates growth and/or reduces transplant shock. Vitamin B_{12} helps protect plants against disease. The detailed of the BBM medium are given in **Appendix B.** The pH of the medium was adjusted to pH 6.7 $\pm$ 0.3 by 0.1 N HCl or NaOH. Then the media was autoclaved at 121℃ for 15 min. The media preparation is shown in **Figure 3-1**.

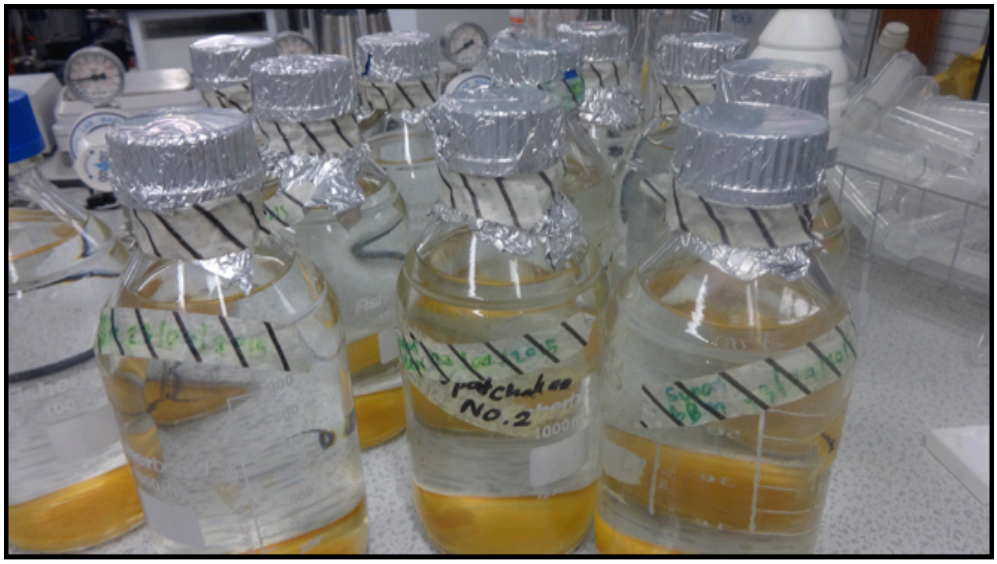


Figure 3-1 BBM medium preparation

3.2.4 Flocculants

There are 2 categories of flocculants used in this study:

1) Metallic salts: 1) Aluminium sulfate hydrate ($Al_2O_{12}S_3.x\ H_2O$) 368458 assay: 98%, MW 342.15 g/mol, CAS 17927-65-0 assay 98% (Sigma-Aldrich) 2) Ferric (III) chloride ($FeCl_3$) 157740 MW 162.20, CAS 770-08-0 mp. 304 ℃ assay 97% (Sigma-Aldrich) 3) Aluminum chloride ($AlCl_3$) 06220 Lot no. BCBN 1502V, CAS 744-70-0 MW: 133.34 g/mol, mp: 190C assay ≥ 99.0% puriss.pa. anhydrous crystalized (Fluka).

2) Bioflocculants: 1) Medium molecular weight chitosan (MW-chitosan) 448877, CAS 9012-76-4 Lot no. SLBG4282 (Sigma-Aldrich) 2) high molecular weight chitosan (HW-chitosan), Sigma-Aldrich CAS no. 419419 coarse ground, 3) crab shell (received from Dr. Robert Edyvean).

Most were "coarse ground" except medium and high molecular weight chitosans (grey fine power), and $FeCl_3$ (black fine powder) (**Figure 3-2**).

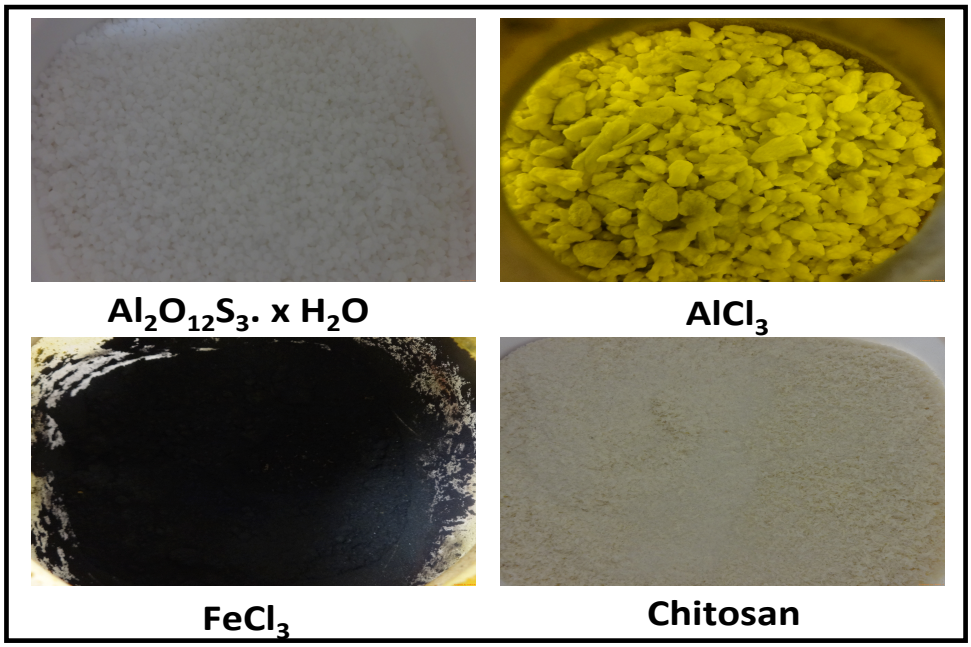


Figure 3-2 Examples of flocculants

Xu *et al.* (2013) found that the optimal dosage of chitosan is 10 mg/g algal dry mass. 1 L algal suspension produces around 0.4818 g dry algal mass (Feng *et al.*, 2011). Thus, 1 g dry mass would be come from 2.0 L algal suspension. Thus, in this study, 5 mg chitosan or crab shell was used per litre algal suspension at pH 7.

Various metallic salts such $FeCl_3$ can be combined with chitosan to provide a high removal efficiency. Different metallic salts, chitosan types, cell characteristics, algal volume, algal density, and characteristic of algal suspension result in different settling characteristics of the algal cells. (Amaral *et al.*, 2005). In addition, characteristics of the water, for example; turbidity, suspended solids, pH, nitrogen and phosphorus affect the settlement (Renault *et al.*, 2009).

3.3 Equipment and methodology

3.3.1 Glassware

Glassware included 25, 50, 100, 500, 1,000 mL volumetric flasks and 500, 1,000 mL Fisher brand flasks which were used to prepare BBM media. Tygon 3350 silicon 3/16 x 5/16 tube, 2-hole rubber stoppers, glass tube and $0.45\mu m$ −SPR 25 air filters were used to complete the basic algal culture vessels (**Figure 3-3**).

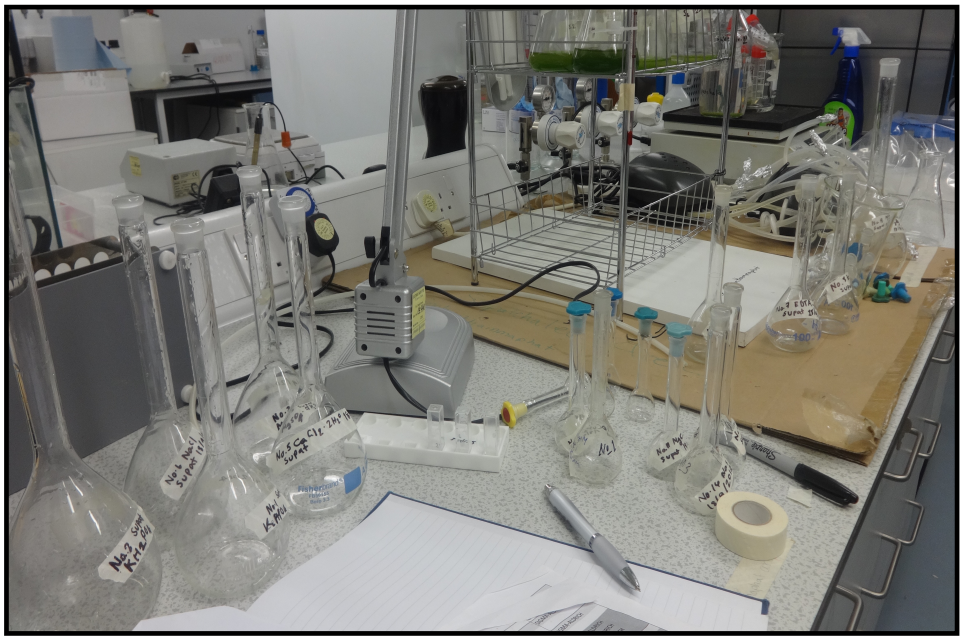


Figure 3-3 Glassware

3.3.2 Microbalance

A 4-digital VWR microbalance, LA 214, S/N IT1303262 Max: 220 g was used to weigh chemicals throughout the study. The calibration check is given in **Appendix C**.

3.3.3 Aeration and flow measurement

600 L/h 4-outlet fish tank pumps and 2.5 inch spargers were used to provide aeration when growing algal stock cultures. CDC Pneumatics 98 SHORE Hardness flow meters calibrated 8 x 5 mm. 13K 150 310245 and 600 C.F.H flow GAP meter scale no. 1/A.D. 341 11/75 were used to check aeration rates. For gas and aeration flow measurement for the photobioreactor system see section 3.4.2.

3.3.4 CO_2 and forms of carbon

It is known that algae can often grow better (in terms of biomass production) when supplied with higher levels of CO_2. Chinnasamy *et al.* (2009) reported that the optimum range of CO_2 for *C. vulgaris* should be under 6% (v/v) but others have reported up to 50% (Blinová *et al.*, 2015). The study of Rendon *et al.* (2013) found that the highest biomass (1.59 gL^{-1}) was obtained from supplying 8.5% CO_2 when algae were grown for 15 days in the various light sources.

CO_2 concentrations between 0-50% v/v may result in different algal growth patterns and changes in the chemical composition of storage products in the algae. However, the optimum values vary widely in the reported literature and will depend on the algal species and factors of the cultivation.

When CO_2 is dissolved in water, it can convert or reverse to various forms as a given by **Equation 3.1**.

$$CO_2 + H_2O \rightleftharpoons H_2CO_3\,(\text{acid}) \rightleftharpoons H^+ + HCO_3^-(\text{base}) \rightleftharpoons 2H^+ + CO_3^{2-}(\text{base}) \quad (3.1)$$

The relative concentrations of the different forms of inorganic carbon are determined by pH. At pH 2, CO_2 converts to H_2CO_3. While HCO_3^- forms around pH 8. On the pH rising to 12, CO_2 becomes CO_3^{2-}. Both alkali and acid conditions will result in algal growth inhibition. In the dark, algal respiration process releases CO_2. New bicarbonate ions are produced that result in a rapid fall in pH which can often be measured in algal solution. CO_2 was taken from the laboratory supply which came from central tanks supplied by BOC.

3.3.5 pH measurement

A HANNA model HI 2211 pH/ORP pH meter was used to monitor pH. The study followed the standard method 4500-H^+ pH value (Rice *et al.*, 2012).

3.3.6 Illumination

23 G fluorescence (max 11 W) tubes were used to provide artificial light for initial algal cultivation and maintenance. The period of light:dark exposure was 24:0 for stock cultures. Six 40 W fluorescent cool white tubes were used in the photobioreactor system with 16:8 hr. light dark regime. Illumination was measured using a light meter sesor biosheric instrument model QSL-2100 S/N 10140 San Diego California, with a programme logger 2100 QSL-S/N 10140 Biopherical Instrument.

3.3.7 Microscope

2 microscopes were used to monitor cell morphology;

1. Algal cells were monitored, and cell counts made, by physical inspection using an AXiostar plus microscopy with 44 1030 A-Plan 40x0.65 Ph2 α/0.17 $\alpha/0.17$ Carl Zeiss lens.

2. Cell size was investigated using Olympus fluoresence microscope model BX 51 S/N BC24020, ProgRes C5, Lense UPlan FLN 100/1.3 oil lens α 0.17/FN 26.5. The image capturing programme was ProgRes 2.6.

3.3.8 Hemocytometry

A hemocytometer (3100 bright-line Hausser Scientific, Horsham, PA USA.) was used with the microscope to measure the cell count following the Louis-Charless Malassez method. (Piccinini *et al.*, 2014; Verso, 1964). The equipment consists of a thick glass microscope slide with a rectangular indentation and a chamber. The grid area is 9 - 1x1 mm. (1 mm^2) squares. It is divided in 3 directions; 0.25 x 0.25 mm. (0.625 mm^2), 0.25 x 0.20 mm. (0.05 $mm.^2$) and 0.2 x 0.2 mm. (0.04 mm^2). The central square is subdivided into 0.05 x 0.05 mm. (0.0025 mm^2) squares.

Dense algal samples were diluted for an accurate count. To ensure that the hemocytometer and the cover slip are clean before use 70% (v/v) ethanol and distilled water was used to wipe them. A fine tipped Pasteur pipette (0.1 μm.) was used to draw an algal solution and drop the cell suspension in both counting chambers. Placing the coverslip ensures a defined volume and then the hemocytometer was placed under a microscope and the number of cells in the squares counted. The depth of the chamber being 0.1 mm^2, the volume of each marked square is 0.01 mm^3. The 40X objective of the Zeiss microscope was used for the counting (Bonner and Hargreaves, 2011).

Cell density can be determined by averaging the cell count over a number of samples. In general, the number of cells in 0.1 mm^3 can be averaged by dividing 5 and then multiply by 25. Converting from cell/0.1 mm^3 to cells/mL is a multiple by 10^4. When performing cell counts, the most accurate count is achieved when 120-200 cells are counted per grid. Calculation of cells per mL is shown in **Equation 3.2**.

$$\text{No. of cell count/mL suspension} = \frac{X \times 25 \times 10^4}{5} \qquad (3.2)$$

where, X is the total counted cells in 5 squares.

3.3.9 Spectrophotometry and OD_{680} measurement

Absorbance or turbidity of a culture is generally determined by using a spectrophotometer or nephelometer (turbidity meter). This study will use both, along with direct cell count to measure cell growth following the standard methodology no. 8111 G biomass monitoring (Rice *et al.*, 2012).

Algal growth rate was measured by optical density using UV Spectrophotometers. A model Ultrospec 2100 pro and Thermo Scientific spectronic 200 version 4.04 were used. The optical density was measured at 680 nm. (OD_{680}). The automatic internal system and calibration compose of 1) memory testing 2) lamp testing 3) auto zero set. The auto programme used was Spectronic 200E. FB 55143 (Fisherbrand) cuvettes were used to hold the samples. A blank (distilled water) was used to adjust the absorbance reading to zero point.

The sample, in a cuvette, is placed in the spectrophotometer. Light of a chosen wavelength is focused on the cuvette (incident light) and a light detector, connected to an amplifier, measures the amount of the light which passes through the cuvette (the transmitted light). Since the cells act as a physical barrier, some of the incident light will be deflected away from the detector. Consequently, the amount of transmitted light will be directly proportional to the distance travelled through the cuvette and the number of cells (**Equation 3.3**).

$$\frac{-dI_t}{dL} = E.I_t.c \qquad (3.3)$$

where, the change in light intensity is I_t through a distance (L) by Beer's Law. E is the molar extinction coefficient and c is the concentration of cells. Integration of the equation is giving by **Equation 3.4** and **Equation 3.5**

$$\ln\frac{I_t}{I_0} = -E.c.L \qquad (3.4)$$

where, the dimension of the cuvette is normally 1 cm. A plot of $\ln\frac{I_t}{I_0}$ versus concentration (c) is a straight line with a slope (– E).

The term $(\frac{I_t}{I_0})$ x 100 is defined as the percentage transmittance, T_r, and the absorbance (A) is equal to log 10 ($1/T_r$), therefore

$$A = \log(1/T_r) = E.c.L. \quad (3.5)$$

3.3.10 Flocculator

Flocculation "Jar tests" were performed using a Stuart SW6 flocculator with 6-1,000 mL glass beakers. The diameter of each beaker is 10 cm. and the height 18 cm. For each batch experiment, all the beakers were simultaneously stirred at the same speed as shown in **Figure 3-4.**

The enhancement of algal flocculation, and thus settling, was studied using additions of the metallic salts and biopolymers given in section 3.2.3. Settlement was measured by optical density (OD) or turbidity of the culture at a set height in the beaker. The details are given in **Chapter 5**.

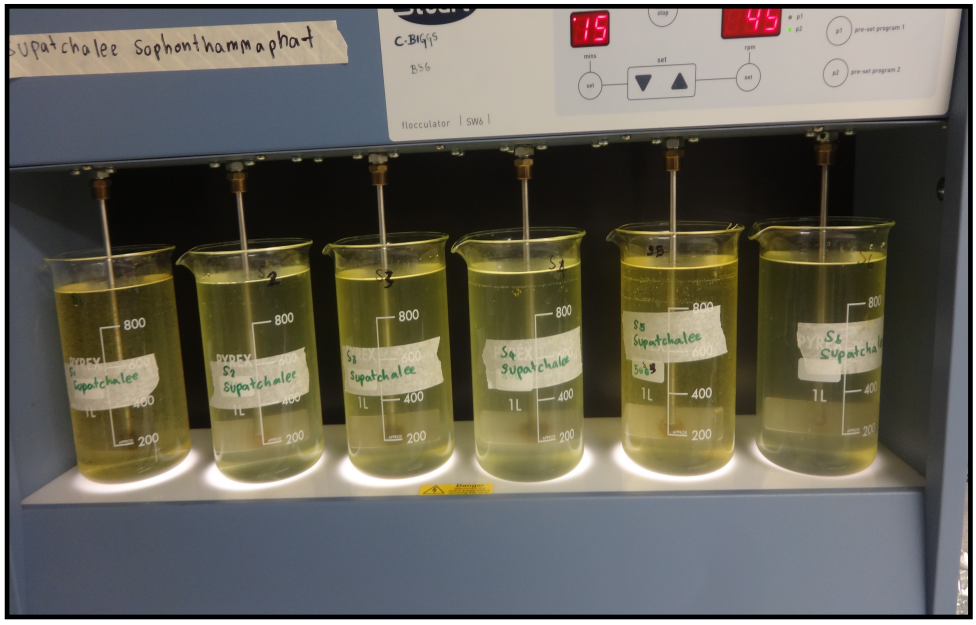


Figure 3-4 Stuart -flocculator consists of six 1 L beakers (10 cm. x 18 cm. (D x H)) which are all stirred at the same set speed.

3.3.11 Nephelometry (Measurement of turbidity)

Turbidity is the amount of cloudiness in the water and can be measured by the amount of light scattered by the particles causing the cloudiness using a nephelometer or turbidity meter by applying the standard 2130 B Nephelometric method (Rice *et al.*, 2012). This can be used to investigate both algae growth in cultures and the settling of algal cells. A nephelometer model: WGZ-20B range 0-20 NTU was used. A light detector is set at 90^{o} to the source beam and particle density is a function of light reflected into the detector (Davies-Colley and Smith, 2001).

3.3.12 TC, TIC, TOC measurement

When adding chitosan in the harvesting process, carbon components and their derivatives might be changed in the liquid and this may affect the use, recycle or reuse of the medium or the harvested cells. Therefore, total carbon, total inorganic carbon and total organic carbon were analysed.

Carbon analysis was by the chemical reaction cuvette method using 380 Hach Lange cuvettes following the DIN 38409-H3 and 5310 C persulphate oxidation method (Rice *et al.*, 2012). In principle, total carbon (TC) and total inorganic carbon (TIC) are converted to carbon dioxide (CO_2) via oxidation and acidification. The CO_2 passes from the digestion cuvette through a membrane into the indicator cuvette. The change of colour of the indicator can be detected by using photometric methods.

Carbon measurements utilised the 380 LCK cuvettes from Hach Lange. The main range used was 2-65 mg/L (380 LCK 2-65 mg/L TOC, HACH LANGE, Scientific lab WAT 3667). However, to study inorganic carbon and organic carbon changes when adding chitosan a wider range of cuvettes were also used (10-73 mg/L C TIC, 2-65 mg/L C TOC)

In the process, the persulphate digestion reagent A (1 dose) is transferred into the TC cuvette. Then, a 2 mL algal sample is pipetted into the TC cuvette. Then, the cuvette is inverted to mix the reagents and sample. The chemical reactions were accelerated by heating in a thermostatically controlled bloc (DR Lange LT100) at 105 ℃ for 120 min. After processing, the colour change in the cuvette tubes were measured using an automatic spectrophotometer system with built-in barcode reader and a light wavelength range of 320 to 1100 nm. (Hach Lange DR 3900). The difference in value between TC and TIC was automatically converted to TOC data.

3.3.13 Data Analysis

Data and information were gathered, then analysed by using Graph Pad Prism Programme 7.0.

The hypothesis was that the probability of the sampling (or observable value) distribution should be closed to the mean ($\bar{x}$)

The descriptive statistics such as mean, standard deviation (S.D., measures the amount of variability or dispersion for a subject of data from

the mean) and standard error of the mean (or SEM, which measures how far the sample mean of the data is likely to be from the true population mean) were determined (**Equation 3.6 and 3.7**).

The mean (μ) is the sum of the observations for example; algal growth samples divided by the number of observations;

$$\bar{x} = \frac{1}{N} \sum_{i=1}^{N} x_i \quad (3.6)$$

where x is sample and $\bar{x}$ is an average of the samples. N is the total number of the samples. x_i is observation. i is a number of observation; 1, 2,3… N is total population (in this study n= observation value,)

The standard deviation (σ) is the square root of the variance

$$S.D. = \sqrt{V(x)} = \sqrt{\frac{1}{N} \sum_i (x_i - \bar{x})^2} \quad (3.7)$$

V is variance of x ($S.D.^2$) which explores how much x is liable to vary from its mean value. N is the number of sample.

S.D. is the most commonly used a measure of the spread of values in a distribution from the mean. Low S.D. shows that the spread of values of the samples is close to the mean.

SEM focuses on confidence intervals. The calculation is given in **Equation 3.8**

$$SEM = \frac{S.D.}{\sqrt{N}} \quad (3.8)$$

This thesis mostly uses S.D. to indicate the spread of the results from the mean. The statistics are presented in tables, bar graphs, line charts, histograms and scattered diagrammes related the data.

3.4 Experimental methods

3.4.1 Stock maintenance and algal inoculation

Cultures of algae from group 1 (*Chlamydomonas reinhardtii* and *Chlorella sp.*) and group 2 (*C.vulgaris*) were grown and kept as stock cultures by repeated sub-culture at 4 ℃. When required experimental cultures were produced from these stocks.

To make a stock or experimental culture, 500 mL Borosilicate Erlenmeyer flasks were sterilised with silicon tubes, rubber stoppers, glass tubes, and tips in place. BBM media was prepared as described in section 3.2.2 above. The volume of the algal inoculum was 2.0 to 20% of the total working volume (400 mL). If the OD_{680} of the algae solution was less than 1.5, 20% v/v inoculum (algal volume/ total working volume) was used, if the OD was around 2.5 then 10% inoculum was used. The final total volume was 400 mL in each 500 mL flask. Aseptic techniques were maintained to avoid contamination. Cultures were grown for 7 days before subculture use elsewhere (e.g. transferring to the photobioreactor). Growth temperature for cultures in the laboratory was 20-25^{o}C.

Aeration of stock cultures was achieved by bubbling air at a flow rate of 0.17 L/min through a stone sparger. The flow rate was regularly checked using a flow meter. An in-line 0.45 μm. (SPR 25) air filter was used for drying the air. Continuous illumination was provided from 11 W artificial lights. The temperature in the laboratory was 20-25^{o}C (**Figure 3-5**).

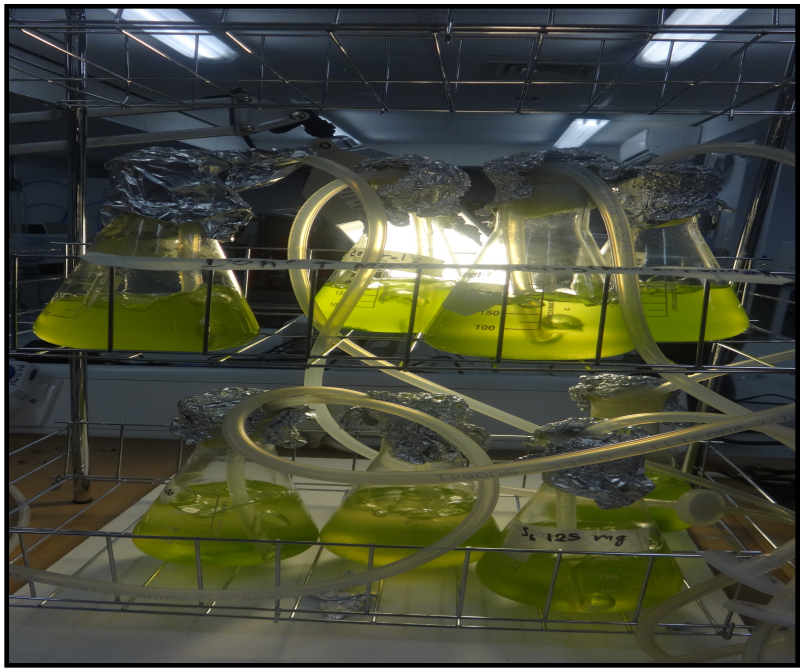


Figure 3-5 Example of intial algal stock cultures of *Chlorella sp.*

3.4.2 Photobioreactor design

1) Photobioreactor draft drawing and construction

The photobioreactor system was first designed in a computer drawing programme (Auto CAD for Mac 2017). After the rough draft, the design was sent to the engineering workshop to approve the raw material specifications and a final design produced. The structures were then built. There are 3 shelves each with four culture vessels. Each shelf has two 40 W fluorescent tubes to provide the light source for 4 vessels (**Figure 3-6 - 3-8**).

Figure 3-6 shows the equipment to connect to the photobioreactor for each of the vessels. It includes 90^{o} fitting nozzles connecting with gas and aeration lines. 2.5 inch spargers, used to bubble the flow of the mixed air and gas through the vessel. The 0.25 inch diameter PVC flow line is linked to a rotameter to check the actual flow rate.

Each photobioreactor consists of a plastic vessel and PVC tubing. A sparger is used to spread the mixed gas through the vessel. At the top of the vessel, there is a sampling line, inlet and outlet connected to the fitting nozzle (**Figure 3-6**).

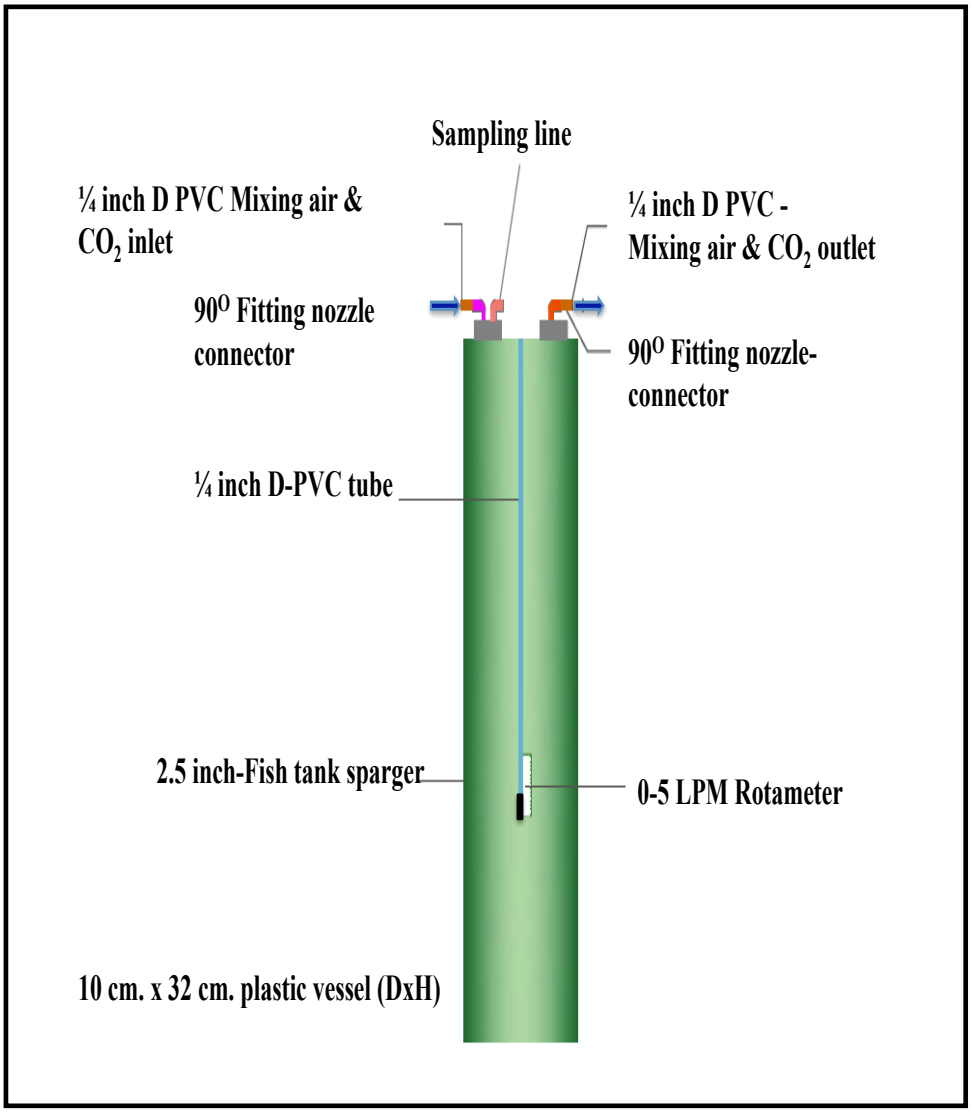


Figure 3-6 Individual photobioreactor vessel design. Each photobioreactor consists of: 1) 10 cm. diameter x 32 cm. height plastic vessel; 2) 1/4 inch PVC tube; 3) 90° Fitting nozzle to connect with gas line; 4) 0-5L Flow meter (rotameter); 5) 2.5-inch air sparger.

The photobioreactor system is 1,600 cm. x 1,600 cm. (D x H). in overall size with 3 shelves on a movable costors. Each shelf has 4-plastic vessels. 1-vessel is used as a control, while the others are replicates of the particular experimental conditions. Compressed air and carbon dioxide are let into a mixing chamber to produce gas mixtures containing different levels of carbon dioxide (0, 6%, 12% 24%, 50% v/v). Rotameters are used to check the flow rate ensuring that the mixing volume is correct. The mixed air and gas flow to the bioreactors at a predetermined rate which is monitored by a rotameter on each vessel (**Figure 3-7**).

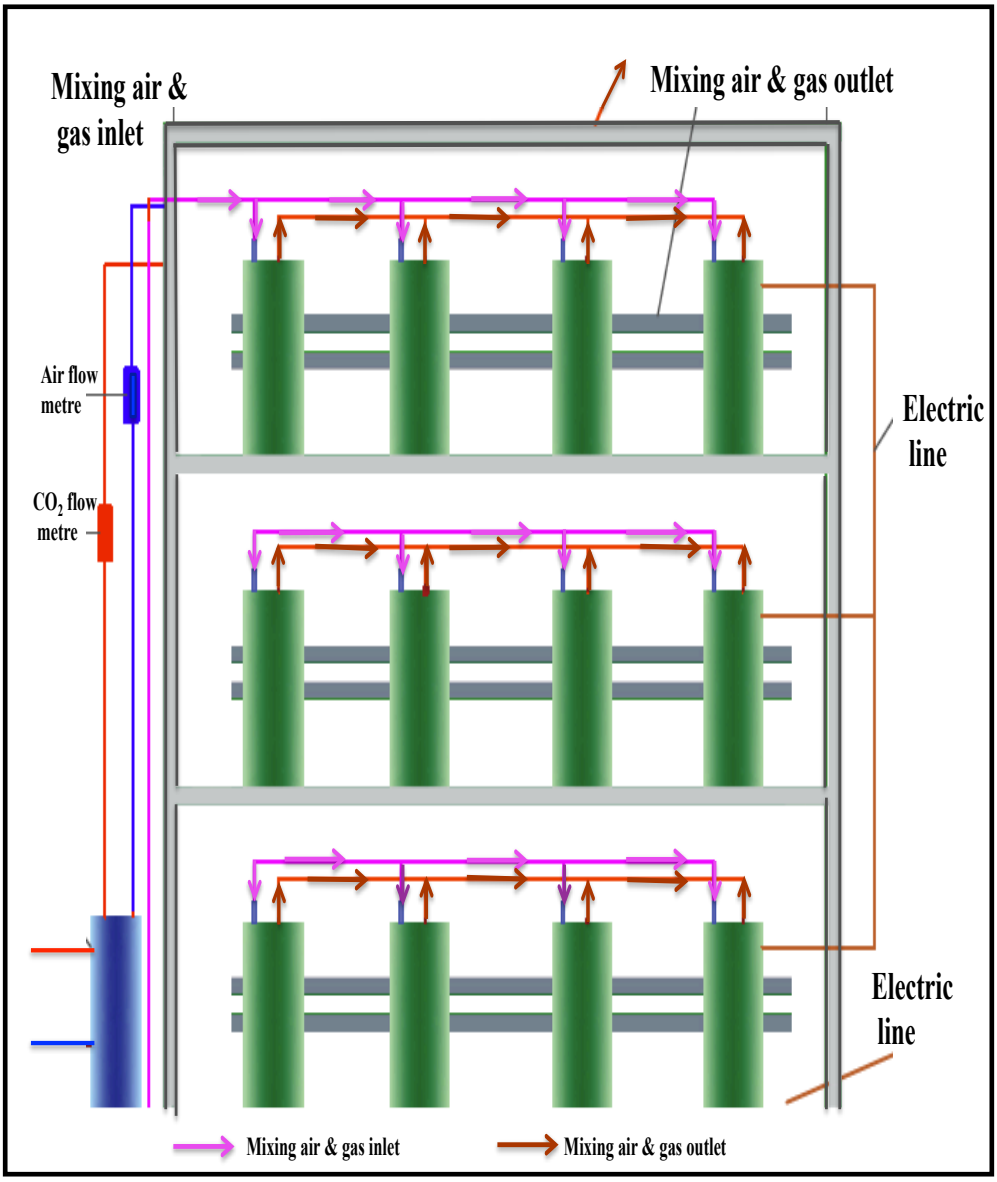


Figure 3-7 3 Dimensional photobioreactor draft drawing: 1) 3 shelf- steel rack with 4-wheels; 2) 12-2L photobioreactors; each 10 cm. diameter and 32 cm. height; 3) 6-40 W artificial light tubes; 4) mixed gas system; 5) digital timer 6) electricity supply.

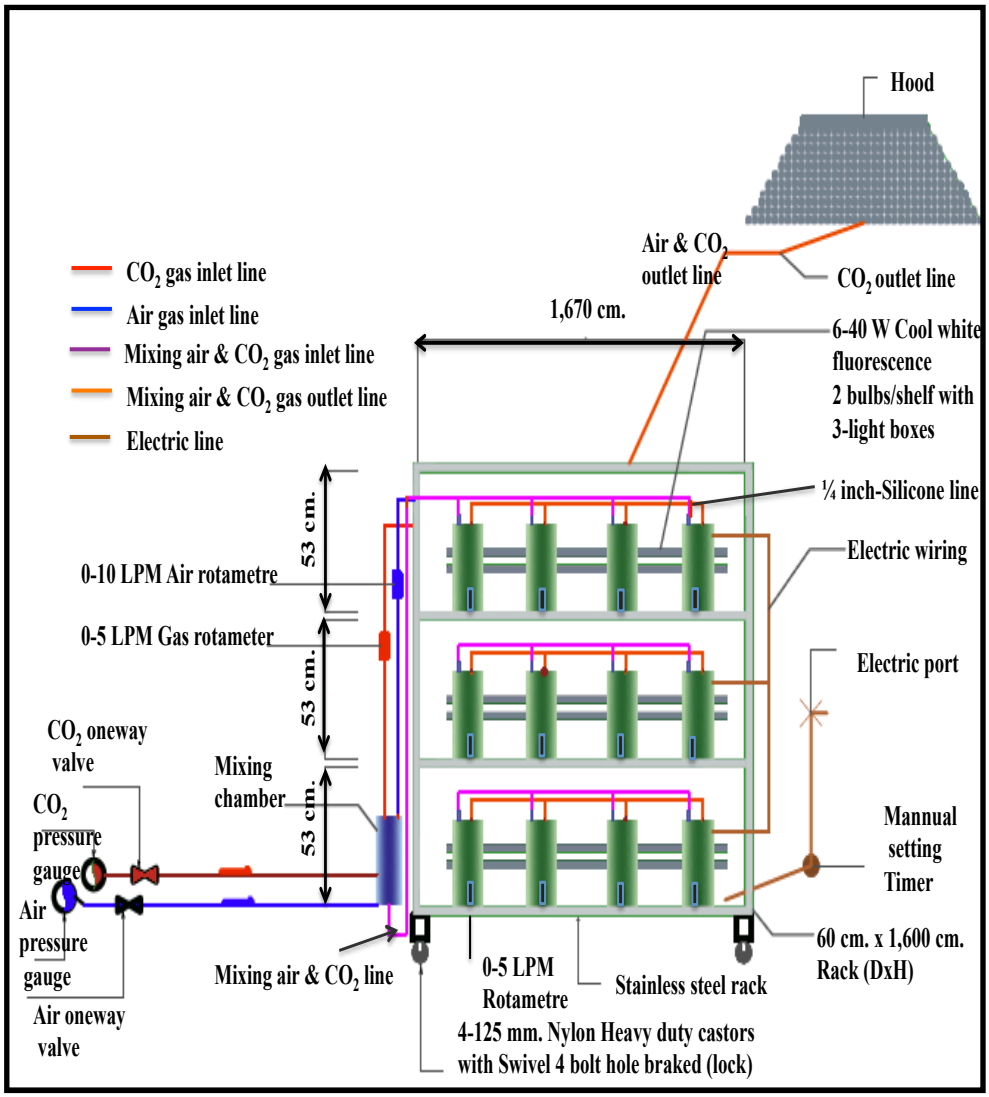


Figure 3-8 The mixed gas and air from the mixed chamber to the photobioreactor. The pink coloured line is the mixed flow inlet. The brown colour is the mixed flow outlet.

2) Photobioreactor air and gas supply details

Compressed air and CO_2 was piped into the laboratory from a compressor/ gas tanks respectively. The flow was controlled using "gas-arc" Lab-master regulators with 20 bar maximum Inlet pressure (**Figure 3-9**).

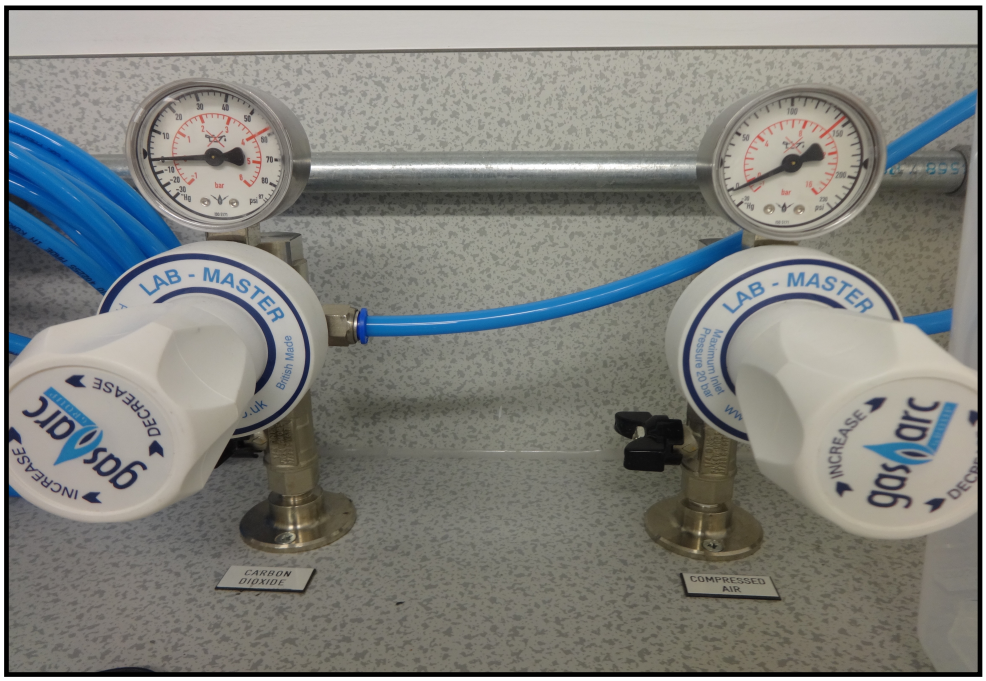


Figure 3-9 Gas (CO_2) and compressed air pipelines with 20 barrel regulators.

3) The mixed chamber for air/ CO_2 mixtures

A mixed chamber (**Figure 3-10**) was constructed to provide a mixed gas to the photobioreactors containing known volumes of CO_2. The mixing chamber is a cylinder 10 cm. x 32 cm. (D x H). The components were: One 0-10 LPM Ki Key instrument rotameter (to measure air flow at the mixing chamber) and one 0-5 LPM Ki Key instrument rotameter to measure CO_2 flow. There were twelve 0-5 LPM Ki Key instrument rotameters to control flow to individual photobioreactors. After the air and gas are mixed, the regulated flow passes via a pipeline and 2.5 cm. sparger (1 sparger for each reactor) to bubble flow through the algal cultures.

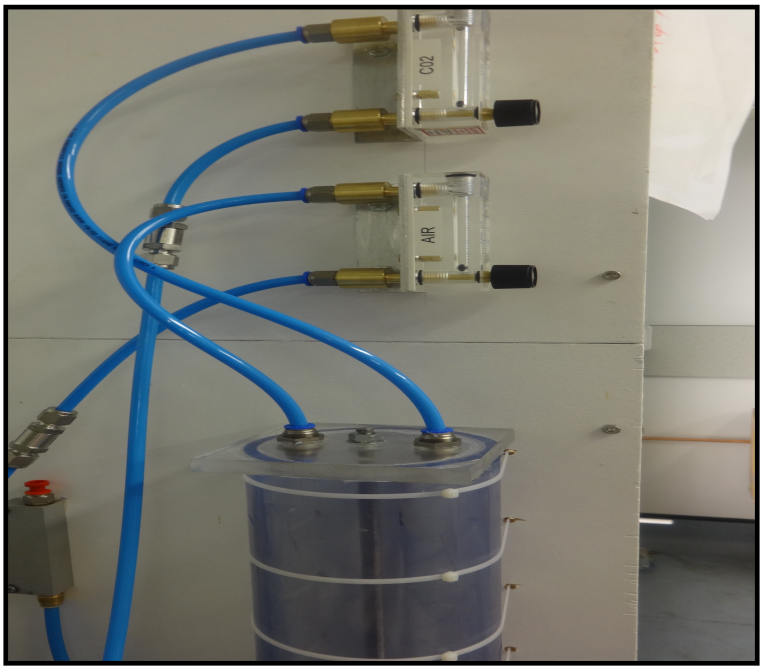


Figure 3-10 Mixing chamber with gas and air flow meters

3.4.3 Photobioreactor set up, commissioning tests and algal culture tests

1) Photobioreactor set up and commissioning tests

Each reactor was commissioned using distilled water to check leakage, and liquid & air flow to ensure that the system worked properly. Electric, light, gas and air were all tested. Performance data was entered into an excel spreadsheet along with photographic records.

The commissioning results found that compressed air and gas could be fed into the column via mixing chamber. However, the flow rate for each photobioreactor may be unstable due to fluctuations in the mixing of gas and air. It was found that the mixing chamber and rotameters should be appropriately designed and selected to control homogeneous circulation and to maintain even flow at the end of the pipeline. This was done by trial and error following the initial calculations to get the specifications given in 3.4.5. There was no water leakage in the photobioreactors. Every sparger showed bubble flow. The electrical wiring passed the safety tests and functioned as expected. Once minor modifications were made the photobioreactor system was ready for algal culture tests.

2) Algal culture tests

After commissioning, the photobioreactor system was verified by undertaking an initial algal culture for 7 days to investigate any problems such as leakage and to ensure that gas line connection, sparger, light etc. can be appropriately run for a batch period (**Figure 3-11** and **Figure 3-12**).

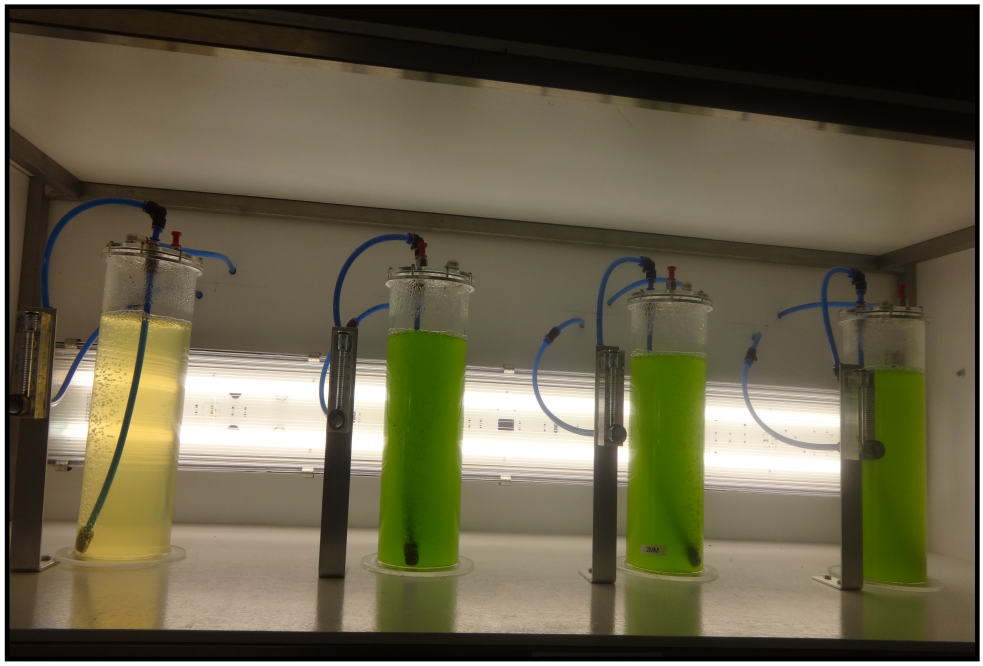


Figure 3-11 Algal cultural test in the photobioreactor on Day 2

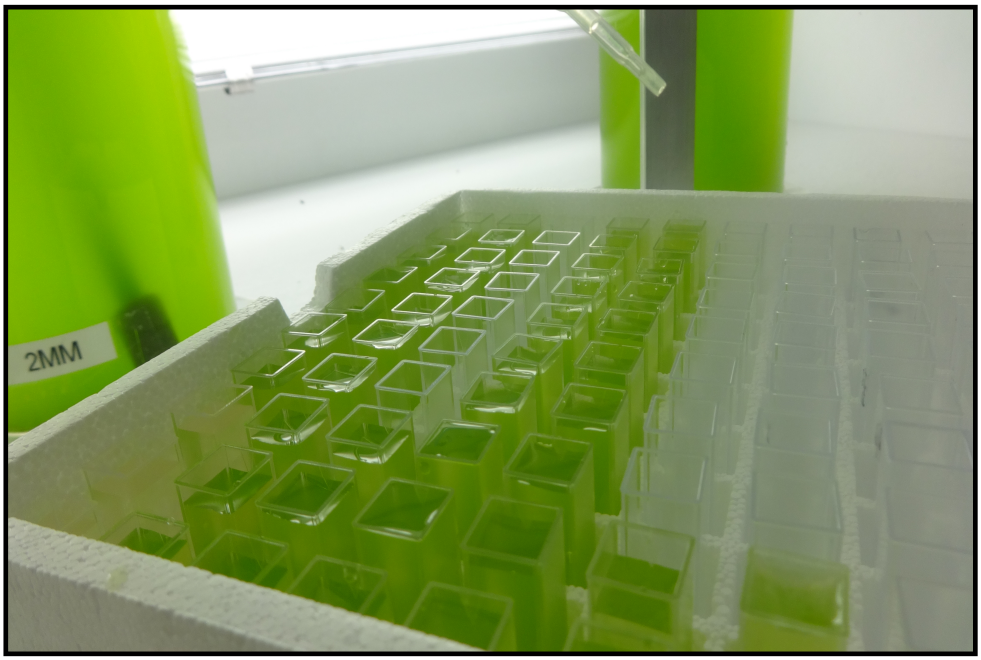


Figure 3-12 Algal samples in cuvettes for turbidity, optical density and cell count measurements

3.4.4 Analysis of algal cultures

Very dense algal solutions need to be diluted by performing serial dilutions before cell counts can be made. Appropriate back calculations are then made to correct for the dilutions.

3.4.5 Growth rate and specific growth rate analysis

Algal cell growth can be related to measured cell mass by wet weight, dry weight, OD, turbidity or cell count. This study uses measurements in terms of units per hour and per day (d^{-1}).

The specific growth rate or SGR (μ) is a measure of the rate of change of a cell mass per unit time (**Equation 3.9)**.

$$\mu = \frac{\ln(X_2 - X_1)}{t_2 - t_1} \quad (3.9)$$

where, X_1 and X_2 are biomass such as measured by turbidity, OD, dry weight. t_1 and t_2 time at initial time and final time respectively in days such t_1 and t_2 are Day 1 and Day 2 (Ji *et al.*, 2013; Maier *et al.*, 2009; Widdel, 2007).

3.4.6 Dry weight analysis

Theoretically, the optical density of 1 unit corresponds to approximately 1 g/L dry cells. However, a study by Feng *et al.* (2011) showed a linear relationship between dry weight (DW) and OD_{658} to be 0.4818 (see **Equation 3.10)**. The study uses this ratio for calculating dry weight because it is close to the range of OD_{680}.

$$DW = 0.4818 \times OD_{658} \quad (3.10)$$

3.4.7 Analysis of the efficiency of sedimentation

In this study, both of OD_{680} and turbidity are used to calculate the percentage removal of algae at setting times of 0,1,12 and 24 hr. as shown in **Equation 3.11** (Xu *et al.*, 2013).

$$\% \text{ removal} = 1 - \frac{OD_{680} \text{ before stirring}}{OD_{680} \text{ after flocculation and setting}} \times 100 \quad (3.11)$$

3.5 The use of CO_2 from flue gas of an electric power plant in Thailand to grow algae *C. vulgaris* – a theoretical consideration

The study estimated the amount of CO_2 from a power plant in Thailand and how this might be used to grow algae in the pilot scale using 6% CO_2 (v/v) (This being the percentage CO_2 released in the flue gas). The CO_2 gas emissions, design, running and maintenance of a photobioreactor system and the economics of the algal biomass produced were investigated. Cost and benefit analyses calculations were made for algae biomass, biodiesel production and value-added products. The details are in **Chapter 7**.

Chapter 4 Preliminary study

4.1 Introduction

A number of factors affect algal growth and this section reports the results aiming to understand the effects of some of these prior to the main experiments. To proof of concept experiments, preliminary studies were carried out to determine effects such as the impact of aeration, mixing and light on the growth rate of *Chlamydomonas reinhardtii*.

Water quality and nutrients are significant in the growth of algae. This study used pond water (from Crookes Valley Pond, Sheffield) to grow the algae because the use of a natural water source may save costs in an industrial system.

The preliminary study investigated:

Experiment 1 Effect of light and aeration

Experiment 2 Effect of mixing medium and various environmental conditions

Experiment 3 Geology and water quality of The Crookes Valley Pond

Experiment 4 Effect of wave length at which optical density (OD) is measured

Experiment 5 Effect of cell selection and screening

Experiment 6 Light measurements

Experiment 7 Effect of serial dilutions

4.2 Materials and Methodology

4.2.1 Experiment 1: Effect of light and aeration

The algal culture was obtained from a stock strain at the University of Sheffield. 250 mL Erlenmeyer flasks were prepared by the sterile technique (Bonner and Hargreaves, 2011). One flask was designed to be a stock. Two flasks were used to cultivate algae by giving an excess of air. Another two flasks were used to grow algae without aeration. The last flask provided a controlled blank (TAP media). The experiment was carried out at a temperature of 25 ±2℃. Algae were kept in the dark for 7 days, and then under continuous light for 7 days using an 11 W fluorescence cool white

G23 tube (**Figure 4-1**). Algal growth was monitored by visual inspection and photography.

Figure 4-1 Preliminary experiment on the impact of aeration and light on the growth rate of *Chlamydomonas reinhardtii*.

4.2.2 Experiment 2: Effect of growth media

In the experiment, the growth of *Chlorella sp.* obtained from stock held by the University of Sheffield was investigated in various media and environmental conditions with and without aeration. 5 media were used; 1) 3N BMM (Bold's Basal Medium), 2) a mixed medium of pond water and BBM, 3) pond water alone, 4) distilled water, and 5) tap water. Grab sampling was used as a simple technique to collect pond water at The Dam House, The University of Sheffield (**Figure 4-2 and Figure 4-3**). The ratio of algal inoculum to the liquid medium was 1:10 in 250 mL Erlenmeyer flasks. Three replicates were carried out under each experimental condition. Culture temperature was 22 $\pm$ 2 ℃ with fluorescent lighting for 366 hr.

Figure 4-2 Pond water source

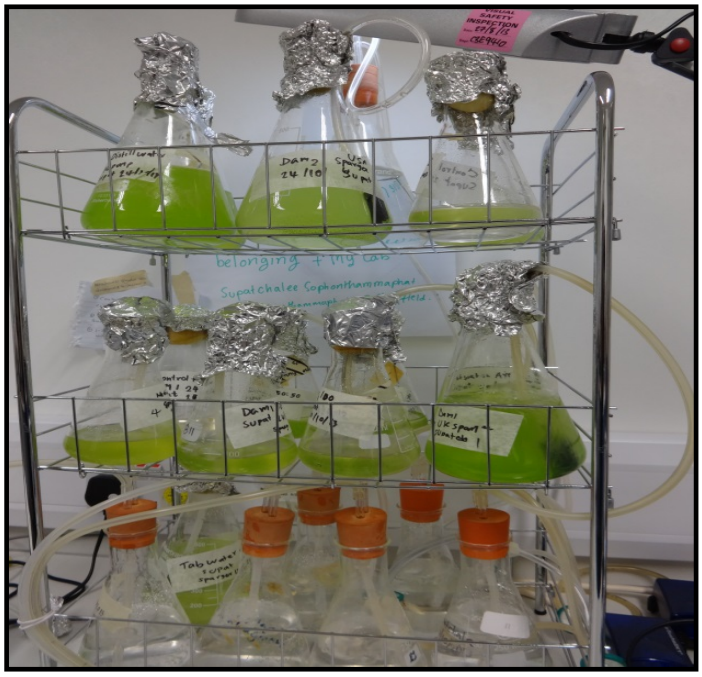


Figure 4-3 Algal cultivation

In the aerated systems, a flow rate of 0.17 LPM (Litre per Minute) was used from a fish tank pump and sparged through a 1 inch artificial stone sparger. Air is passed through a 0.25 inch autoclaved silicone line via 0.22 μm. filter to trap any contamination and humidity. Algal growth rates were monitored at OD_{680} by UV spectrophotometer (Amersham Biosciences model Ultrospec 2100 pro). It is noted that: n=3 (3 sampling replications). The total sample (N= 330) 330 samples (3 sampling replications x 10 timing point x 11 media types) were tested.

4.2.3 Experiment 3: Geology and water quality of The Crookes Valley Pond

A literature review, site visit, and water quality measurements identified the parameters of the pond water and how appropriate it may be for grown algae. This study used a water test kit (Nutrafin Test) to determine pH, ammonia, nitrite, nitrate, phosphate, calcium, iron, general hardness, and carbonate hardness.

The methodologies are described in **Table 4-1**. All samples were tested in the laboratory at a temperature of 25 ℃. For each parameter, 3 replicate samples were used and the data from each was averaged. The total sampling tests comprised 27 samples (3 replicates x 9 parameters).

Table 4-1 Parameters and methodologies

Parameter	Methodology
1. pH	Colourimetric modified method based on Water Quality Monitoring –A Practical Guide to the Design and Implementation of fresh water quality studies and monitoring programme on behalf of United Nations Environment Programme and the World Health Organisation, 1996 UNEP/WHO Chapter 6 Field Testing Methods
2. Ammonia	Indophenol modified method based on The Standard method for the examination of water and wastewater American health public association (APHA) 19th edn, 1995 (Eaton et al., 1995; Yun et al., 1997)
3. Nitrite	Diazotization modified method based on The US.EPA. Diazotization method which approved for wastewater analysis, Federal Register, 44(85), 25505 (May 1, 1979)
4. Nitrate	Diazotization modified method based on The US.EPA. Diazotization method which approved for wastewater analysis, Federal Register, 44(85), 25505 (May 1, 1979)
5. Phosphate	Ascorbic acid modified method based on The Standard method for the examination of water and wastewater American health public association (APHA) standard method 1st edn part 4500-P E, 1905
6. Calcium	EDTA Titrimetric modified method base on The standard methods for the examination of water and wastewater (APHA) 17th edn, 1989 part 3500 D (Lenore *et al*., 1989)
7. Iron	Tripyridyltriazine (TPTZ) modified method based on The standard methods for the examination of water and wastewater (APHA) 17th edition, 1989 part 2340 C (Lenore *et al*., 1989)
8. General hardness	EDTA Titrimetric modified method based on The standard methods for the examination of water and wastewater (APHA) 17th edn, 1989 part 2340 C (Lenore *et al*., 1989)
9. Carbonate hardness (KH)	Titrimetric modified method based on The standard methods for the examination of water and wastewater (APHA) 20th edn, 1999 part 4500-CO_2-C

4.2.3.1 pH

The study followed the manufacturer guidelines for operating the test kit instrument. A colourimetric method is used to measure pH. The indicators bromthylmol blue, thymol blue and meta cresol purple react in water to create a distinct colour for each pH level. In practice 3 drops of reagent-A7813 (<0.1% sodium hydroxide) was added to 5 mL of pond water and shaken to mix well. The colour was matched with the colour chart.

4.2.3.2 Ammonia

The indophenol modified standard method for the examination of water and wastewater, 19th edition, American health and Public Health Association (APHA) 1995 was applied in this study (Eaton *et al*., 1995; Yun *et al*., 1997). In principle, when phenol is combined with hypochlorite it reacts in the presence of ammonia to create indophenol blue. The colour intensity is proportional to the total amount of ammonia.

In practice, 5 mL samples of pond water were taken and seven drops of 5-7% sodium hydroxide (Reagent no.1-A7856) were added and the tube shaken. Then 7 drops of a mixture of 5-10% of sodium hydroxide and 0.1-1.0% of sodium hypochlorite (Reagent no. 2-A7857) were pipetted into the test tube and mixed again. 3 drops of a phenol reagent no.3-A5878 (which consists of 5-10% Phenol, 15-30 Ethyl Alcohol, Denatured and 0.1-1.0% Sodium Nitroprusside) was added and the tube shaken. The colour that develops after 20 mins can be matched against a colour chart.

4.2.3.3 Nitrite

The study used a modified diazotization method, nitrite in the water samples is treated with 4-aminobezesulfonic acid to create varying red shades. Intensity of colour is proportional to the nitrite present.

5 drops of 4-aminobezulfonic acid (Reagent no.1-A7826 composed of 0.1-1% Sulfanilic acid and 1-5% Hydrochloric Acid) and Reagent no. 2-A 7827 (60-100% of 1,2-Propanediol, 10-30% Acetic acid, and 1-5% of N-(1-Naphtyl)-Ethylenediamine Dihydrochloride) were added to 5 mL pond water in a test tube and shaken. A colour appears after 2 minutes, which can be matched with a chart.

4.2.3.4 Nitrate

A modified diazotization method was used in this work. Nitrate (NO_3^-) is transformed with a reducing agent to produce a coloured compound proportional to the amount of nitrate present. 5 drops of 4-amonobenzulfonic acid (A mixture of 0.1-1% Sulfanilic acid and 1-5% Hydrochloric acid, Reagent no 1-A7846) was added to 5 mL pond water in a test tube and shaken. Then, 5 drops of a solution consisting of 60-100% of 1,2-Propannediol, 10-30 Acetic acid, and 1-5% N- (1-Naphtyl)-Ethylenediamine Dihydrochloride (Reagent no. 2-A7847) was added and shaken followed by 3 drops of 75% of a mixture ethoxydiglycol (0.1-1% Zinc metal, 60-100% Ethoxydiglycol and 10-30% Propylene Glycol (Reagent no. 3-A7848). This reagent is carefully added with the tube at a 45 angle tilt, then closed and shaken. The colour that develops after 5 mins is compared with the colour chart.

4.2.3.5 Phosphate

The study used a modified ascorbic acid method. In an acid medium, ammonium molybdate reacts with phosphate in water to produce a complex phosphomolybdate. The reductive agent produces a blue colour in proportion to the phosphate concentration.

3 drops of a mixture of 5% sulfuric acid and 1-5% Ammonium Molybdate (Reagent no.1-A7841) was added to 5 mL pond water in a test tube and shaken. Then, 3 drops of 0.1-1% antimony potassium tartrate (Reagent no. 2-A7842) was added and the test-tube shaken. 3 drops of a mixture of 30-60% Ethoxydiglycol and 10-30% Propylene glycol (Reagent no. 3-A7843) was then added. After a final shaking and allowing 2 minutes for the colour to develop a level of phosphate can be matched on a colour chart.

4.2.3.6 Calcium

Although algae can get carbon from the atmosphere, they still need some nutrients, for example carbonate, some trace metals, nitrogen, phosphate, magnesium and calcium to support their growth. Even small

quantities of calcium in the form of calcium chloride and calcium nitrate can significantly affect algal growth.

A modified EDTA titrimetric method was used following the standard methods for the examination of water and wastewater 17th edition, 1989 part 3500 D (Lenore *et al*., 1989). In principle, EDTA (ethylenediaminetetraacetic acid or its salt) is added to water which contains both calcium and magnesium. Firstly, calcium can be determined directly with EDTA when the pH is sufficiently high (pH 12-13). Then, the magnesium is largely precipitated as the hydroxide form. In contrast, an indicator is used to combine with calcium only. It changes colour when calcium forms a complex.

5 mL of pond water was added to each of 3 replicate test-tubes. The following reagents were added to the samples with the sample being shaken between each addition: 7 drops of 7-13% Sodium hydroxide (Reagent no. 1-A7851 in the test kit); 3 drops of a mixture of Murexide (ammonium purpurate) and Eriochrome Blue Black R in 60-100% propylene glycol in 60-100% propylene glycol, Reagent no. 2-A 7852), 1 drop of EDTA (Reagent no. 3-A7853, a mixture of 1-5% EDTA di-tri potassium salt, 0.1-1% EDTA and 0.1-1% Potassium Hydroxide). A pink colour is produced after adding 1 drop. Then further drops of EDTA are added and stopped when the colour changes from light pink to violet. Then, the sample is compared with the paper chart and read with a light source. The result is found by multiplying the total number of drops by 20 to determine calcium in mg/L (ppm).

4.2.3.7 Ferric Iron

A modified tripyridyltriazine (TPTZ) method was used (from the standard methods for the examination of water and wastewater 17th edition, 1989 part 3500 (Lenore *et al*., 1989). A quantitative distinction between ferrous and ferric iron can be obtained with a special procedure using bathophenanthroline and other organic complexing reagents such as ferrozine or tripyridytriazine (TPTZ). They are capable of determining iron concentrations as low as 1 μg/L. When iron is treated with tripyridytriazine and then placed into contact with hydroxylamine, a blue colour is produced. The intensity is proportional to the amount of iron in the sample.

3 drops of hydroxylamine hydrochloride, (Reagent No.1-A7836 for this test in the kit) were added to 5 mL of pond water in test tubes and shaken. After one minute the test colour was matched to a colour chart and the result determined.

4.2.3.8 General hardness (GH)

General hardness is a measure of all the dissolved salts in a water as represented by calcium (Ca) and magnesium (Mg). The concentration of dissolved salts affects osmotic systems.

A modified Ethylenediaminetetra acetic acid (EDTA) titrimetric method from the standard methods for the examination of water and wastewater 17th edition, 1989 past 2340 C (Lenore *et al*., 1989) was used in this work.

EDTA and its sodium salt form a chelated soluble complex when added to metal cations. If a small amount of a dye such as Eriochrome Black T or Calmagite is added to an aqueous solution containing calcium and magnesium ions at a pH of 10.0 $\pm$ 0.1 the solution becomes wine red. If EDTA is added as a titrant, the calcium and magnesium will be complexed. The colour will change from wine red to blue. Magnesium ions must be present to yield a satisfactory end point. The sharpness of the end point increases with increasing pH. However, the pH cannot be increased too far because of the danger of precipitating $CaCO_3$, or $Mg(OH)_2$. The specified pH of 10 $\pm$ 0.1 is a satisfactory compromise. A limit of 5 min is set for the duration of the titration to minimise the tendency toward $CaCO_3$ precipitation.

The reagent reacts with calcium and magnesium present in the water sample. The indicator changes colour when the calcium and magnesium from a complex. 5 mL the pond water was placed in a clean test tube. One drop of GH reagent-A7832 (30-60% methanol, 30-60% Triethanolamine, and 1-5% EDTA mono-di sodium salt) is added to the test tube which is then shaken to mix. A pink colour should result. The reagent was continued to be added, one drop at a time and with the contents shaken after each addition until the colour changes from pink to blue. The total number of drops multiplied by 20 to determine the general hardness in mg/L (ppm) as carbonate ($CaCO_3$).

4.2.3.9 Carbonate hardness (KH)

Carbonate hardness (commonly referred to as alkalinity) is a measurement of the capacity for water to neutralise acid. A titrimetric method using an acid reagent which changes colour when all alkalinity is neutralised was used. 5 mL of pond water was placed in a test tube and one drop of KH reagent A7831 from the kit (30-60% ethyl alcohol, Denatured) was added and the tube shaken. A blue colour results. The reagent was then added one drop at a time with the contents of the tube shaken after each drop. Additions were stopped when the colour changed from blue to a yellow or lime colour. Multiplying the total number of drops by 10 determined the carbonate hardness in mg/L (or part per million, ppm).

4.2.4 Experiment 4: Effect of wave length at which optical density (OD) is measured

Growth rate was measured by OD at various wave lengths (550, 650, 680, 700, and 770 nm.). Algae were grown in the various media as in the previous experiments (without cleaning cells) and optical density was measured on day 4 of the culture.

4.2.5 Experiment 5: Effect of cell selection and screening

Impurities and contamination may affect algal growth. The aim of this study was to purify algal cells by simple methods of screening and cell selection. A comparison of the growth rate of both new cells, and aged reused cells was determined.

20 mL algae old stock solution from the last experiment were re-inoculated into 200 mL working volume of 3 N BBM media in 250 mL Erlenmeyer flasks. Air was supplied at 0.17 LPM (L/M) via a 0.22 μm filter and bubbled through the media using sparging stones. Light was by 11 W fluorescent tubes. 3 groups of algae were grown;

1) Algae were inoculated using old cell stock and no treatment;

2) Cellulose filtration by using a cellulose “extraction thimble” size 25 x 100 mm. (**Figure 4-4**) and;

3) Fabric filtration and centrifugation at 3,000 rpm for 5 min with the supernatant being discarded (**Figure 4-5**). A rough estimate of the pore size of the fabric filters is 2.0 mm.

The temperature was controlled at 22 ±2 ℃. Algal growth rate was measured at OD $_{680}$ by a UV spectrophotometer (Spectronic 200).

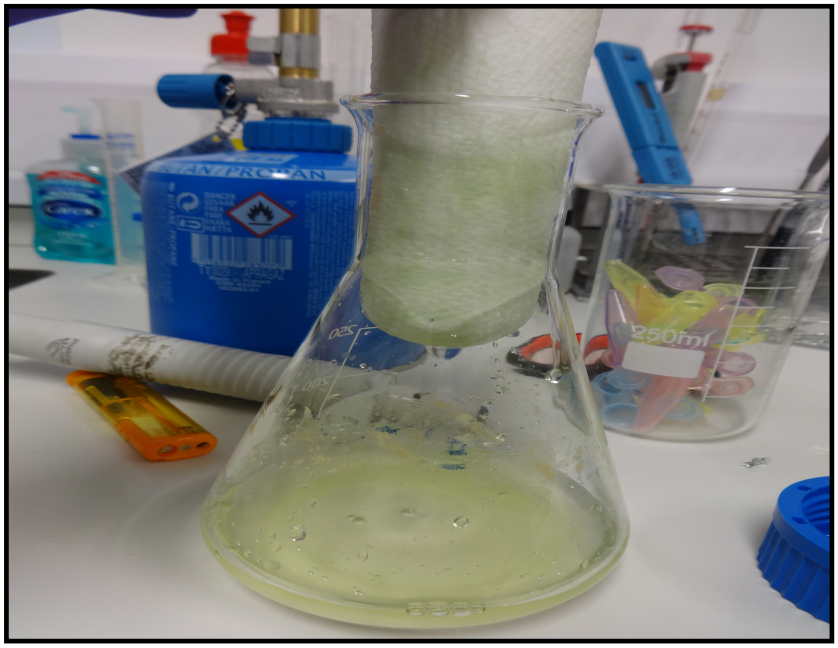


Figure 4-4 Cellulose filtration

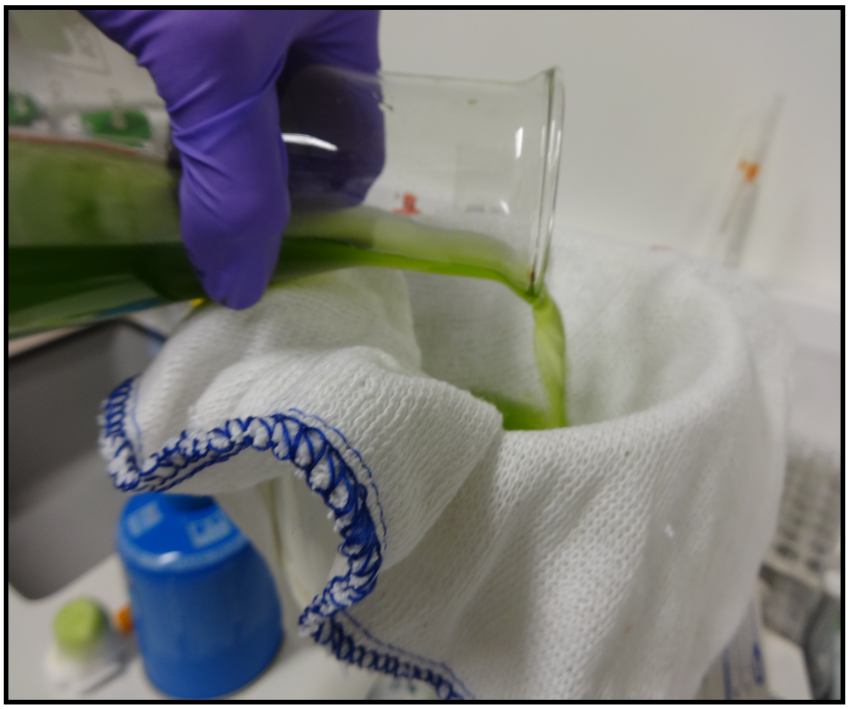


Figure 4-5 Fabric filtration

4.2.6 Experiment 6: Light measurements

Light is an important factor in algal growth as shown in the literature review (**Chapter 2**). To low or too great light intensity may cause growth inhibition. To set up an appropriate area for growing algae, light was measured by sampling 10 points (n=10) around the innoculum/stock culture area and 10 points on the photobioreactor shelves using light meter sensor biosheric instrument model QSL-2100 S/N 10140 San Diego California with programme logger 2100 QSL-S/N 10140 Biopherical Instrument. The results were recored as an average in unit of micro Einsteins.$m^{-2}.s^{-1}$ ($\mu E.m^{-2}.s^{-1}$ or μ mol.$m^{-2}.s^{-1}$ equivalent).

4.2.7 Experiment 7: Effect of serial dilutions

When a culture is growing strongly there is often a need to make a dilution to enable the measurement technique to be viable. Serial dilutions tests aim to check the accuracy of the laboratory practice. Experiments were conducted to investigate how using a serial dilution affects measurement of cell growth. A known volume from a culture of *Chlorella vulgaris* was taken at different stages in its growth cycle and was initially measured without dilution by optical density (OD_{680}). A serial dilution was then made of the sample and measurements made at dilution factors of 1:10 and 1:100 (both of which are likely to be needed later in this study). These were then "back-calculated" to give a measurement for the original culture.

4.3 Results

4.3.1 Experiment 1: Light and aeration

As would be expected, algae in the aerated system grow slowly in dark conditions and rapidly once light is provided. Algae grow better with aeration than without it.

4.3.2 Experiment 2: Effect of mixing medium and various environmental conditions

Algal growth was monitored at OD_{680} of a total of 330 samples (3 replications for each sample). At 24^{th} hr., the mean of the blanks is 0.000-0.004. The standard deviation is 0.000. The mean OD of algal grown in 100 mL-BBM media at time 24 - 366 hr. is 0.174-0.582 $\pm$0.000. While the mean OD of the pond water is 0.008-0.166 at the same period of time. The

distilled water is 0.207 – 0.303, and tap water is between 0.208-0.295. Most of the samples give a standard deviation (S.D.) close to zero except the samples from pond water with aeration (S.D. is 0.002-0.100) and tap water with aeration, which is at 0.015-0.071. This indicated that OD in both of them fluctuates to some degree.

The results show that algae grown in 3N BBM with aeration (green line in **Figure 4-6**) show a normal growth curve. Algal in 3 N BBM media with no aeration (blue line) grow more slowly (**Table 4-2** and **Figure 4-6**). Mixed media (pond water and 3 N BBM media) resulted in a fluctuating growth (purple line). This may indicate the breakdown of more complex organic material periodically releasing nutrients for the algae. Other cultures (in distilled water and tap water) either grew much more slowly or not at all, with a number of growth curves showing decreasing cell numbers from the innoculum.

Table 4-2 The mean and standard deviation of algal growth over time with various growth media.

Growth media	Time (hour)									
	24	72	120	144	168	229	264	288	312	366
1) Blank control (Distilled water)	0.003 ± 0.000	0.004 ± 0.000	0.004 ± 0.000	0.003 ± 0.000	0.002 ± 0.000	0.001 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
2) 100 mL media	0.178 ± 0.000	0.174 ± 0.000	0.193 ± 0.000	0.265 ± 0.000	0.278 ± 0.000	0.344 ± 0.000	0.401 ± 0.000	0.457 ± 0.000	0.504 ± 0.000	0.582 ± 0.000
3) 100 mL media + aeration	0.238 ± 0.000	0.238 ± 0.000	0.291 ± 0.000	0.356 ± 0.000	0.451 ± 0.000	0.589 ± 0.000	0.692 ± 0.000	0.829 ± 0.000	0.895 ± 0.000	0.875 ± 0.000
4) 50 mL Pond water + 50 mL media	0.139 ± 0.000	0.256 ± 0.000	0.160 ± 0.000	0.223 ± 0.000	0.198 ± 0.000	0.198 ± 0.000	0.012 ± 0.000	0.219 ± 0.000	0.254 ± 0.000	0.186 ± 0.000
5) 50 mL Pond + 50 mL media + aeration	0.238 ± 0.000	0.165 ± 0.000	0.164 ± 0.000	0.206 ± 0.000	0.234 ± 0.000	0.545 ± 0.000	0.440 ± 0.000	1.000 ± 0.000	0.946 ± 0.000	2.104 ± 0.000
6) 100 mL Pond water	0.166 ± 0.000	0.074 ± 0.000	0.008 ± 0.000	0.023 ± 0.000	0.018 ± 0.000	0.028 ± 0.000	0.012 ± 0.000	0.138 ± 0.000	0.092 ± 0.000	0.082 ± 0.000
7) 100 mL Pond water + aeration	0.242 ± 0.000	0.118 ± 0.000	0.100 ± 0.000	0.062 ± 0.000	0.098 ± 0.000	0.069 ± 0.000	0.050 ± 0.000	0.013 ± 0.000	0.040 ± 0.000	0.038 ± 0.000
8) 400 mL Pond water + aeration + sparger	0.236 ± 0.002	0.158 ± 0.041	0.112 ± 0.035	0.127 ± 0.041	0.128 ± 0.006	0.125 ± 0.083	0.113 ± 0.100	0.150 ± 0.137	0.145 ± 0.049	0.062 ± 0.057
9) 400 mL Distilled water	0.291 ± 0.000	0.262 ± 0.000	0.226 ± 0.000	0.253 ± 0.000	0.247 ± 0.000	0.303 ± 0.000	0.287 ± 0.000	0.287 ± 0.000	0.262 ± 0.000	0.207 ± 0.000
10) 400 mL Tap water	0.234 ± 0.000	0.234 ± 0.000	0.208 ± 0.000	0.265 ± 0.000	0.228 ± 0.000	0.252 ± 0.000	0.282 ± 0.000	0.287 ± 0.000	0.296 ± 0.000	0.295 ± 0.000
11) 400 mL Tap water + aeration + sparger	0.200 ± 0.028	0.184 ± 0.052	0.145 ± 0.060	0.141 ± 0.066	0.147 ± 0.071	0.144 ± 0.065	0.066 ± 0.012	0.064 ± 0.019	0.065 ± 0.021	0.062 ± 0.015

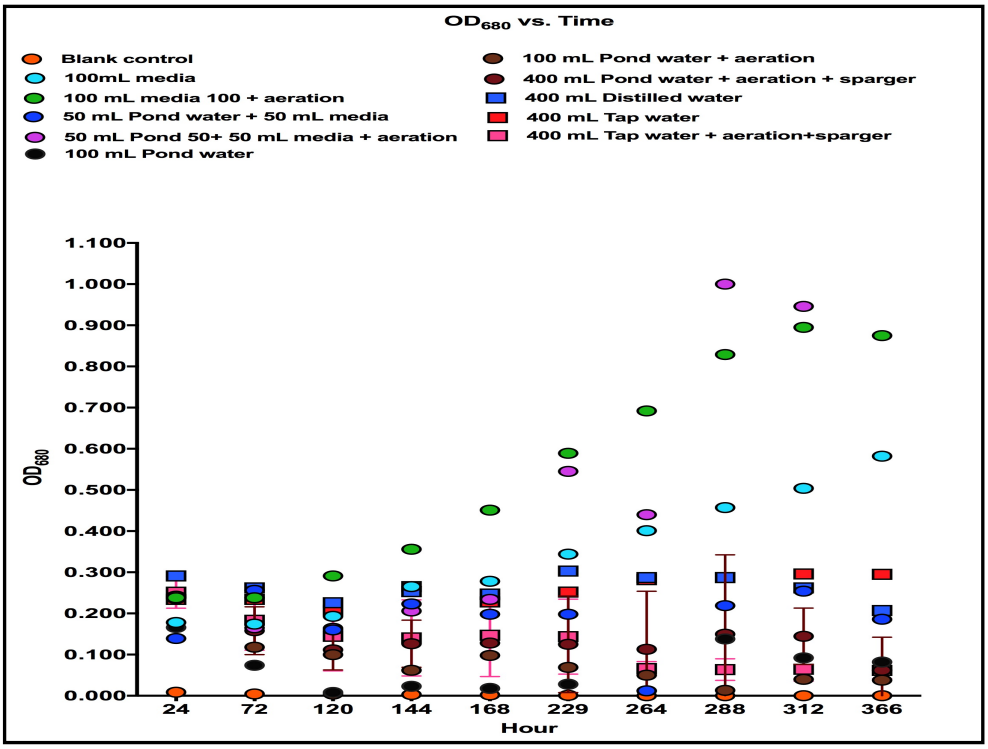


Figure 4-6 Algal growth by optical density against time

As may be expected, cultures grown with pond water in the medium were found to be contaminated by bacteria (**Figure 4-7** and **Figure 4-8**) and aggregation (floc formation) was seen. Such aggregation has both advantages and disadvantages. In addition, pond water at The Crookes Valley Park is less nutrient enriched and may have components that inhibit algal growth.

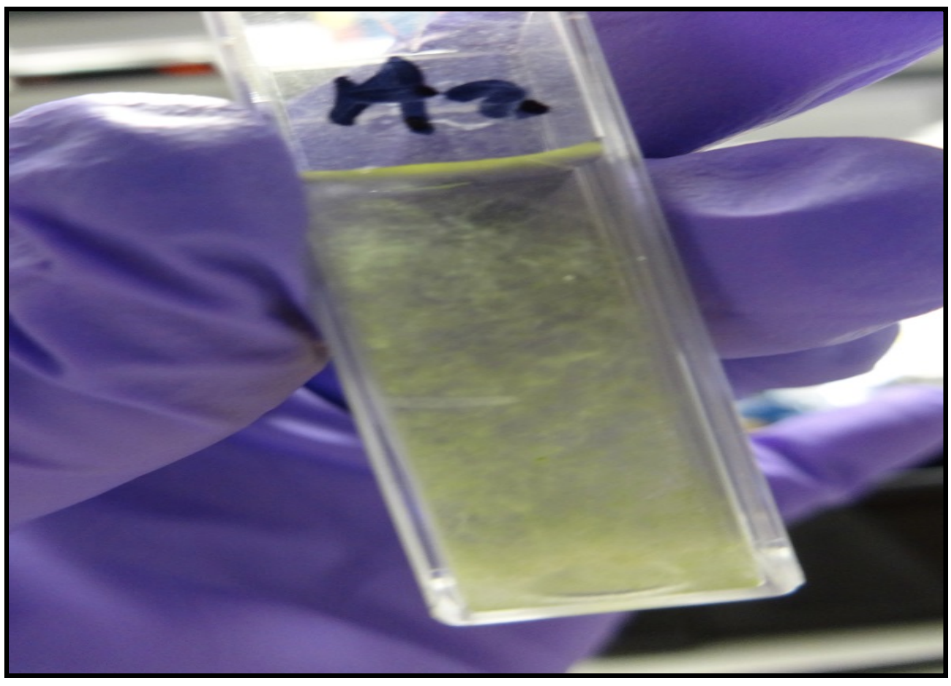


Figure 4-7 Effect of the impurities in the medium mixed with pond water

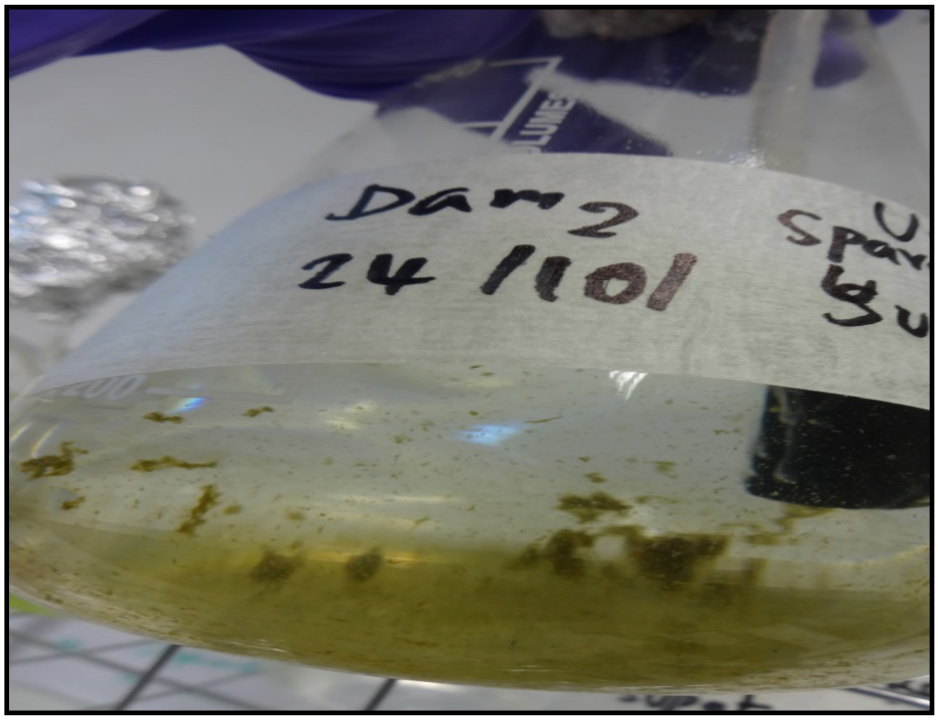


Figure 4-8 Algal sample of *Chlorella sp*. from The University of Sheffield

4.3.3 Experiment 3: Water quality of The Crookes Valley Pond

The experiments monitor the water quality of The Crooks Valley Pond. The study found that the pond water is slightly alkaline (pH in BBM media is a more suitable for algal growth (6.7 ± 0.3) than the pond water at pH 8.2). Nitrite, nitrate, and phosphate are in the range of the WHO drinking water standard except for ammonia as given in **Table 4-3**. The study found the BBM media has a higher concentration of essential elements to grow algae than the pond water (for example; nitrate and phosphate).

Table 4-3 The mean physico-chemical characteristics of The Crookes Valley Pond water (n=3). This noted that the S.D. is closed to zero.

Parameter	Unit	The Crookes Valley Pond water	WHO (2017)	BBM media
1.pH	-	8.2	Not of health concern at levels found in drinking-water	6.7 ± 0.3
2. Ammonia (NH_4)	mg N/L	0.8	Below than 0.2 (for ground water and surface water)	-
3. Nitrite	mg N/L	0.1	3 mg/L (short-long term effect)	-
4. Nitrate	mg N/L	5	50 mg/L (short-long term effect)	25 g/L ($NaNO_3$)
5. Phosphate	mg/L	0.25	No guideline value is propose	7.5 g/L(K_2HPO_4) 17.5 g/L (KH_2PO_4)
6. Calcium	mg/L	40	No guideline value is proposed	2.5 g/L ($CaCl_2$)
7. Iron	mg/L	0	0-50 (in natural fresh water)	4.98 g/L $FeSO_4.7H_2O$ with 1 mL H_2SO_4
8. General hardness	mg/L	66.7	No guideline value is proposed	No guideline value is propose
9. Carbonate hardness (KH)	mg/L	53.3	No guideline value is proposed	No guideline values is propose

4.3.4 Experiment 4: Effect of wave length at which optical density (OD) is measured

The results show that measuring algal growth using different optical densities in the range of 550-770 nm will give different results (**Table 4-4** and **Figure 4-9**).

The minimum OD values in every wave length is in 50 mL pond water with 50 mL media (0.189-0.198). The maximum values are 100 mL - pond water with adding aeration (0.327-0.339).

OD measured at 680 nm. tends to provide the highest values. It also shows that the distilled water cultures give the most consistent results across the wavelengths. This could indicate that the variation is due to the media rather than the algae (though there is still a slight increase at 680 nm.). The study also confirms that algae grown in BBM medium show higher productivity than pond water, distilled water and tap water. The difference between medium 1 and 2 is around 0.028. While the difference in OD measurements within the pond water groups is 0.071. Distilled water shows

a range of 0.020 within the group and the tap water group shows a range of 0.074 OD units.

Table 4-4 Measurement of algal growth by optical density at day 4 of cultures at a number of wave lengths.

Growth medium	Wave length (nm.)				
	550	650	680	700	770
100 mL media	0.189	0.181	0.198	0.190	0.189
100 mL media + aeration	0.104	0.140	0.145	0.141	0.137
50 mL Pond water + 50 mL media	0.016	0.025	0.028	0.031	0.026
50 mL Pond + 50 mL media + aeration	0.070	0.064	0.069	0.060	0.054
100 mL Pond water	0.200	0.200	0.208	0.202	0.198
100 mL Pond water + aeration	0.339	0.338	0.342	0.336	0.327
400 mL Pond water + aeration + sparger	0.300	0.301	0.303	0.299	0.294
400 mL Distilled water	0.226	0.234	0.252	0.239	0.226
400 mL Tap water	0.069	0.067	0.079	0.070	0.067
400 mL Tap water + aeration + sparger	0.201	0.202	0.208	0.205	0.184

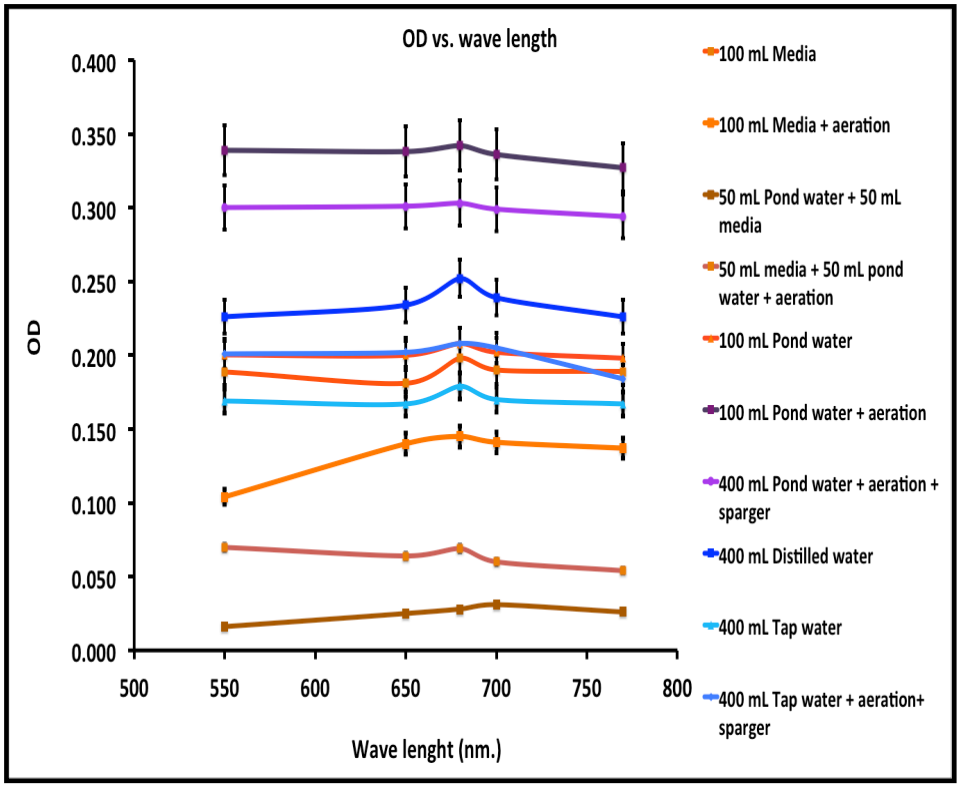


Figure 4-9 optical density of algal cultures measured at a range of wavelengths on Day 4 of the culture.

4.3.5 Experiment 5: Effect of cell selection and screening

The results of different pretreatment of the algal cells prior to culturing (**Table 4-5**) found that those cells which had no treatment (Group 1) have an OD_{680} mean ranging from 0.053 on Day 1 to 0.974 on Day 16. The standard deviation is between 0.001-0.103. For Group 2, Cellulose filtered cells, the mean OD_{680} is 0.044 on Day 1 and 1.00 on Day 16. The standard deviation is between 0.000-0.067. For Group 3, the fabric filtered and centrifuged cells, the OD_{680} is 0.031 on Day 1 and 0.444 on Day 16. The standard deviation is between 0.000-0.202.

The study shows that both new and older algal cells grow well when there has been no treatment of the cells. Cells screened using cellulose filtration show slower initial growth but have caught up with un-screened cells on Day 13. Screening cells by using cellulose filtration give better growth than separation by fabric screening and centrifugation. This could be due to detrimental effects of centrifugation on the cells resulting in cell death and thus fewer cells to start the culture.

Table 4-5 and **Figure 4-10** show that, following slow but steady growth in the first seven days, growth under all conditions increased. After 16 days, algae that had not been filtered and algae that had been separated using cellulose filters showed a sharp increase in growth. The highest average growth was at Day 16. Cells that had been centrifuged showed the least growth while showing a similar pattern of growth to cellulose filtered and unfiltered cells.

Table 4-5 Mean and standard deviation of optical density of algal cultures following different treatments.

Day	Group 1: No treatment	Group 2: Cellulose filtration	Group 3: Fabric filtration + Centrifuge
	Mean ±S. D.	Mean ±S. D.	Mean ±S. D.
1	0.053 ±0.003	0.044±0.004	0.031±0.001
2	0.060 ±0.010	0.049±0.003	0.034±0.001
3	0.078 ±0.001	0.060±0.000	0.038±0.008
4	0.101 ±0.015	0.079±0.001	0.039±0.008
5	0.117 ±0.011	0.087±0.009	0.047±0.000
6	0.159 ±0.008	0.113±0.023	0.052±0.007
7	0.183 ±0.009	0.130±0.011	0.061±0.018
8	0.274 ±0.018	0.153±0.000	0.064±0.021
9	0.418 ±0.023	0.221±0.004	0.076±0.033
10	0.496 ±0.029	0.267±0.006	0.104±0.066
11	0.574 ±0.062	0.328±0.001	0.134±0.092
12	0.716±0.095	0.476±0.016	0.176±0.134
13	0.782±0.103	0.671±0.013	0.265±0.189
14	0.897±0.062	0.874±0.067	0.314±0.202
15	0.917±0.062	0.996±0.006	0.357±0.197
16	0.974±0.023	1.000±0.000	0.444±0.173

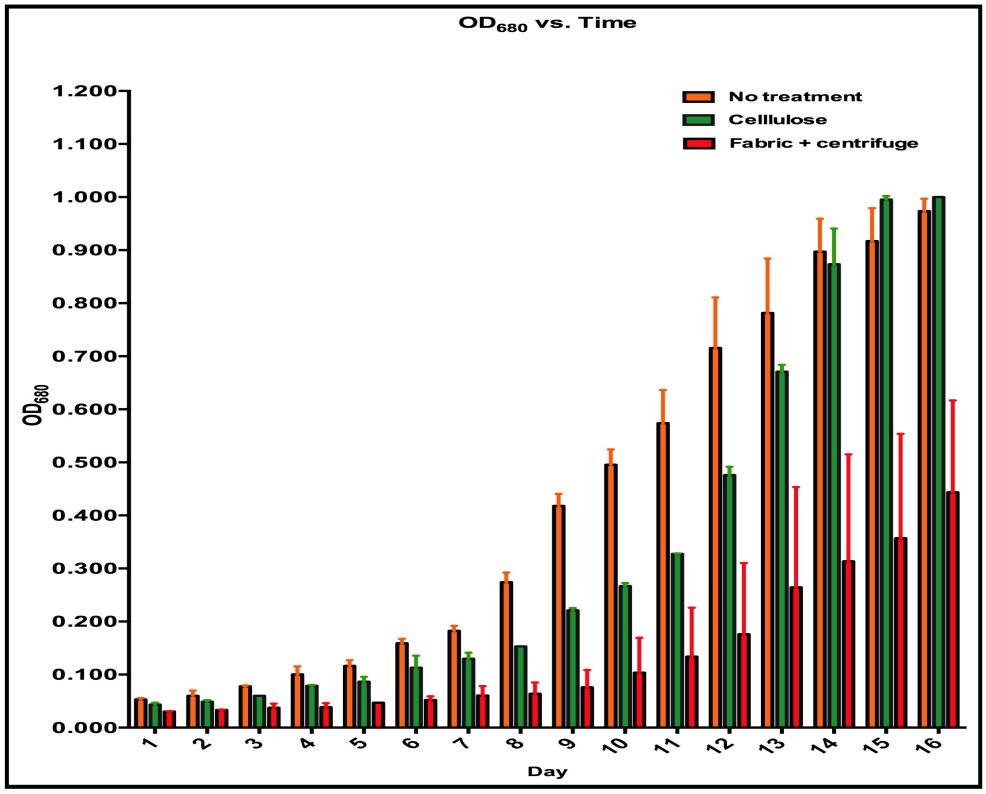


Figure 4-10 The optical density (OD_{680}) by using no treatment algal cells (Group1: orange colour), Group 2: celloluse filtration (green colour), fabric filtration and centrifuge (Group 3: red colour)

4.3.6 Experiment 6: Light measurements

The G 2311 W light source for stock cultures gives an average light of 60 $\mu Em^{-2}s^{-1}$. For the photobireactor the illumination was around 500 $\mu Em^{-2}s^{-1}$. At the inoculum area, the standard deviation (SD) is around 0.436 $\mu Em^{-2}s^{-1}$. While at the photobioreactor is 2.377 $\mu Em^{-2}s^{-1}$(see **Table 4-6** and **Figure 4-11**).

Table 4-6 The illumination of stock algal cultures and in the photobioreactor (n=10)

Sample	Inoculum (μE/m^2/s)	Photobioreactor (μE/m^2/s)
1	60.81650	496.49700
2	60.79365	502.59860
3	60.34567	502.99307
4	60.79032	498.35105
5	60.94364	497.33350
6	60.89793	498.49705
7	61.04367	499.79032
8	59.99870	502.99345
9	60.09851	500.00451
10	59.87500	501.33590
Mean ± S.D.	60.56036±0.4361	500.03944±2.3784

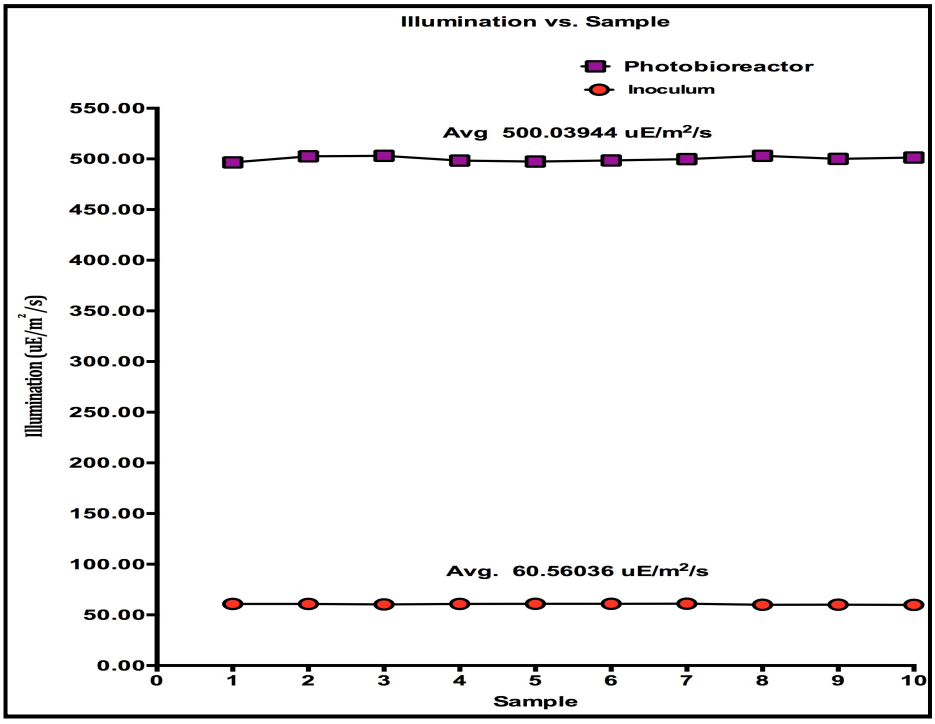


Figure 4-11 Illumination of the stock/inoculum area (red line around 60 μE.m^{-2}.s^{-1}) and in the photobioreactor (purple line, around 500 μE.m^{-2}.s^{-1}).

4.3.7 Experiment 7: Serial dilutions

The results are as shown in **Figure 4-12** and **Figure 4-13** compared to "expected data" i.e. the data obtained from the undiluted samples.

1. The relationship between expected data (X-axis) and actual data for a dilution of 1:10 (Y-axis) is shown in **Figure 4-12** The linear relationship is Y = 1.000* X-0.0003116. The slope is 1.000 ± 0.0002098 at 95% confidence, and a P value < 0.0001 R^2 is 1.000.

2. A serial dilution of 1:100 gave a relationship of the actual value (Y) and the expected data (undiluted data X). The slope is 1.000 ± 0.0004172 at 95% confidence interval. R^2 is 1.000 (**Figure 4-13**).

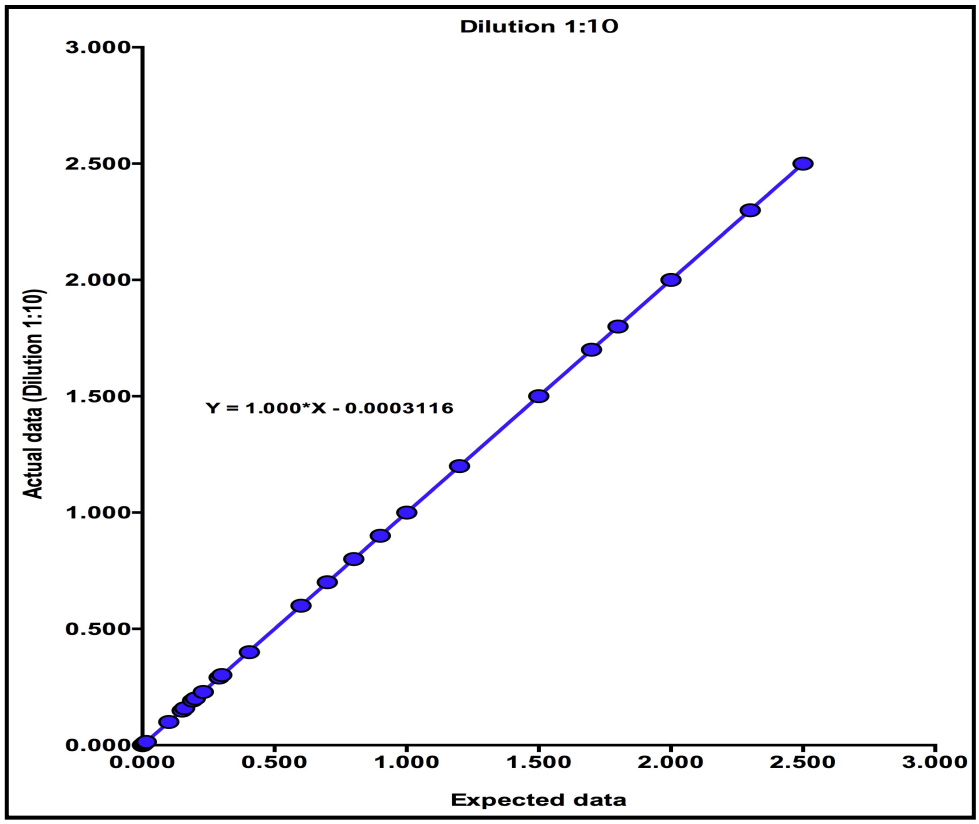


Figure 4-12 Comparison of optical density measured at a dilution of 1:10 and converted to the equivalent at zero dilution with "expected data" i.e. measurement at zero dilution (The units are optical density units).

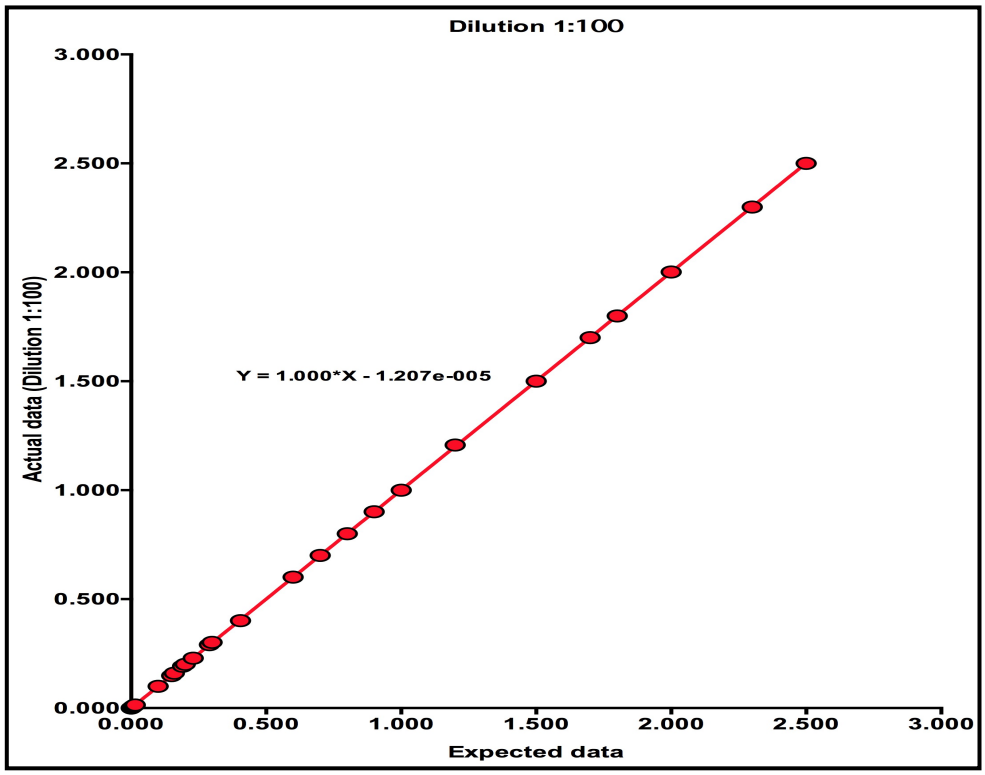


Figure 4-13 Comparison of optical density measured at a dilution of 1:100 and converted to the equivalent at zero dilution with "expected data" i.e. measurement at zero dilution (the units are optical density units).

4.4 Discussion

4.4.1 Experiment 1: Light and aeration

These results are in agreement with many other researchers, for example Blinová *et al.* (2015) who show that light and CO_2 are significant to algal biomass yield and Chen *et al.* (2011) who found that artificial light such LED is suitable for culturing algae. This means that there should be no detriment to using the artificial light system for algal growth in the photobioreactors for the rest of the experiments.

4.4.2 Experiment 2: Effect of mixing medium and various environmental conditions

Macronutrients such as nitrogen, phosphorus, sulphur, potassium and micronutrients (i.e. iron, magnesium) in the media are important for cell growth (Juneja *et al.*, 2013; Walker, 1953). BBM media is suitable for algae. It provides more (and more accessible) essential elements than pond water, tap water, and distilled water. Some studies such as that of Ramaraj *et al.* (2016) used BBM media to culture *C. vulgaris* in their research. Another

study by Feng *et al.* (2011) found that *C. vulgaris* can grow in artificial wastewater medium where it achieved a highest lipid content of 42%.

The study of Abdel-Raouf *et al.* (2012) reported that cultivation of algae in wastewater must consider wastewater quality, pH, and temperature in any application. Blair *et al.* (2014) looked at the effects of 3N BBM growth medium with varying media components of $CaCl_2$, NaCl, $NaNO_2$, $MgSO_4$, KH_2PO_4, K_2HPO_4, with each at 25%, 50%, and 100% of 3 N BBM on the growth of *C.vulgaris* measured by optical density. The study found that, at 50%, the medium provided an exponential growth rate on Day 12, while at 25% the media only provided a stationary growth phase. The maximum growth rates were 0.0475 $gL^{-1}d^{-1}$ (Day 4), 0.0525 $gL^{-1}d^{-1}$ (Day 8), 0.0893 (Day 7) for media with 100%, 50% and 25% components respectively. The study concluded that the media components are significant to the growth rates of the algae.

4.4.3 Experiment 3: Geology and water quality of The Crookes Valley Pond

4.4.3.1 pH

The water samples from The Crookes Valley Pond showed a pH of 8.2. indicating that the pond water is slightly alkaline. This alkalinity may derive from minerals, food and animal waste dissolved in the water. An healthy aquarium situation is considered to be between pH 6.5 and 9.0 (Wurts and Durborow, 1992).

While studies, such as that of Gong *et al*. (2014) found that high pH resulted in high yield at various light intensities (Gong *et al*. found that growth of *C. vulgaris* was highest at pH 10 for light intensities of 3960, 7920 and 11920 lux), it is likely that the further the pH is away from neutral the longer algae will take to adapt to it (extending the lag phase).

Therefore, consideration should be made on adding acid buffer to the pond water to adjust it to pH 7 when growing algae.

4.4.3.2 Ammonia

Generally, ammonia exists in two forms when dissolved in water. The first is free ammonia or ammonia-nitrogen (NH_3-N) which is very poisonous to fish. The second is known as an ionised ammonia (NH_4^+). It is

not quite as harmful as the free form. Unionised ammonia (NH_3) is the most toxic. It disrupts the ability to regulate water and salts. It also can damage delicate gill tissue.

Ammonia can be used by algae and other plants. It is the most common form of nitrogen in aquatic system which is an essential micronutrient to grow algae. The study of Chen *et al.* (2012) found that ammonia can provide for a high growth rate in first of 3 days of cultivation of the marine algal strains *Nanochloropsis oculata, Isochrysis aff., agalbana, Chaetoceros muelleri and Tetraselmis chui*. However, dry weight productivity also depends on a favourable temperature for each species.

Caicedo *et al.* (2000) found that the duckweed (*Spirodela polyrrhiza*) showed maximum growth when culturing with 3.5-20 mg/L ammonia. At higher concentrations (over 20 mg/L), nitrogen exists in a changed form which results in a pH change. Both increasing level of ammonia and the resultant pH affect result in declining growth rate. This study showed that a low level of supplement is more advantageous than a high level.

The study found that average ammonia is about 0.8 mg/L. The nitrogen cycle begins with ammonia. The waste products of fish, plants, and invertebrates, along with dead organisms (dead leaves and fish) and any uneaten food are decayed by bacteria producing ammonia and other dissolved products.

The Crookes Valley Pond is also an open area. Ammonia can be directly excreted by animals such as dog, fish and birds. Other sources of ammonia are, for example; atmospheric deposition and leaching from fertilisers using in agriculture.

Ammonia is changed by aerobic bacteria (*Nitrosomonas spp.*) to nitrite. Another aerobic bacterium, (*Nitrobacter spp*.) changes the nitrite to nitrate which is a food for pond plants and algae. Ammonia, nitrite, and nitrate thus fluctuate in the pond according to the activities of the bacteria.

In addition, photosynthesis and respiration may create a cyclical change in pond pH. The pond water becomes most acidic just before the periods of darkness ends and is in the most alkaline form after several hours of daylight. The presence of unionised ammonia (toxic form) increases as pH rises and decreases as pH falls.

Ammonia concentration is less than 0.2 mg/L in unpolluted groundwater. It can be as high as 12 mg/L in surface water (Yuncong and Kati, 2011). Above 1.2 mg/L in alkaline water (mostly above pH 8.0) it becomes more toxic to aquatic organisms. In this study found that ammonia at The Crookes Valley Pond is 0.8 mg/L in a water with pH at 8.2. This makes it boarder line toxic to fish. However, algal growth will tend to remove ammonia.

The study of Feng *et al*. (2011) found that *C. vulgaris* grown in an artificial wastewater in a column aerated photobioreactor showed a nutrient removal efficiency of 86% (COD), whilst NH_4^+ is 97% removed and total phosphate (TP) is 96% removed. *C.vulgaris* UTEX 259 cultivated in wastewater with 15% (v/v) CO_2 from flue gas gave an ammonia removal rate of 0.92 g NH_3 $m^{-3}h^{-1}$ (Yun *et al*., 1997).

4.4.3.3 Nitrite

Nitrite is the most ephemeral compound in an environment. It is a less stable intermediary form of nitrogen which found during nitrogen transforming processes. Although nitrite is not as toxic as ammonia. It can be harmful to fish.

Algae and other plants assimilate nitrate reductase (NR) using either nicotinamide adenine dinucleotide (NADH) or nicotinamide adenine dinucleotide phosphate (NADPH) as an electron donor. WHO (see **Table 4-3**) and Hagen (2008) (mentioned in the instruction of The Nutrafin product) state that Nitrite in the water should not be more than 3 mg/L because it results in a fish stress. However, nitrate has to be above 50 mg/L to cause such stress. This indicates that nitrite is highly reactive and more toxic than nitrate.

Nitrite is generated through ammonia oxidation by nitrifying bacteria such as *Nitrosomonas spp*. At low concentrations, nitrite is then converted to nitrate. A concentration of over 0.3 mg/L, NO_2 is considered potentially dangerous to fish and algae. This study found only 0.1 mg/L of nitrite.

Photosynthetic reduction of nitrate has been found in intact cells of green algae and other organisms. Kessler and Zumft (1973) studied an effect of nitrite and nitrate on chlorophyll fluorescence in green algae (*Chlorella* and *Ankistrodesmuc braunii*). It was found that the effects of nitrite and

nitrate on chlorophyll fluorescence is a very sensitive indicator for the redox photosynthesis.

4.4.3.4 Nitrate

Nitrate is formed a natural by-product of the bacterial reduction or removal of ammonia, also nitrite in the pond ecosystem. All algae and other plants need nitrogen as a nutrient source. High nitrate concentration indicated a rise in pollution level in the water and can lead to the uncontrolled algal growth (an algal "bloom"). The study of Fried *et al.* (2003) found that increasing algal growth depends on the nitrogen (as nitrate) added. However, at a high concentration of 9.61 $x10^{-4}$ mol nitrate inhibited growth. In 2011, Sydney *et al.* (2011) found that *C.vulgaris* (LEM 07), *Botryococcus braunii* (LEM 14) and *Isolated strain LEM-IM 11* have a removal rate of 16.78-22.21 nitrate $mg.d^{-1}$.

Nitrate concentration is usually less than 2 mg/L in natural ground water. It can be over 100 mg/L in the contaminated water (Yuncong and Kati, 2011). In this study, nitrate is 5 mg/L. It is a regular level for surface water.

Su *et al.* (2012) found that nutrient removal under different conditions (no mixing, mixing rate of 100 rpm, and mixing rate of 300 rpm) provide a different result of nitrate and nitrite concentrations. Mixing at 100 rpm assimilates nitrate to peak up on Day 7 from around 7 mL^{-1} to 12 mgL^{-1} and nitrite removal changes from 1.5 mgL^{-1} to 8 mgL^{-1}.

In 2015, Taziki *et al*. (Taziki *et al.*) found differences in nitrate removal by different microalgae strains (*Chlamydomonas reinhardtii, Chlorella vulgaris, Scenedemus rubescence and phormidium spp.*). The important parameters are initial nitrate concentration, light intensity, pH and temperature (Su *et al.*, 2012; Taziki *et al.*, 2015).

4.4.3.5 Phosphate

Phosphate in water is normally found as orthophosphate (HPO_4^{2-} and PO_4^{3-}). The majority of phosphate originates from fish feces and the decay of

organic matter such as plant, fish and other organisms. It is an essential nutrient for algae.

The study found that phosphate in the pond water is 0.25 mg/L. Phosphate concentration is usually less than 0.03 mg/L in uncontaminated surface water. It can be over 0.1 mg/L in contaminated water. High PO_4^-P in water often causes algal blooming and eutrophication (Paerl *et al*., 2001). This has been a serious problem in aquatic systems (Yuncong and Kati, 2011). The Crookes Valley Park pond thus has a high level of phosphate.

4.4.3.6 Calcium

The concentration of calcium is usually less than 20 mg/L in ground water. But it can be as high as 200 mg/L to 400 mg/L in seawater. This study found that calcium in the pond water is about 40 mg/L which is within normal levels for surface water. The study of Zhao *et al.* (2011) found that a cell densities of the unicellular *Microcystis aeruginosa* strain (*FACHB-469*) declined when grown without calcium and magnesium for 5 days.

4.4.3.7 Iron

Iron is one of the essential nutrients for plants, animals, and humans. High concentrations in water may cause rusty colour and metallic taste but it is not considered toxic. The US.EPA. sets in the secondary drinking water standard for iron as 0.3 mg/L (Yuncong and Kati, 2011). Iron is an important component for proper metabolism in most terrestrial and aquatic plants. It is required for a number of enzymes which controls photosynthetic processes. Iron is also linked with plant respiration, chlorophyll synthesis and plant protein production. It is an essential element in the formation of plant cell membranes.

In general, iron exists in two forms-free (non-chelated) and chelated iron. Firstly, non-chelated iron needs to be distinguished from the less dangerous chelated form of iron. Non-chelated iron can be toxic at levels of 0.3 mg/L and above.

In water samples, iron may occur in solution from organic matter, as inorganic or organic iron complexes, or relatively coarse suspended particles. It may be either ferrous or ferric, suspended or dissolved.

The study found that iron in pond water is 0 mg/L. The average ferric concentration is around 0.7 mg/L in surface water. In general, the concentration of 0.1-10.0 mg/L appears in the ground water. (Yuncong and Kati, 2011). There is, however, no iron in the Crookes Valley Pond water sample. Effect of iron on growth and lipid accumulation in marine microalgae strain (*C. vulgaris*) was investigated by Liu *et al.* (2008). It was found that addition of chelated $FeCl_3$ in the late growth phase increases the final cell density. Total lipid content in cultures supplemented with 1.2 $x10^{-5}$ mol/L $FeCl_3$ was up to 56.6% biomass by dry weight.

4.4.3.8 General hardness

The study found that general hardness in the pond water is 66.7 mg/L. The study of Kothari *et al*. (2012) found that, in general, wastewater has a wide range of general hardness between 52-345 mg/L. For surface water a hardness over 190 mg/L carbonate hardness is unusually high. In this study, the pond water has a general hardness at 66.7 mg/L.

4.4.3.9 Carbonate hardness

The study found that carbonate hardness in pond water is 53.3 mg/L which is in a moderate alkalinity level. A desirable alkalinity for fish is of 75 to 200 mg/L. Below 20 mg/L the water cannot support good phytoplankton growth. They do not commonly experience dramatic pH increases because of intense photosynthesis (Wurts and Durborow, 1992). This will have little effect on algal growth.

Without the assessing the water parameters, it is difficult to make a proper estimation about the physical and chemical characteristics of water using for algal growth. Therefore, the study of water quality of The Crookes Valley Pond is very important.

4.4.4 Experiment 4: Effect of wave length at which optical density (OD) is measured

Growth rate was measured by OD at various wave lengths (550, 650, 680,700, and 770 nm.). Different wavelengths give different values which may relate to chlorophyll (Das *et al.*, 2011; Yentsch and Menzel, 1963). OD_{680} has been used to measure growth rate such as in the study of SorokinKrauss (1958). OD measured at 680 nm. tends to provide the highest values when compared to the other wavelengths and thus will be used in all the studies in this work.

4.4.5 Experiment 5: Effect of cell selection and screening

Cell selection and screening is important to healthy cell growth. Strong algal cells should be selected for reproduction when they are mature. An aged or weak cell does not grow or reproduce well and poor cultures tend to form clusters of weak cells and often sediment.

Weak cell or unhealthy cells risk bacterial or virus infections. They may then cause a spoiled suspension leading to failure of the culture. Therefore, cleaning cells or screening, using a simple methods in the laboratory may be needed to maintain good algal growth in batch systems. It was found that after 4-5 generations cell cultures should be cleaned.

4.4.6 Experiment 6: Light measurements

The study found that at inoculative area the intensity is close to the another researcher which reported that the light intensity for inoculum algal cell in flask were used around 60-130 $\mu E.m^{-2}s^{-1}$ (see **Chapter 2** and Andersen (2005)). This is similar to the study of Scragg *et al.* (2002) which used a light intensity 130 $\mu E.m^{-2}.s^{-1}$ when growing *C. vulgaris Beijerinck*. They showed that algae can grow well at 0.4 d^{-1} with a cell count of 5.7 $x10^7$ cell/mL. The study of Chiu *et al.* (2009) used a light for their photobireactor of 300 $\mu molm^{-2}s^{-1}$ (or $\mu Em^{-2}s^{-1}$). The maximum yield was between 2.369-3.461 g/L. Lopez *et al.* (2006) used irradiance of 50-2,000 $\mu Em^{-2}s^{-1}$ for a bubble column photoreactor. The study found an optimal yield of 130 $\mu molm^{-2}s^{-1}$. Dauta *et al.* (1990) found that 300 $\mu molm^{-2}s^{-1}$ was best for *C.vulgaris* when studying light intensities between 5-800 $\mu molm^{-2}s^{-1}$. Therefore, the optimal illumination depends on algal strain, the density of algae in suspension, the volume of algal culture,

and vessel size. A large working volume needs more light intensity to process the photosynthesis and to scale up their yield than a small volume.

4.4.7 Experiment 7: Serial dilutions

Although both 1:10 and 1:100 dilutions gave R^2 values of 1.000, indicating an excellent correlation between measurements at dilution with measurements undiluted, the slope of the 1:10 dilution plot shows slightly less error than at a 1:100 dilution. This is to be expected as a repeated serial dilution can accumulate errors causing the data to drift away from the actual value. However, the results are remarkably good and with careful attention to technique and the use of suitable replicates these results show that serial dilution should introduce little error.

4.5 Conclusions

The experiments found that algae grown in 3 N BBM media with aeration follow a typical growth curve. Algae grown without aeration grew much more slowly than with aeration. Mixing the media with pond water results in variable growth. Some experimental regimes showed negative growth. In these preliminary experiments, it was found that the main causes of inhibition are a lack of enriched elements (nutrients), uncontrolled water quality, non-circulating flow and contamination (bacterial infection). The study showed that future experiments must provide a suitable light regime, appropriate nutrients, and good mixing and aeration to give the best growth rates.

When using the pond water in the lab to grow algae its pH should be adjusted to close to 7.0 before introducing the algae Some essential elements should be added to provide a complex nutrient to support algal growth. This is because when comparing the pond water with BBM media, the study found that, as would be expected, the pond water has a lower nutrient level than BBM media.

Other aspects of the pond water quality may affect algal growth. In particular the presence of microorganisms such coliform bacteria, *E.coli*, protozoa and viruses might inhibit (or even promote) algal growth.

The geological area, land and water use, the physical properties of the pond, pollution by industrial and municipal wastes and by agricultural activities which discharge into the water all affect the pond water and thus any algae grown in it. The study demonstrates the need to fully analyse any water that is to be used to grow microalgae and to ensure that all aspects are adjusted to maximize production (absolute biomass or products from the algae).

The study found that a wavelength of 680 nm. gave the highest values when measuring the optical density of cultures of *Chlorella sp.*The study concludes that cellulose filtration is the best technique to purify cell populations (particularly aged populations) before re-inoculation. Of course, such a technique will not remove all contamination (bacteria etc.) but can be used to wash away a lot of detrimental soluble and small particulate material.

The study found that G23 11 W – fluorescent light gives an average illumination of 60 $\mu Em^{-2}s^{-1}$ for the stock cultures. At the photobireactor platforms, two 40W-fluorescent lights produce around 500 $\mu Em^{-2}s^{-1}$. To set up optimal light conditions for algal growth, the choice of artificial illumination should take account of algal strain, algal density, vessel volume and light scattering.

The serial dilution experiments demonstrate a very good relationship between diluted (and "calculated back") and undiluted measurements indicating that, with good laboratory practice and replicate experiments, good data can be obtained even if dilution is required for the measurements.

Chapter 5 Results : The effect of carbon dioxide on growth rate of *C. vulgaris*

5.1 Introduction

Increasing the CO_2 concentration in the air supplied to algal cultures is known to enhance the photosynthesis process, and the production of carbohydrate and lipids in the algal cell (Tsuzuki *et al.*, 1990; Widjaja *et al.*, 2009).

C.vulgaris TISTR 8580 isolated from Thailand was used in this study. Experiments were conducted to investigate;

1 The characteristics of *C.vulgaris* strain from Thailand

2. Determination specific growth rate using optical density OD_{680}

Then, algae in exponential growth rate were transferred to the photobioreactor. To scale up algae were plant in 2L-photobioreactor. 1 batch uses 4 photobioreactors (8 L/batch). The effect of various CO_2 concentrations was monitored on algal growth rates in a photobioreactor system. Three main methodologies (turbidity, OD_{680}, and cell count) were used to monitor algal growth (Ra and Rajendranb, 2013; Schoen, 1988). In addition, environmental conditions such media preparation, pH, and addition of $NaHCO_3$ were investigated to determine optimal conditions to grow algae and to obtain a good yield. Experiments were conducted to determine the:

1. Effect of 6%, 12%, 24%, and 50% CO_2 concentration in air on algal growth rates;

2. Effect of CO_2 concentration on specific growth rates;

3. Effect of CO_2 concentration on a dry weight;

4. Effect of CO_2 on algal culture colour;

5. Effect of CO_2 induced pH change on algal cultures;

6. Effect of $NaHCO_3$ concentrations on pH change in BBM medium and;

7. Effect of $NaHCO_3$ on pH and algal growth in the photobioreactors

5.2 Methodology

5.2.1 Physical investigation of microalgae strains *C. vulgaris TISTR 8580* strain from Thailand

A *Chlorella vulgaris* strain was obtained from Thailand and is the main algal species and strain to be used in the work described in this thesis as the ultimate goal is to set up algal biomass facilities in Thailand. Characteristics of this *Chlorella vulgaris* were investigated using light microscopy (Axiostar plus, Carl Zeizz eye lens 44 42 32 Zeizz A-plan 100x). Cell size measurement was also monitored. The standard aseptic technique is used to prepare cell slides.

5.2.2 Determination of specific growth rate using optical density OD_{680}

To study a specific growth rate using OD_{680}, 80 mL *C.vulgaris* stock solution was inoculated into 800 mL working volume of 3 N BBM media in 1,000 mL Erlenmeyer flasks. The ratio inoculum:media was 1:10. Air was provided at 0.17 L/M via 0.22 μm filter. Media was enhanced by adding 1 mL of vitamin B_1, B_{12} in 1 L media and 0.001 M. $NaHCO_3$. Light was provided by 24 hours illumination using 11 W fluorescent tubes. Cultures were grown for 7 days. Photosynthetic active radiation (PAR) was measured to be 60 $\mu Em^{-2} s^{-1}$ by a light meter.

Initially, blank samples (air sparged media alone) were monitored by OD_{680} for 7 days in 4 replications (n=4). The total is 35 flasks (N =35) in total were used, comprising the 28-algal samples (4 flasks x 7 days =28) and 7 blanks were measured for each level of CO_2. The data were collected and presented in the form of growth curves.

5.2.3 Effect of 0% 6%, 12%, 24% and 50% CO_2 on algal growth

The growth of *Chlorella vulgaris* cultures in the photobioreactor was measured by turbidity, optical density (OD_{680}) and cell count over 7 days (Day 0 - Day 7) and biomass yield was calculated.

5.2.3.1 Algal growth rates

The study investigated the effect of air (assumed as 0% CO_2), 6%, 12%, 24% and 50% CO_2 on growth (5 categories).

The blank samples (no algae) were monitored for turbidity when adding air and 0%, 6%, 12%, 24% and 50% CO_2 (v/v).

The blanks gave a total of 600 samples (n = 15, (n is the number of each observable group) x 5 categories x 8 days (Noted that: day 0 was measured). There are the same number of algal samples giving 1,200 turbidity samples. These samples were also used for OD. For thecell count only 5 samples were processed for each concentration (n=5).

The photobioreactor was run 1 batch at each CO_2 concentration. Each experiment was run for 7 days except for the 6% CO_2 experiment which was continued for 10 days.

The data were statistically processed in Graph pad programme (Prism 7 version 7.0 c, 1st March 2017 OS X Mac) and then presented in suitable tables, growth curves, and bar charts.

5.2.3.2 Biomass yields

Biomass yield was calculated using turbidity, OD_{680} and cell number.

1) Yield per day

The biomass yield is found by summing the subtracted values of the integrals of the biomass (area under the graph) on Day 1-0 (bar 1), Day 2-1 (bar 2), Day 3-2 (bar 3) until day 7 (bar 7).

2) Cumulative sum yield during Day 1-4, Day 1-7, and total yields for 4 and 7 day harvesting over 28 days.

The area under a graph of a biomass yield is found by summing the values of the integrals of the biomass on Day 1-4 (bar 1) and Day 1-7 (bar 2). Then, the value of bar 1 is multiplied by 7 (bar 3) and bar 2 multiplied by 4 (bar 4) to determine the yield in 28 days.

3) Total yield with harvesting at different days over 28 and 30 days

Calculations were made to determine algal yield when harvesting every 5 or 6 days over a 30 day cycle. The cumulative turbidity for Days 1-4 and Days 1-7 in the various CO_2 concentrations (0%, 6%, 12%, 24% and 50%) were used for these calculations. The data was also used to estimate the yield over a total of 28 days for different harvest times.

5.2.4 Effect of CO_2 concentration on specific growth rates

The increase in cell mass per unit time is the growth rate. The specific growth rate (SGR) is given by μ. In this study, the unit time is a day. Specific Growth Rate is a way of measuring how fast the cells are dividing in a culture. It is defined on the basis of the doubling rate.

5.2.5 Effect of CO_2 concentration on dry weight

Optical density can be converted to dry weight by using OD_{680} multiplied by a specific ratio of 0.4818 (see **Chapter 3**). The yield in dry weight of *C.vulgaris* grown in the different concentrations of CO_2 (v/v) was.

5.2.6 Effect of CO_2 on algal culture colour

On a commercial scale an indication of good algal growth, or of something going wrong with a culture, is needed as quickly as possible. One way of achieving this is by visual inspection. This study looks at the colour of algal cultures under various growth conditions, using the British Standard RAL Classic colour chart.

5.2.7 Effect of CO_2 induced pH change on algal cultures

CO_2 is known to affect pH with the formation of carbonic acid (and hence lowered pH). Such a change can be detrimental to the growth of algae. This study examines the effect of CO_2 concentrations on pH change in the media and algal cultures of a photobioreactor system. Aeration with varying CO_2 concentrations (6%, 12%, 24%, and 50%) was supplied to the algal cultures grown in the photobioreactor system and the pH of the culture measured.

5.2.8 Effect of $NaHCO_3$ concentrations on pH change in BBM medium without algal growth

Sodium bicarbonate ($NaHCO_3$) is commonly used as a pH buffering agent. pH in BBM medium as prepared is 6.7 ± 0.3. Experiments were conducted into adding $NaHCO_3$ at concentrations of 0, 0.1, 0.01 and 0.001 mol (M) to determine how such additions would affect pH and if such pH control could help algal growth. 4 groups of treatment were investigated;

Group 1 Comparing autoclaved and un-autoclaved BBM

Group 2 The medium were separated into 3 sub-groups:

1) BBM media adding 0.1 M $NaHCO_3$

2) BBM media adding 0.1 M $NaHCO_3$ and then autoclaving

3) BBM media, autoclaving, and then adding 0.1 M $NaHCO_3$,

Groups 3-4 are the same as Group 2, but using 0.01, and 0.001 M. $NaHCO_3$, respectively.

There were thus 11 sub-groups with 3 replicated samples measured every day for 7 days (231 samples)

5.2.9 Effect of $NaHCO_3$ on pH with algal growth in the photobioreactors

The effect of $NaHCO_3$ on pH change with algal growth as measured by optical density, turbidity and cell count were investigated over 7 days using 6% CO_2 (v/v) aeration for seven culture variations as given below:

1. Autoclaved BBM media and;
2. BBM medium, adding 0.1 M $NaHCO_3$, and autoclave;
3. BBM medium, autoclave, and adding 0.1 M $NaHCO_3$;
4. BBM medium, adding 0.01 M $NaHCO_3$, and autoclave;
5. BBM medium, autoclave, and adding 0.01 M $NaHCO_3$;
6. BBM medium, adding 0.001 M $NaHCO_3$, and autoclave and;
7. BBM medium, autoclave, and adding 0.001 M $NaHCO_3$

Again there were 3 replicates of each variable.

5.3 Results

5.3.1 *Chlorella vulgaris* strain from Thailand

This strain of *C. vulgaris* was found to be a single celled freshwater algae with green colour as shown in **Figure 5-1**. The cells appear oval in shape. In standard culture, they look to have storage products (oil/lipids) inside the cell making them possible candidates for lipid and biomass production. Cell size was measured using Olympus microscope model BX 51 S/N BC24020, ProgRes C5, Lense UPlan FLN 100/1.3 oil lens α 0.17/FN 26.5. A camera was connected to the microscope and the capturing programme ProgRes 2.6 was used to capture a picture. Setting size on screen was 1:1. The cells were found to be between 3-4 μm in diameter as shown in **Figure 5-2**.

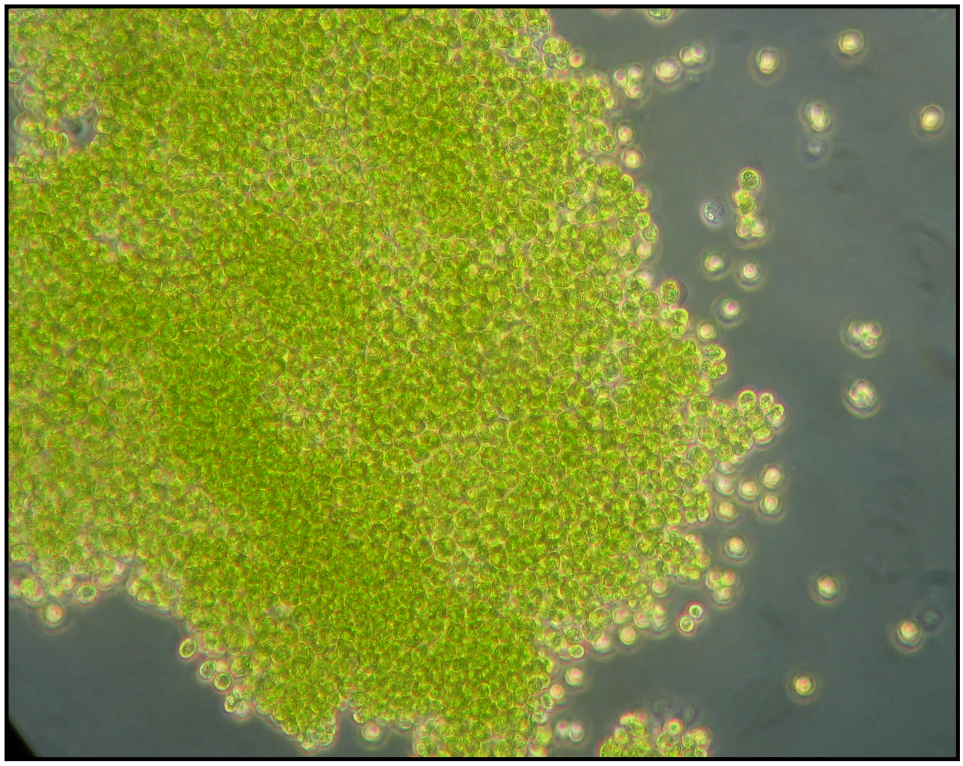


Figure 5-1 Light microscope photograph of *C.vulgaris* strain from Thailand using light microscopy.

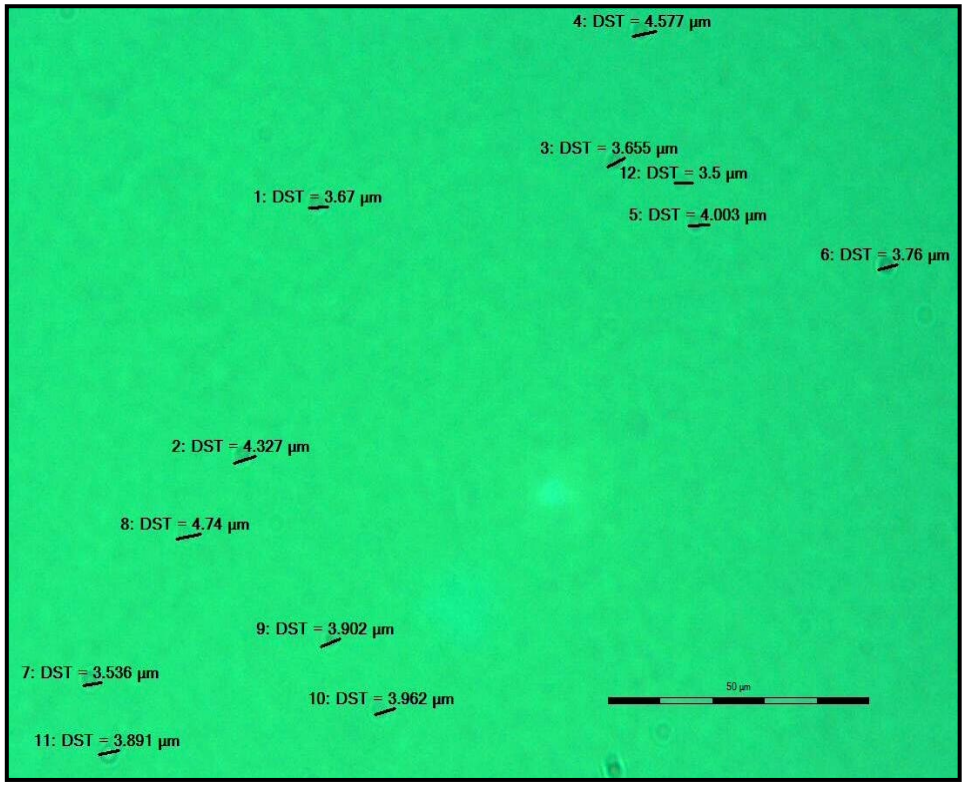


Figure 5-2 Fluorescence micrograph of *C.vulgaris* showing size between 3-4 μm.

5.3.2 Determination of specific growth rate using optical density OD_{680}

The results show that, during the second day, the algae grow rapidly. By Day 7, the optical density is close to 1.8 (**Table 5-1** and **Figure 5-3**). The results indicate that 6-7 day old algal cultures with OD_{680} 1.2-1.8 should be used to inoculate larger reactors. A study by Blair *et al.* (2014) found that between day 0 and 4, *C. vulgaris* is in the lag phase, whilst the stationary phase starts around Day 8-10. This study gives a highest specific growth rate of 0.860 d^{-1}on Day 2. It means that on this day the algae are growing very fast (exponential growth phase the log difference in the OD between day 1 and 2 is 0.860).

Table 5-1 Optical density (OD_{680}) and specific growth rate (μ) measurements for the growth of *C. vulgaris* (n=4).

Day	Blank	Flask					
		Flask 1	Flask 2	Flask 3	Flask 4	Mean ± S.D.	Specific growth rate (μ)
0	0.009	0.107	0.096	0.092	0.095	0.098 ± 0.007	0.000
1	0.004	0.157	0.151	0.148	0.150	0.152 ±0.004	0.441
2	0.004	0.366	0.356	0.350	0.360	0.358 ±0.007	0.860
3	0.003	0.409	0.498	0.431	0.448	0.447±0.038	0.221
4	0.002	0.641	0.692	0.695	0.640	0.667±0.031	0.401
5	0.001	0.892	0.885	0.883	0.866	0.882±0.011	0.279
6	0.000	1.200	1.190	1.193	1.195	1.195±0.165	0.304
7	0.000	1.870	1.770	1.750	1.865	1.814 ± 0.479	0.418

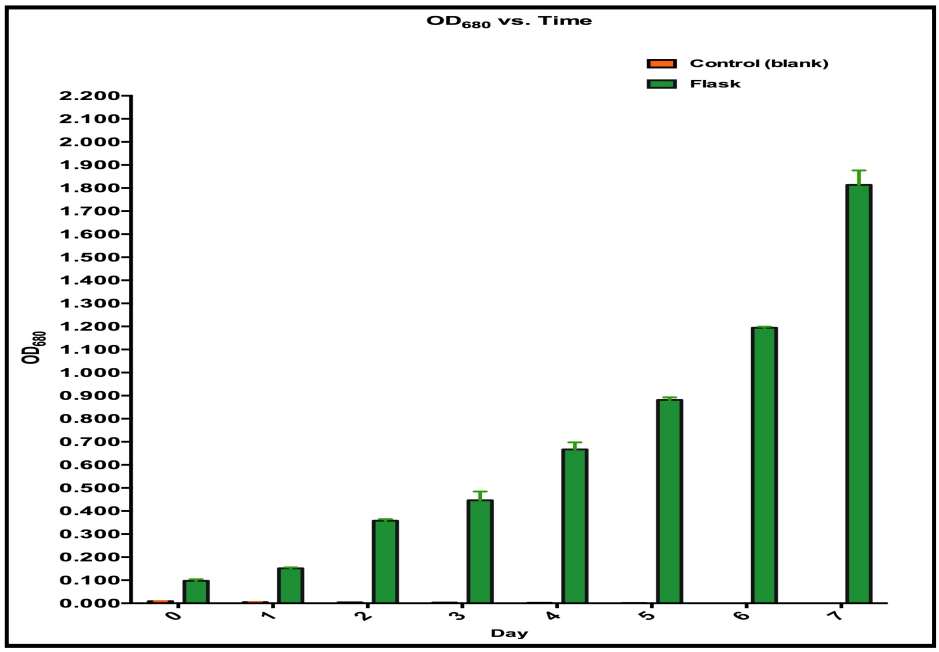


Figure 5-3 Mean growth of *Chlorella vulgaris* with time measured by optical density (OD_{680}).

5.3.3 Effect of CO_2 on algal growth

5.3.3.1 Algal growth rates as measured by turbidity

The mean and S.D. of the turbidity of control blanks (air only, no CO_2 and no algae) over 7 days is 0.000 ± 9.138. While Algae grown under these conditions (air, assumed as 0% CO_2) give turbidity measurements of 29.369-182.179 ± 0.012-6.263.

At 6% CO_2 in the air supply, the turbidity is 29.375-565.797 ±0.208-2.615. At 12% CO_2, turbidity is 9.289-458.718 ± 0.006-6.754. At 24% CO_2 it is 21.113-365.743 ± 0.022-5.136 and at 50% CO_2 it is 26.347-129.764 ±0.065-3.084.

The study found that algae cultured while sparged with air alone (assumed as 0% CO_2 (v/v)), and with CO_2 at 6%, 12%, 24%, and 50% (v/v) gave different yields (**Figure 5-4)**. Growth rates show a similar pattern in 6, 12 and 24% CO_2 in the first 5 days. Algae grown in 12% CO_2 (v/v) gave the highest yield at day 5 when compared with the others. Algae cultured in 50% (v/v) CO_2 showed less growth than in air alone.

From day 5, algae grown in 6% CO_2 (v/v) continued growing strongly, in contrast, the growth of algae in 12%, and 24%, dropped off.

Algae cultured in 50% CO_2 (v/v) peaked at Day 6 to near the growth rate of algae grown in the air but then fell considerably during day 7.

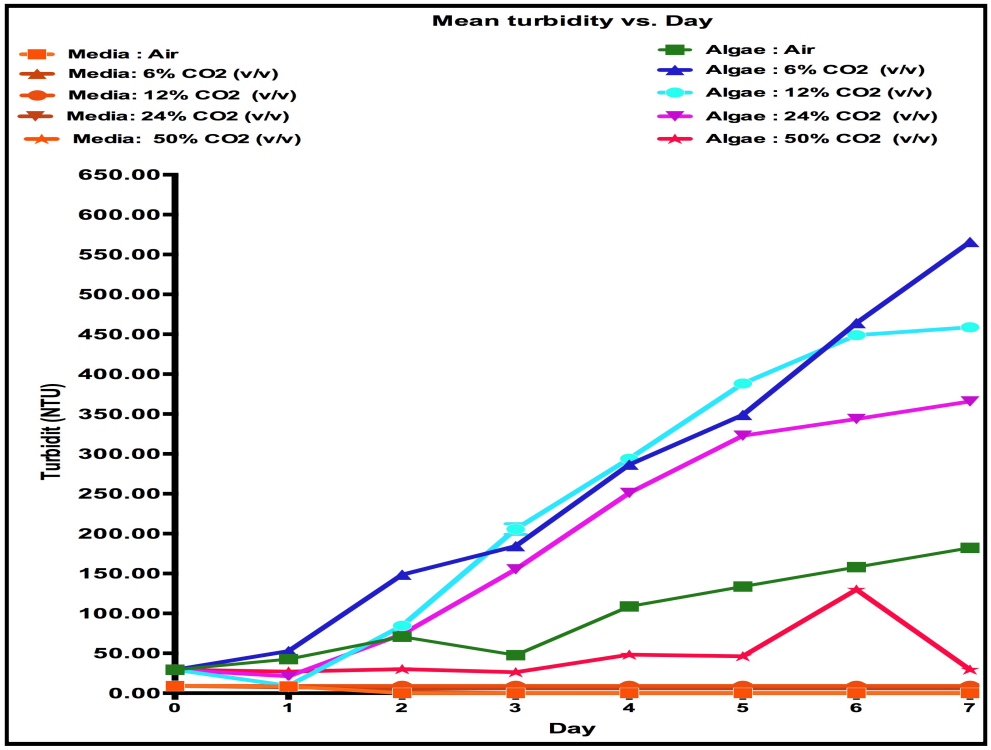


Figure 5-4 Turbidity vs. time for the growth of *C. vulgaris* cultured under different levels of CO_2 concentration in the air supplied (n=15).

5.3.3.2 Algal growth rates as measured by optical density (OD_{680})

The results found that mean ± S.D. of the blanks (no added algae and only feeding air and air plus 6%, 12%, 24% and 50% CO_2 to the photobioreactor) are between 0.000-0.026 ± 0.000-0.009 on Day 0-Day 7.

The mean ± S.D. of algae grown in the air is 0.069-2.817 ± 0.002-0.019. While algae grown in 6% is 0.269-9.705± 0.008-0.075. At 12% CO_2 is of 0.029-7.551± 0.001-0.070. Algae grown in 24% and 50% CO_2 are 0.269-7.873± 0.001-0.448 and 0.269-3.705± 0.007-0.047 respectively.

The highest OD_{680} is 9.705 ± 0.044 of algae grown in 6% CO_2 on Day 7. At 12% CO_2, the peak is at 7.551± 0.144 on Day 5. For 24% CO_2, it is 7.873± 0.102 on Day 5. For 50% CO_2 (v/v) it is 3.705 ± 0.047 on Day 6.

Figure 5-5 shows the algal growth rates as measured by optical density. The results show the same trends as when measured by turbidity but with more marked differences between 6%, 12% and 24% CO_2 (v/v). Unlike the turbidity measurements, the optical density measurements indicate

a fall in growth after day 5 for all conditions except algae grown in 6% CO_2 (v/v).

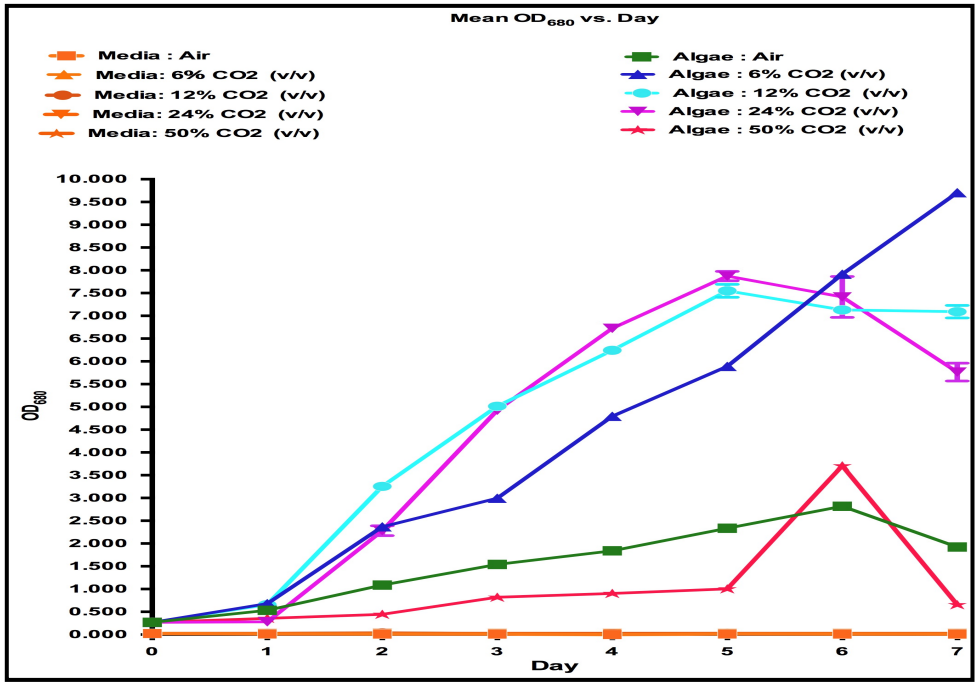


Figure 5-5 OD_{680} vs. time for *C. vulgaris* cultured under different levels of CO_2 concentration in the sparging air.

5.3.3.3 Algal growth rates as measured by cell count

As would be expected, when measuring the growth of algal cultures by cell count, the trends are the same as for turbidity and optical density except the results are even more marked for cell count than for optical density (which itself shows more marked trends than turbidity). During the first 4 days, algae grown in 6% CO_2 (v/v) is 27.660 $x10^5 \pm 0.055$ and 21.640 $x10^5 \pm 0.055$ for 24% CO_2 (v/v). Algae grown in air alone show a steady, but smaller growth, while those grown in 50% CO_2 are only just showing any growth at all.

After day 4, algae cultured in 24% CO_2 (v/v) exhibit a marked drop, in contrast to 6% and 12% CO_2 which continue to grow well. Algae grown in 50% CO_2 (v/v) are hardly growing at all. On Day 6-7 algae cultured in 24% CO_2 continue to decline (19.440 $x10^5 \pm 0.055$ to 16.100 $x10^5 \pm 0.071$) while algae cultured in 12% CO_2 (v/v) now begin to decline.

At Day 7 algae grown without CO_2 show continuing gradual growth while those grown with 50% have dropped to almost zero (**Figure 5-6**). The

highest growth rate is in the 6% CO_2 at 55.600 x10^5 ± 0.071 or around 5.5x10^6cell/mL. While at 12% CO_2 is 43.124 x 10^5 ± 0.071 cell/mL. 24% is 1.611x10^6 ±0.729 and 50% is 2.020x10^5±0.447 cell/mL.

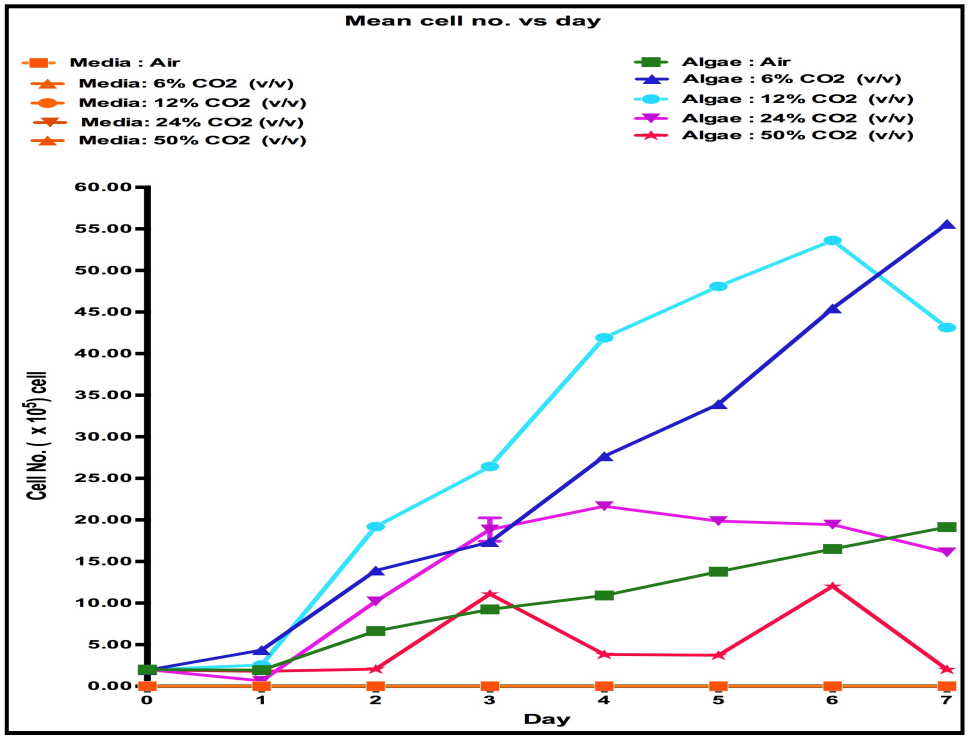


Figure 5-6 Cell number vs. time for *C. vulgaris* cultures under various levels of CO_2 concentration in the sparging air (n=5).

5.3.3.4 Algae grown for ten days in 6% CO_2 (v/v)

As algae grown in 6% CO_2 (v/v) showed the highest yield at day 7 with no sign of any fall off in growth, a study was undertaken to investigate what would happen over a longer time period. The results (**Figure 5-7**) show the highest growth is on Day 7 with an OD of 9.705± 0.044. After this, the growth curve begins to fall with the fall becoming more marked with time. On Days 8, 9 and 10, the means of OD_{680} are 9.541± 0.001, 9.408±0.001 and 8.492 ± 0.001 respectively.

The results in **Figure 5-7** show a typical growth curve for batch culture, with a shorter lag time due to a strong inoculum, strong growth from day 1 to day 7 and then a fall off as nutrient and other factors limit growth.

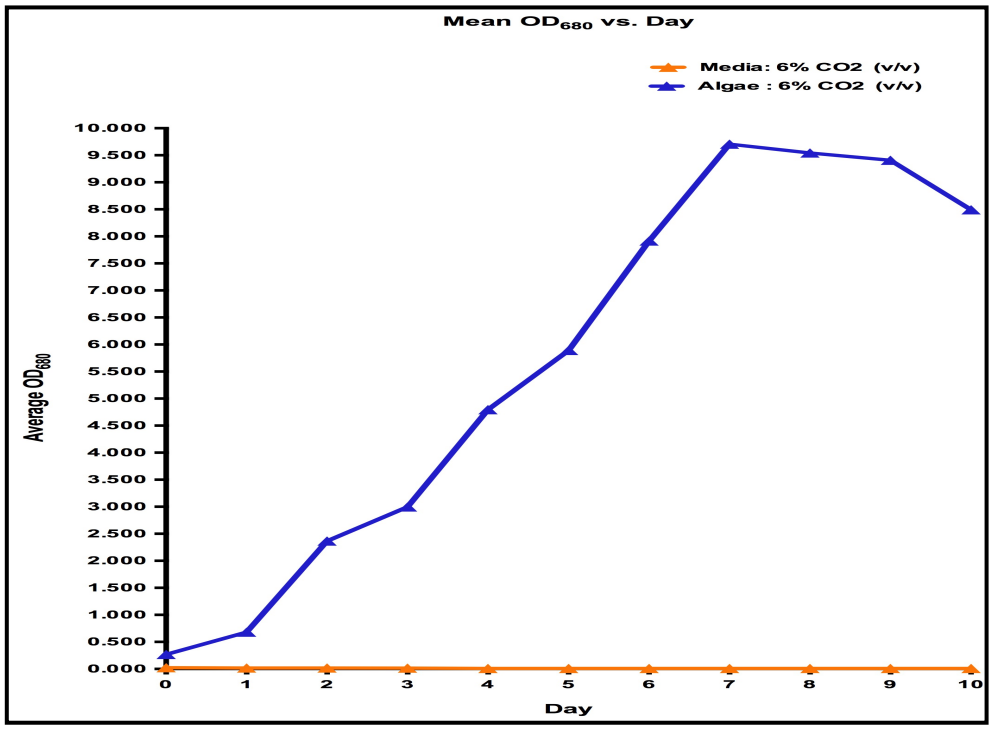


Figure 5-7 OD_{680} vs. time for *C. vulgaris* cultured for 10 days with 6% CO_2 concentration (v/v) in the sparging air.

5.3.3.5 Biomass yields

1) Yield as estimated by turbidity

1.1) Yield per day

The biomass yield is found by summing the subtracted values of the integrals of the biomass (area under the graph) on Day 1-0 (bar 1), Day 2-1 (bar 2), Day 3-2 (bar 3) and so on until Day 7 (bar 7). The bar chart (**Figure 5-8**) shows that algae grown in 6% CO_2 give the highest yield on Day 6. At 12% highest yield is on Day 3. Air alone, 24% CO_2 and 50% CO_2 give lower yields.

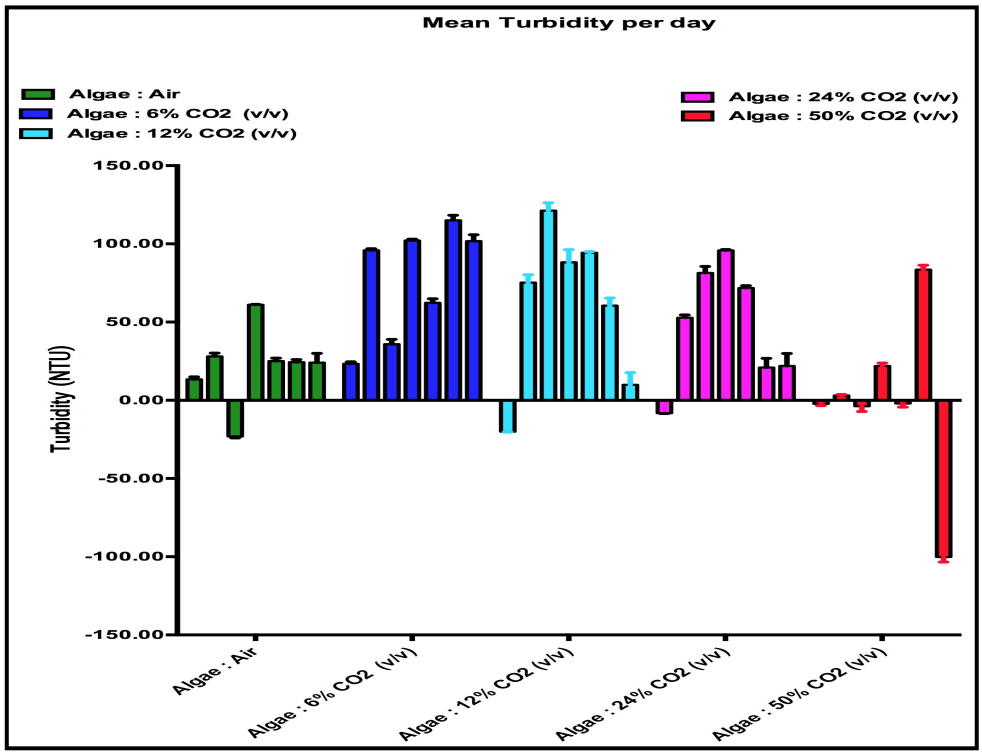


Figure 5-8 The mean of turbidity change (yield) per day (growth rate) for *C.vulgaris* grown sparged with air containing various concentrations of CO_2.

Algae cultured in air alone showed an increase in yield from day one to day two (13.385± 1.616 NTU in day one and 28.033 ± 2.100 NTU in day two). However, on Day 3 the yield measurement is negative (-23.151 ± 0.901 NTU). The highest yield is on Day 4 at 61.076± 0.198 NTU.

Algae grown in 6% CO_2 (v/v) give a good yield on Day 6 with turbidity values of 115.063 ± 3.201 NTU. On Day 7 and Day 4, the values are still high at 101.787 ± 4.031 and 102.175 ± 0.816 NTU. However, the data fluctuates. Looking at days 1, 3, 5 and 7 would produce an almost straight line of increasing yield, while the data for days 2, 4 and 6 give a separate pattern. This is not the case for other CO_2 levels and may be due to how the algae access nutrients at different CO_2 levels.

Algae cultured at 12 % CO_2 (v/v) show a fall in turbidity (negative yield) of 20.077 ± 0.216 NTU in Day 1. This may be caused by the introduction of the higher CO_2 concentration (it occurs to some extent at all the higher CO_2 levels) and may be due to algae needing to adapt to the new conditions. During Days 2-5, the algae grow very well showing an increase in yield from 75.232 to 121.241 ± 0.770-8.096 NTU. However, the daily yield declines to around 60.499 ± 4.901 and 9.933± 7.771 NTU on Day 6

and 7 respectively, indicating that either nutrients are depleted or that the algae eventually find the higher CO_2 levels detrimental.

Algae grown with 24% CO_2 (v/v) show lower yields than at 6 and 12% (v/v) CO_2. On Day 1, the turbidity shows a negative yield. On days 2, 3 and 4 the algae grow well. The highest turbidity is 95.784 ± 0.613 NTU on Day 4. The yields during Day 6-7 drop to 20.934-21.939 ± 5.894-7.962 NTU respectively.

At 50% CO_2 (v/v), algae cannot adapt and struggle to cope with the conditions. There is a little growth on Day 4 but this falls away on Day 5. There is more growth on Day 6, but then there is a drop (negative yield) of around 100 NTU in day 7.

1.2) Cumulative sum yield during Day 1-4, Day 1-7, and total yields for 4 and 7 day harvesting over 28 days.

Figure 5-9 illustrates cumulative turbidity during Day 1-4 and Day 1-7 in various CO_2 concentrations (0%, 6%, 12%, 24% and 50%). The data was also used to estimate the yield over 28 days for different harvest times.

For algae grown in air, the first and second bar show total yield for 4 days and 7 days. The mean ± S.D. on Day 1-4 is 79.342 ± 0.278 NTU and Day 1-7 is 152.810 ± 6.263 NTU. The third and fourth bar show the total yield when harvesting every 4 days or 7 days for 28 days. The values are 555.394 ± 1.946 NTU and 611.240 ±25.053 NTU.

The results show that yields of algae grown in 6, 12, and 24% CO_2 (v/v) are markedly high. While algae cultured in air alone or 50% CO_2 (v/v) give a much lower yield.

The study found that when comparing harvesting at 4 days with harvesting at 7 days, algae grown in 6% CO_2 should be harvested every 7 days (4 times/month) which gives the highest total yield at 2,145.688 ± 9.973 NTU equivalent. Harvesting every 4 days gives a lower yield at 1,800.601 ± 9.376 NTU equivalent. Algae grown in 12% CO_2 (v/v) and 24% CO_2 (v/v) give the best yields when harvested every 4 days (1,852.340 ± 14.927 NTU and 1,552.040 ± 17.346 NTU) respectively.

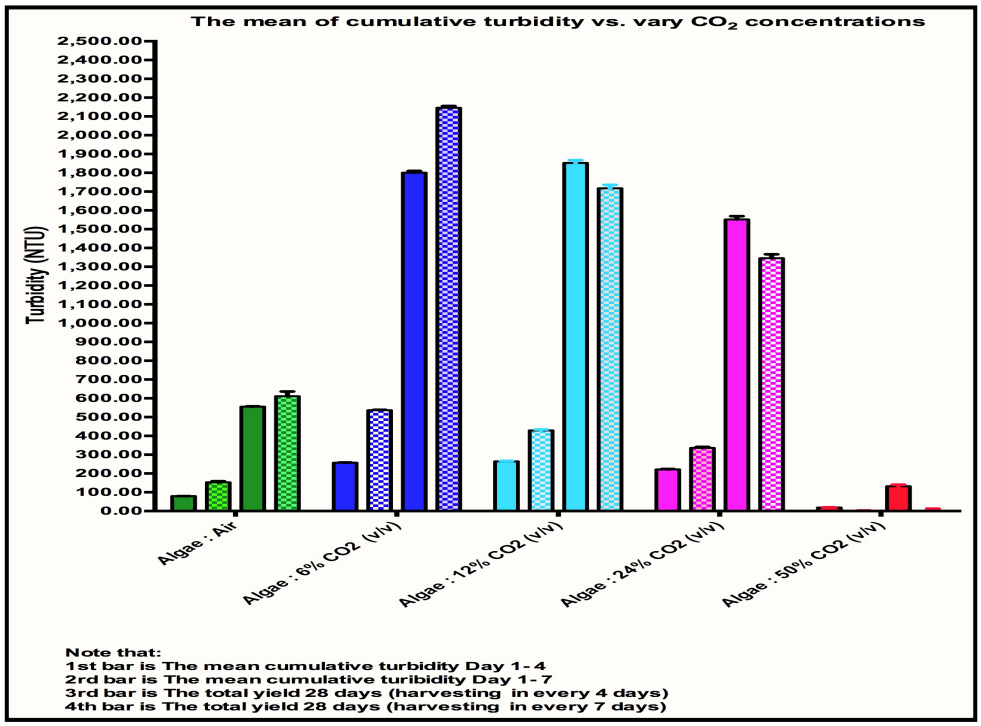


Figure 5-9 Cumulative algal yields when harvesting at 4 and 7 days as measured by turbidity.

1.3) Total yield with harvesting at different days over 28 and 30 days

Further calculations compared algal yield when harvesting every 5 or 6 days over a 30 day cycle. The results found that at 6% CO_2 (v/v) algae are best harvested every 6 days with a cumulative yield, as measured by turbidity, of 2,173.173 $\pm$ 10.680 NTU, whilst at 12% CO_2 the algae are best harvested every 5 days (2,153.512 $\pm$ 8.204 NTU).

It is clear that algae grown in 24% CO_2 (v/v) gives less yield than 6% and 12%. The highest value for 24% CO_2 (v/v) is 1,760.992 $\pm$ 5.831 NTU when harvested every 5 days. Algae cultured in air and 50% CO_2 (v/v) have much lower yields (**Figure 5-10**).

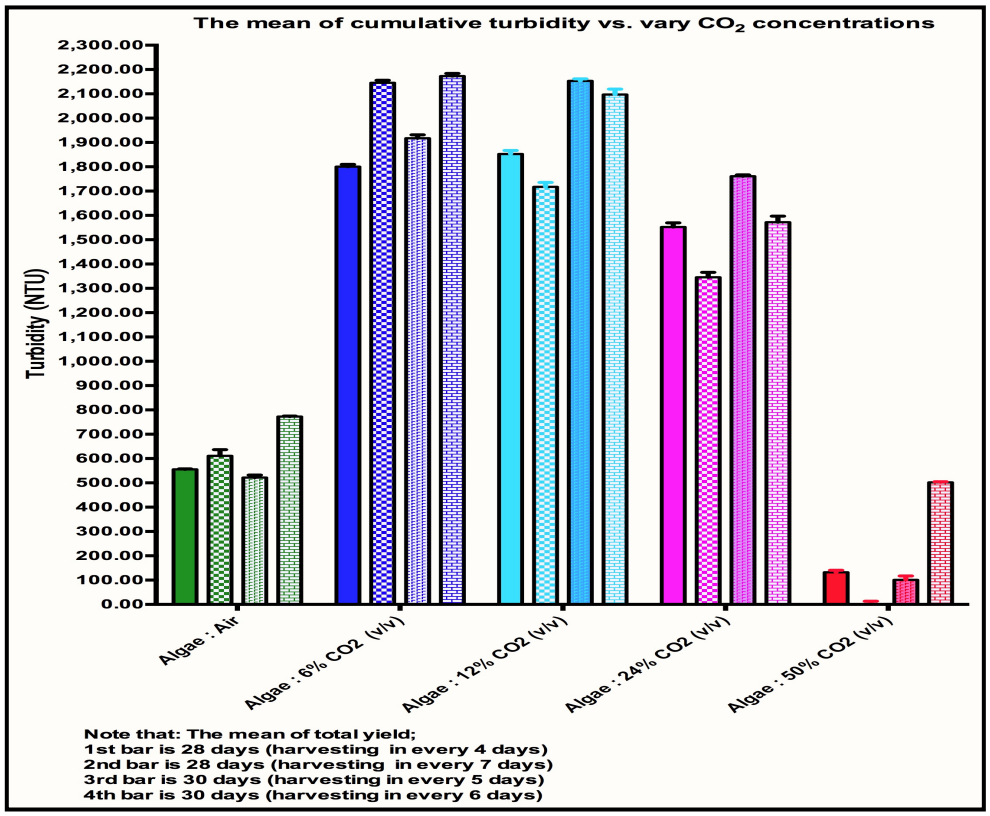


Figure 5-10 Cumulative yield, as measured by turbidity, for harvesting at different times over 28 and 30 day cycles. Note that: The cumulative turbidity of algae grown in 6% and 12% CO_2 (v/v) show a higher yield when compared with algae grown in air, 24% and 50% CO_2. At 6% CO_2 harvesting algae every 6 days (5 times in 30 days) is slightly better than harvesting algae every 7 days (4 times in 28 days). At 12% CO_2 algae are best harvested every 5 days (6 times in 30 days).

2) Yield as estimated by optical density at 680 nm.

2.1) Yield per day

Figure 5-11 shows the results of yield as measured by optical density. They are very similar to the results from turbidity measurements but any changes are somewhat more marked. One difference from the turbidity measurements is that there is no "negative yield" i.e. drop in OD, on day one.

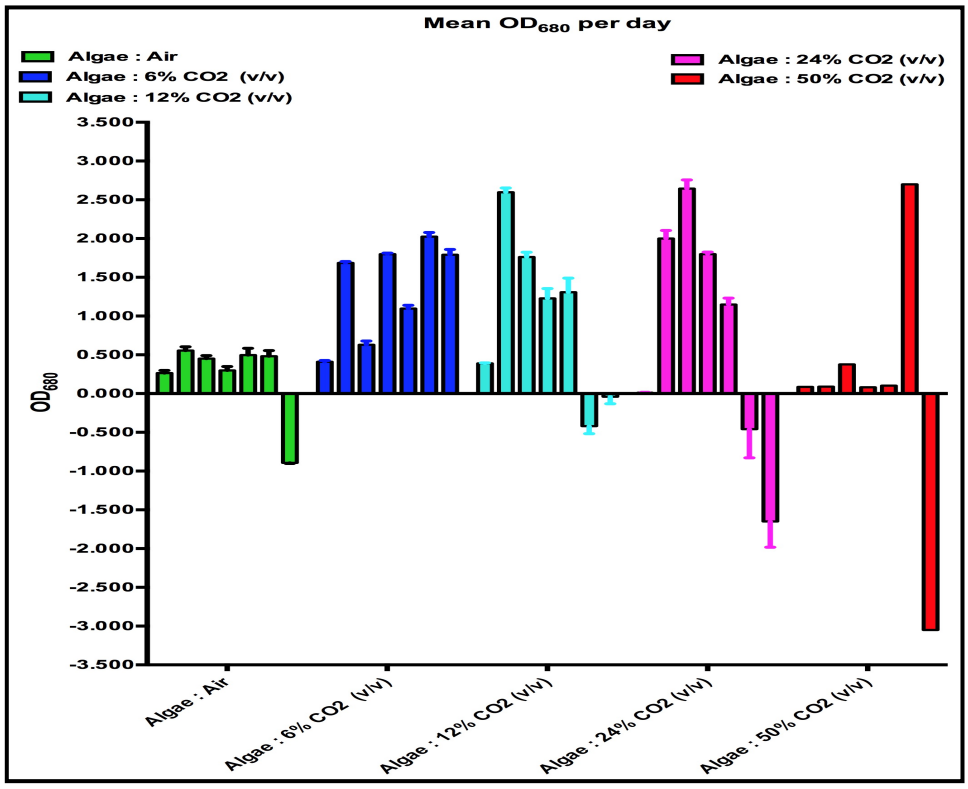


Figure 5-11 Mean optical density change per day for *C. vulgaris* cultures with various levels of CO_2. The first bar was calculated by subtracting OD_{680} value of Day 1 from Day 0 (bar 1), the next by subtracting Day 2 from Day 1 (bar 2) and so on by similar calculations.

Algal yield in Day 7 is all minus except for algae grown in 6% CO_2. At 50% CO_2, the yield is very low during Day 1-5 (OD_{680} = 0.081-0.103). At Day 6 there is a sharp rise (2.699), and then a marked drop on Day 7 (-3.049). This is due to algae adapting during the initial stage (0-5 days) to the high CO_2 level. Then, they grow and die quickly because the reproducing and new algae cannot tolerate these high CO_2 levels. Algae grown at lower CO_2 levels show a steady increase in yield which is greater than algal grown without added CO_2 or with 50% CO_2. Thus, it begins to become apparent that an optimal yield is somewhere between 6% and 24% CO_2.

2.2) Cumulative yields over 4 and 7 Days, and total yields for 4 and 7 day harvests over 28 days

Cumulative yield, as measured by OD_{680} (Mean ± S.D.) for harvesting at 4 or 7 days and every 4 or 7 days over a period of 28 days clearly shows that algae cultured in 6% CO_2 (v/v) are best harvested every 7 days (4 times in 28 days) with a total OD of 41.970± 0.258. In contrast, algae grown in 12

and 24% CO_2 (v/v) give a higher yield when harvested every 4 days with a total are 41.823 ± 0.415 and 45.194 ± 0.159 respectively in 28 days. Algae grown in air and 50% CO_2 (v/v) give a very low yield (4.434±0.105) in comparison (**Figure 5-12**).

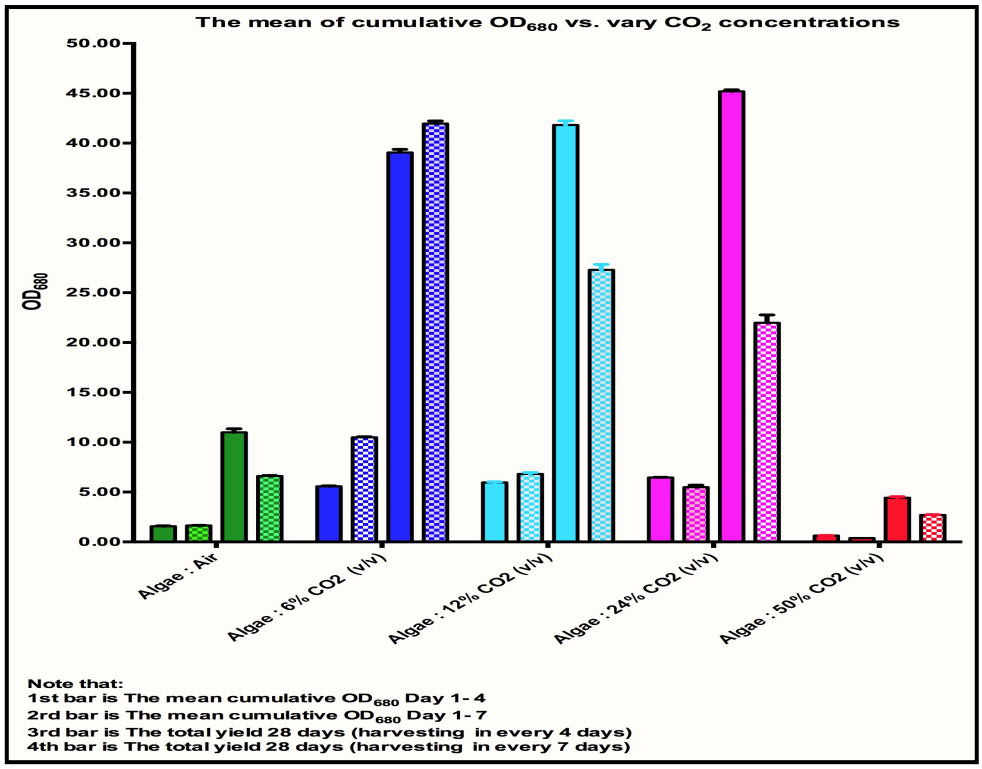


Figure 5-12 Cumulative yield, as measured by OD_{680}, for 4 days and 7 days culturing and for harvesting every 4 or 7 days over a 28 day period.

2.3) Total yield for harvesting at 5 and 6 days when compared to 4 and 7 days over 28 days and 30 days

Further calculations show that harvesting algae grown in 6% CO_2 give the best yield as measured by OD_{680} at 6 days (43.509 ± 0.193) while algae grown in 12 % and 24% CO_2 (v/v) show the best yield when harvested every 5 days (43.680 ± 0.861 and 45.626 ± 0.613) (**Figure 5-13**). However, these yields are only a little better than harvesting every 4 days (it is 41.823 of 12% CO_2 and at 24% is around 45.194) and it should be remembered that an extra two days growth has been added here.

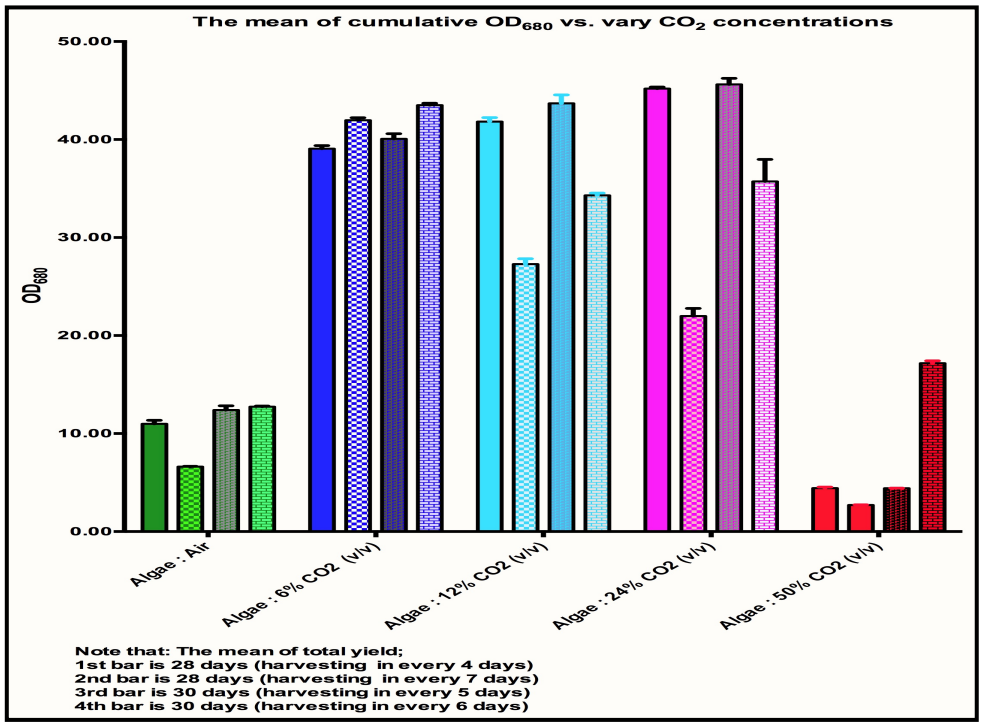


Figure 5-13 Cumulative yield, as measured by $OD_{680,}$ for 4, 5, 6 and 7 days culturing over 28 and 30 day periods.

3) Yield as estimated by cell number

3.1) Yield per day

It is very clear that the higher the level of added CO_2, the shorter the batch life cycle of the algal culture (**Figure 5-14**). Algae grown in 6% gives a more stable yield than 12% and 24% CO_2 and is thus more suitable for a commercial scale up. The highest for 24% CO_2, algal cell number is 9.700 $x10^5$ cell/mL, whilst at 12% CO_2 (v/v) is 2.09 x 10^6 cell/mL on Day 3.

However, on Day 4, the mean of cell number for 12% CO_2 (v/v) feeding is only $1.796x10^5$ cell/mL. This may be a high concentration CO_2 limits a reproduction of the living cells.

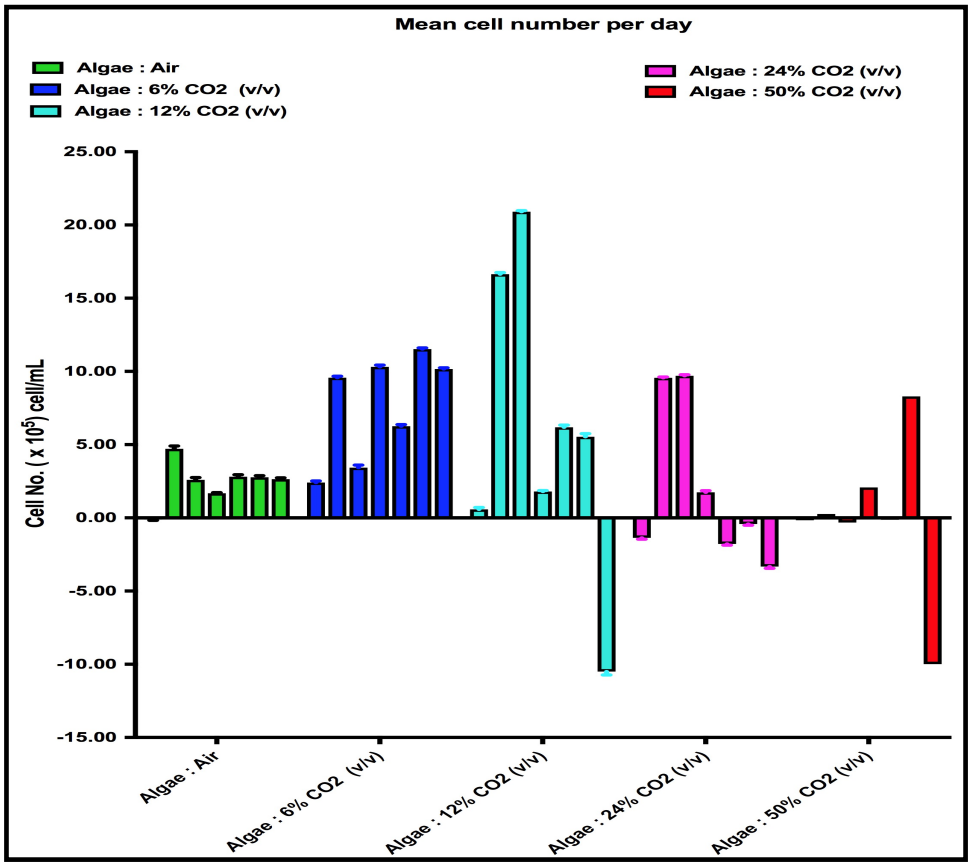


Figure 5-14 Mean cell count change per day for *C. vulgaris* cultures supplied with various levels of CO_2.

3.2) Cumulative yields over 4 and 7 Days, and total yields for 4 and 7 day harvests over 28 days

The cumulative yields, as measured by cell count, show very clearly that the best yield for algae grown in 6% CO_2 (v/v) is obtained by harvesting every seven days which gives a total of $2.15 \times 10^7 \pm 0.333$ cell/mL over 28 days. For 12% and 24% CO_2 (v/v), the best algal yields are by harvesting every 4 days at $2.79 \times 10^7 \pm 1.526$ cell/mL and $2.94 \times 10^7 \pm 2.185$ cell/mL respectively. In contrast, algae cultured in the air give a yield of ($6.879 \times 10^6 \pm 0.490$) and algae cultured in 50% CO_2 (v/v) give a very low yield by cell number of $1.26 \times 10^6 \pm 0.976$ cell/mL, both for harvesting every 4 days (**Figure 5-15**).

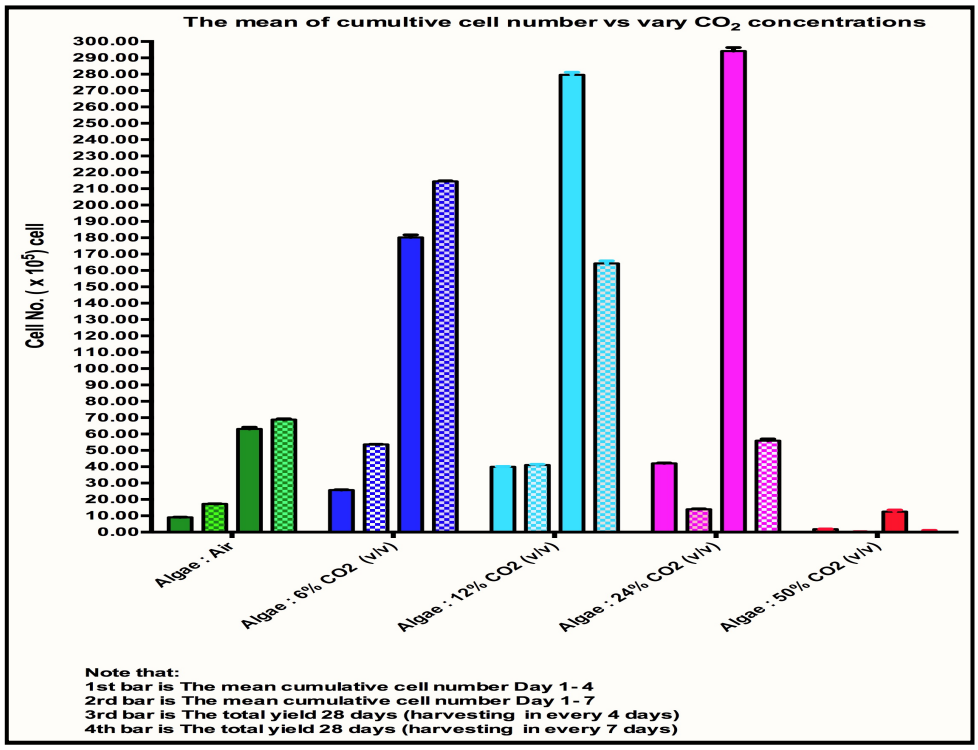


Figure 5-15 Cumulative yield, as measured by cell count for 4 days and 7 days culturing and for cumulative harvesting every 4 or 7 days over a 28 day period.

3.3) Total yield for harvesting at 5 and 6 days when compared to 4 and 7 days over 28 days and 30 days

Apart from 6% CO_2 (v/v) where it is very close between six and seven days (and probably there is no difference given that the 6 days cumulative figure is for an extra two days growth, Day 6 is 2.17 $\pm$1.239 x10^7 cell/mL and Day 7 is 2.145 $\pm$0.333 x10^7 cell/mL).

The total over 28 or 30 days when harvested every 5 or 4 days. The yield for 12 and 24% CO_2 (v/v) is no better for harvesting every 5 days than harvesting every 4 days. At 12%, harvesting every 5 days is around 2.76 x10^7 and harvesting every 4 days is 2.79 x10^7 cell/mL. Similarly, at 24%, harvesting every 5 days is 2.03 x10^7 and harvesting every 4 days is 2.94 x10^7cell/mL. The greatest yield is still for 24% CO_2 (v/v) at 2.94 x$10^7\pm$ 2.185 cell/mL harvesting in every 5 days (**Figure 5-16**).

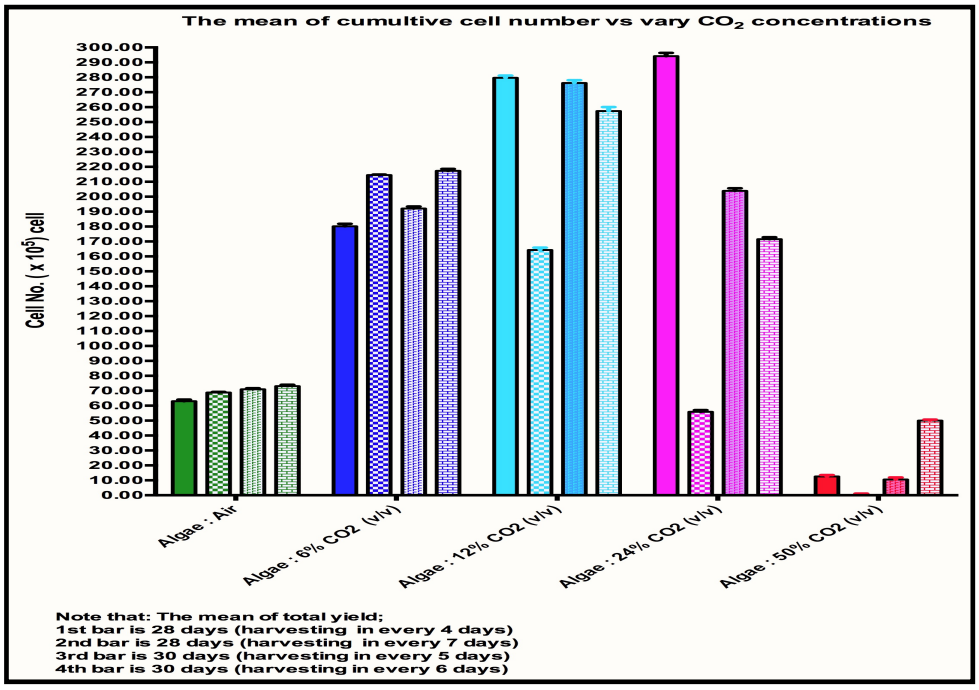


Figure 5-16 Cumulative yield, as measured by cell count for 4, 5, 6 and 7 days culturing over 28 and 30 day periods.

4) Algae grown in 6% CO_2 (v/v)

Using optical density, this study investigated yield of algae grown in 6% CO_2 (v/v) over approximately 1 month (27 to 30 days) with a range of different times to harvest. **Figure 5-17** shows the cumulative yield (by OD_{680}) for harvesting at 3,4,5,6,7 and 9 days and the cumulative yields for these harvest times over 30, 28, 30, 30, 28 and 27 days respectively.

The highest total mass as estimated by OD measurement is 38.225$\pm$ 0.173 when harvesting every 6 days for 30 days. However, this in only slightly more than harvesting every 7 days (37.743 $\pm$ 0.174) which may be explained by the extra two days growth (30 days given for every 6 days and 28 days for the 7 day harvest). If the yield were to be calculated over 42 days then the 7 day harvest would yield 56.58 while the 6 day harvest would yield 53.52 showing that, in the long run, a seven day harvest regime would be best especially given that a harvest is costly and the fewer the harvests the better.

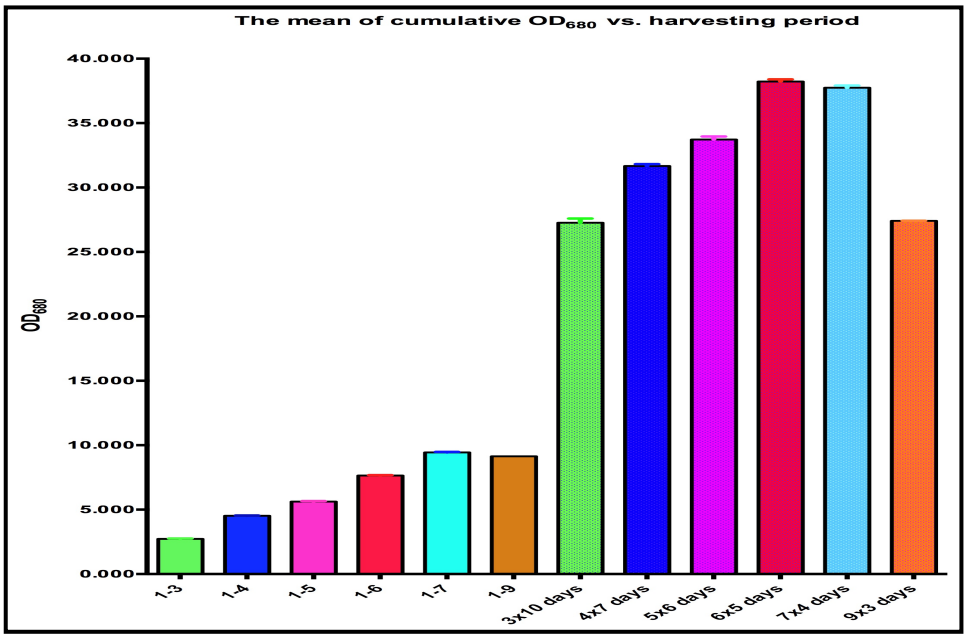


Figure 5-17 The mean of cumulative algal harvesting for *C. vulgaris* grown in 6% CO_2 (v/v)

5.3.4 Effect of CO_2 concentration on specific growth rates

The increase in cell mass per unit time is the growth rate. The specific growth rate (SGR) is given by μ. The unit is per unit time (e.g. day or hour) and can be used to identify the exponential growth phase. The specific growth rate constant is a way of measuring how fast the cells are dividing in a culture. It is defined on the basis of the doubling rate. This study determined the specific growth rates using algal growth as measured by OD_{680}, turbidity, and cell count. The unit of SGR is recorded per day (d^{-1}).

5.3.4.1 Specific growth rate as measured by turbidity

Specific growth rate (μ) as measured by turbidity is shown in **Table 5-2 and Figure 5-18**. During Day 1, algae grown in air and 6% CO_2 show a slightly positive growth rate (0.586 ±0.021). Cultures with higher CO_2 levels (12%, 24% and 50%) of CO_2 showed a negative growth rate (-1.150 to -0.081 ± 0.010-0.042).

During Day 2, algae growth rates were positive. The SGR is 1.036 ± 0.021 at the 6% CO_2 level. Algae cultured in 12% CO_2 (v/v) gave the greatest specific growth rate on Day 2 at 2.207± 0.079. However, at this level of CO_2 the SGR fluctuates, having been negative on Day 1 and dramatically declining to 0.890 ± 0.039 on Day 3.

On Day 3, all of the algae cultures show a drop in specific growth rate. At 6% CO_2 the SGR drops to 0.213±0.020. After Day 3 the specific growth rates continue to fall for algae grown in 12% CO_2 and over while the fall occurs after Day 4 for air and 6% CO_2, the exponential growth phase or the peak growth rate is on Day 2 in every CO_2 concentration.

Table 5-2 Specific growth rate (μ) (unit is per day, d^{-1}) for cultures of *C.vulgaris* grown in various levels of carbon dioxide as estimated by turbidity (Mean ± S.D.) n = 15.

Day	Algae: Air	Algae: 6% (v/v)	Algae: 12% (v/v)	Algae: 24% (v/v)	Algae:50% (v/v)
Day 0	0.000	0.000	0.000	0.000	0.000
Day 1	0.377±0.038	0.586±0.021	-1.150 ±0.022	-0.329±0.010	-0.081 ±0.042
Day 2	0.503 ±0.046	1.036±0.021	2.207±0.079	1.253 ±0.021	0.108±0.022
Day 3	-0.398±0.015	0.213 ±0.020	0.890 ±0.039	0.744 ±0.041	-0.137±0.124
Day 4	0.823 ±0.005	0.440 ±0.008	0.357±0.038	0.480 ±0.008	0.609±0.007
Day 5	0.207±0.013	0.200 ±0.008	0.277±0.005	0.250 ±0.008	-0.046±0.052
Day 6	0.163 ±0.013	0.287±0.010	0.143 ±0.013	0.063 ±0.018	1.037±0.061
Day 7	0.143 ±0.032	0.197±0.010	0.020 ±0.017	0.060 ±0.025	-1.485 ±0.104

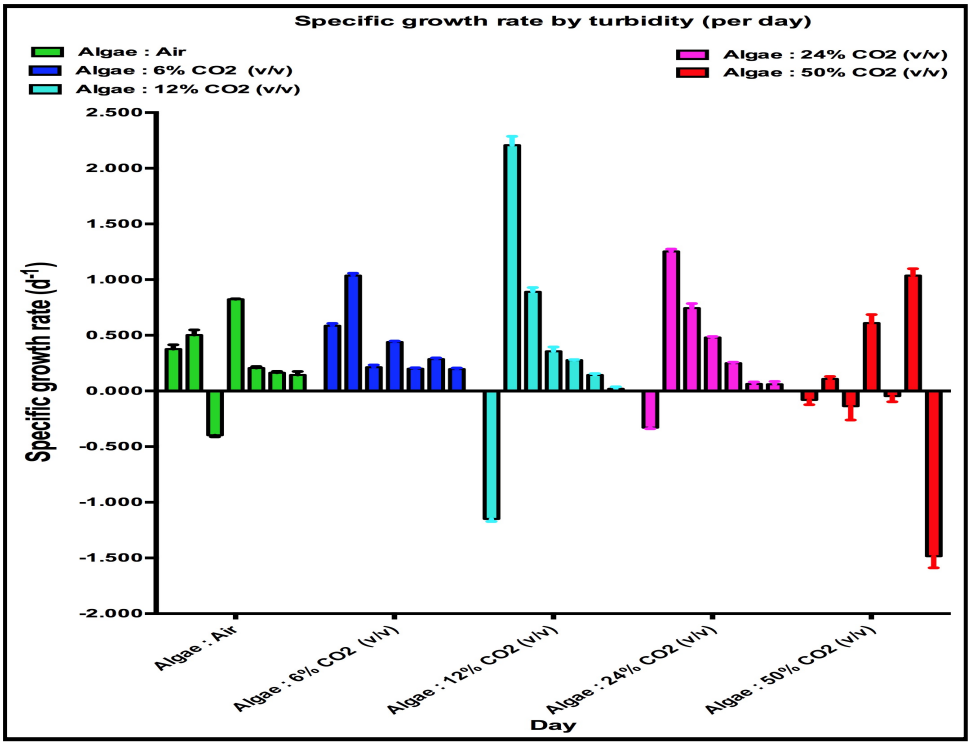


Figure 5-18 Specific growth rate for cultures of *C.vulgaris* grown in various levels of carbon dioxide as measured by turbidity.

5.3.4.2 Specific growth rate as measured by OD_{680}

Specific growth rates calculated using optical density data show the same trends as those calculated using turbidity data but there were negative growth rates in day 7 except for algae grown in 6% CO_2 (v/v). This is due to algae reaching the decline phase of their growth curve. This result the negative values (**Table 5-3** and **Figure 5-19**).

Table 5-3 The mean and S.D. of specific growth rate for cultures of *C.vulgaris* grown in various levels of carbon dioxide as estimated by OD_{680}, n=15.

Day	Algae : Air	Algae: 6% (v/v)	Algae: 12% (v/v)	Algae: 24% (v/v)	Algae:50% (v/v)
Day 0	0.000	0.000	0.000	0.000	0.000
Day 1	0.684±0.072	0.927±0.021	0.893±0.012	0.044±0.015	0.279±0.025
Day 2	0.714±0.071	1.247±0.020	1.599±0.025	2.092±0.037	0.224±0.037
Day 3	0.348±0.032	0.236±0.018	0.432±0.015	0.771±0.048	0.613±0.024
Day 4	0.177±0.028	0.470±0.007	0.219±0.023	0.311±0.004	0.094±0.030
Day 5	0.239±0.041	0.206±0.008	0.190±0.025	0.157±0.010	0.108±0.017
Day 6	0.188±0.032	0.295±0.008	-0.057±0.012	-0.062±0.052	1.305±0.015
Day 7	-0.381±0.003	0.204±0.008	-0.006±0.013	-0.250±0.044	-1.732±0.018

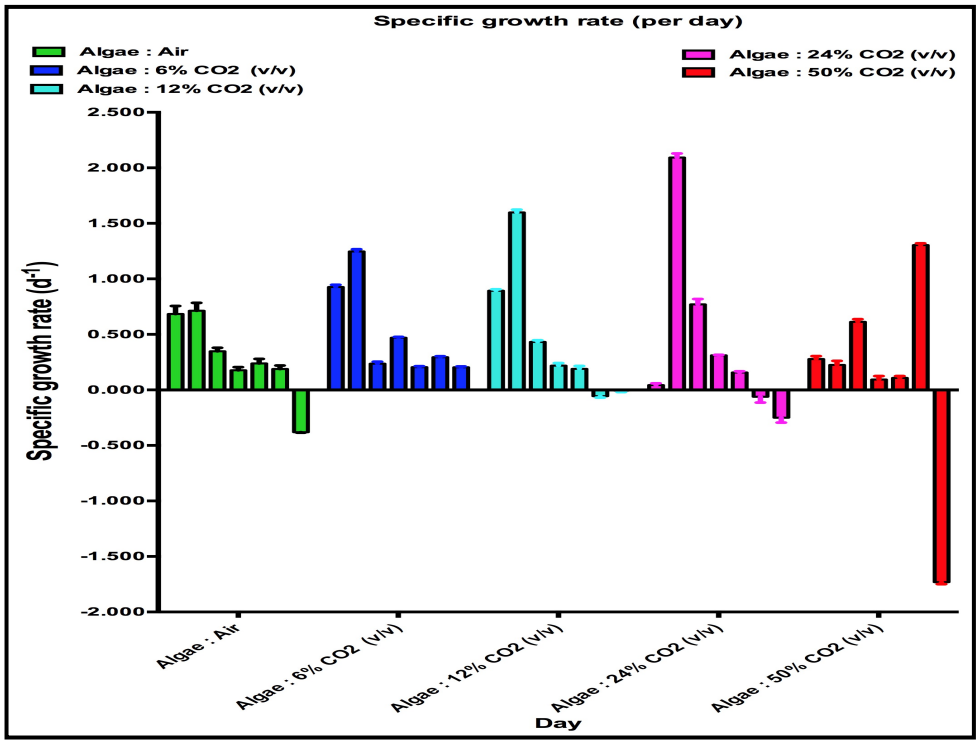


Figure 5-19 Specific growth rate for cultures of *C.vulgaris* grown in various levels of carbon dioxide as measured by OD_{680}.

5.3.4.3 Specific growth rate as measured by cell count

The mean and standard deviation (Mean ± S.D.) of specific growth rates calculated from the cell count data were similar to the other two calculations but perhaps closer to those calculated from turbidity data than from optical density (**Table 5-4** and **Figure 5-20**).

Table 5-4 Specific growth rate for cultures of *C.vulgaris* grown in various levels of carbon dioxide as measured by cell count.

Day	Algae : Air	Algae: 6% (v/v)	Algae: 12% (v/v)	Algae: 24% (v/v)	Algae:50% (v/v)
Day 0	0.000	0.000	0.000	0.000	0.000
Day 1	-0.032 ±0.060	0.806±0.052	0.254 ±0.068	-1.144 ±0.093	-0.086±0.080
Day 2	1.208±0.036	1.160 ±0.010	2.014 ±0.024	2.770 ±0.078	0.134±0.047
Day 3	0.328±0.023	0.220 ±0.012	0.736±0.005	0.668±0.008	-0.168±0.053
Day 4	0.168±0.004	0.468±0.008	0.042 ±0.004	0.084 ±0.005	0.784 ±0.027
Day 5	0.228±0.011	0.204 ±0.005	0.135±0.004	-0.086±0.005	-0.026±0.025
Day 6	0.184 ±0.004	0.292 ±0.004	0.112 ±0.004	-0.024 ±0.005	1.170 ±0.024
Day 7	0.148±0.004	0.200 ±0.000	-0.220 ±0.007	-0.188±0.008	-1.782 ±0.025

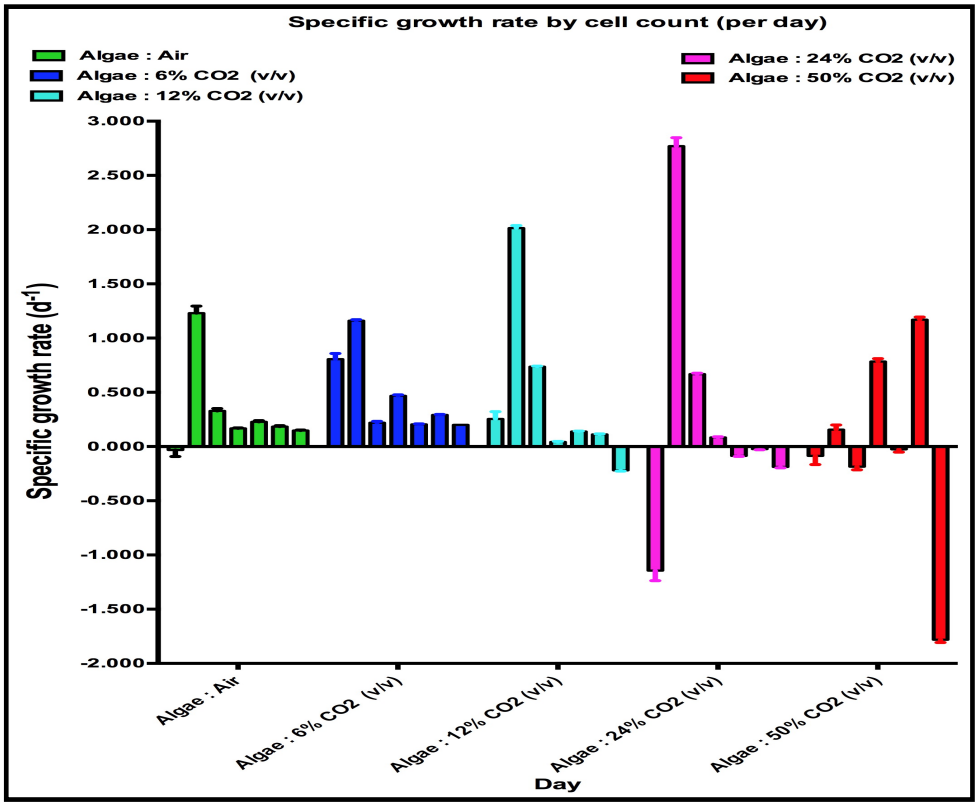


Figure 5-20 Specific growth rate for cultures of *C.vulgaris* grown in various levels of carbon dioxide as measured by cell count.

5.3.5 Effect of CO_2 concentration on dry weight

5.3.5.1 The mean of dry weight during Day 0 (start up) to Day 7

The study found that during Day 1, the trend is a slow increase in dry weight. The increase is more rapid in days 2-4 after which the rates for algae cultured at 12%, and 24% CO_2 (v/v) decline while those for 0% and 6% continued to increase (albeit at very different levels). On Day 7, all the algal curves decline except for algae cultured with 6% CO_2 (v/v) which increases.

The highest dry weight per day was 1.36 g/L on Day 6 for air alone, 4.68 g/L on day 7 for 6% CO_2, 3.64 g/L on Day 5 for 12% CO_2 and 3.79 g/L on Day 5 for 24% CO_2. In contrast, the highest dry weight at 50% CO_2 is only 1.79 g/L on Day 6 (**Table 5-5** and **Figure 5-21**).

Table 5-5 The mean and standard deviation of dry weight with the standard deviation (g/L) of *C.vulgaris* as estimated from OD_{680} measurements (A total dry weight). A dry weight for each concentration (n)=15.

Day	The mean and standard deviation of dry weight (g/L) during Day 0-7				
	Algae : Air	Algae: 6% (v/v)	Algae: 12% (v/v)	Algae : 24% (v/v)	Algae: 50% (v/v)
Day 0	0.130 ±0.005	0.130 ±0.001	0.130 ±0.001	0.130 ±0.001	0.130 ±0.005
Day 1	0.257±0.015	0.328±0.008	0.317±0.004	0.135±0.002	0.171 ±0.006
Day 2	0.524 ±0.015	1.140 ±0.012	1.568±0.024	1.099±0.052	0.215±0.006
Day 3	0.742 ±0.007	1.443 ±0.015	2.417±0.034	2.374 ±0.005	0.396±0.003
Day 4	0.886±0.024	2.310 ±0.009	3.008±0.028	3.240 ±0.011	0.435±0.011
Day 5	1.125±0.036	2.838±0.019	3.638±0.069	3.794 ±0.049	0.484 ±0.005
Day 6	1.357±0.005	3.813 ±0.016	3.436±0.022	3.572 ±0.216	1.785±0.022
Day 7	0.927±0.004	4.676±0.021	3.416±0.066	2.778±0.094	0.316±0.003

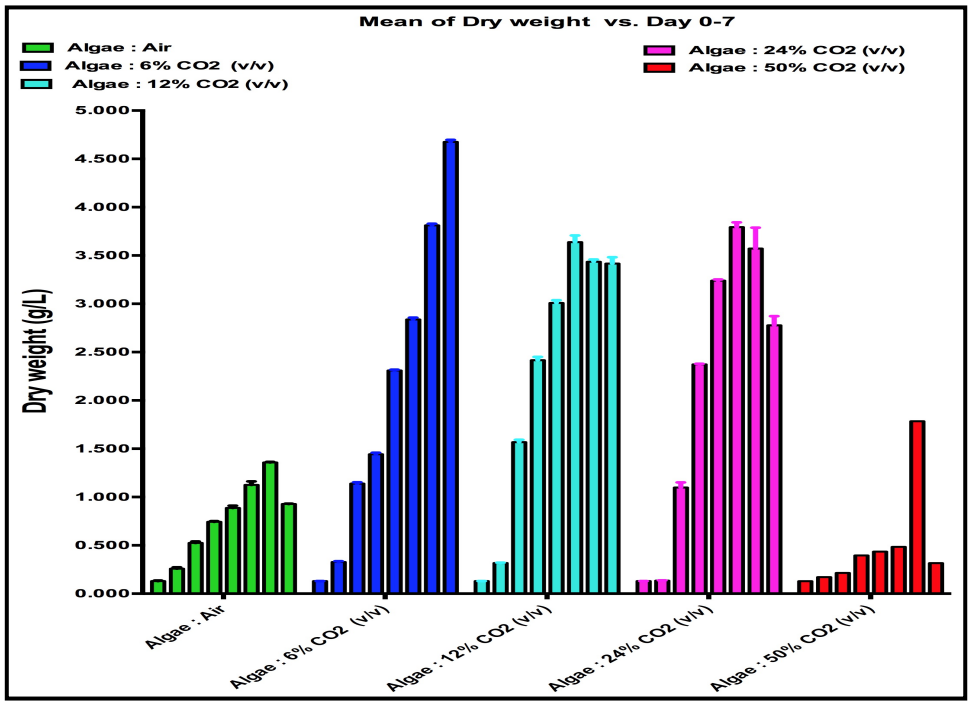


Figure 5-21 The mean of dry weight (g/L) during Day 0-7 of *C. vulgaris* as estimated from OD_{680} measurements multiplied by 0.4818.

5.3.5.2 Dry weight per day

It is useful to understand when in the cycle there is the most biomass increase. This can be seen from a determination of the dry weight per day (n=15). The values were calculated by the dry weight Day 1 minus Day 0 and Day 2-Day 1 and so on until Day 7 (**Table 5-6 and Figure 5-22**). The dry weight of algae grown with 6% CO_2 (v/v) shows three peaks, one on Day 2, one on Day 4 and one on Day 6 (0.867-0.975 $gL^{-1}d^{-1}$).

Algal cultures grown in 12% and 24% CO_2 (v/v) show single peaks on day 2 at 1.251 $gL^{-1}d^{-1}$ for 12% CO_2 and on day 3 for 24% at 1.274 $gL^{-1}d^{-1}$. Algae grown with air alone shows no marked peaks (-0.430 to 0.267 $gL^{-1}d^{-1}$), while algae grown in 50% CO_2 (v/v) shows a peak at Day 6 and then a crash in Day 7.

Table 5-6 The mean of dry weight per day ($g.L^{-1}d^{-1}$) of *C. vulgaris* grown in various levels of carbon dioxide (n=15)

Day	The mean of dry weight per day ($g.L^{-1}d^{-1}$)				
	Algae : Air	Algae: 6% (v/v)	Algae: 12% (v/v)	Algae : 24% (v/v)	Algae: 50% (v/v)
Day 1	0.127±0.016	0.198±0.008	0.187±0.003	0.006±0.002	0.042 ±0.004
Day 2	0.267±0.024	0.812 ±0.008	1.251 ±0.027	0.964 ±0.050	0.043 ±0.007
Day 3	0.218±0.018	0.303 ±0.024	0.848±0.030	1.274±0.053	0.181 ±0.004
Day 4	0.144 ±0.024	0.867±0.006	0.592 ±0.061	0.867±0.013	0.039±0.013
Day 5	0.239±0.042	0.528±0.021	0.630 ±0.088	0.553 ±0.040	0.050 ±0.007
Day 6	0.232 ±0.035	0.975±0.026	-0.202 ±0.047	-0.221 ±0.178	1.301 ±0.023
Day 7	-0.430 ±0.004	0.863 ±0.033	-0.019±0.044	-0.795 ±0.161	-1.469±0.023

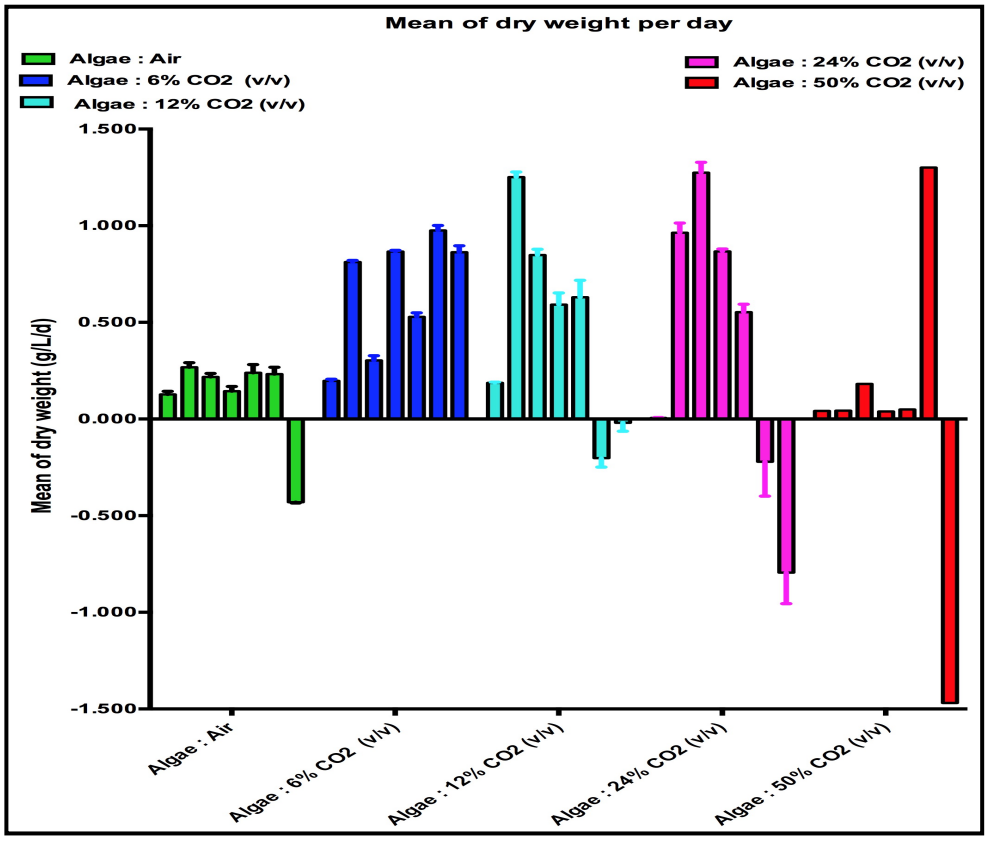


Figure 5-22 The mean of dry weight per day ($g.L^{-1}d^{-1}$) of *C. vulgaris* grown in various levels of carbon dioxide. Note that: The data were calculated by the mean of dry weight day 1 minus day 0 (bar 1), day 2 minus day 1 (bar 2), and so on until 7 days (bar 7). Some data shows a negative value because the dry weight is declining rapidly. This is due to algae dying or giving less yield when compared to the previous day.

5.3.5.3 The cumulative dry weight during a 28 day cycle

The data (**Table 5-7**) show that, for algae grown with air alone the cumulative dry weight is less on Day 7 than on day 6. At 6%, cumulative dry weight peaks on Day 7 at 4.55 g/L.

For algae grown in 12% CO_2 (v/v), the cumulative dry weight peaks on Day 5 while for 24% and 50% CO_2 the peak is on Day 6.

Table 5-7 Cumulative dry weight (g/L) during Day 1-7

Day	Cumulative dry weight during (g/L)				
	Algae : Air	Algae: 6% (v/v)	Algae: 12% (v/v)	Algae : 24% (v/v)	Algae: 50% (v/v)
Day 1	0.14	0.20	0.19	0.01	0.04
Day 2	0.41	1.01	1.44	0.97	0.08
Day 3	0.63	1.31	2.29	2.25	0.26
Day 4	0.77	2.18	2.88	3.12	0.30
Day 5	1.01	2.71	3.51	3.12	0.35
Day 6	1.24	3.69	3.31	3.45	1.65
Day 7	0.81	4.55	3.29	2.66	0.18

The study estimated the yield over several harvests by simulating a harvesting period in every 3-7 day for 28-30 days. The data show that, for optimal yield, algae grown with air alone should be harvested every 6 Day[s] with a cumulative dry weight 6.20 g/L. in 30 days.

At 6% CO_2 the yield is 18.45 g/L per month for harvesting every 6 days for 30 days, but a single harvest on Day 6 is 3.69 while it is 4.55 on Day 7, the yield is 4.55. The total is 18.20 g/L when collecting on Day 7 for 28 days.

If the calculation is continues to 42 days the yield is 25.83 when harvested every 6 days and 27.30 when harvested every 7 days. Thus a harvest every 7 days is more efficient at this CO_2 level. At 12% CO_2, the highest yield is 22.90 g/L when harvesting every 3 days. However, harvesting every 3 days would be costly.

For 24% CO_2 the highest yield is 22.50 g/L for harvesting every 3 days. 50% CO_2 gives a very low yield (**Table 5-8**).

To sum up, algal grown in 6% CO_2 should be harvested every 7 days while for 12 and 24% CO_2 algae should be harvested every 3 days. However, if these higher CO_2 percentages have to be used (e.g. for more efficient CO_2

removal from the flue gas) then the harvest time could be lengthened to 5 days without too high a loss of algal yield. It should also be noted that a biomass yield estimated by OD does not allow for dead cells floating in the algal suspension. Therefore, cell count or colour of the algal suspension may help to decide the best harvesting interval.

Table 5-8 Mean cumulative dry weight (g/L) for harvesting Day 3-7 and the culturing period of a 28 day.

Harvesting period (Day x times))	Cumulative dry weight Day 3-7 (g/L)									
	Algae : air		Algae: 6% CO_2 (v/v)		Algae: 12% CO_2 (v/v)		Algae: 24% CO_2 (v/v)		Algae: 50% CO_2 (v/v)	
	Single harvest	28-30 day Cumulative	Single harvest	28-30 day cumulative	Single harvest	28-30 day cumulative	Single harvest	28-30 day cumulative	Single harvest	28-30 day cumulative
3 (x 10) = 30 day	0.63	6.30	1.31	13.10	2.29	22.90	2.25	22.50	0.26	2.60
4 (x 7) =28 day	0.77	5.39	2.18	15.26	2.88	20.16	3.12	21.84	0.30	2.10
5(x 6) = 30 day	1.01	6.06	2.71	16.26	3.51	21.06	3.12	18.72	0.35	2.10
6 (x 5) =30 day	1.24	6.20	3.69	18.45	3.31	16.55	3.45	17.25	1.65	8.25
7 (x 4) =28 day	0.81	3.24	4.55	18.20	3.29	13.16	2.66	10.64	0.18	0.72
Day 6 X7 = 42 day			3.69	25.83						
Day 7x6 = 42 day			4.55	27.30						

5.3.6 Effect of CO_2 on algal culture colour

During day 1, when air with CO_2 at 0% 6%, and 12% (v/v) was sparged into BBM media alone, the solution colour gradually changed from Green beige (RAL1001) to Beige (RAL 1002). Adding 24% and 50% CO_2 (v/v) resulted in a slightly darker colour (**Figure 5-23**). At the same time, all of the algal cultures at any CO_2 concentration were slightly coloured Yellow green (RAL 6018).

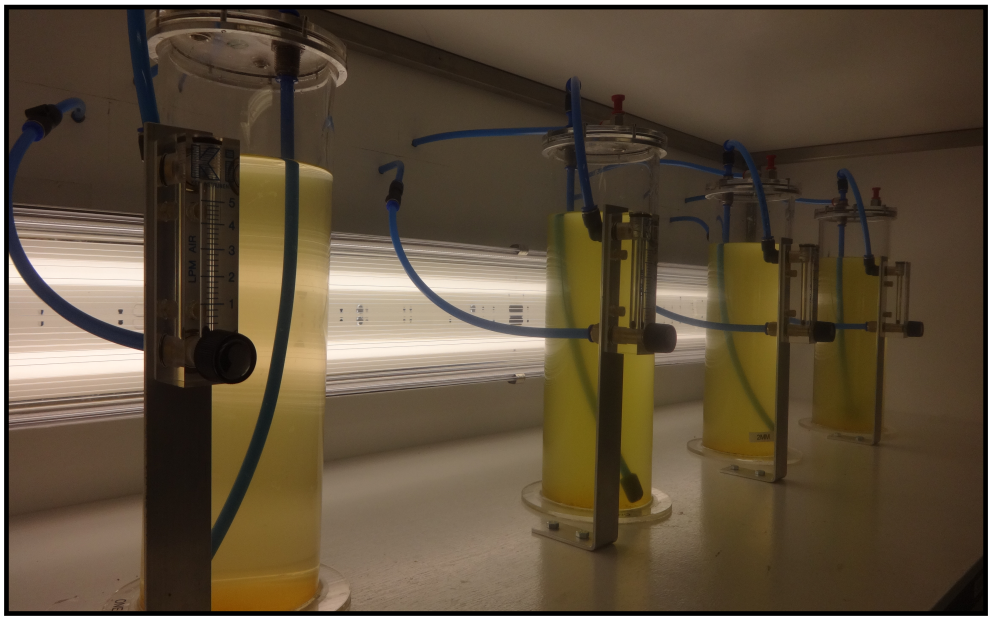


Figure 5-23 BBM media alone (left) and algae in photobioreactors at Day 1.

During Day 2, the colour of BBM media alone is darker than Day 1. All the *C. vulgaris* cultures changed to May green (RAL 6017) as shown in **Figure 5-24**.

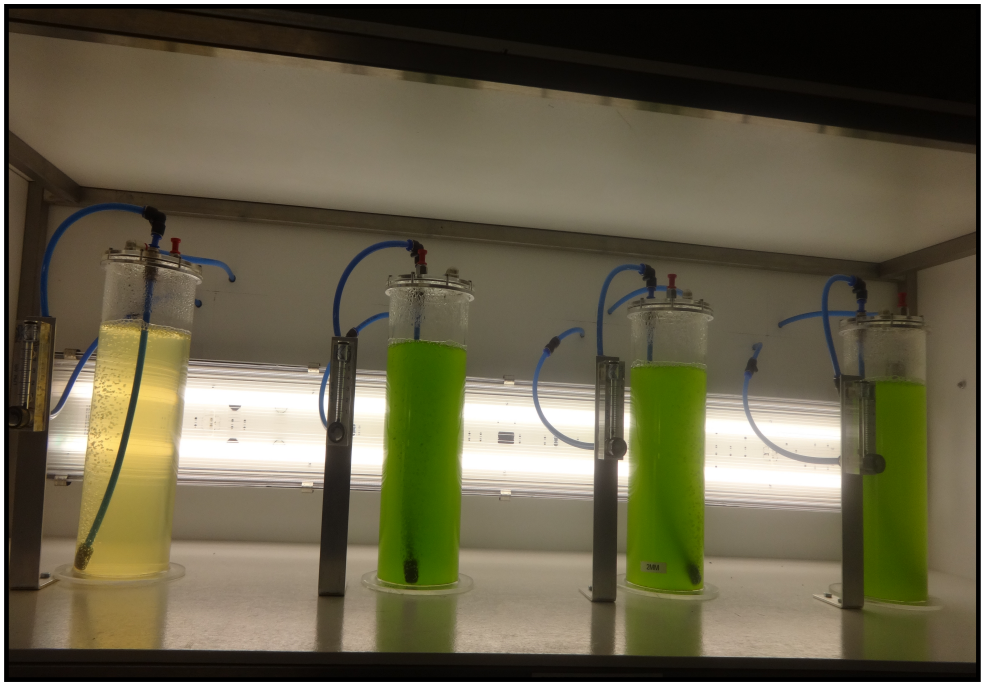


Figure 5-24 BBM media alone (left) and algal cultures in 6% CO_2 (v/v) on Day 2

After 7 days, the colour of BBM alone with 0%, 6%, 12% added CO_2 (v/v) had changed from Beige (RAL 1001) to Sand yellow (RAL 1002). While media exposed to 24% and 50% CO_2 (v/v) are Brown beige (RAL 1011).

The colours of the algal cultures grown in 0%, 6%, and 12% CO_2 (v/v) had become leaf green (RAL 6002) with the 0% cultures lighter than the others. However, the colour of algae cultured in 24% and 50% added CO_2 (v/v) had changed to Yellow green (RAL 6018) as shown in **Figure 5-25**.

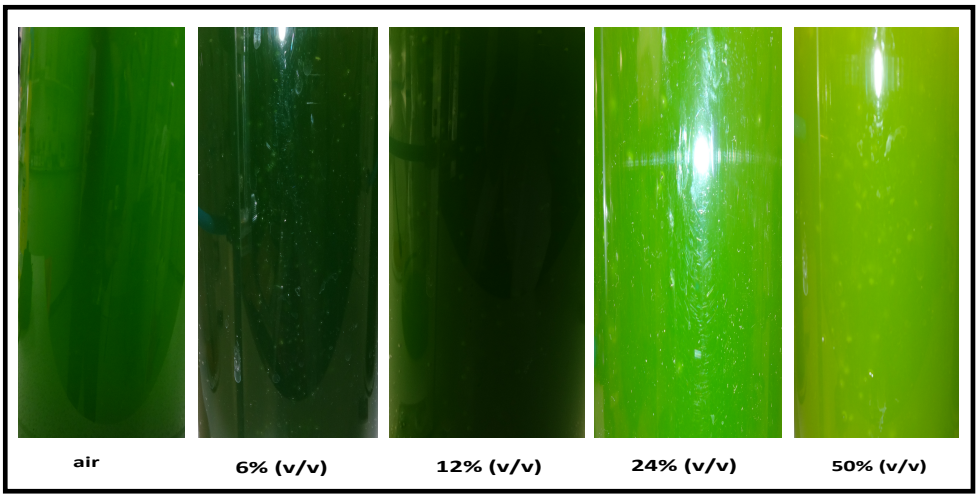


Figure 5-25 Colour of cultures of *C. vulgaris* grown in different levels of CO_2 for seven days.

Sometimes, a photobioreactor system had a problem, for example, a broken sparger, unstable flow rate, or cell contamination. These effects could be quickly seen by the colour of the culture and its uniformity **(Figure 5-26)**.

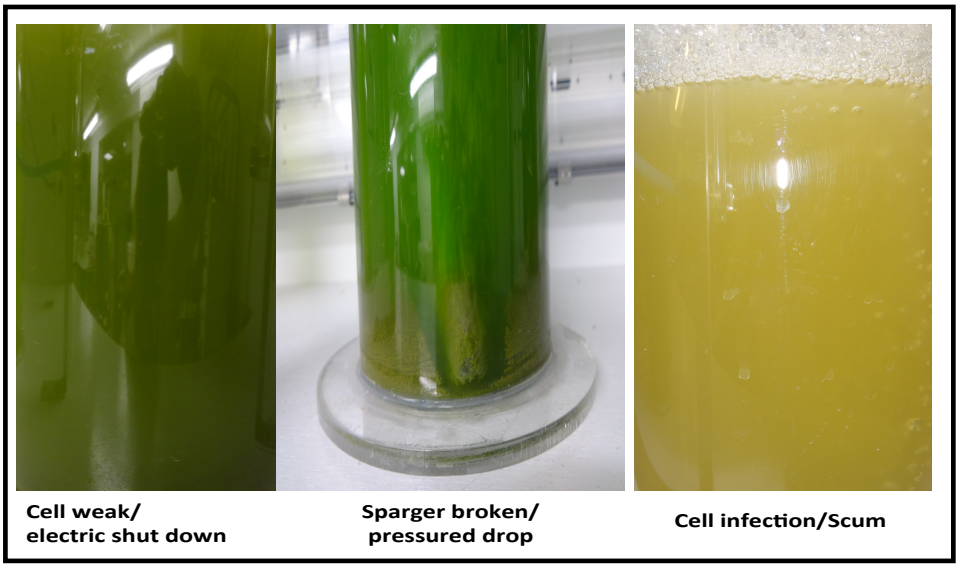


Figure 5-26 Examples of colour changes due to problems affecting the algal cultures.

5.3.7 Effect of CO_2 induced pH change on algal cultures

5.3.7.1 pH in medium alone

pH in media without added CO_2 increases over the first two days and then is stable at around pH 7.99 for Day 3-7. pH in media with added CO_2 (6%, 12%, 24% and 50%) drops on day 1 and then is stable for 12% and 24% at around pH 6 and at around pH 5.4 for 50% CO_2. The pH drop for 6% shows a more gradual drop to the same eventual level as for 12% and 24% (**Figure 5-27**).

5.3.7.2 pH in algal cultures

pH in algal cultures grown with sparging air with no added CO_2 increases from 7.24 to 9.94 over the first two days after which the pH is stable but rises slightly in day 7 (pH 10.53).

While the pH in algal cultures on Day 7 is always higher than the equivalent media alone, this difference is very small for 24% (pH 5.75$\pm$ 0.009), and 50% (pH 5.66 $\pm$ 0.011) and larger for 6% (pH 6.68 $\pm$ 0.038) and 12% (pH 6.54 $\pm$ 0.014). The pH in the algae cultures with all levels of CO_2 (v/v) decreased rapidly during Day 1. Then, on Day 2, there is a slight shift upwards after which the pH stabilises throughout the rest of the experiment (**Figure 5-27**). The pH for 6% and 12% is very similar, while that for 50% is the lowest and very close to the 50% media alone (at around pH 5.35) with the results for 24% between these two (but with a drop to the level of 50% at day 7).

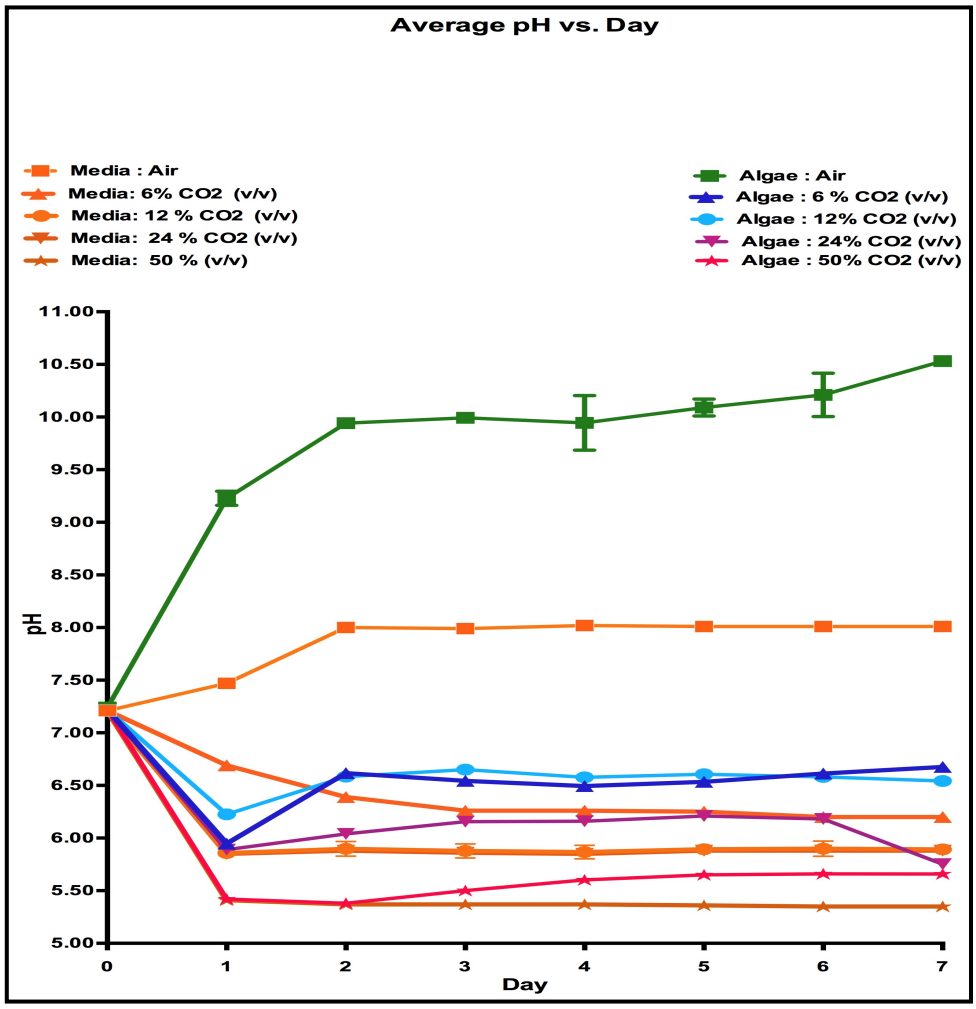


Figure 5-27 Average pH vs. time (days) for *C.vulgaris* cultures and media alone under various levels of CO_2

5.3.8 Effect of $NaHCO_3$ concentrations on pH change in BBM medium

At a confidence interval of 95%, the study found that:

Group 1: pH in autoclaved media is higher than pH in the non-autoclaved medium. pH in media dosed with chemical and then autoclaved has a slightly higher pH than in media autoclaved before adding the chemical.

Between **Groups 2-4**, medium with the addition of 0.1 M. $NaHCO_3$ (**Group 2**) gives the highest pH (as would be expected). The pH when adding 0.01 M and 0.001 M are lower but still around 1 pH unit above the media alone **Figure 5-28**.

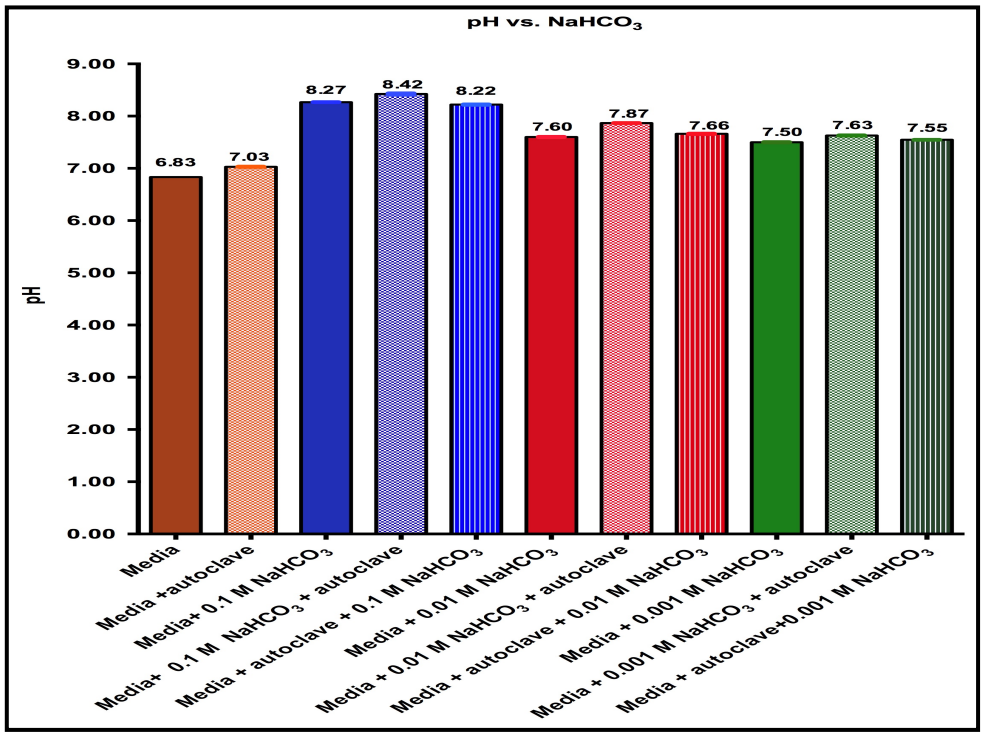


Figure 5-28 Average pH of media preparations with varying bicarbonate doses and autoclave treatments

5.3.9 Effect of $NaHCO_3$ on pH and algal growth in the photobioreactors

5.3.9.1 Effect of $NaHCO_3$ on pH change

The study found that during Day 1, pH in every culture rises except the control (no algae, pH 7.03). After this rise, there is a very gradual fall between Day 2 and 7 (6.03-7.03), apart from the culture with no added $NaHCO_3$ where the pH continues to rise slightly during day 2 and then falls gradually for the rest of the experiment.

The results fall into four distinct groups; there is no effect which way round the autoclaving was done but there is a difference in pH in the algal cultures between the three levels of added $NaHCO_3$. pH in algal cultures grown in 0.1 M $NaHCO_3$ give the highest final value of around pH 8.5, while the values for algae grown with 0.01 M $NaHCO_3$ is around pH 8.25 and for 0.001 M $NaHCO_3$ it falls more sharply to pH 7.5. For the cultures with no added $NaHCO_3$ the final pH is around 7.00. The latter results are indicative of the algae having greater control of the pH in the media and/or the added CO_2 counteracting the effects (**Figure 5-29**).

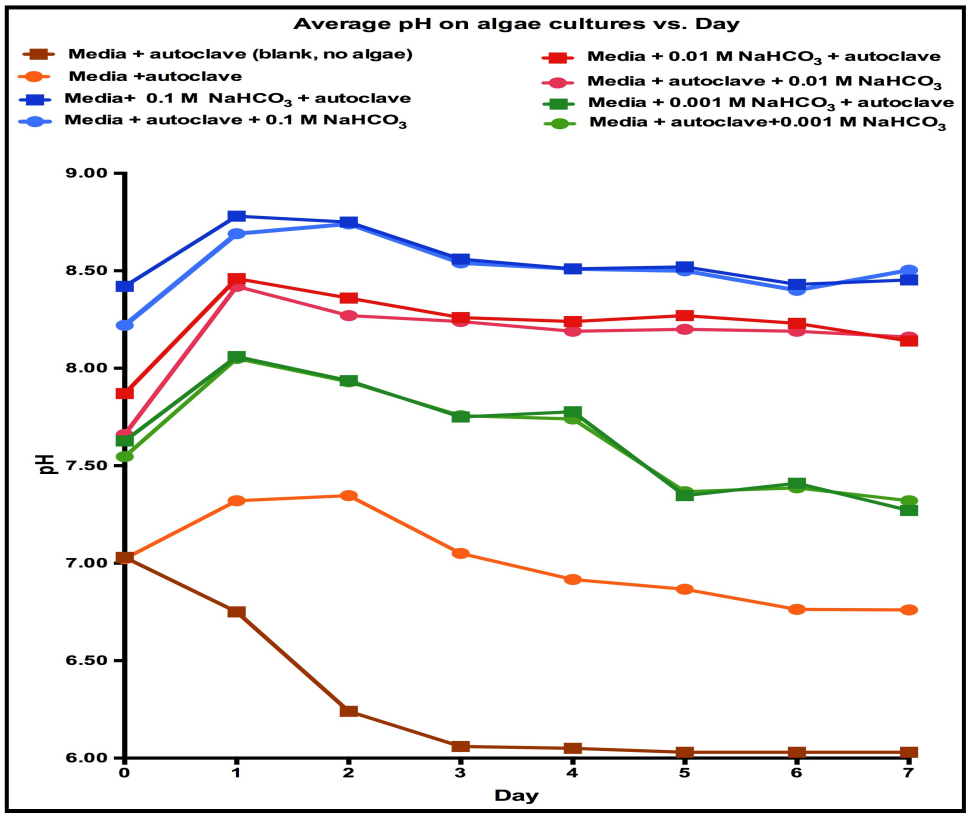


Figure 5-29 pH change in algae cultures fed with 6% CO_2 (v/v) with various additions of $NaHCO_3$ (n=3).

5.3.9.2 Effects of $NaHCO_3$ on algal growth rates as measured by optical density, turbidity, and cell count

The study found that algae grown in media with no added $NaHCO_3$ and with adding 0.001 M $NaHCO_3$ give a higher yield than algae grown in media with 0.1 and 0.01 M $NaHCO_3$. The trends of turbidity, OD_{680} and cell count are all similar (**Figure 5-30 – 5-32**).

During Day 1 and 2, algae grow slowly under every condition. In Day 2-5 the trend is increasing growth but most marked in the media without addition and with the addition of the least (0.001 M) $NaHCO_3$. In Day 6-7 growth is steady for these but falling for the higher levels of added $NaHCO_3$.

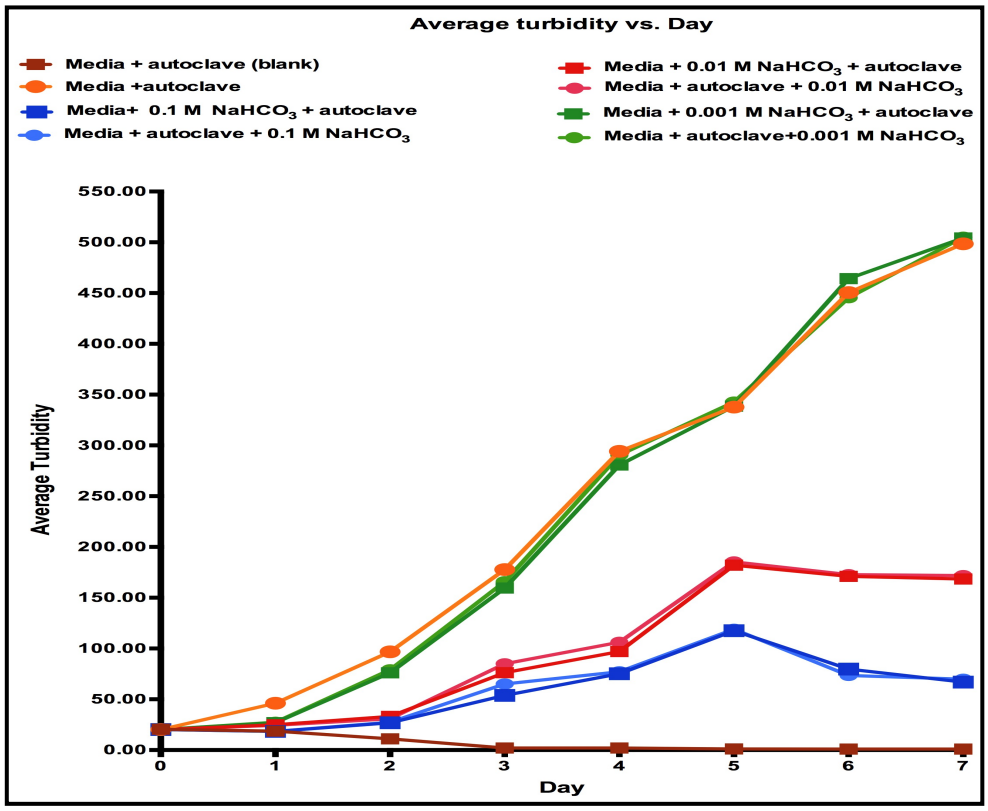


Figure 5-30 Average turbidity in algal solutions with varying additions of $NaHCO_3$ to the medium.

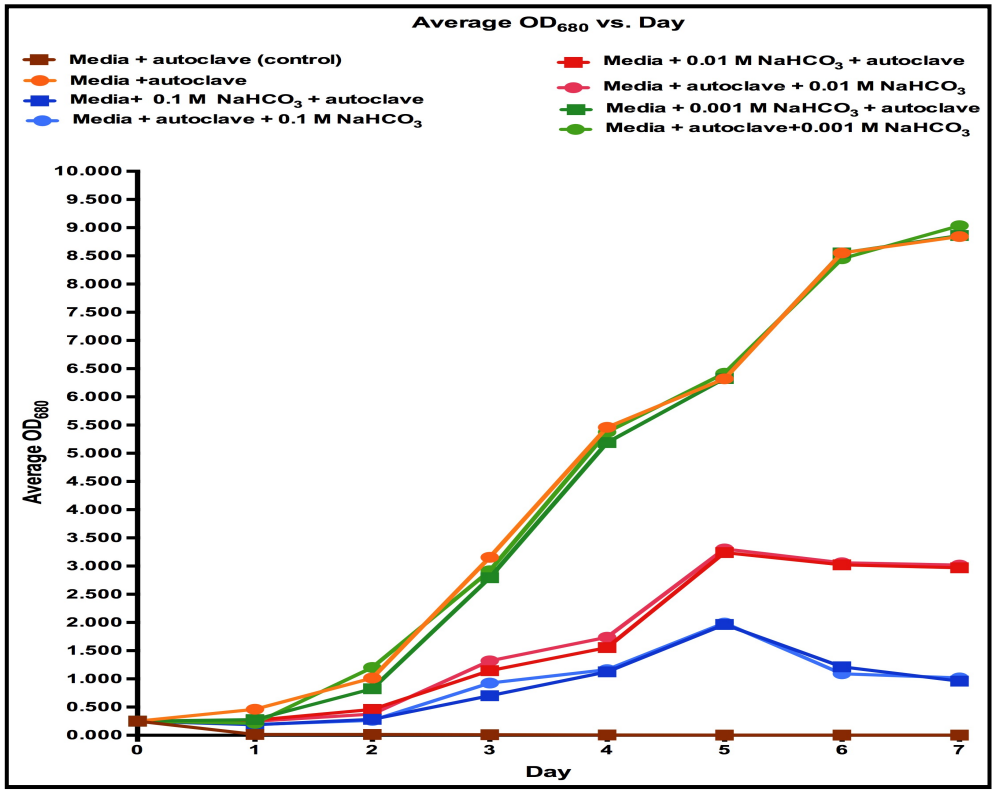


Figure 5-31 Average optical density in algal solutions with varying additions of $NaHCO_3$ to the medium.

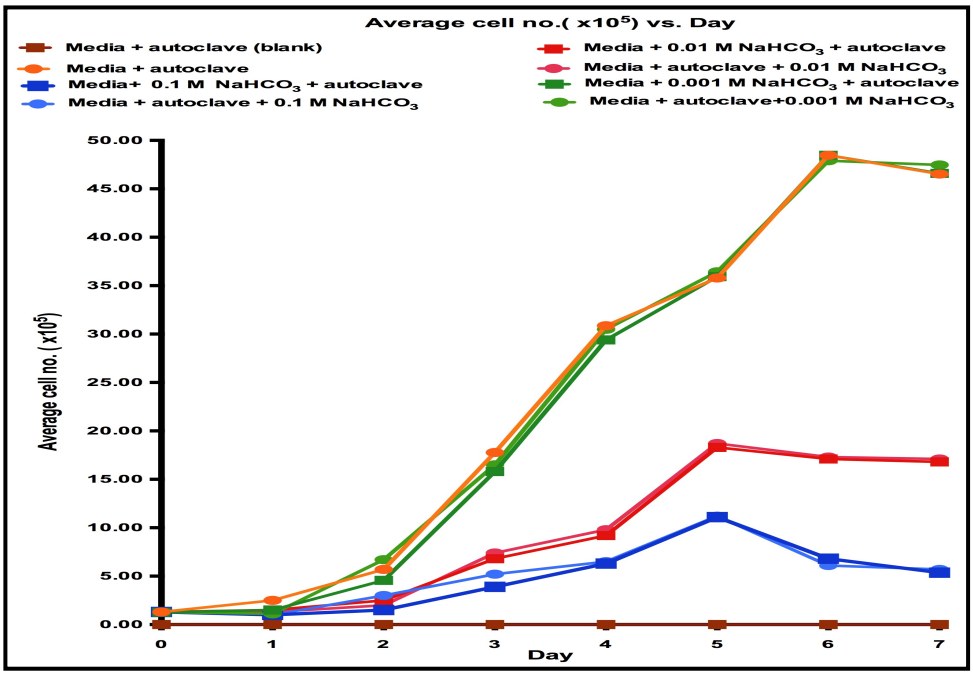


Figure 5-32 Average cell number in algal solutions with varying additions of $NaHCO_3$ to the medium.

5.4 Discussion

5.4.1 Isolation and Identification of *Chlorella vulgaris* strain from Thailand

C. vulgaris is the most suitable algae to be used in the study because it is a non-edible biofuel feedstock. It can be easily grown in a range of media and tolerates a wide range of environmental conditions. The cells have a lipid that can produce biodiesel (Sawaengsak *et al.*, 2014). A high carbohydrate content has a potential for bioethanol fermentation and other uses. In addition, they can live in and capture high CO_2 concentrations (Honda *et al.*, 2012) and are ecofriendly (Sirajunnisa and Surendhiran, 2016).

Tongprawhan *et al.* (2014) found that *C. vulgaris 8580* grown in modified Chu 13 media with normal air sparging (0.03% CO_2) yielded close to 2.0 $x10^7$ cell mL^{-1} with a specific growth rate (μ) of 0.071 ± 0.009 and dry cell weight (DCW) of 351.1 ± 43.7 mgL^{-1}.The cells had 28.5 ± 3.3 % DCW lipid content and a lipid productivity of 4.17 ± 0.52 $mgL^{-1}d^{-1}$.

Under mixotrophic conditions (in BBM medium, pH 6.8, temperature 25℃, 3,000 lux light intensity, light:dark period of 16:8) Rattanapoltee and Kaewkannetra (2014) found that *C.vulgaris TISTR 8580* contains palmitic

acid, oleic acid, linoleic acid, and linolenic acid at 35.33%,19.01%, 19.21%, and 11.68 % respectively and can tolerate a temperature of 37 ℃ with light conditions of 120 $\mu E^{-2}sec^{-1}$ in BG 11 (Charuchinda *et al.*, 2015) which is an advantage for using this strain in Thailand (Prommuak *et al.*, 2012).

5.4.2 Determination of specific growth rate using optical density OD_{680}

This study found the highest growth rate (0.860 d^{-1}) was on Day 2 (exponential growth phase the log difference in the OD between day 1 and 2 is 0.860). This rapid growth is due to the cells consuming the enriched nutrients and dividing rapidly as the environment is favourable. Similarly, Blair *et al.* (2014) found the highest specific growth rate on Day 2 of 0.137 d^{-1} with a biomass yield of 0.0088 g/L.

5.4.3 Effect of CO_2 concentration on algal growth

5.4.3.1 Algal growth rates

The graphs of growth rate as measured by turbidity, optical density (OD_{680}), and cell count show the same trends but with different emphasis. Over days 1-5, algae cultured in 6%, 12%, 24% (v/v) grow better than algae cultured in air alone or 50% CO_2 (v/v). It can be assumed that algae grown with air alone show the "baseline" condition and that the addition of CO_2 allows the algae better use of the photosynthetic pathway for more growth (it is assumed that the culture media provides adequate nutrients). It appears that the algae are readily adapted for growth at the higher CO_2 levels. When the CO_2 level becomes too high (somewhere between 24 and 50% CO_2) the conditions become detrimental and cannot be tolerated by the algae. During Day 6, algae growth in every CO_2 concentration dropped except for algae grown in 0% and 6% CO_2 (v/v). Where the algae can tolerate the CO_2 levels (i.e. below 50%), increasing CO_2 tends to increase the cycle of algal growth. Algae cultured in 12% and 24% grow and then die more quickly than those in 0% or 6% added CO_2. When algae are consuming more nutrients, they emit waste into the system. Increasing fouling waste, and cell decay in cultures with high CO_2 concentration may eventually affect cell growth. Algae grown in high CO_2 concentrations may also have shorter life cycles.

5.4.3.2 Biomass yields

The study found that algae grown in air and 50% CO_2 (v/v) gave a much lower yield than algae grown in 6%, 12%, and 24% (v/v) CO_2. This confirms that the addition of carbon dioxide, at some level between 6 and 24% is suitable for the growth of *C.vulgaris* and will considerably increase yield. It is interesting to find that, when cumulative yield is looked at over a period of time and using various times to harvest, the results can be different than first thought.

There are some differences depending on the measurement technique. Thus when measuring yield by optical density and cell count, the highest yield comes from cultures grown in 24% CO_2 (v/v) and harvested every 4 days while, if measured by turbidity, the best yield is for cultures grown in 6% CO_2 (v/v) and harvested every 6 days, closely followed by cultures grown in 12% CO_2 (v/v) and harvested every 5 days.

The results also show that, while a particular configuration of harvesting and 24% CO_2 (v/v) may give the greatest yield, the considerable lower yields for other harvest times at this level of CO_2 indicate a potentially unstable system which may have considerable problems in an industrial application. Thus this study has narrowed down the optimal range of CO_2 to 6% and over but certainly below 24%. The region between 12 and 24% would need to be studied further to refine this range.

Li *et al.* (2011) cultured algae (*S. Obliquus*) to capture CO_2 from flue gas combustion. It was found that CO_2 levels of 12 to 14% CO_2 (v/v) is best suited to this process.

Bhola *et al.* (2011) studying the effects of various parameters on the biomass yield of *C.vulgaris* found growth was best at 4% CO_2 when harvested on Day 15 (1,222 mg/L). They also found that CO_2 levels of 6-15% CO_2 (v/v) gave a lower biomass yield. This is in contrast to this work and indicates the need to look closely at growth rates and harvest times during scale-up. The harvest time that gives the best yield with a feed of air alone may not be a suitable harvest time when air enhanced CO_2 with is supplied.

5.4.4 Effect of CO_2 concentration on specific growth rates

The specific growth rate data indicates that adaptation is taking place during day one with the algal inoculum taking longer to adapt to higher CO_2 conditions than to lower CO_2 conditions. Algae take time to adapt to new environmental conditions such as; medium, light, mixing system, temperature and pH. This may cause growth inhibition with a delay of reproduction. Different CO_2 concentrations result in different specific growth rates, some of which are more predictable than others (the results indicate that under greater CO_2 stress the algae show greater swings in specific growth rate, possibly as they try different metabolic routes to adapt to the conditions).

Algae grown with air alone show a low cell number at Day 1. In contrast, this specific growth rate (μ) is different from that calculated by turbidity and optical density because during the first day the algae are adapting, the green colour and turbidity increase, but the cells are very small and are not easy to count in the microscope. The study of Scragg *et al.* (2002) found that *C. vulgaris* grown in a tubular photobioreactor gave a growth rate at 0.40 d^{-1} when culturing in Watanabe's medium at flow rate 0.31 L/m.

Algae grown in 6% CO_2 (v/v) do not show any negative data. This indicates that, at this level of CO_2, the system in the photobioreactor is stable and the CO_2 level is such that the algae can adapt easily. There is a rise in specific growth rate. Algae can grow quickly and maintain their life cycle longer than under other conditions.

The data show that algae find it difficult to adapt themselves at first (1-2 days) to conditions with high CO_2 levels such as 24% and 50%. Another study mentioned that algae grown in 50% CO_2 (v/v) grow slowly because they cannot survive too high CO_2 levels (Chinnasamy *et al.*, 2009; Van Den Hende *et al.*, 2012).

5.4.5 Effect of CO_2 concentration on dry weight

Commercially, dry weight yield is probably the best measure for algal biomass culture. Algae grown in a media sparged with compressed air alone cannot provide the high biomass yield that can be achieved when CO_2 is added. However, too much CO_2 becomes detrimental to the algae and the yield drops. To complicate matters, between these two extremes the length

of time the cultures are maintained before harvesting becomes important. If the culture time is to be 7 days then 6% CO_2 is the best option. For a shorter culture time (4 days) then 12 or 24% CO_2 is best. While 24% CO_2 may show a slightly better yield over 12% at 4 days there may be a commercial advantage in using the lower level of CO_2. Looking to commercial exploitation the number of cultures within a set time period and their total yield needs to be considered.

The study found that the highest dry weight for 28-30 days gives a yield around 18.45 g/L from algae grown in 6% CO_2 (v/v) on Day 7. Algae grown in 12% CO_2 (v/v) give a highest yield of 22.90 g/L on Day 3. However, this data is only for dry biomass yield. There are other considerations such as the health of the cells and the amount of any desired component that should also be considered. The study found that a long term period (42 days) at 6% should be harvested on Day 7 (18.20 g/L) with an accumulative yield of 27.30 g/L. Similarly, at 12% CO_2 harvesting is best every 5 days (21.06 g/L).

On a commercial scale, many factors have to be taken into consideration including the stability of the algal culture system in the face of high levels of added CO_2. This, together with a rapid turn round of cultures may lead to culture crashes not found in more steady and stable longer culture times. Three main factors must be considered; these are 1) the operation of a sustainable photobioreactor system; 2) a cell cycle, which maintains healthy algae and 3) harvesting costs.

C. vulgaris cultures in 6% and 12% CO_2 (v/v) are more stable than 24%. To scale up a photobioreactor system, the concerns are about the sustainability of the reactor system while maintaining yield. This study can be compared with reports in the literature review of Chen *et al.* (2011), where *C. vulgaris* in this study shows a higher yield (0.98-1.25 $gL^{-1}d^{-1}$) at 6-12% CO_2 (v/v) than *C. vulgaris* No. 259 (0.01 $gL^{-1}d^{-1}$) grown in air flow rate 200 mL/min with media and 1% glucose (Liang *et al.*, 2009) and *C. vulgaris* F&M-M49 (0.20 $gL^{-1}d^{-1}$) (Rodolfi *et al.*, 2009), and *C. vulgaris CCAP 211/11b* (0.17 $gL^{-1}d^{-1}$) when growing algae at an enriched air given by Rodolfi *et al.* (2009). Different cultural conditions result in different growth rates.

The study by Yoo *et al.* (2010) found around 0.104 $gL^{-1}d^{-1}$ (or 104 mg. dry weight/$L^{-1}d^{-1}$) of *C.vulgaris* KCTC AG10032 could be obtained from a culture with ambient air containing 2% CO_2 and flue gas 0.3 $vv^{-1}m^{-1}$ in BG 11 in a lag phase for 1 week inoculation. Then, increasing CO_2 concentration to 10% CO_2 was used to continue algae culture for 14 days.

Chinnasamy *et al.* (2009) grew *C. vulgaris* by varying CO_2 between 0.036 to 20% (v/v) and temperature at 30, 40 and 50 ℃. The study found that the highest chlorophyll of 11 μg/mL and biomass 210 μg/mL (dry weight) was obtained by adding 6% CO_2. Algae grew well in 30 ℃. Chiu *et al.* (2008) found that, for *Chlorella sp*. grown in 2%,5%, 10% and 15% CO_2, the specific growth rate was 0.58 - 0.66 d^{-1} and dry weight was 0.76-0.87 g/L. Chiu's study stressed that the algal growth rates depended on CO_2 concentration (shown in **Table 5-9)**.

Table 5-9 The biomass production and specific growth rate for different CO_2 concentration as found by Chiu *et al.* (2008).

CO_2 aeration	Biomass (Dry weight, g/L)	Specific growth rate (μ, d^{-1})
Low density inoculum (8x 10^5 cell/mL)		
Air	0.537 ± 0.016	0.230
2%	1.211 ± 0.031	0.492
5%	0.062 ± 0.027	0.127
10%	0.010 ± 0.003	-
15%	0.537 ± 0.001	-
Low density inoculum (8x 10^6 cell/mL)		
Air	0.682 ± 0.007	0.248
2%	1.445 ± 0.015	0.605
5%	0.899 ± 0.003	0.343
10%	0.106 ± 0.001	-
15%	0.099 ± 0.001	-

5.4.6 Effect of CO_2 on algal culture colour

For uninoculated media, the study found that, after 7 days, the colour of the BBM media had become slightly yellow when exposed to 0% 6% and 12% (v/v) added CO_2. At higher (24% and 50% CO_2 levels) the colour is darker, probably due to a stronger acidic environment.

C.vulgaris grows well under added CO_2 concentrations of 0%, 6%, and 12% (v/v). The cultures are dark green in Day 2-4. After 7 days, a dense algal suspension is formed with 6 and 12% CO_2 the darkest. These results are similar to the study of Amoroso *et al.* (1998) who found that

Chlamydomonas reinhardii and *Dunaliella tertiolecta* grow well when sparged with air enriched with 5% CO_2 (v/v) when compared to lower or higher CO_2 concentrations. At 24% and 50% (v/v) added CO_2, the cultures are less green after 7 days. The conditions may not be suitable because the algae cannot handle the high CO_2 concentrations (hypertonic solution) or tolerate the acidity, either for the length of time (7 days) or at all.

An unexpected or unusual colour change can indicate a problem with the cell culture and three examples are shown in **Figure 5-26**. These can be due to physical infrastructure breakdown, or to infection. Cell weakness may occur because of a lack of air/CO_2 supply to the culture.. Contamination of a culture by aggressive bacteria will kill the algae, causing yield depletion and producing a yellow culture with a scum on the surface. A surface scum or floating mat is also an indication of an imbalance in nutrients.

Sometimes, overpopulation may lead to food competition and unhealthy cells which leads to culture failure. In very high densities, algae blooms may result in yellow, brown, and black toxic conditions. The sources of the problem are, for example; excessive nitrogen, phosphorous, carbon or potassium, or decomposing algal cells.

5.4.7 Effect of pH of media and algal suspension of *C.vulgaris* grown at various CO_2 levels

5.4.7.1 pH in the media alone

When sparging air alone in the media, the pH is slightly base (alkaline) because the media solution has a component of enriched carbonate dissolved in the BBM media. Both CO_2 sources from medium and marginally from the air can be aggregated to influence the change of carbonate to bicarbonate (deprotonated form). This is indicated by an increasing pH during Day 1-2. Once these changes have taken place the pH is nearly stable.

The addition of CO_2 (6%, 12%, 24% and 50%) produces a weakly acidic solution (due to the formation of carbonic acid) during day 1 after which the pH stabilises at a lower level dependent on the percentage of CO_2.

5.4.7.2 pH in algal cultures

pH in algae cultures without additional CO_2 increases during day 1-2. Then, it is stable at around pH 10 with a slight increase in days 6 and 7. While this can be considered the "natural" (or a baseline) state of growth in a culture, it can be seen from the results in section 5.3.3 that these are not the most productive conditions and that algae grow considerably more productively in a media that is more acid. There is a balance to be found between the eventually detrimental effects of more and more acidic conditions and the increased availability of CO_2 allowing higher growth.

pH in algae cultures grown in 50% CO_2 (v/v) is lower than 24%, 12%, and 6% respectively. The results from 24% and 50% CO_2 may indicate the limiting acid level which becomes detrimental to algal growth.

During Day 2, the pH increases slightly, indicating that the algae rapidly adapt to the higher CO_2 levels and begin fixing more CO_2 and entering an exponential growth phase. During Days 3-6, the pH is stable. This is because CO_2 supply equals algal demand which leads to a saturated system. On day 7, pH can show a slight fall and this corresponds to the beginning of a drop off in growth at most of the CO_2 levels. It seems that the increasing population becomes limited by the supply of nutrients.

5.4.8 Effect of $NaHCO_3$ concentrations on pH change in BBM medium

As expected, pH in media with added $NaHCO_3$ is higher than the pH of the media alone. pH in autoclaved media is higher than unautoclaved media. This is because, when the medium is heated under a pressure of approximately 15 psi, at 121^{o}C for 15-20 min, dissolved CO_2 in the solution is expelled out of the solution. By Henry's law, the solubility of a gas decreases when the temperature increases (degassing process in an autoclave). This allows the medium to be more alkaline.

pH in BBM medium with the addition of 0.1 M $NaHCO_3$ (Group 2) results in a higher pH value when compared with Group 1, 3, and 4 because of the considerably higher chemical addition. This may help to design how much $NaHCO_3$ is required to achieve the desired pH.

5.4.9 Effect of $NaHCO_3$ on pH and algal growth in the photobioreactors

5.4.9.1 Effect of $NaHCO_3$ on pH change

The results show that during days 1-3, pH in the media with no algae declines and then stabilises at near pH 6. This is due to the partial pressure of CO_2 gas changing its form to become a carbonic acid as shown in **Equation 5.1**.

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \quad (5.1)$$

In contrast, pH in the medium alone and in algal cultures with added bicarbonate increases on adding 6% CO_2 (v/v) into the photobioreactor system. Here the algae use CO_2 in the photosynthesis process for their growth as shown in **Equation 5.2**. They release O_2 into the photobioreactor system, this bonds with hydrogen to form water which can reduce acid conditions. In addition, air aids to balance CO_2 in the system and some of CO_2 becomes an alkaline form (HCO_3^-). The result is that alkali (base) is initially more dominant than acid leading to an increasing pH in the algal culture.

$$6CO_2 + 6H_2O + \text{light} \rightarrow C_6H_{12}O_6 + 6O_2 \quad (5.2)$$

From Day 2 the pH in algae cultures grown in every condition drops because the algae grow rapidly and need more CO_2 to support their growth. CO_2 and $NaHCO_3$ in the media are changed from the alkaline to the acid form. This balances the system to a near neutral equilibrium. With time, demand for CO_2 tends to equal supply resulting in less pH change.

5.4.9.2 Effects of $NaHCO_3$ on algal growth rates as measured by optical density, turbidity, and cell count

For 6% CO_2 (v/v) there is no advantage in terms of growth of adding $NaHCO_3$ to the medium. As a level of 0.001 M $NaHCO_3$ is not detrimental to growth there could be some advantage in buffering the system to a slightly more alkaline pH at this level but it seems that the effect of adding higher levels of $NaHCO_3$ is to prevent the algae accessing the additional carbon provided by the CO_2. There is no difference between adding chemicals before or after autoclaving the media. It appears therefore that

there is little or no value in adding additional chemicals to try to maintain a higher pH when using CO_2 enhanced air sparging in these algal cultures.

5.5 Conclusions

A *Chlorella vulgaris* strain was obtained from Thailand and is the main algal species and strain to be used in the work described in this thesis as the ultimate goal is to set up algal biomass facilities in Thailand.

The study found that this *C.vulgaris* grows well when a seven day inoculum is introduced into suitable media under correct environmental conditions. Algae have a high exponential growth rate. The highest specific growth rate was 0.860 d^{-1} on Day 2.

For seven days growth, algae cultured with 6-12% CO_2 (v/v) provide a consistent system with high biomass yields. If growth is to be for a shorter time before harvest (3-4 days) then it would be worth considering up to 24% CO_2.

The study found that, while *C. vulgaris* can grow well in a range of levels of added carbon dioxide (certainly between 6 % and 12% CO_2 (v/v) and possibly up to near 24% CO_2 (v/v)), the yield also depends on when the algae are harvested i.e. how long they are allowed to grow. Added to this must be, on the commercial scale, any uncertainties of growth at too high CO_2 (v/v) values and the cost of harvesting. Thus while these results give a good indication, a cost-benefit analysis would be needed to determine the best economic balance between the level of carbon dioxide provided and harvest times. Certainly, algae grown in air alone gives much lower yields than added CO_2 and the results show that 50% CO_2 (v/v) is detrimental to algal growth.

The relationship between specific growth rate of *C.vulgaris* grown with air sparging and with the addition of various CO_2 concentrations found that algae cultured with 6% and 12% CO_2 (v/v) give high growth rates. These levels of CO_2 are more stable than the other conditions (air alone, 24%, and 50% CO_2 (v/v) which fluctuate. The results for algal cultures grown in 24% CO_2 are interesting as the overall results indicate that this may be close to the upper limit of CO_2 tolerance for this algae.

The study found that, for a cumulative yield of several harvests to 28 or 30 days, algae grown in 6% CO_2 give the highest weight when harvested on Day 6 or 7 (18.20-18.45 g/L). Algae grown in 12% CO_2 give the high yield on Day 3-5 at 20.16-22.90 g/L and for 24% CO_2 (v/v) slightly less yield at 18.72-22.50 g/L at the same period.

Algae cultured with air only and with 50% CO_2 show lower or, in the case of 50% CO_2, a very poor yield.

C.vulgaris is shown in this study to be able to survive at a wide range of pH from 5 to above 10. However, there is a trade-off between increasing production by increasing CO_2 supply and the detrimental effects of acidification due to increasing CO_2 supply.

Bulk colour can be used as a guide to quickly identify the efficiency of photosynthesis in a photobioreactor system. These experiments found that using colour alone as a guide, 6% and 12% (v/v) CO_2 appear most suitable for culturing *C. vulgaris*. Problems with an algal culture through a system failure, imbalance of nutrients or contamination can also be spotted by colour changes.

The greater the amount of $NaHCO_3$ added the higher the pH. and adding the chemical before autoclaving the media gives the greatest increase in pH. However, adding over 0.01 M $NaHCO_3$ is detrimental to the growth of the algae.

Chapter 6 Results: The use of chitosan and metallic salts to aid algal sedimentation

6.1 Introduction

To study the effect of metallic salts (alum, ferric chloride, aluminium chloride), and biopolymer (medium molecular weight chitosan, high molecular weight chitosan and crab shell) on algal settling, these flocculating agents were added to algal cultures in the exponential growth phase and to dead cell suspensions. Sampling and measurement were carried out at a half of vessel height. Experiments used various flocculating agent dosages, and two stirring speeds. Control experiments were also undertaken. Turbidity and OD_{680} were used to measure settlement. The results have been analysed in 5 categories.

1) Effect of dose and mixing speed when adding medium molecular weight chitosan;

2) A comparison of metallic salts and biopolymer addition on algal settlement;

3) Determination and comparison of turbidity optical density, and pH measurements between and within replicates in groups of experiments investigating the settlement of living alga suspensions, dead algal suspensions and controls (medium alone);

4) Determination of percentage removal and;

5) Effect on carbon measurements (TC, TIC, TOC) after adding chitosan

6.2 Methodology

6.2.1 Effect of dose and mixing speed when adding medium molecular weight chitosan

The flocculation tests were undertaken for a number of different mixing times and speeds (rpm.). These were a rapid mixing of 1-120, 5-120, 5-150, 5-200 min-rpm followed by slow circulation for 30 min at 45 rpm. Settling time before measurement was 10 min. Chitosan was dosed at concentrations of 0, 25, 50, 100, 150, 200, 250, 300, 350, 400 and 450 mg/L into 1,000 mL

algal culture and the contents stirred for the time and speeds given above. Then, the turbidity of the algal cultures was measured after 10 minutes settling. For each chitosan concentration, 18 sampling replications were tested, n=18. The total is 792 samples (18 samples x 11 concentrations x 4 mixing types). The study also measures pH as the same number of samples (N=792).

6.2.2 A comparison of metallic salts and biopolymer addition on algal settlement

The sedimentation of *Chlorella vulgaris* was investigated using 6 flocculants: three metallic salts; $Al_2(SO_4)_3x\ H_2O$, $FeCl_3$, and $AlCl_3$, and three biopolymers: medium molecular weight (MW) chitosan, high molecular weight (HW) chitosan and crab shell. These were added at 600, 800 and 1,000 mg/L.

Prior to sedimentation, the cultures were stirred at 200 rpm for 30 min and then at 45 rpm for 30 min. Turbidity was sampled over time at a point half way up the vessel. Percentage removal was calculated and compared to controls.

Sedimentation was monitored by measuring turbidity and optical density (OD_{680}). pH was also recorded. There were six sampling times (see **Figure 6-1**):

1. Before adding the flocculants (**Point A**, timeline is at 0 hr.);

2. 5 min after adding the flocculants (**Point B**, timeline is at 0.083 hr.);

3. After stirring for 1 hr. (settling time is 0, **Point C**, timeline is 1.083 hr.);

4. After a settling time of 1 hr. (**Point D**, timeline is at 2.083 hr.);

5. After a settling time of 12 hr. (**Point E**, timeline is at 13.083 hr.);

6. After a settling time of 24 hr. (**Point F**, timeline at 25.083 hr.)

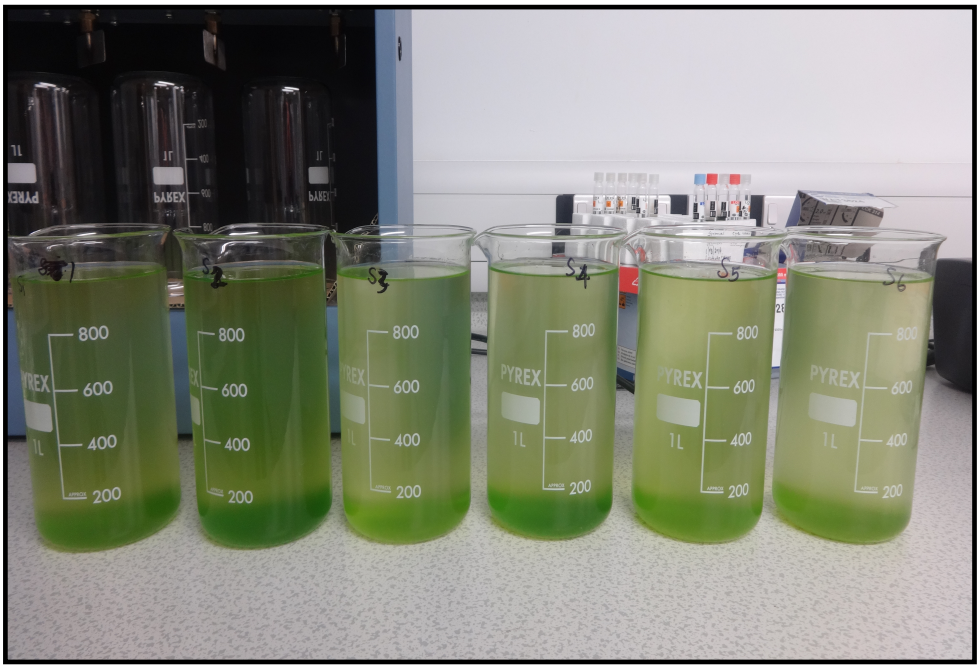


Figure 6-1 Photograph of algal settlement following flocculant addition, circulation and settling. The algal suspensions were sampled at the middle of beaker to measure 4 parameters (pH, turbidity and optical density) at 0,1,12 and 24 hr.

Three categories were investigated;

1. Medium groups; no flocculant addition and adding chemicals at 600 mg /L, 800 mg/L, 1,000 mg/L;

2. Living algal groups; no flocculant addition and adding chemicals as in group 1;

3. Dead cell group: no flocculant addition and adding chemicals as in group 1.

The flocculants tested were: $FeCl_3$, $AlCl_3$, MW chitosan, HW chitosan and crab shell.

For each flocculant there are 360 samples (5 sampling replications x 12 sub groups x 6 testing points). Thus, a total is 2,160 samples for the six flocculants. Optical density (OD_{680}) and pH were also tested. Therefore, this experiment had a total of 6,480 samples (2,160 samples x 3 parameters; Turbidity, OD and pH).

6.2.3 Determination and comparison of turbidity optical density, and pH

To determine and compare the turbidity, optical density (OD_{680}) and pH, the mean and standard deviation of the data were analysed both between groups (Media, algal living cells, dead cells) and within the groups as shown in **Table 6-1**. The study compared between and within replicates in groups of experiments investigating the settlement of living alga suspensions, dead algal suspensions and controls (medium alone).

Table 6-1 Group analysis

Item	Group 1	Group 2	Group 3
1	Media	Algae	Dead cell
2	Media + alum 600 mg/L	Algae + alum 600 mg/L	Dead cell + alum 600 mg/L
3	Media + alum 800 mg/L	Algae + alum 800 mg/L	Dead cell + alum 800 mg/L
4	Media + alum 800 mg/L	Algae + alum 800 mg/L	Dead cell + alum 800 mg/L
5	Media + $FeCl_3$ 600 mg/L	Algae + $FeCl_3$ 600 mg/L	Dead cell + $FeCl_3$ 600 mg/L
6	Media + $FeCl_3$ 800 mg/L	Algae + $FeCl_3$ 800 mg/L	Dead cell + $FeCl_3$ 800 mg/L
7	Media + $FeCl_3$ 1,000 mg/L	Algae + $FeCl_3$ 1,000 mg/L	Dead cell + $FeCl_3$ 1,000 mg/L
8	Media + $AlCl_3$ 600 mg/L	Algae + $AlCl_3$ 600 mg/L	Dead cell + $AlCl_3$ 600 mg/L
9	Media + $AlCl_3$ 800 mg/L	Algae + $AlCl_3$ 800 mg/L	Dead cell + $AlCl_3$ 800 mg/L
10	Media + $AlCl_3$ 1,000 mg/L	Algae + $AlCl_3$ 1,000 mg/L	Dead cell + $AlCl_3$ 1,000 mg/L
11	Media + MW chitosan 600 mg/L	Algae + MW chitosan 600 mg/L	Dead cell + MW chitosan 600 mg/L
12	Media + MW chitosan 800 mg/L	Algae + MW chitosan 800 mg/L	Dead cell + MW chitosan 800 mg/L
13	Media + MW chitosan 1,000 mg/L	Algae + MW chitosan 1,000 mg/L	Dead cell + MW chitosan 1,000 mg/L
14	Media+ HW chitosan 600 mg/L	Algae + HW chitosan 600 mg/L	Dead cell + HW chitosan 600 mg/L
15	Media+ HW chitosan 800 mg/L	Algae + HW chitosan 800 mg/L	Dead cell + HW chitosan 800 mg/L
16	Media+ HW chitosan 1,000 mg/L	Algae + HW chitosan 1,000 mg/L	Dead cell + HW chitosan 1,000 mg/L
17	Media + crab shell 600 mg/L	Algae + crab shell 600 mg/L	Dead cell + crab shell 600 mg/L
18	Media + crab shell 800 mg/L	Algae + crab shell 800 mg/L	Dead cell + crab shell 800 mg/L
19	Media + crab shell 1,000 mg/L	Algae + crab shell 1,000 mg/L	Dead cell + crab shell 1,000 mg/L

For the data within groups, the mean and standard deviation for every concentration of each individual flocculant added to the medium, living algae, and dead cells was calculated (see **Figure 6-2**).

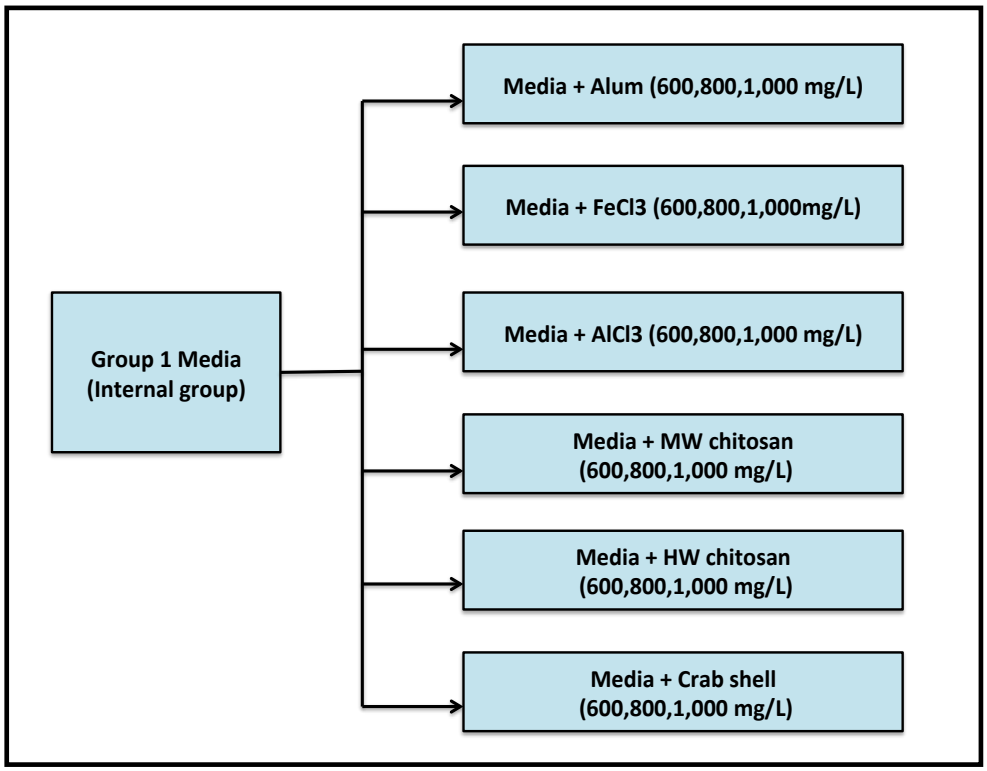


Figure 6-2 Data analysis between internal groups

6.2.4 Determination of percentage removal

The calculated percentage biomass removal is based on turbidity and on optical density at OD_{680} (see section 3.4.7).

6.2.5 Effect on carbon measurements (TC, TIC, TOC) after adding chitosan

6.2.5.1 Effects of 150 mg/L MW chitosan on TC, TIC and TOC

This study looks at how adding granular chitosan to an algal culture may influence the carbon component in the suspension and from this the degree to which the chitosan can be considered an "inert" addition can be judged. MW chitosan at 150 mg/L was added to algal cultures. The cultures were then mixed at 120 rpm for 5 min followed by 45 rpm for 30 min. Samples were taken and analysed for TC and TIC (and by subtraction TOC) and compared to samples taken before the addition of the chitosan. The sampling replications are 3 (n=3).

6.2.5.2 Effect of chitosans and crab shell on TC, TIC, and TOC change

Algal cultures to which 600 and 800 mg/L of MW chitosan, HW chitosan and crab shell had been added were selected for further carbon analysis. Sampling was made for 3 replicates of each of the following:

1. Blank media; before and after stirring
2. Algae; before and after stirring

3 After adding 600 and 800 mg/L MW chitosan and stirring for 1 hr.

4. After adding 600 mg/L HWchitosan and stirring for 1 hr
5. After adding 600 and 800 mg/L Crab shell and stirring for 1 hr.

6.3 Results

6.3.1 Effect of dose and mixing speed when adding medium molecular weight chitosan.

6.3.1.1 Dosage and mixing

The results show that when MW chitosan is added at concentrations between 0 and 150 mg/L, the mean turbidity ranges between 300.61-330.28 NTU (**Figure 6-3**). It remains nearly constant indicating that such small amounts of chitosan are not enough to network algal cells into settling flocs.

Between 150-300 mg/L, the turbidity increases due to algae and chitosan particles creating new bonds and flocs. The highest turbidity is for 200 and 250 mg/L chitosan at 5-200 min-rpm (380.50 NTU). Between 300-450 mg/L of chitosan, the turbidity slightly decreases at all speeds as some agglomerates are already sedimenting (some were also observed to be floating to the surface). In this case, turbidity is 289.44 NTU for 350 mg/L of chitosan at 5 -120 min- rpm and 280.44 NTU for 450 mg/L at 1-120 min-rpm. The detailed results are given in **Appendix D**

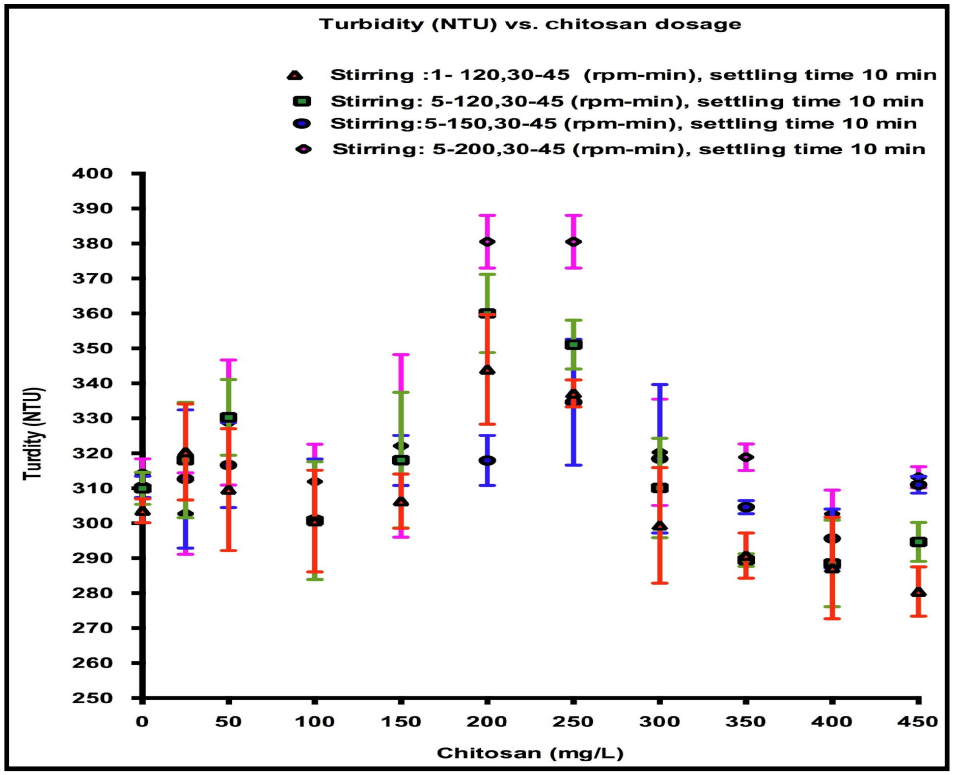


Figure 6-3 Mean turbidity of MW-chitosan dosed algal cultures after stirring for various times and 10 minutes settling. Error bars represent the standard deviation.

6.3.1.2 pH measurement

The initial pH of the cultures averaged pH 7.81 and the pH increased on addition of chitosan at 100 mg/L and above to between 7.96 and 8.10, while at 25 and 50 mg/L the increase was only between pH 7.86 and 7.99 (**Figure 6-4**).

Lower speed stirring (1-120, 5-120 min-rpm) has less effect on pH change but, at 150 mg/L of chitosan and above, there was little change in pH at any speed.

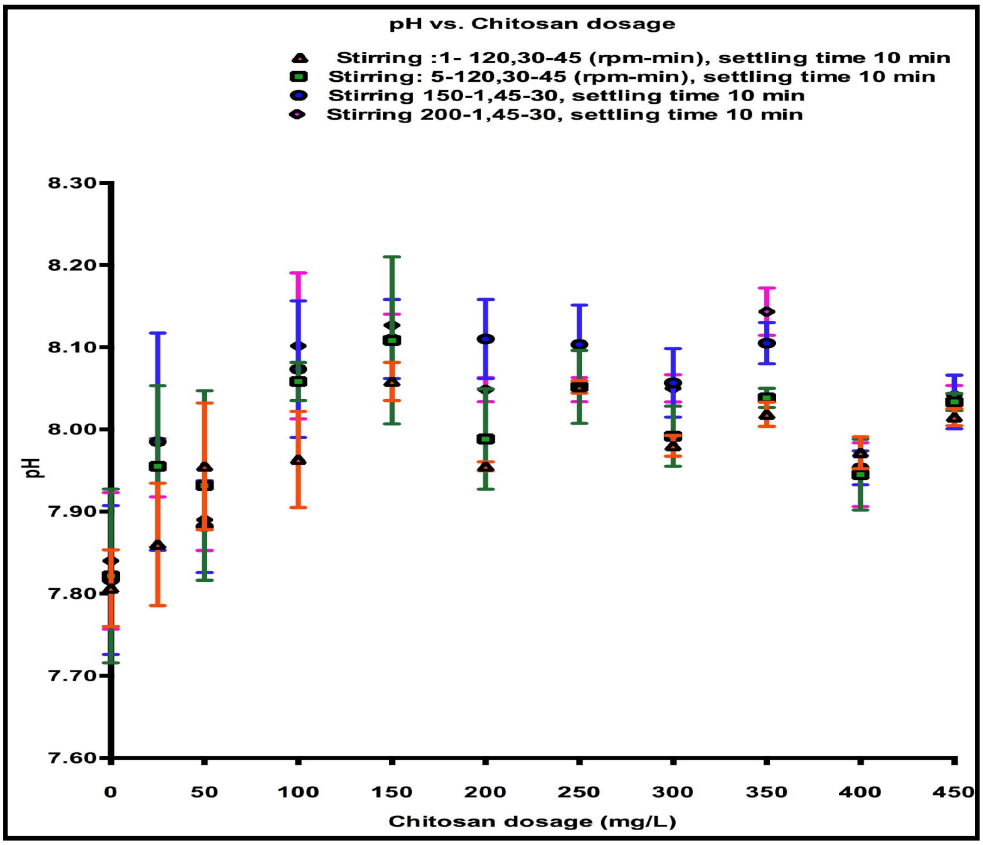


Figure 6-4 The mean and standard deviation of pH on adding MW-chitosan doses of 0-450 mg/L.

6.3.2 A comparison of metallic salts and biopolymer addition on algal settlement

6.3.2.1 Turbidity measurements

1) Aluminium sulphate anhydrate (Alum)

After an initial rise in turbidity in the first hour (between when the flocculants were added and immediately after stirring), there is a rapid drop in turbidity over the first hour of settlement (timeline between 1.083-2.083 hr.) at all alum dosages when compared to the non-dosed controls. After this, there is a much more gradual or no decline in turbidity. The turbidity is between 10 and 20 NTU after one hour's settlement and less than 10 NTU for a sedimentation time of 12 hr. Turbidity is nearly zero at a settling time of 24 hr. At this point, algae have visibly settled to the bottom of the vessel (see **Appendix E**).

The turbidity of both living and dead algae cells behave in a similar way on adding flocculant while the controls show a much more gradual decline in turbidity and are therefore settling very slowly (**Figure 6-5**).

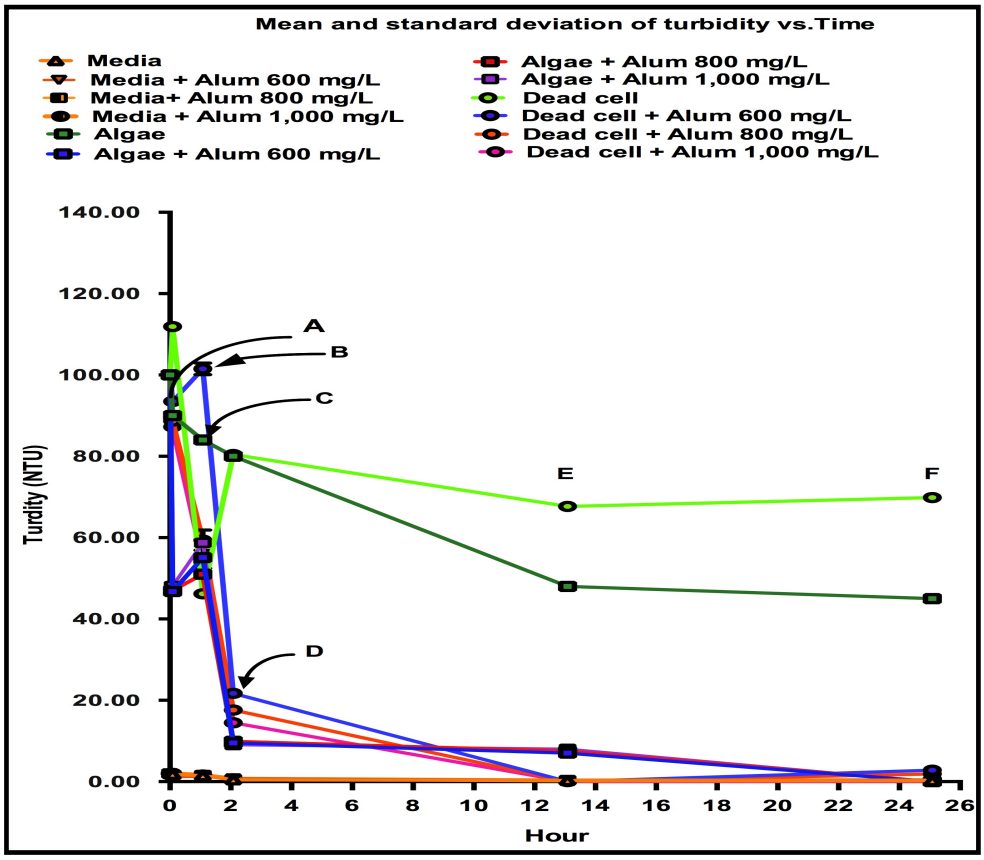


Figure 6-5 The relationship between turbidity and time during the settlement of living and dead algal suspensions treated with various levels of alum. A-E are the sampling points listed in section 6.5.2. Error bars (standard deviation) are very small and do not show up (0.000 -1.508).

Figure 6-6 shows the turbidity measurements in more detail before adding alum (Point A), 5 min after adding alum (Point B) and after 1 hr. stirring (Point C). The results show a difference between live and dead cells. Live cells show a rapid drop in turbidity in the first 5 minutes followed by a very slight rise during stirring (from 100 NTU to 48 NTU). Dead cells show a much smaller initial drop in turbidity (around 100 NTU to 84 NTU) followed by a continuing drop for 800 and 1,000 mg/L alum additions (17.61 $\pm$ 0.424 and 14.46 $\pm$ 0.633 NTU).

The lowest alum addition results in a slight rise similar (but at a different turbidity starting point) to live cells. At the end of the one hour stirring time (point C), all the suspensions with alum additions are at a similar turbidity except for the dead cells with 600 mg/L alum which has a higher turbidity (101.49 $\pm$ 1.445 NTU). This falls rapidly over the first hour of settling (to point D) and, with the turbidity of other treated cultures

falling, but not as rapidly, the turbidity levels at point D are all similar at around 20 NTU while both the live and dead cell initial controls are at 100 NTU.

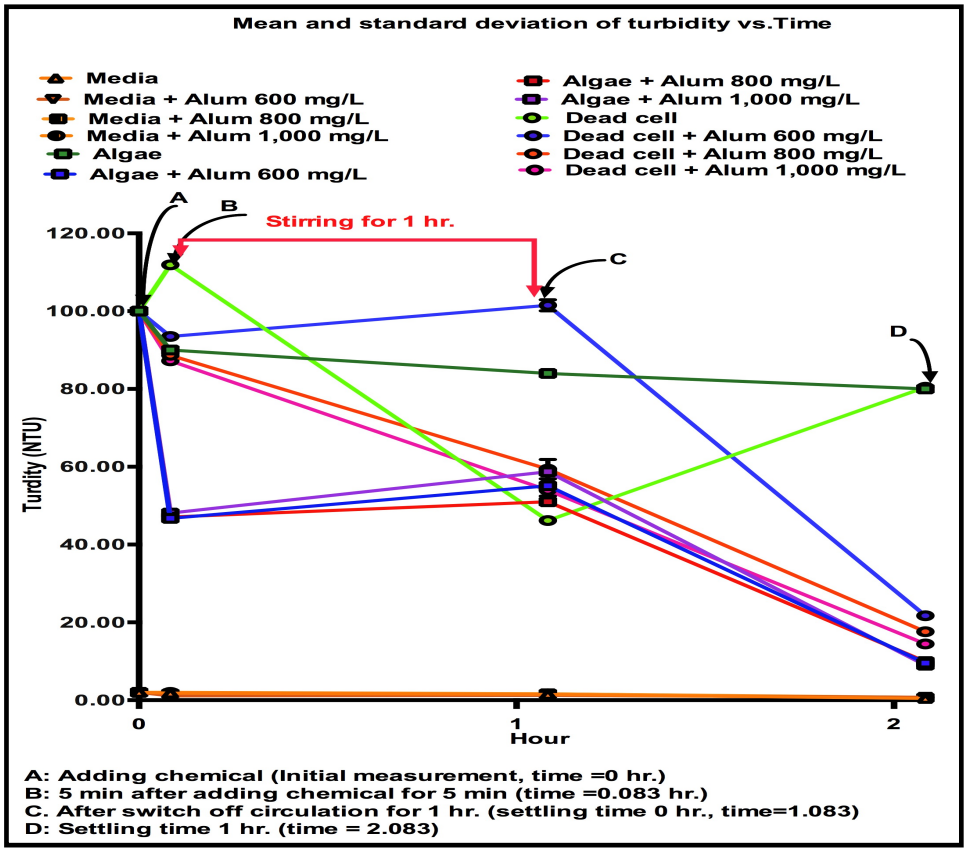


Figure 6-6 Turbidity over the time period 0-2.083 hr. for live and dead algal cell suspensions treated with various amounts of alum. This detail shows the time line before adding chemical (timeline = 0), 5 min after adding the chemical (timeline =0.083 hr.), after 1hr. of stirring (timeline = 1.083), and one hour of settling (timeline = 2.083 hr.).

2) Ferric chloride

The study shows turbidity measurements of algal cell suspensions after adding various amounts of ferric chloride. At 1,000 mg/L of ferric chloride, the turbidity initially rises (from 100 NTU to 128.00 ± 0.018 NTU). In contrast, at 600 mg/L it is nearly stable (100.20 ±0.015), and at 800 mg/L the turbidity declines from the outset (**Figure 6-7**).

While the turbidity for 600 and 800 mg/L additions has already declined and remains relatively stable, the turbidity for 1,000 mg/L addition rapidly declines to very low levels after settling time of 12 hours (13.22 ± 2.127) and to nearly zero after 24 hr. (0.124±0.017).

While there is considerable variation in the first hour (before and after stirring, see **Figure 6-8** for detail); during settling there is a distinct difference in behaviour between live and dead cells. At the start of stirring the turbidity of live cell suspensions is high and then falls rapidly in the first 12 hours while the turbidity of dead cells has already fallen and remains roughly stable over time. The result is that the turbidity after 24 hours of settlement is much lower (near to zero) for the live cells while that for dead cells is around 35 NTU. The overall standard deviation for each of the measurements is in the range of 0.01-0.15 NTU unit.

When looking at the first two hours data in more detail (**Figure 6-8**) there is no discernable trend apart from the dead cells ending up with a lower NTU than live cell cultures.

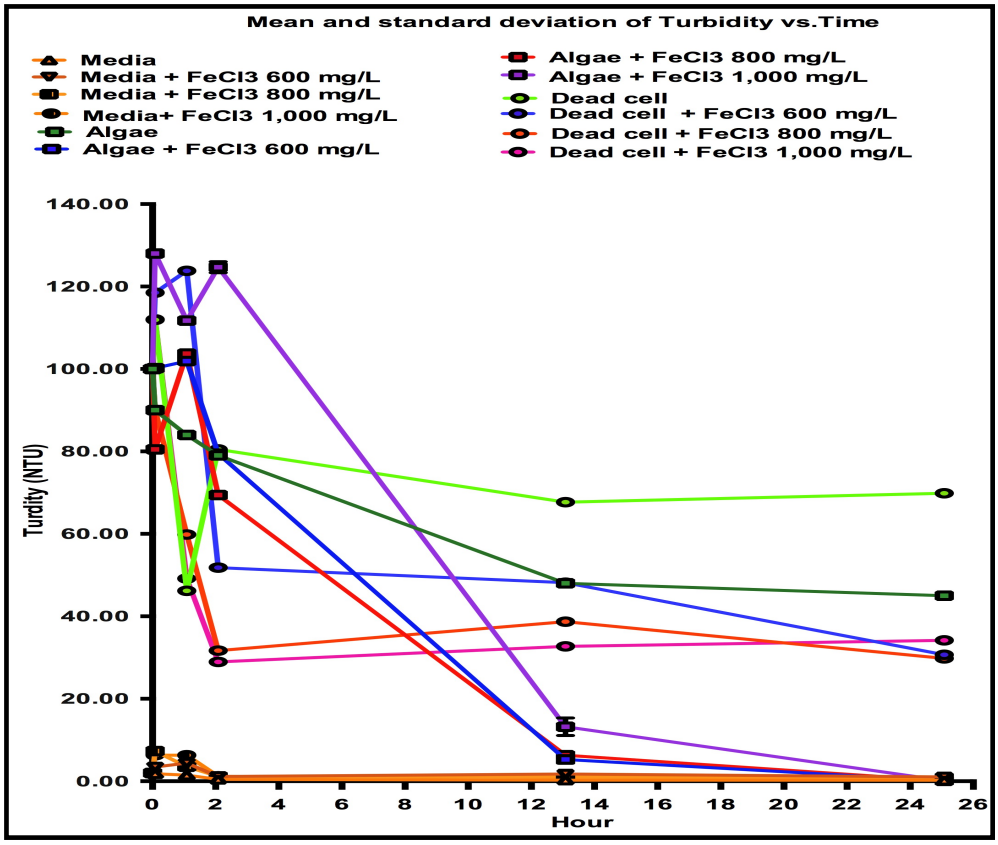


Figure 6-7 Mean and standard deviation of turbidity with time for the settlement of algal cell suspensions dosed with ferric chloride.

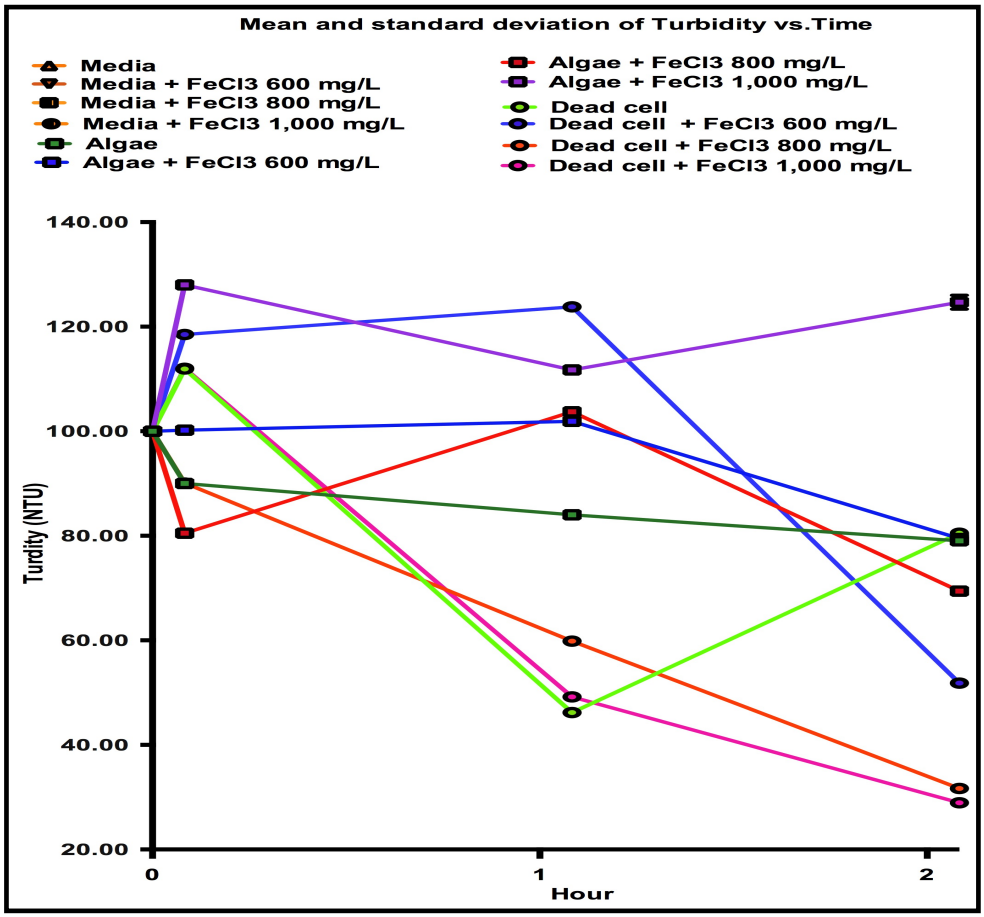


Figure 6-8 Detail of the mean and standard deviation of turbidity with time (0-2.083 hr.) for the settlement of algal cell suspensions dosed with ferric chloride.

3) Aluminium chloride

Figure 6-9 and **Figure 6-10** illustrate that, on addition of aluminium chloride, turbidity falls sharply between 0 and 2.083 hr. (from 100 NTU to around 1-3 NTU). Again there is an initial rise in turbidity for the dead cell suspensions which is not seen in the live cultures. By 2 hr. the turbidity of live cell cultures is close to zero and remains so for the rest of the experiment. The turbidity of dead cell suspensions falls rapidly (from an initial high) over the first hour of settling then more gradually over the next 12 hours and either keeps falling (for the two higher doses) or remains stable – even increasing a little – for the lowest dose (**Figure 6-9**).

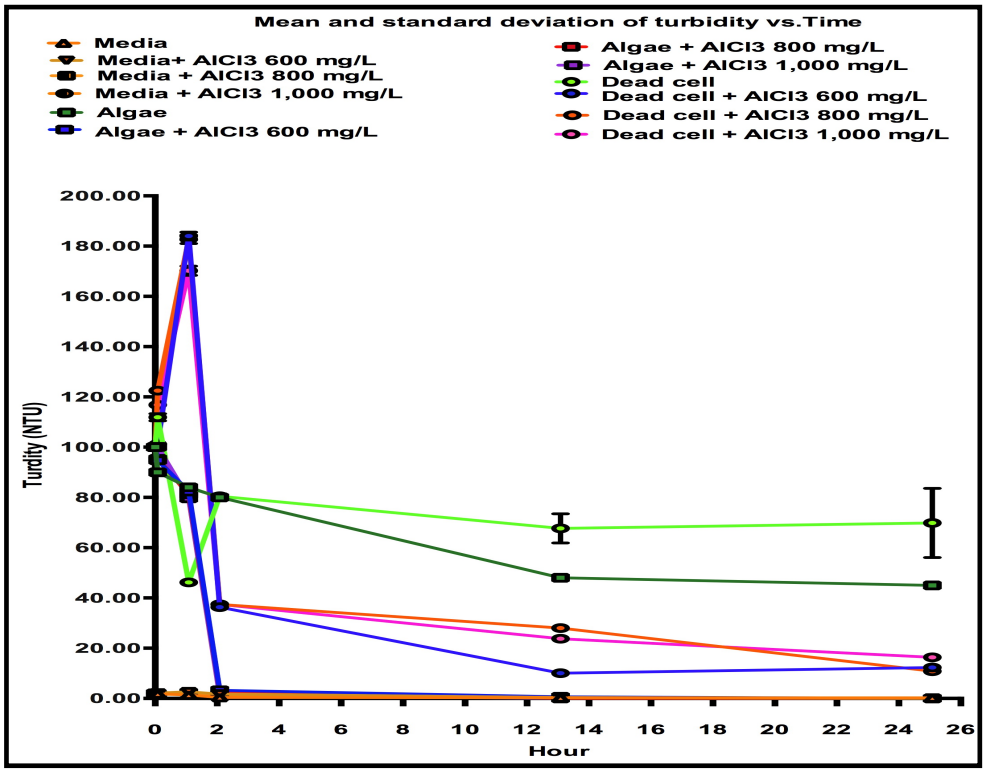


Figure 6-9 Mean and standard deviation of turbidity with time for the settlement of algal cell suspensions dosed with aluminium chloride.

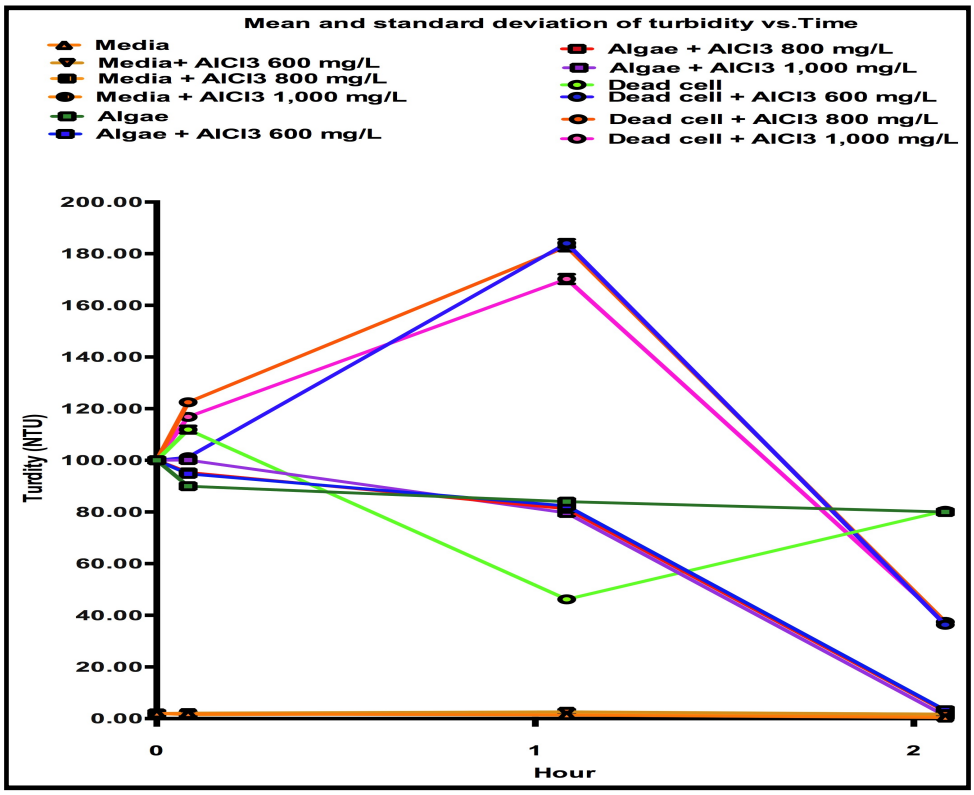


Figure 6-10 Detail of the mean and standard deviation of turbidity with time (0-2.083 hr.) for the settlement of algal cell suspensions dosed with aluminium chloride.

4) MW (medium molecular weight) chitosan

The data (**Figure 6-11**) shows that, 5 min after adding MW chitosan to algal suspensions at levels of 600 and 800 mg/L, the turbidity falls from 100 NTU to 80.07± 0.0186 and 83.03 ± 0.170 NTU. Dosing with 1,000 mg/L only gives a slight decline to 94.97± 1.943 NTU. For dead cell suspensions, adding 600, 800, and 1,000 mg/L MW chitosan results in values of 94.20±0.456, 90.04±0.131, 90.78 NTU ±0.233 respectively after 5 minutes.

Algal suspensions with no MW chitosan declined to 90.01 ±0.098 NTU. In contrast, the turbidity of dead cell suspensions with no added chitosan increases to 111.91±0.126 NTU (see **Figure 6-11** and **Figure 6-12**). When stirring is stopped, all cultures containing algae, dead or alive, treated or untreated are between 80-90 NTU. The only exception is for dead cells without any added chitosan where the turbidity is less than 50 NTU.

After 12 hr. (**Figure 6-11**) the turbidity of algal cell suspensions with added chitosan (600-1,000 mg/L) drops to between 55.52-56.70 NTU for living cells and 36 NTU for dead cells with standard deviations of between 0.11-1.959 NTU units. With no added chitosan, dead cells, originally at 67.69± 0.150 NTU drop to 48.00± 0.530 NTU.

It is between 12 and 24 hours that the most dramatic changes take place. After settling time of 24 hr., algae and dead cell controls (with no added MW chitosan) are stable at 45.00±0.136 NTU and 69.85±0.179 NTU respectively. The dead cell suspensions with additions of chitosan of 600, 800, and 1,000 mg/L are stable with means of 33.07±0.226, 33.62±0.057, 36.96±1.067 NTU respectively. However, living algal suspensions with additions of 600, 800, and 1,000 mg/L MW chitosan show a drop in turbidity to 7.070, 6.140, 6.480 NTU (standard deviations between 0.11-0.673 NTU). Media and media with added chitosan are close to zero throughout.

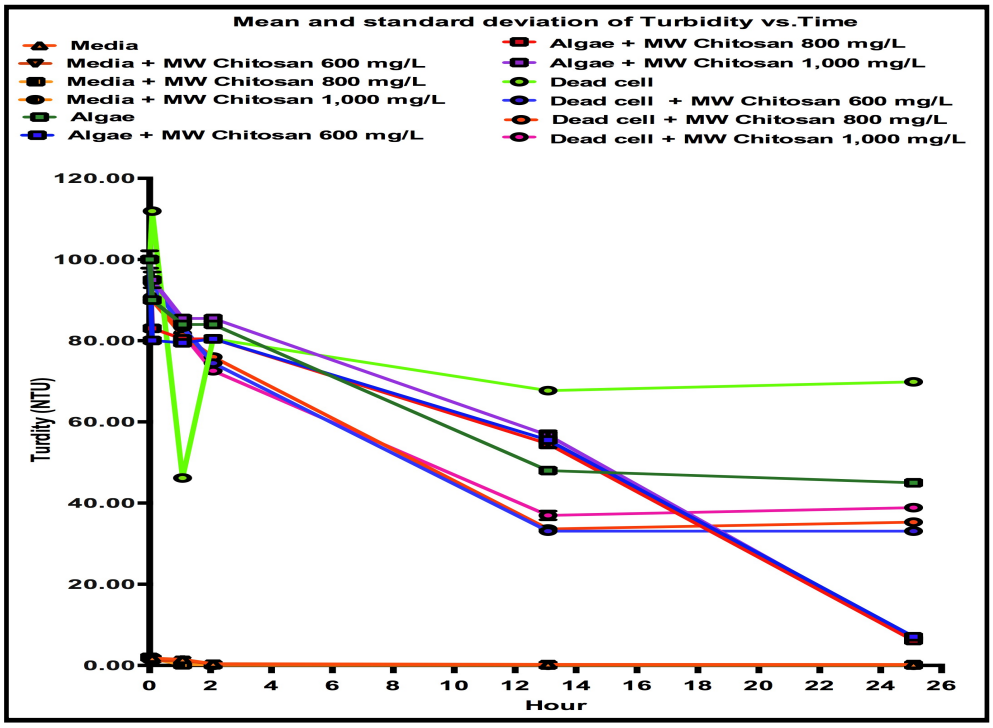


Figure 6-11 Mean and standard deviation of turbidity with time for the settlement of algal cell suspensions dosed with MW chitosan. Note that the plots of live algae with various additions of chitosan are overlaid.

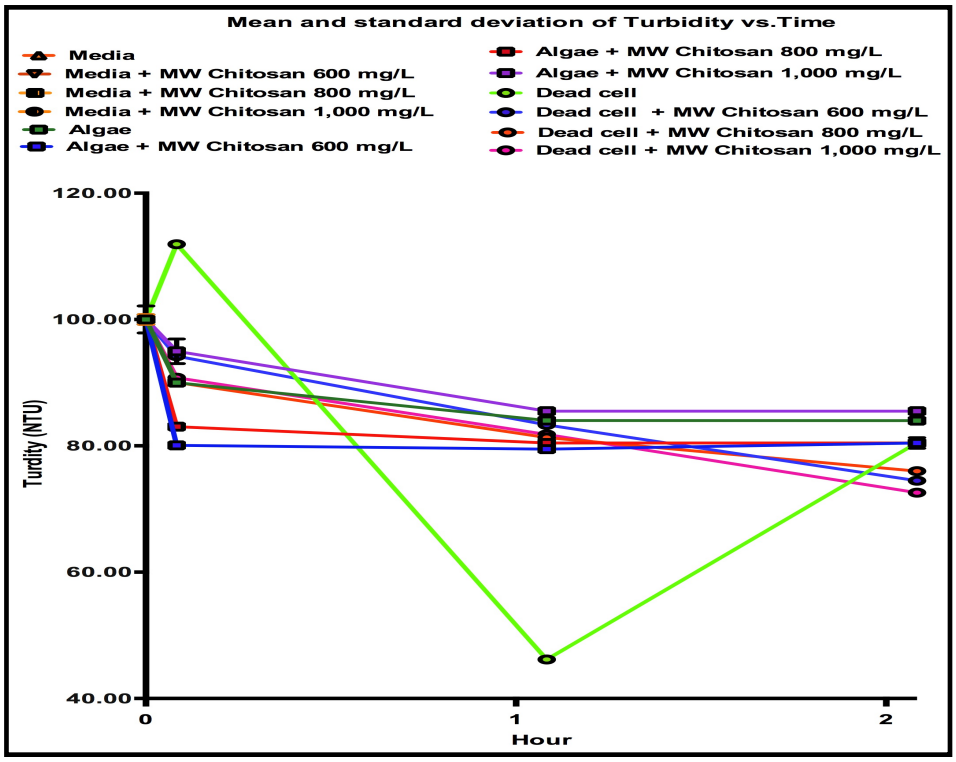


Figure 6-12 Detail of the mean and standard deviation of turbidity with time (0 - 2.083 hr.) for the settlement of algal cell suspensions dosed with MW chitosan.

5) High molecular weight (HW) chitosan

The turbidity and optical density measurements for algal cultures treated with high molecular weight chitosan are shown in **Figure 6-13** and, in more detail, in **Figure 6-14.** After the first 2 hours of variable turbidity the sinking rate, as measured by the decline in turbidity, up to 12 hours is similar for both live and dead cells (though starting from different levels of turbidity at 2 hours). The turbidity for live cells continues to fall at about the same rate between hours 12 and 24 with very similar results for all levels of HW chitosan addition. However, the turbidity for dead cell suspensions tends to plateau. The overall result is that most live and dead cell suspensions end up at a similar turbidity (around 50 NTU – the outlier being dead cell suspensions with 1,000 mg/L HW chitosan which end up at about 35 NTU). As algae without addition of any HW chitosan actually out perform live cells with added chitosan this is in marked contrast to the effects of MW chitosan and illustrates the need to select the type of chitosan carefully.

When looking in detail at the first two hours (stirring and the first hour of settling) it is difficult to see any trends. The live cell cultures tend to be stable during stirring and their turbidity rises during the first hour of settlement.

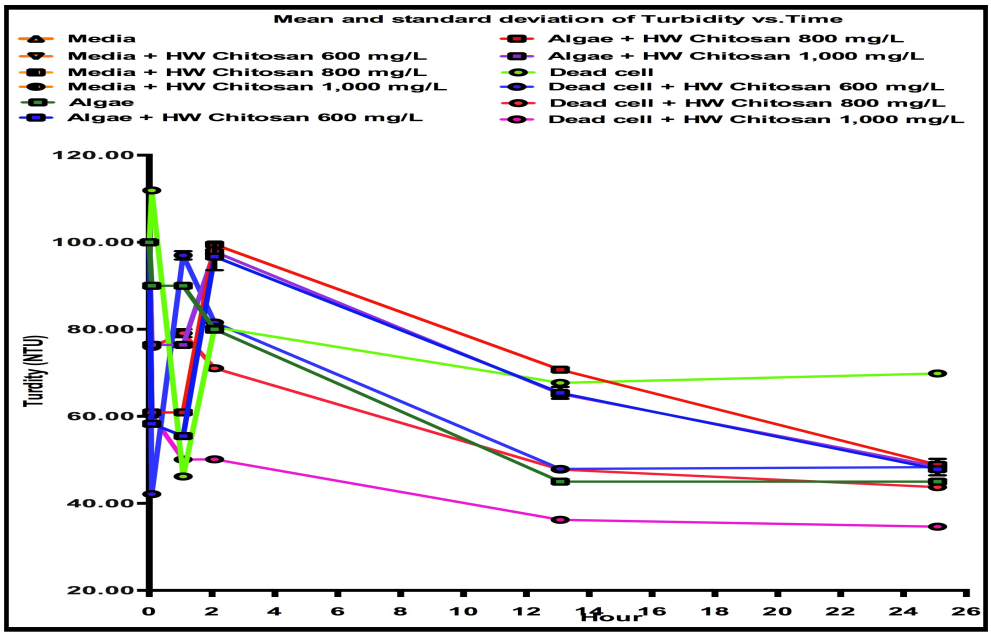


Figure 6-13 Mean and standard deviation of turbidity with time for the settlement of algal cell suspensions dosed with HW Chitosan.

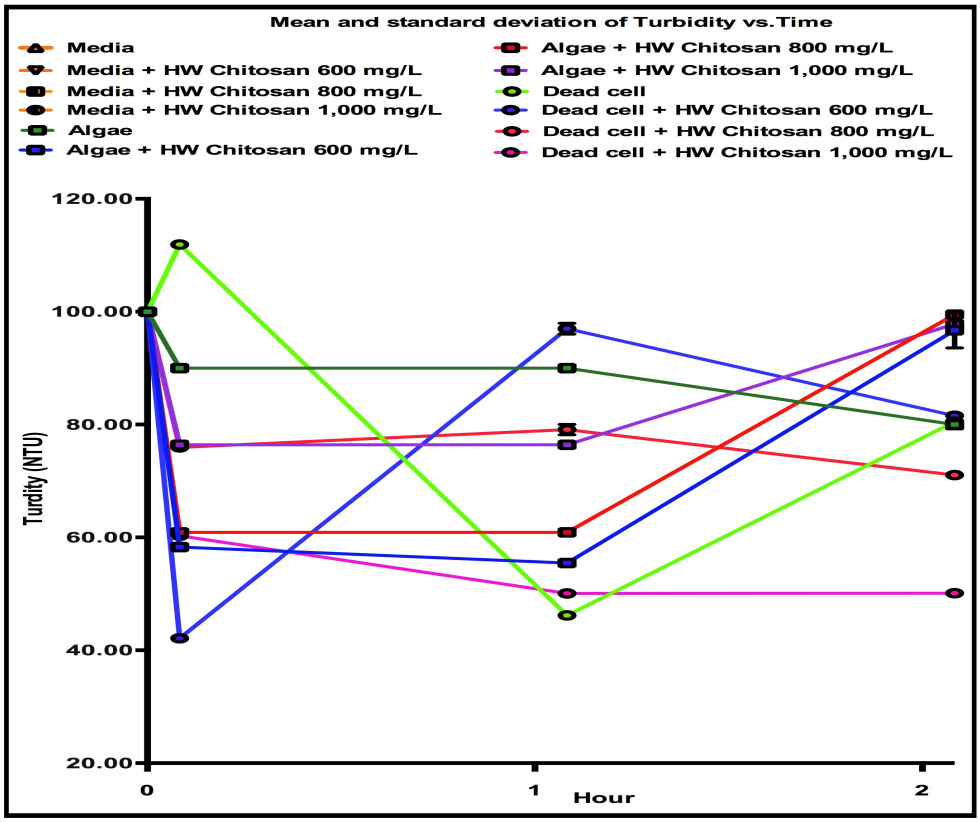


Figure 6-14 Detail of mean and standard deviation of turbidity with time (0-2.083 hr.) for the settlement of algal cell suspensions dosed with HW Chitosan.

6) Crab shell

Turbidity results are shown in **Figures 6-15** and **6-16.** In the first two hours dead cells with the highest loading of crab shell show an increase in turbidity and these stay the highest. Live cells show an increase in turbidity during the first hour of settling while dead cells show a fall in turbidity.

Between the settling times of 1 and 12 hr. all the experiments show a decline in turbidity to around the same point with dead cells without crab shell and dead cells with 1,000 mg/L crab shell a little higher. After 12 hours living cells show a continued decline to near zero while the others tend to be stable. Both media control and media with added crab shell remain at near zero for the whole experiment. The finding that the best settlement and a near zero turbidity after 24 hours is with living cell suspensions could be useful for an industrial application both as a low cost means of encouraging settlement and as a means of separating live from dead cells.

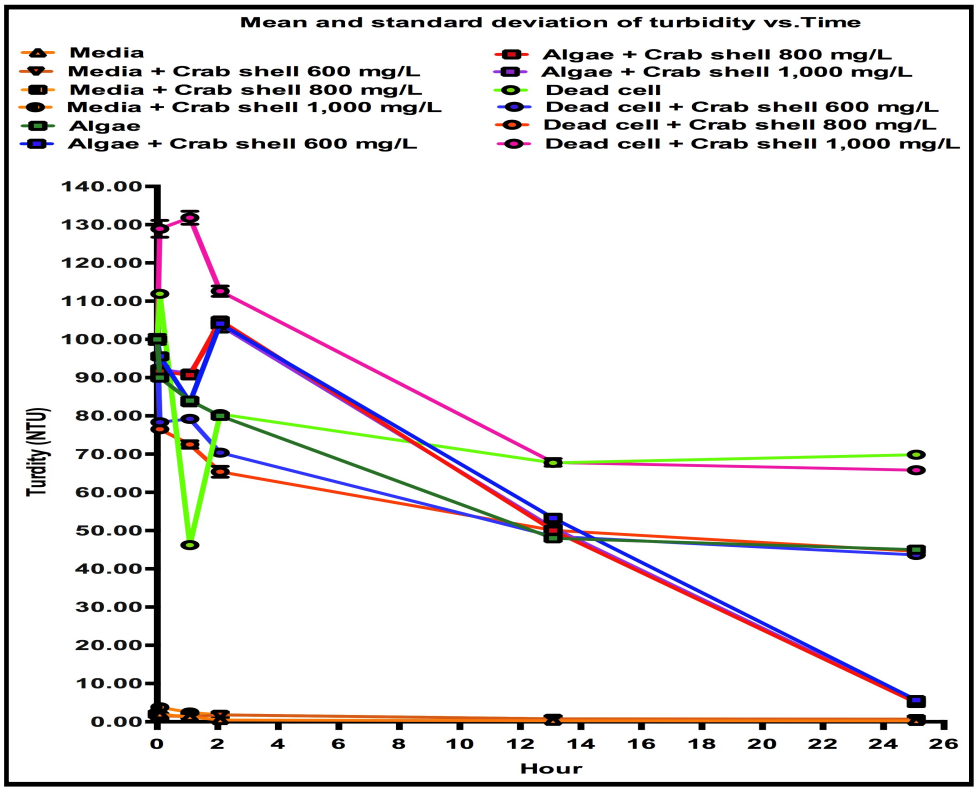


Figure 6-15 Mean and standard deviation of turbidity with time for the settlement of algal cell suspensions dosed with crab shell.

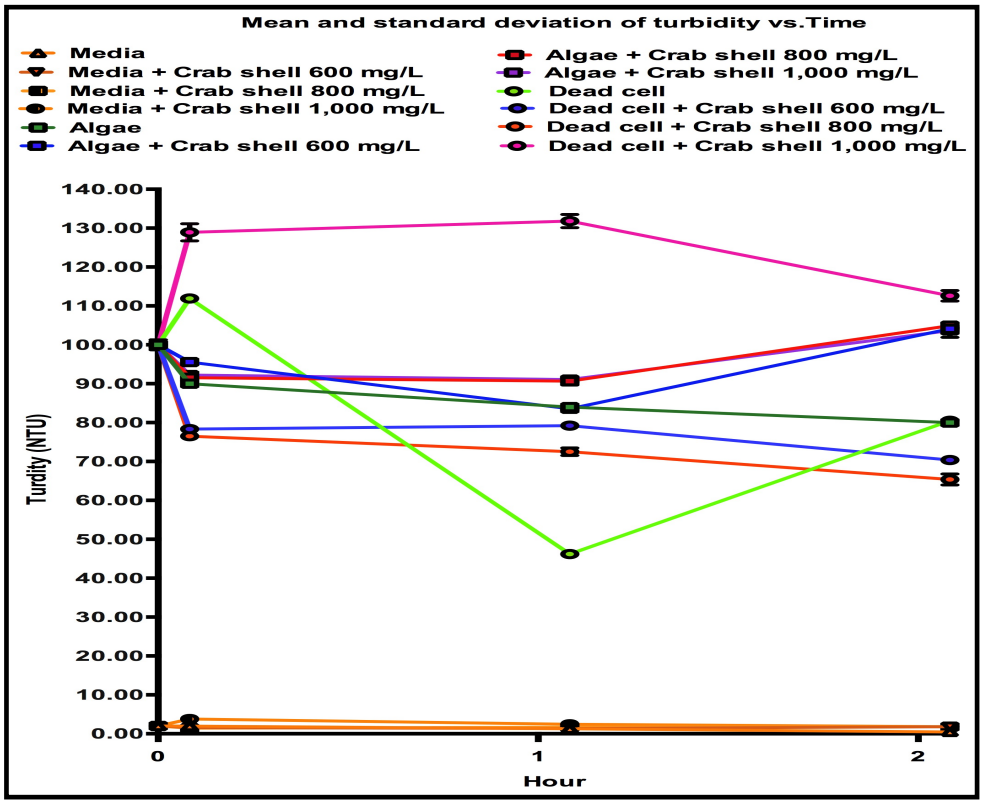


Figure 6-16 Detail of Mean and standard deviation of turbidity with time (0-2.083 hr.) for the settlement of algal cell suspensions dosed with crab shell.

6.3.2.2 Optical density measurements

1) Aluminium sulphate anhydrate (Alum)

Figure 6-17 shows the results from the same experiments but measured by optical density (OD_{680}). The data shows that the optical density of algal suspensions dramatically decline after dosing with 600, 800, 1,000 mg/L of alum, stirring and leaving to settle for 1 hr. (0.136 ± 0.017, 0.026 ± 0.046 and 0.235 ± 0.000). At 12 hr. and 24 hr. the OD_{680} of the suspensions is close to zero. With both living and dead cells, the addition of alum causes a much more rapid sedimentation than cells suspensions without the flocculant, though it is interesting to note that the difference is less marked when measuring by OD than by turbidity (**Figure 6-5**). The OD of dead cells is lower than for living algal cells. Both gradually decrease with longer settling times but living cells are still not fully sedimented after 24 hr.

Like **Figure 6-17, Figure 6-18** shows the results of optical density measurements between 0-2.083 hr. As would be expected, the results show similar trends as for turbidity, both with time and between live and dead cells and alum additions when compared to controls.

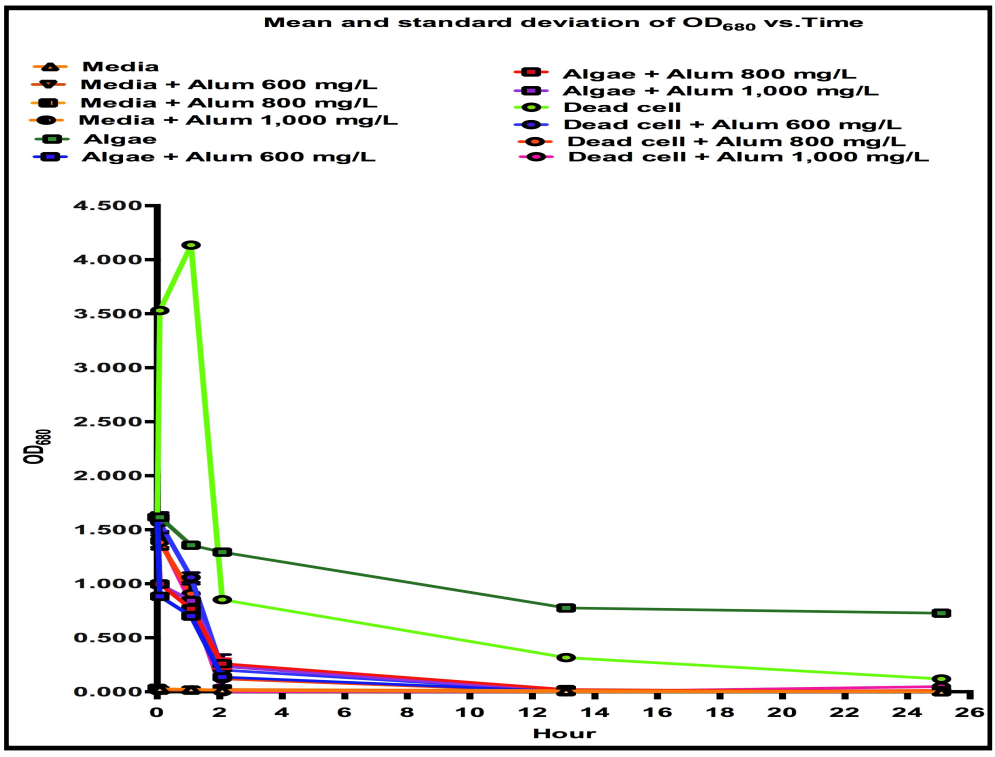


Figure 6-17 The relationship between optical density (OD_{680}) and time for living and dead algal suspensions with various levels of added alum.

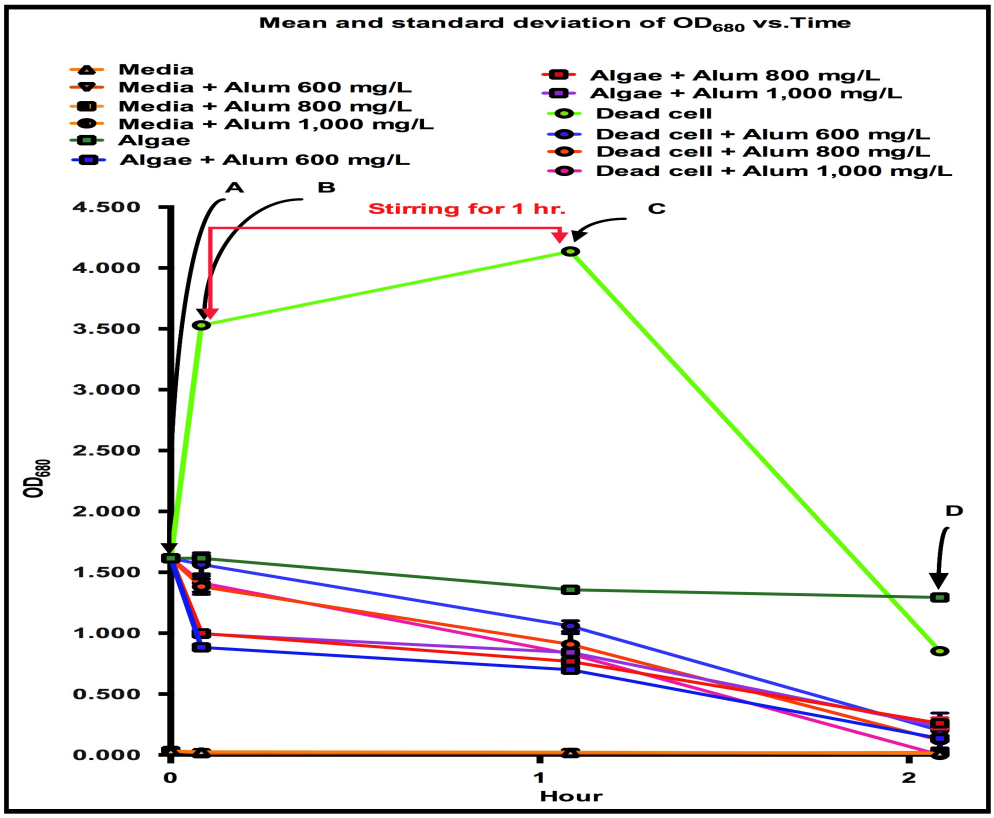


Figure 6-18 The relationship between optical density (OD_{680}) and time (between 0 -2.083 hr.) for living and dead algal suspensions with various levels of added alum.

2) Ferric chloride

While the overall trend of optical density measurements (**Figure 6-19)** is similar to **Figure 6-7** there is difference in the detail with the treated dead cells showing higher optical density than live algal suspensions at 2 hours and then remaining relatively stable for the rest of the experiment (though with a large SD after 24 hours settling) while the live cells, starting from a lower OD show a decline to zero after 12 hours settling and remain at around zero for the rest of the experiment. An addition of between 800 and 1,000 mg/L of ferric chloride is required to ensure good and rapid sedimentation.

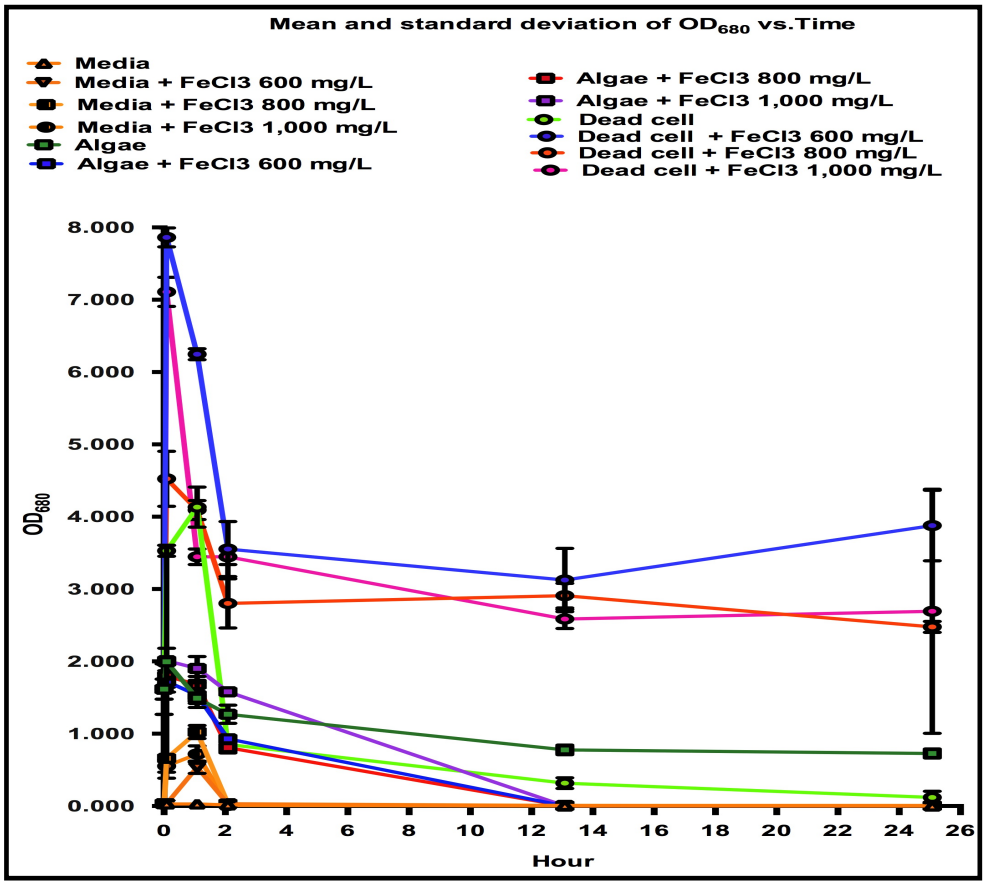


Figure 6-19 Mean and standard deviation of optical density (OD_{680}) with time for the settlement of algal cell suspensions dosed with ferric chloride.

When looking at the first two hours in detail (addition of ferric chloride, stirring and the first hour of settlement (**Figure 6-20)** it can be seen that, while there is a small rise in OD on adding ferric chloride to the live cell suspensions similar to the medium control, there is a considerable rise in OD for dead cell suspensions and while the OD remains essentially the same before and after stirring for live cells, there is a drop in OD during stirring for dead cells. This initial increase in OD for dead cells is dependent on the amount of ferric chloride added. Somewhat surprisingly, the less $FeCl_3$ added, the greater the rise in OD.

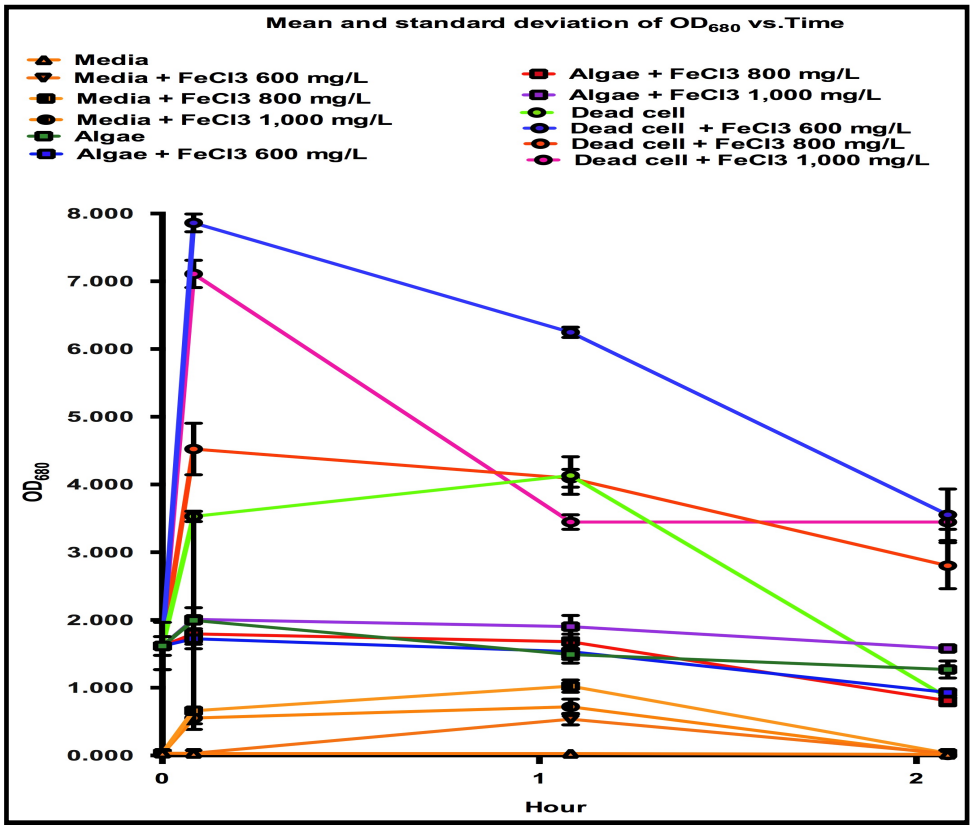


Figure 6-20 Detail of the mean and standard deviation of optical density (OD_{680}) with time (between 0-2.083 hr.) for the settlement of algal cell suspensions dosed with ferric chloride.

3) Aluminium chloride

The mean and standard deviation of optical density (OD_{680}) vs time (**Figure 6-21**) and the detail for addition of aluminium chloride, stirring and the first hour of settlement (**Figure 6-22)** shows considerable fluctuations over the first two hours (ending up at near zero for living cell suspensions). Those (dead cell) suspensions that are not at zero by this time fall to near zero after 12 hours (**Figure 6-21**) and remain at zero or just above for the rest of the experiment. The exception is the lowest aluminium chloride addition (600 mg/L) to the dead cell suspensions which, while showing a fall over the first 12 hours of settling, does not reach zero and then shows a rise to the same OD as untreated dead cells at 24 hours.

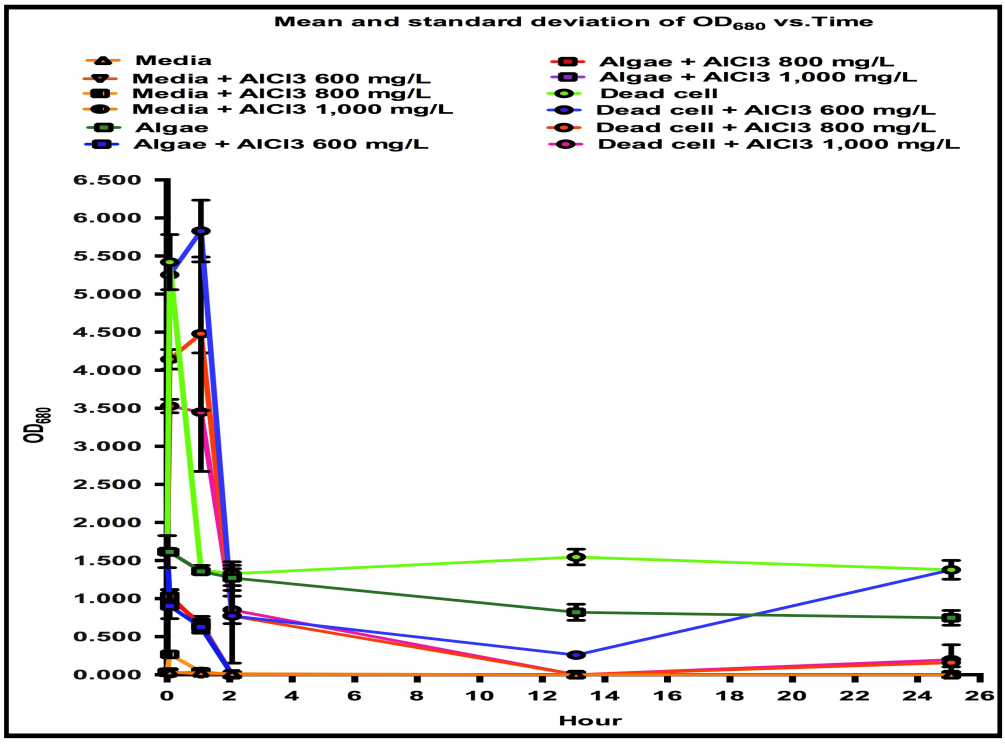


Figure 6-21 Mean and standard deviation of optical density (OD_{680}) with time for the settlement of algal cell suspensions dosed with aluminium chloride.

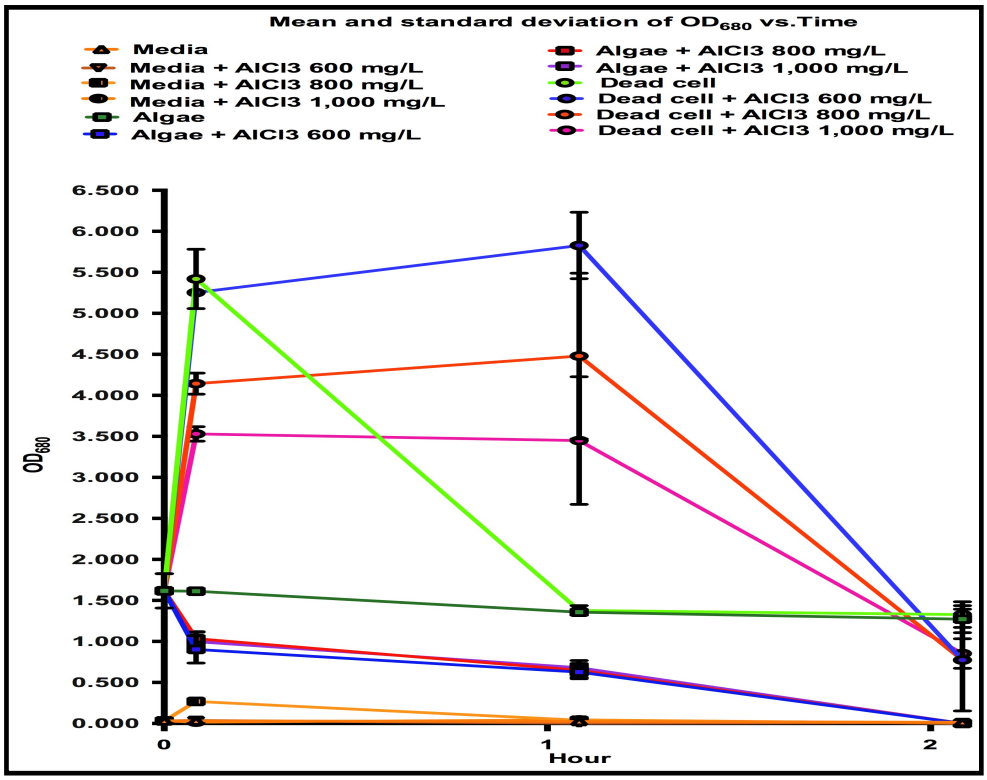


Figure 6-22 Detail of mean and standard deviation of optical density (OD_{680}) with time (0-2.083 hr.) for the settlement of algal cell suspensions dosed with aluminium chloride.

Looking at the first two hours in detail, while the OD of the living cells drops slightly after 5 minutes, the OD of dead cells increases markedly (5.253± 0.000), even for the control which has no added aluminium chloride. This may indicate that the initial stirring caused components of the dead cells to break up increasing the OD.

However, while the OD of the dosed dead cell suspensions remains at these high levels during stirring, the control rapidly falls back to the OD of un-dosed living algal cells and these controls are the same after 1 hour settling. During stirring, the OD of live cell suspensions continues to fall and does so over the first hour of settling. At the one hour settling point (timeline = 2.083) the undosed algal controls have the highest OD at around 1.272± 0.165 for the living algae and 1.328± 0.157 for the dead cells. The dosed dead algal cell suspensions have the next highest OD at around 1.546± 0.103 unit while, the live cell cultures are lowest with a near zero OD.

4) MW (medium molecular weight) chitosan

The results illustrated in **Figure 6-23** and in detail for the first two hours in **Figure 6-24** show similar results to turbidity measurements but with less distinction between the different groups (living and dead cells and media alone). **Figure 6-24** particularly shows the different behaviours of dead cells to which no MW chitosan has been added. The OD rapidly rises 3.529±0.048 and to 4.134 (with standard deviation of 0.018) at the end of stirring. However, after 1 hour settling the OD for these untreated dead cells is the lowest of all except media alone.

The results of turbidity and optical density measurements on adding chitosan show the same trends. The algae gradually sink over the first 12 hours after stirring is stopped. However it is only the cultures to which chitosan has been added that show a continued fall to near zero at 24 hours.

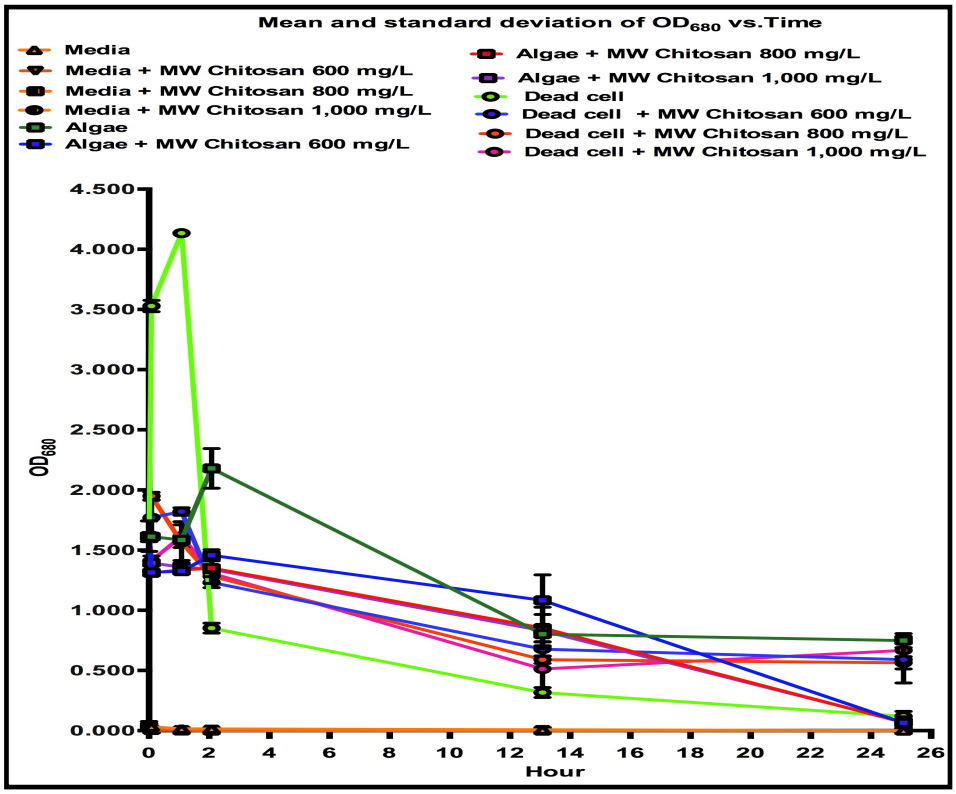


Figure 6-23 Mean and standard deviation of optical density (OD_{680}) with time for the settlement of algal cell suspensions dosed with MW Chitosan.

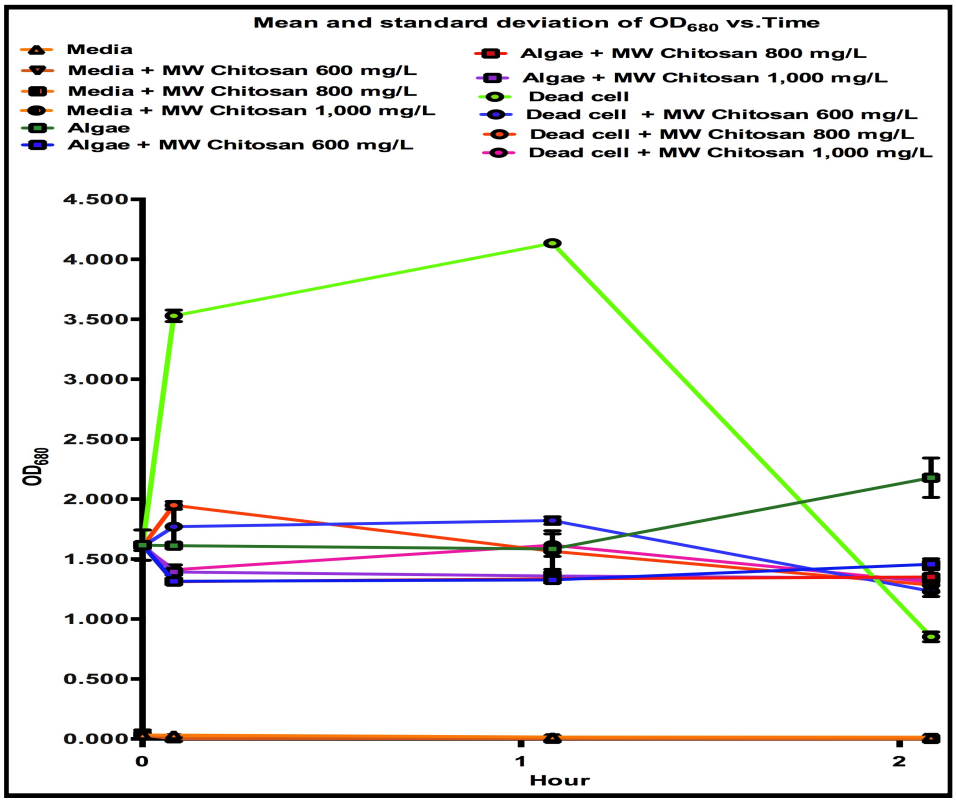


Figure 6-24 Detail of the mean and standard deviation of optical density (OD_{680}) with time (0-2.083 hr.) for the settlement of algal cell suspensions dosed with MW Chitosan.

5) High molecular weight (HW) chitosan

The results of optical density at 680 nm. show that, 5 min after adding high molecular weight chitosan, living algal cell suspensions decline in OD from 1.616 to 1.000-1.500 and remain stable during stirring. This is followed by an increase in OD for live cell suspensions and a slight decrease in OD for dead cell suspensions for the first hour of settlement (4.135). During this time, untreated dead cells, which showed a considerable increase in OD during the first 5 minutes and during stirring show a rapid fall in OD to the level of the lowest treated dead cell suspensions. During the remainder of the settling time, the OD of untreated dead cells falls steadily to near zero after 24 hours while all the other cultures fall or remain stable ending in the range 0.637-0.870. There is no difference between the settlement of untreated live cell suspensions and those treated with any of the three levels of HW chitosan after 24 hr. settling. This reinforces the conclusion that, unlike MW chitosan, HW chitosan is not suitable as an aid to harvesting algal cells (**Figure 6-25 – 6-26**).

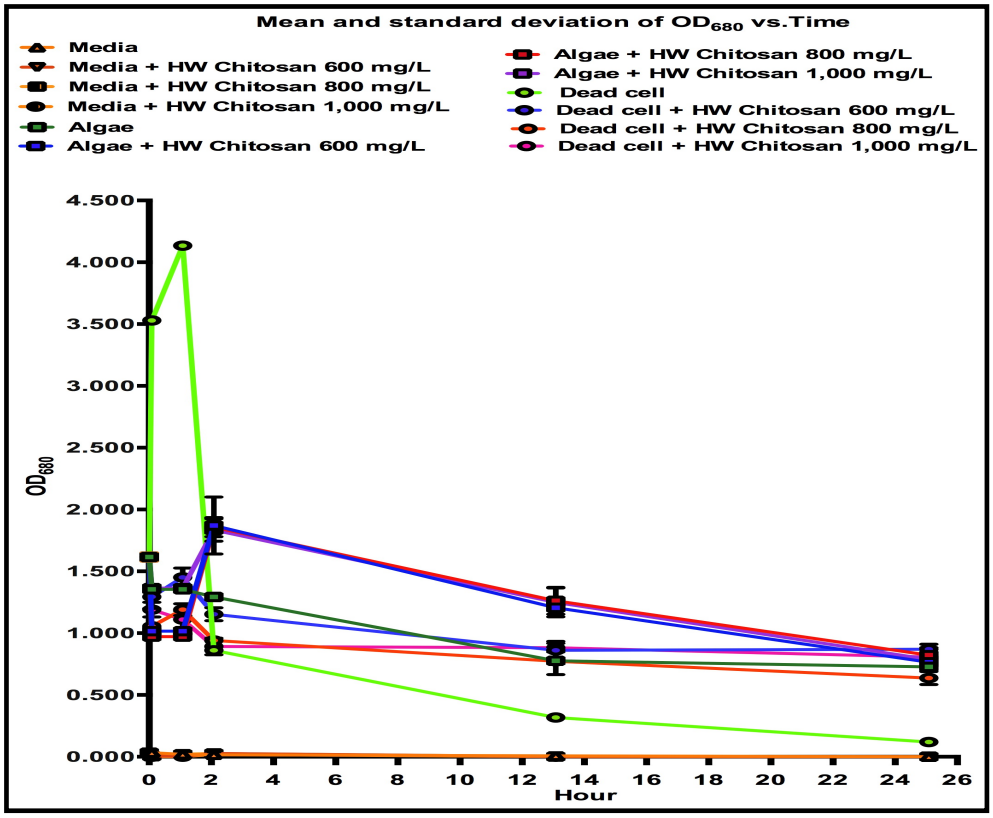


Figure 6-25 Mean and standard deviation of optical density (OD_{680}) with time for the settlement of algal cell suspensions dosed with HW Chitosan.

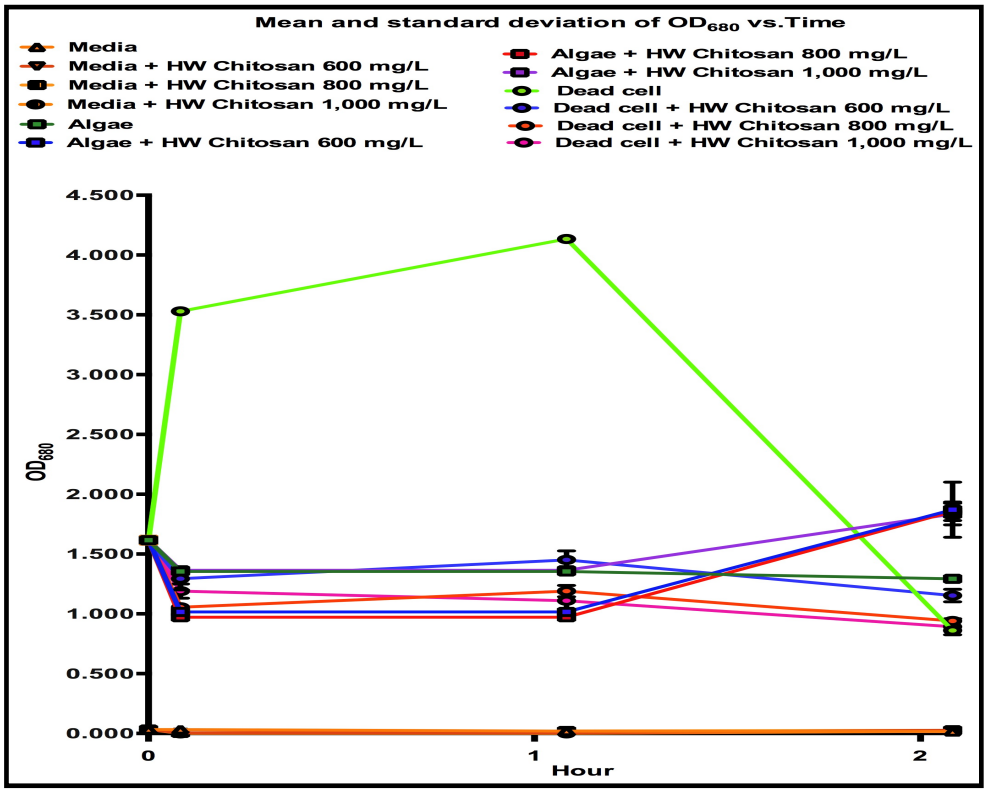


Figure 6-26 Detail of mean and standard deviation of optical density (OD_{680}) with time (0-2.083 hr.) for the settlement of algal cell suspensions dosed with HW Chitosan.

6) Crab shell

The results of optical density measurement of the effects of crab shell on the settlement of algae are shown in **Figures 6-27** and **6-28**. 5 min after adding crab shell, the OD of both living and dead cell suspensions have declined. The only exception is dead cell suspensions with added crab shell at 1,000 mg/L (OD is 2.068± 0.044). After these falls, treated dead cell suspensions, which have decreased the most, remain stable throughout mixing and the first hour of settlement while the OD of live algal suspensions rise somewhat (and the dead cell suspensions treated with 1,000 mg/L crab shell eventually fall to the same level as the treated live suspensions).

After the first hour of settling, treated live algal suspensions show a consistent fall in OD to near zero after 24 hours. The results are very similar for all the dosing levels. Dead cell suspensions are more variable and tend to be at their lowest after 12 hours settling after which they either increase or remain stable to 24 hours (0.353-0.594± 0.227-0.324).

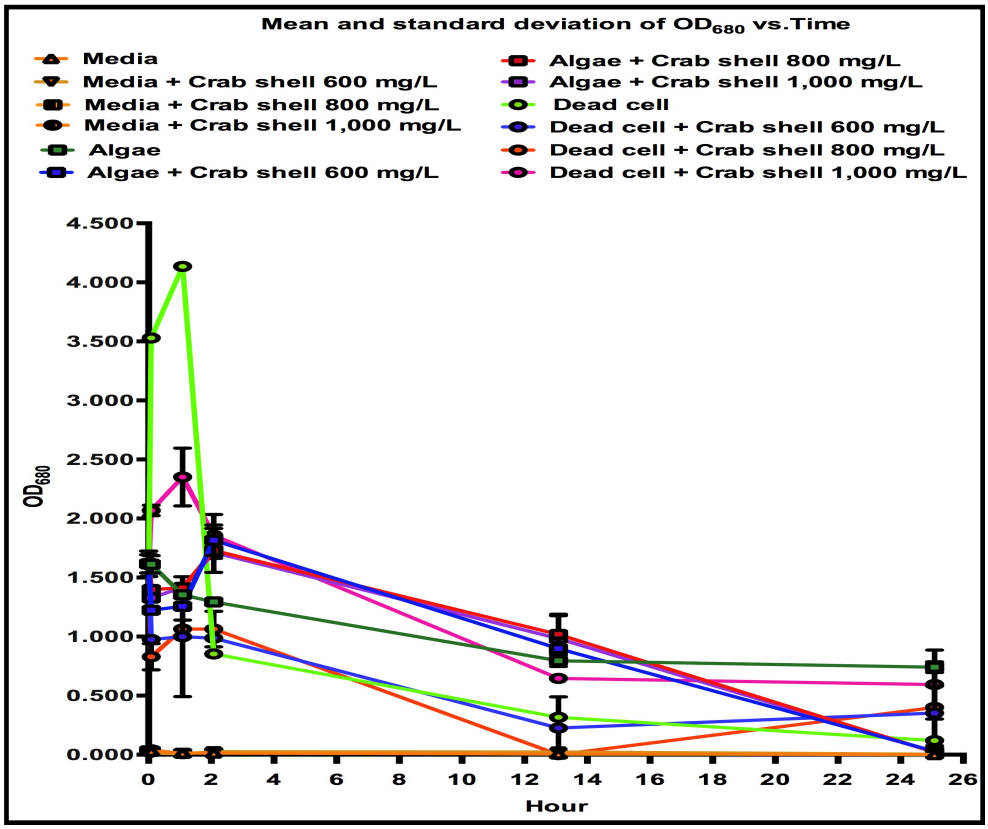


Figure 6-27 Mean and standard deviation of optical density (OD_{680}) with time for the settlement of algal cell suspensions dosed with crab shell.

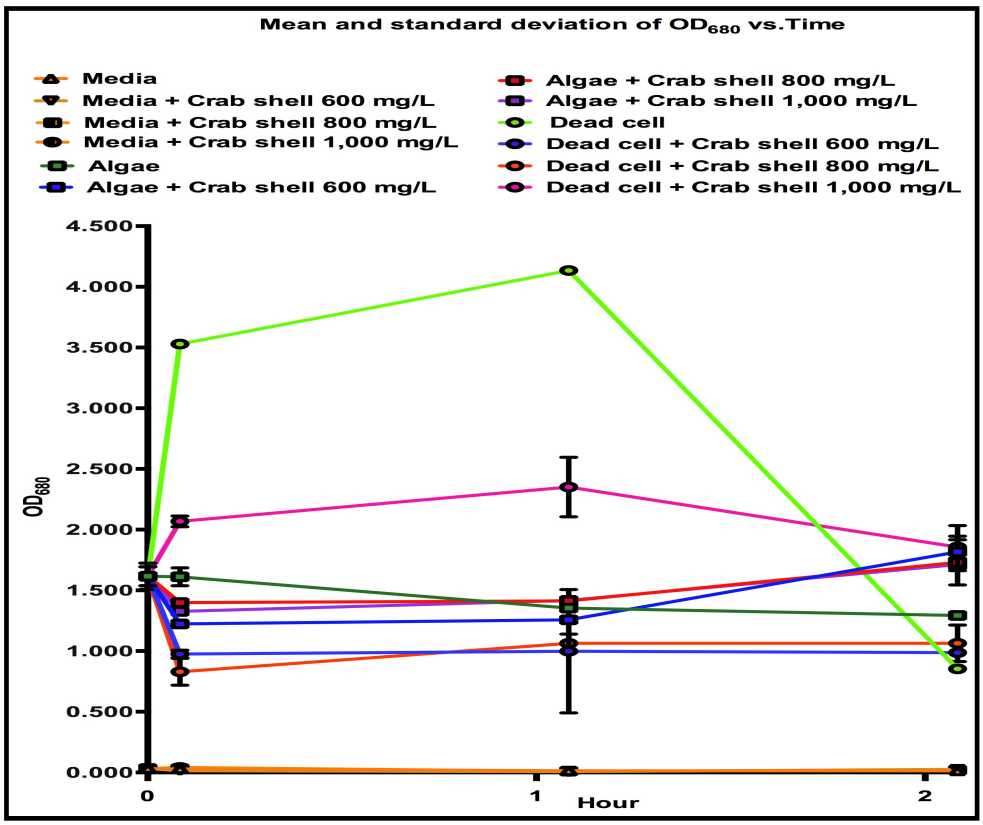


Figure 6-28 Detail of mean and standard deviation of optical density (OD_{680}) with time for the settlement of algal cell suspensions dosed with crab shell.

6.3.2.3 pH

1) Aluminium sulphate anhydrate (Alum)

Figure 6-29 shows the relationship between pH and time after adding alum. The pH drops on adding alum and stirring. For living cells, the initial average pH of 6.90 drops to between 5.08± 0.007 and 5.32 ± 0.005 while for dead cells it drops to between 3.28±0.011 and 3.42±0.005. Once stirring stops the pH remains stable for the rest of the experiment. Media, living algae, and dead cell controls (without alum dosing) show no change in pH. The standard deviations of the results are very small (0.000-0.011).

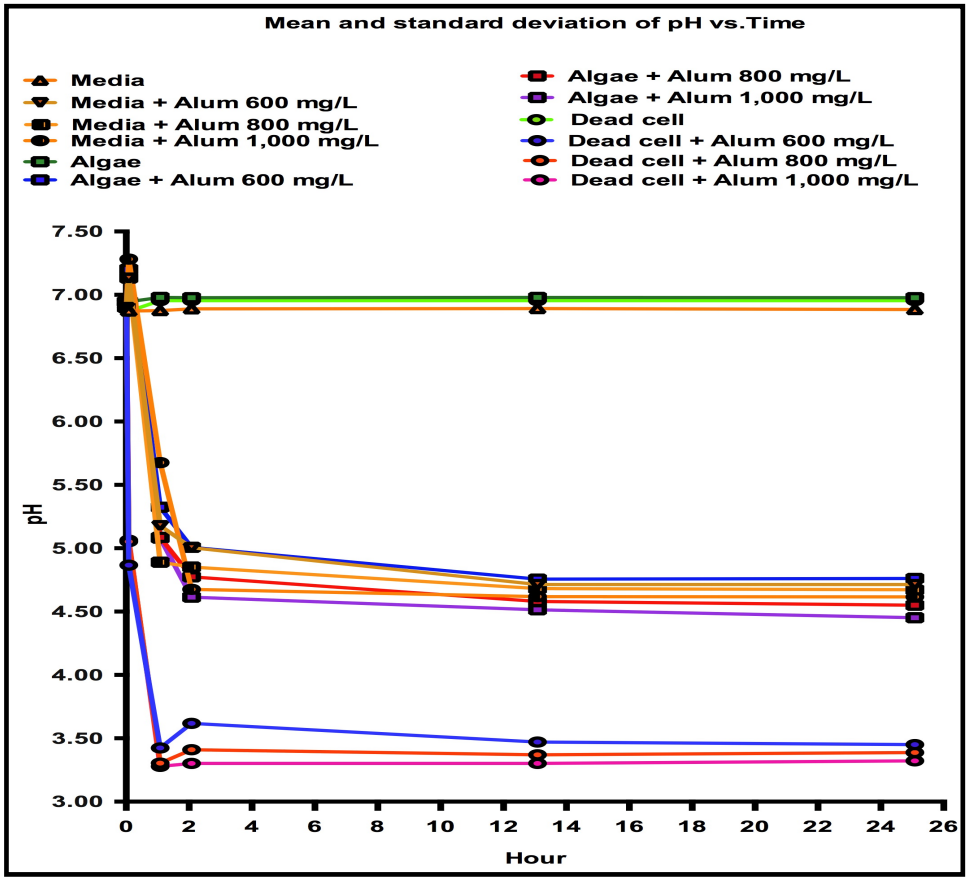


Figure 6-29 The relationship between pH and time for the addition of various amounts of alum to algal suspensions.

Figure 6-30 shows in detail the pH changes between 0-2.083 hr. 5 min after adding alum, the pH of medium alone and of living algae dosed with alum at 600, 800, and 1,000 mg/L is slightly raised from 6.90 to 7.14-7.20. Medium, algae, and dead cells without added chemical are stable at just under pH 7.00 while the pH of dead cells with added alum has dropped to around pH 5.0.

After stirring for 1 hr., the pH in all experiments, except controls with no added alum, have fallen further. The dead cells with doses of alum of 600, 800, and 1,000 mg/L give lower pH values (pH 3.28± 0.011- 3.42±0.005) than living cells (pH 5.08± 0.007- 5.32±0.005). In the first hour of settling the living algae show a small further fall in pH while the dead cells show a slight rise.

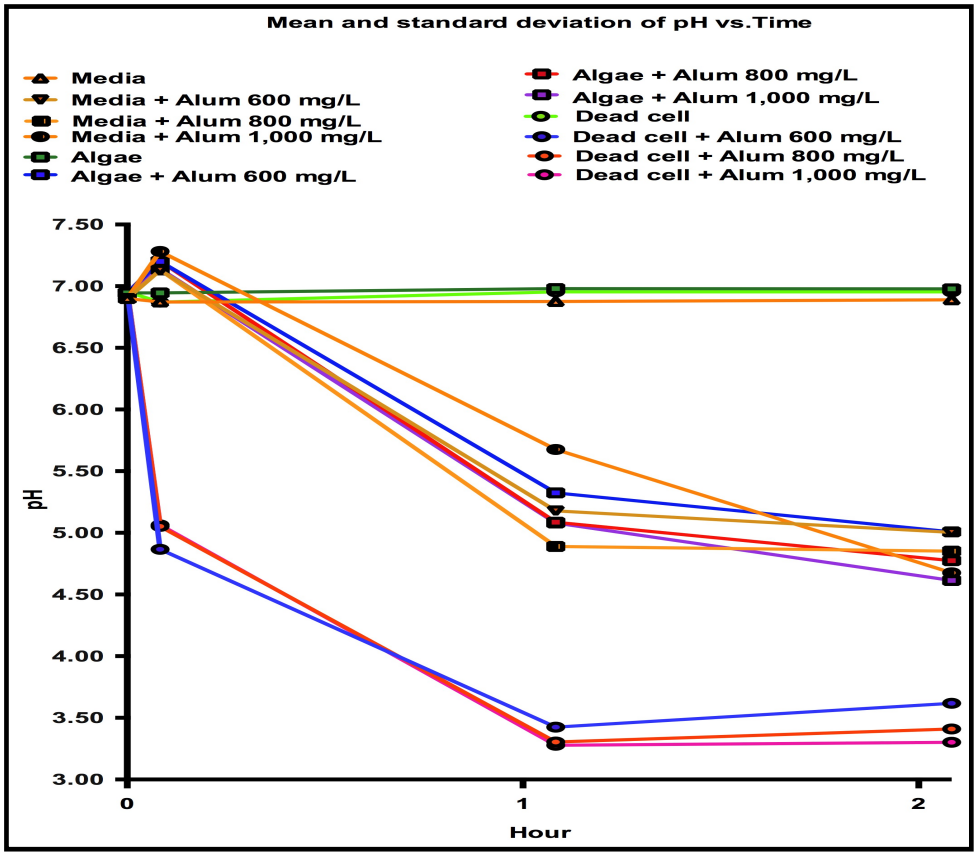


Figure 6-30 pH and time between 0-2.083 hr. when dosing alum into algal suspensions

2) Ferric chloride

Figure 6-31 shows the effect of adding ferric chloride on pH. After circulation, the pH declines from an initial value of 6.90 to 3.42-3.80 in live algal cultures dosed with ferric chloride of 600,800 and 1,000 mg/L. The standard deviation is to 0.000-0.014 and the amount of ferric chloride added makes little difference. The pH for dead cell suspensions drops to pH 1.55-1.70. The standard deviation is between 0.00-0.01. Controls of media alone and both living and dead cells show no change in pH.

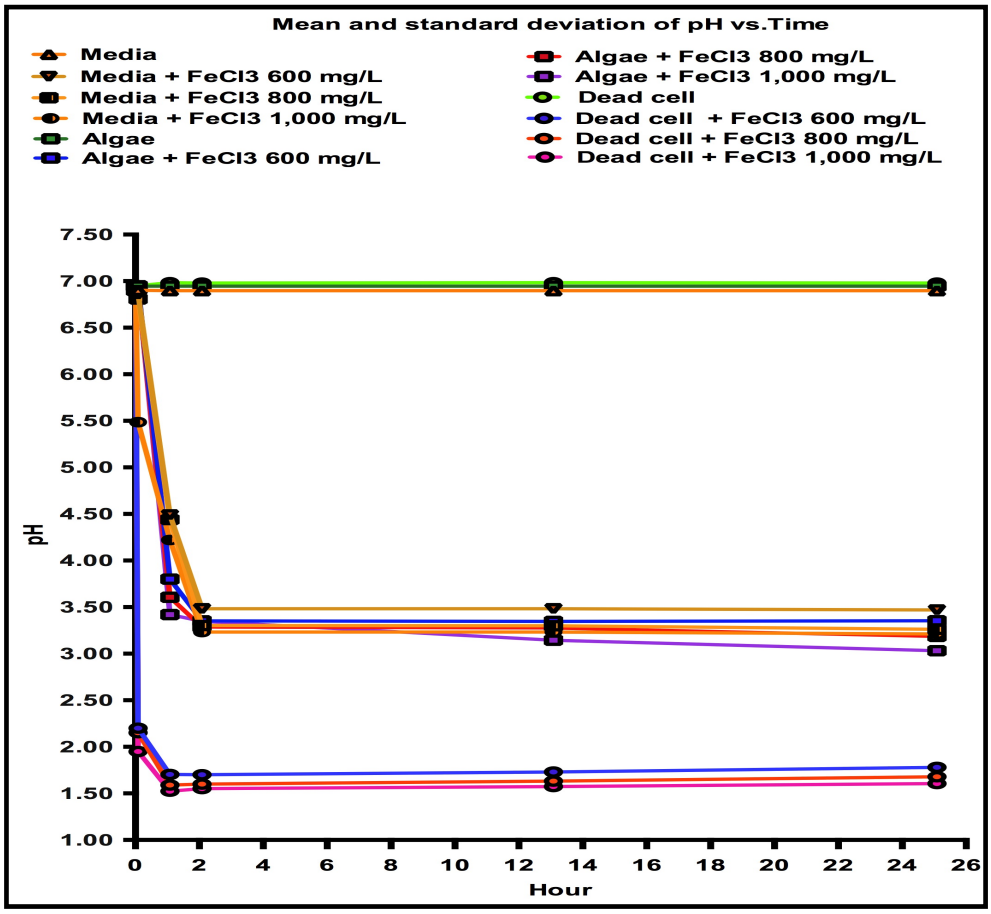


Figure 6-31 The relationship between pH and time for live and dead cell suspensions dosed with various levels of ferric chloride.

When looking at the data in more detail over the first two hours it can be seen (**Figure 6-32**) that, while the pH falls on the addition of ferric chloride, the pH of dead cell suspensions falls fastest, the pH of live algal suspensions falls less quickly while the pH of the medium alone falls the slowest. However, the outcome after one hour stirring and one hour settling is that the treated medium and the treated live cell suspensions are at a similar pH while the dead cell suspensions are at a much lower pH. There must be some component of the dead cells that is released by the addition of ferric chloride that makes the conditions more acid. This could be an important factor in harvesting operations where too acid conditions could be detrimental to the integrity or to the amount of the desired products that are recoverable.

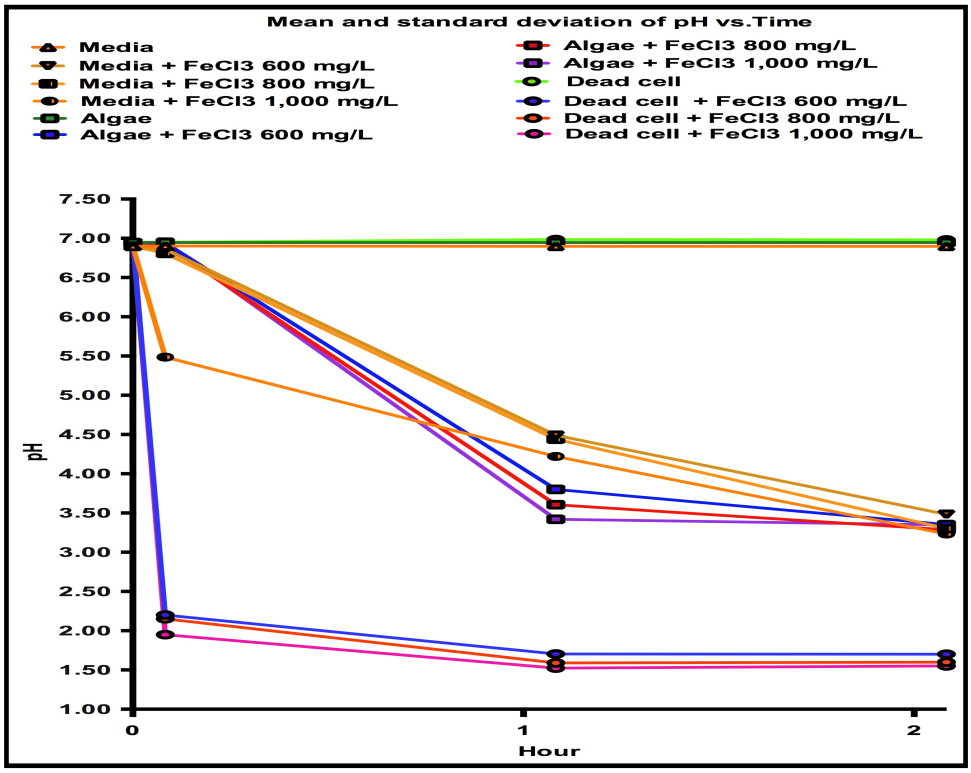


Figure 6-32 pH over the timeline of 0-2.083 hr. for live and dead cell suspensions dosed with ferric chloride.

3) Aluminium chloride

The results in **Figure 6-33** show that the pH of the algal cultures drops after adding $AlCl_3$ at 600, 800 and 1,000 mg/L and stirring for 1 hr., the mean of pH in the live algal suspensions changes from 6.95 to 5.04. For dead cells, adding $AlCl_3$ at 600, 800, and 1,000 mg/L causes the pH to drop to 5.62, 5.52, and 5.64 respectively (**Figure 6-34**). However, the pH quickly stabilises after stirring is stopped and remains stable for the rest of the experiment. The pH of the controls remains just under pH 7.0 while that of the dead cells with aluminium chloride is between pH 5.5 and 6.0 and for live cells and medium alone with added aluminium chloride the pH is between 4.0 and 4.5.

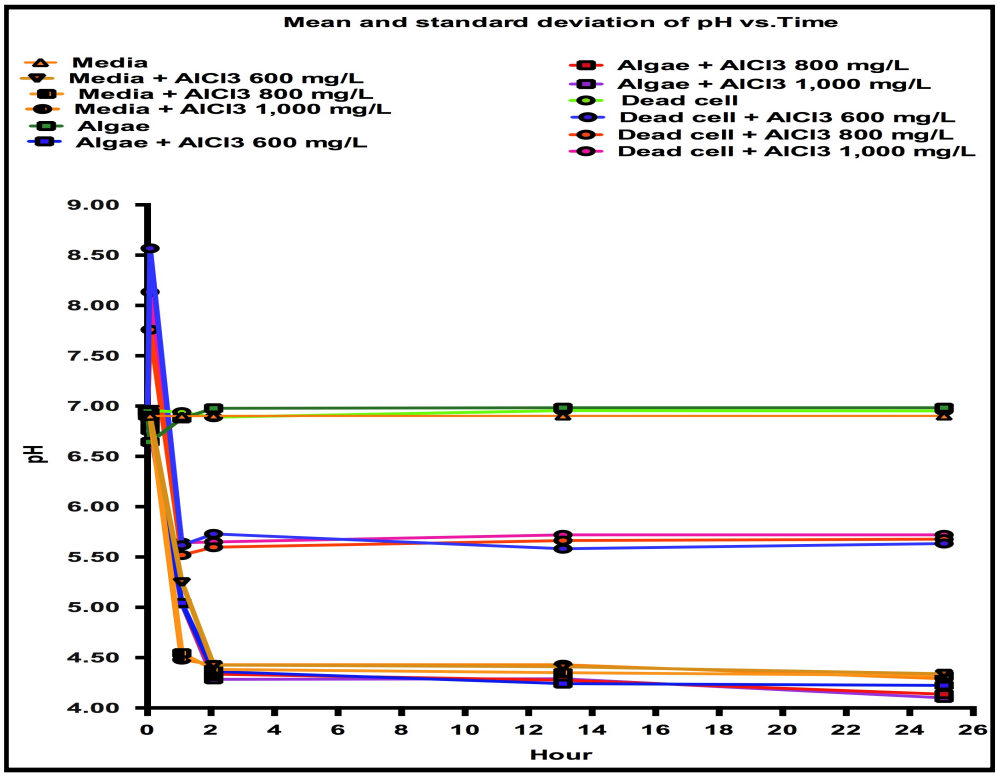


Figure 6-33 The mean and standard deviation of pH vs time for live and dead algal cell suspensions and controls on the addition of aluminium chloride.

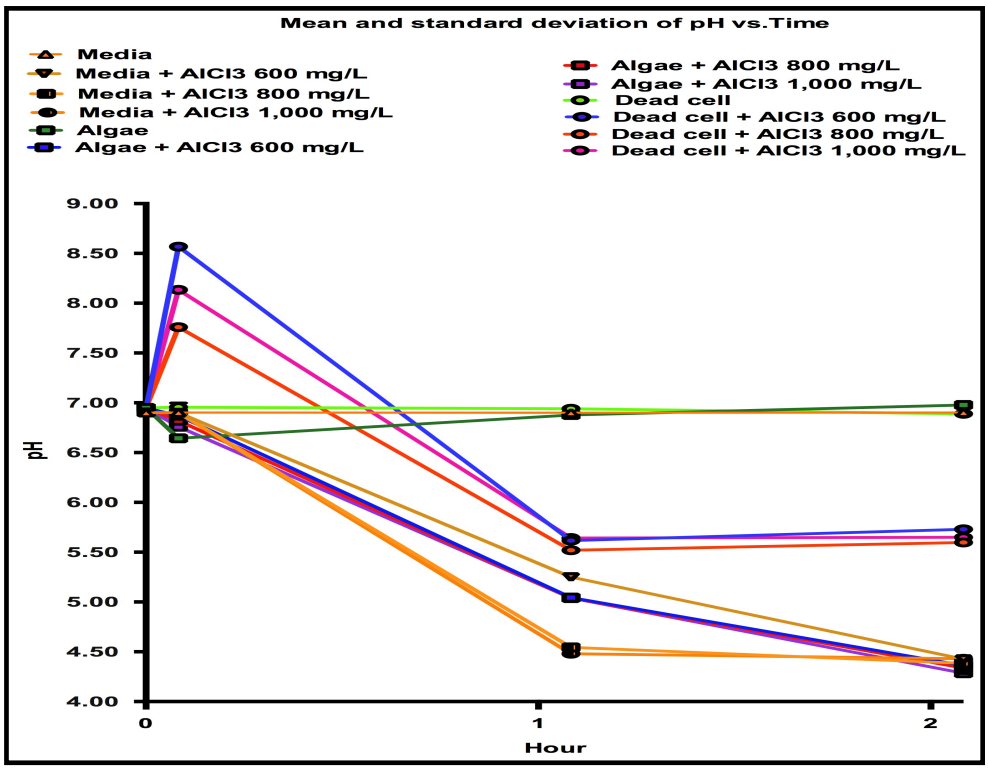


Figure 6-34 Detail of the mean and standard deviation of pH vs time (0-2.083 hr.) for live and dead algal cell suspensions and controls on the addition of aluminium chloride.

4) MW (medium molecular weight) chitosan

The results show that 5 min after adding 600, 800, and 1,000 mg/L of MW chitosan to the medium alone, the pH increases from 6.95 to 8.35-8.38. The algal cultures after 1 hr. stirring show a pH rise from 6.95 to 7.74, 7.90, and 7.79 respectively. Dead cell suspensions with additions of MW chitosan show a small rise in pH to between 7.00-7.06. There is no pH change in media, living cell, and dead cell suspensions without adding the chitosan.

On addition of chitosan and during stirring and the first hour of settling, dead algal suspensions with chitosan additions and dead algae, live algae and the media without chitosan tend to show the same stable pH while living algal suspensions with added MW chitosan increase in pH and then fall to the same level (around pH 7.00). Media alone with MW chitosan additions shows the greatest initial pH increases (8.37, 8.34, 8.37) but these also fall to the same level as the others after one hour of settling (**Figure 6-35 – 6-36**).

After this the only group to show any major changes in pH are the live algae suspensions where the pH falls to just under 6.5 at 12 hours (6.46, 6.45 and 6.46) and a little lower than this after 24 hours (6.34, 6.41 and 6.44).

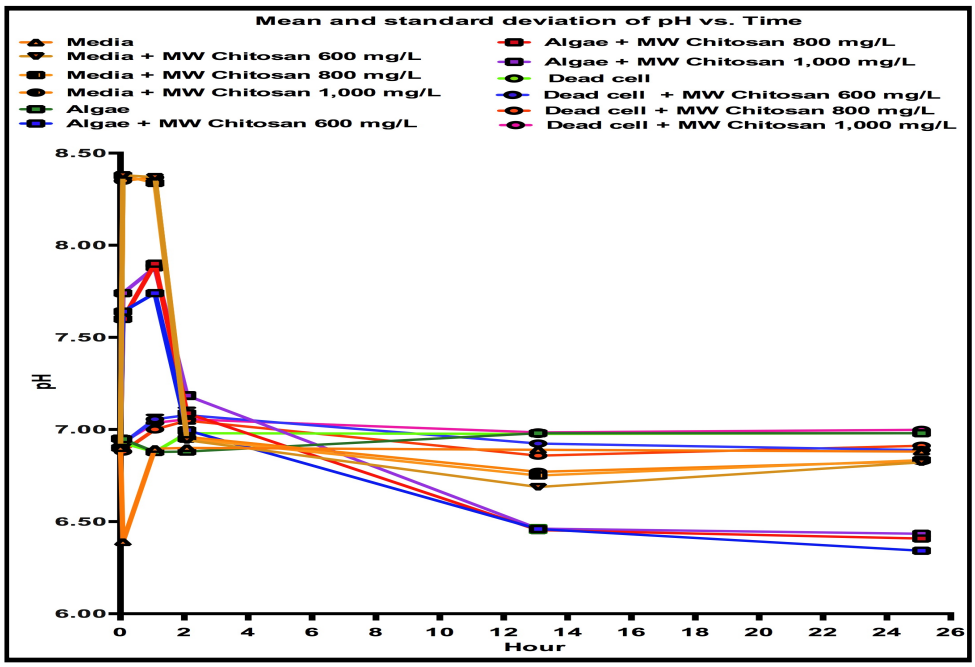


Figure 6-35 pH vs time for living and dead algal cell suspensions with addition of MW chitosan.

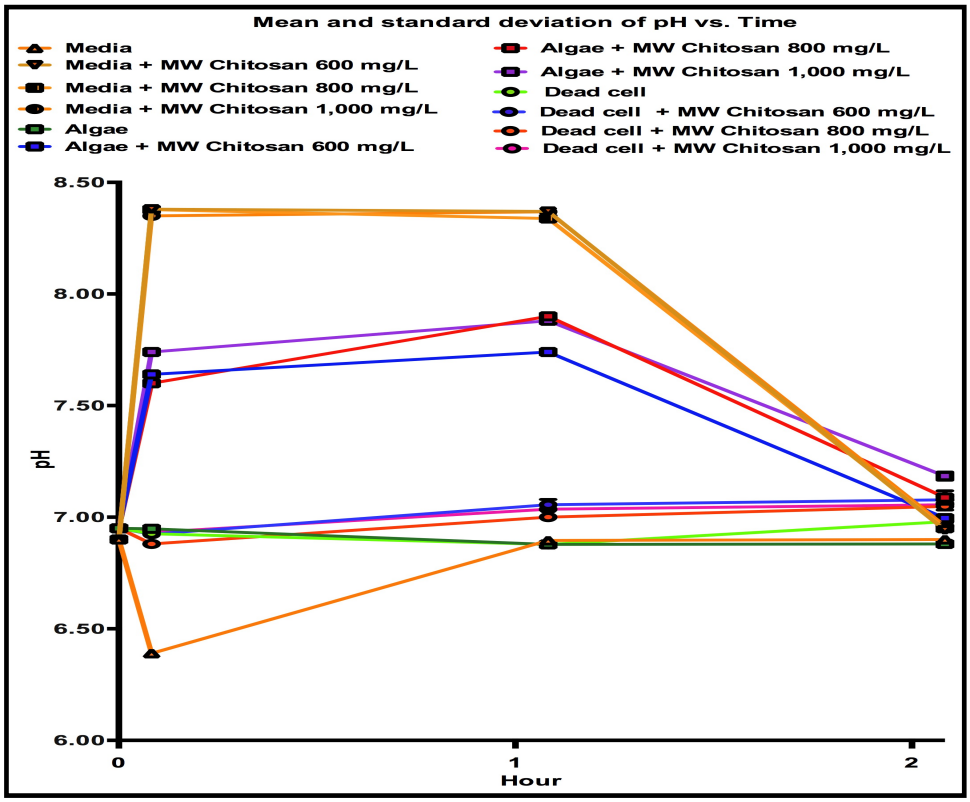


Figure 6-36 Detail of pH changes of living and dead algal cell suspensions with added MW chitosan over a timeline of 0-2.083 hr.

5) High molecular weight (HW) chitosan

The pH of the settling suspensions (**Figure 6-37**) show a slight overall fall for live cells but a much more marked pH fall (some 2.5 pH units) for dead cells. Whereas the fall in pH for the live cells is gradual over the first 12 hours and then tends to be stable to 24 hours, the fall in pH for the dead cells is seen in the first 5 minutes of adding the HW chitosan and again during the first hour of settling (**Figure 6-38**).

Thus there are two groups. The pH remains around 7.00 in the medium and living cell suspensions with and without added chitosan. The other group, consisting of dead cell suspensions with added HW chitosan, falls from around 7.00 to about 6.00 in the first two hours and then to 4.89-5.23 by 24 hours.

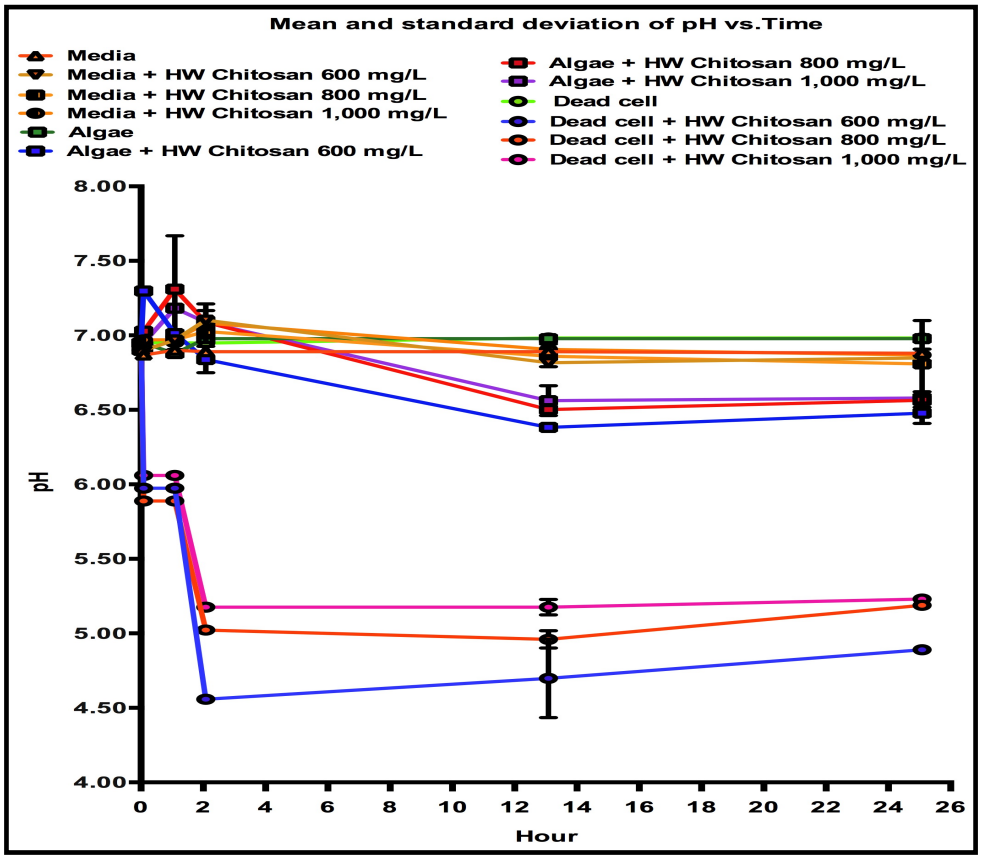


Figure 6-37 pH vs time for live and dead algal suspensions treated with HW chitosan.

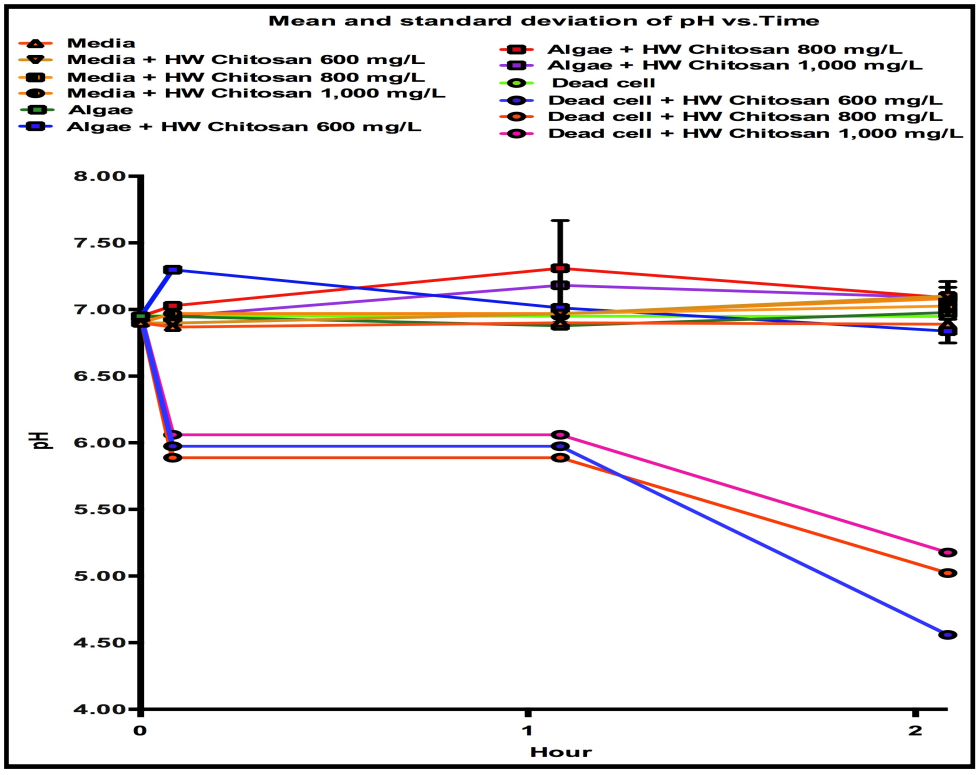


Figure 6-38 Detail of pH vs time (0-2.083) for living and dead cell suspensions treated with HW chitosan.

6) Crab shell

The addition of the highest level of crab shell to the medium alone and all levels of crab shell to living algal cultures causes an immediate rise in pH from about 6.9 to around 8.5. This pH rise is maintained throughout stirring for the live cells but drops rapidly in the first hour of settling (the pH of the medium alone with 1,000 mg/L crab shell added drops back to near the original pH during stirring). The pH of dead cell suspensions and controls tends to stay the same over the first 2 hours (see **Figure 6-39** and **Figure 6-40**). During the first 12 hours settling the pH stabilises and remains stable until the end of the experiments for all treatments except for the treated dead cell suspensions which show a gradual fall in pH ending up about 0.5 pH units below the live cells and controls.

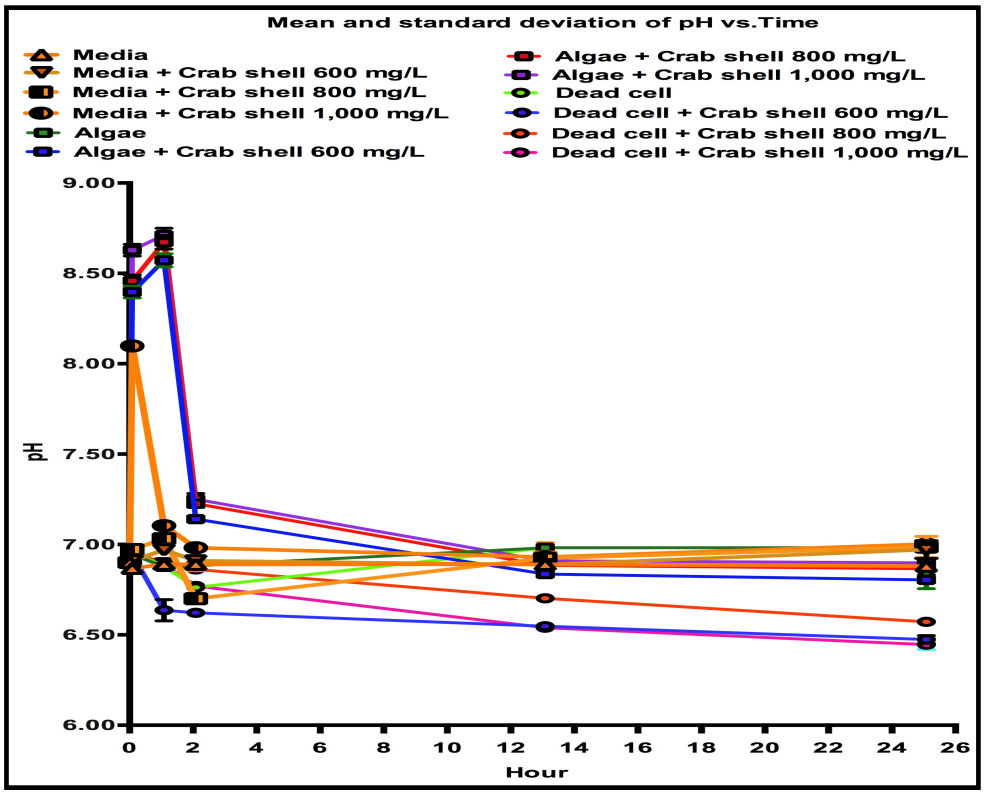


Figure 6-39 pH changes vs time for living and dead algal suspensions treated with crab shell.

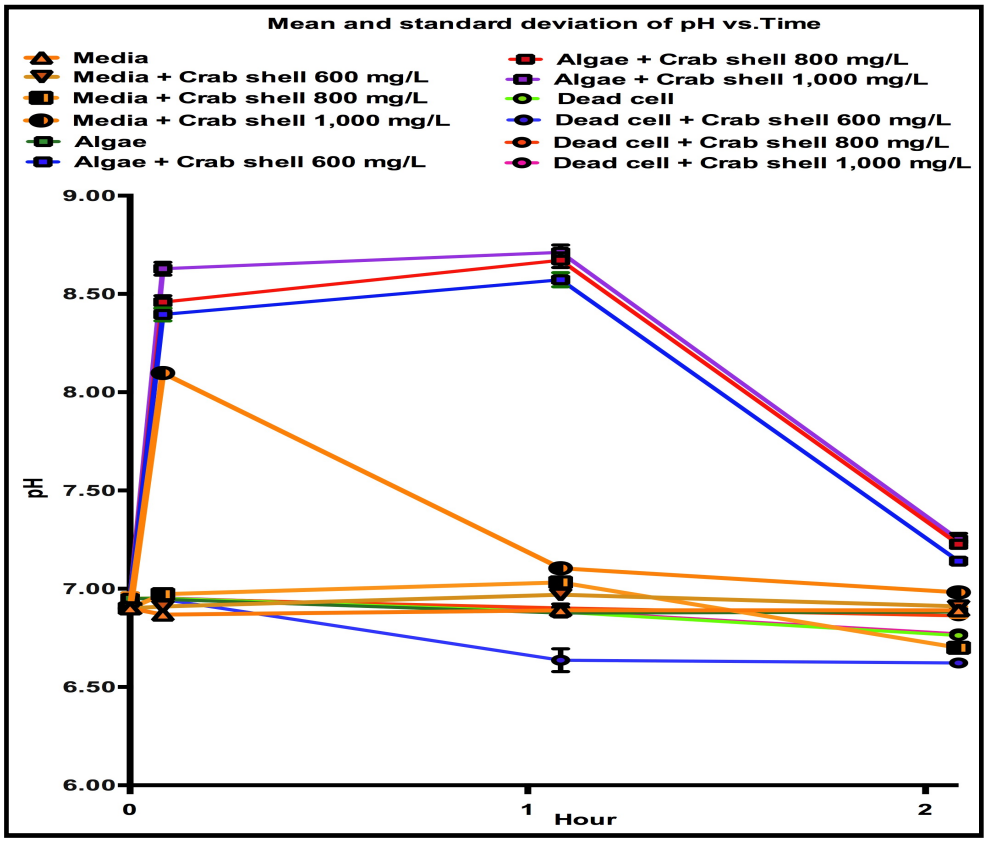


Figure 6-40 Detail of pH vs time for 0-2.083 hr. for living and dead algal suspensions treated with crab shell.

6.3.3 Determination and comparison of turbidity optical density, and pH

6.3.3.1 Turbidity

1) Between groups (Untreated media, living and dead cell suspensions)

As would be expected, the medium gives a lower turbidity and smaller standard deviations than living and dead algal cell suspensions. Living algal cultures show smaller standard deviations than dead cell suspensions in the first two hours but the dead cell suspensions show a slightly smaller range in standard deviation at 12 and 24 hours of settling (see **Appendix E**). After 12 hours of settling the mean turbidity of both living and dead cell suspensions have declined and are very similar. At 24 hours, while both continue to decline, living algal cultures give the lowest turbidity (**Figure 6-41**).

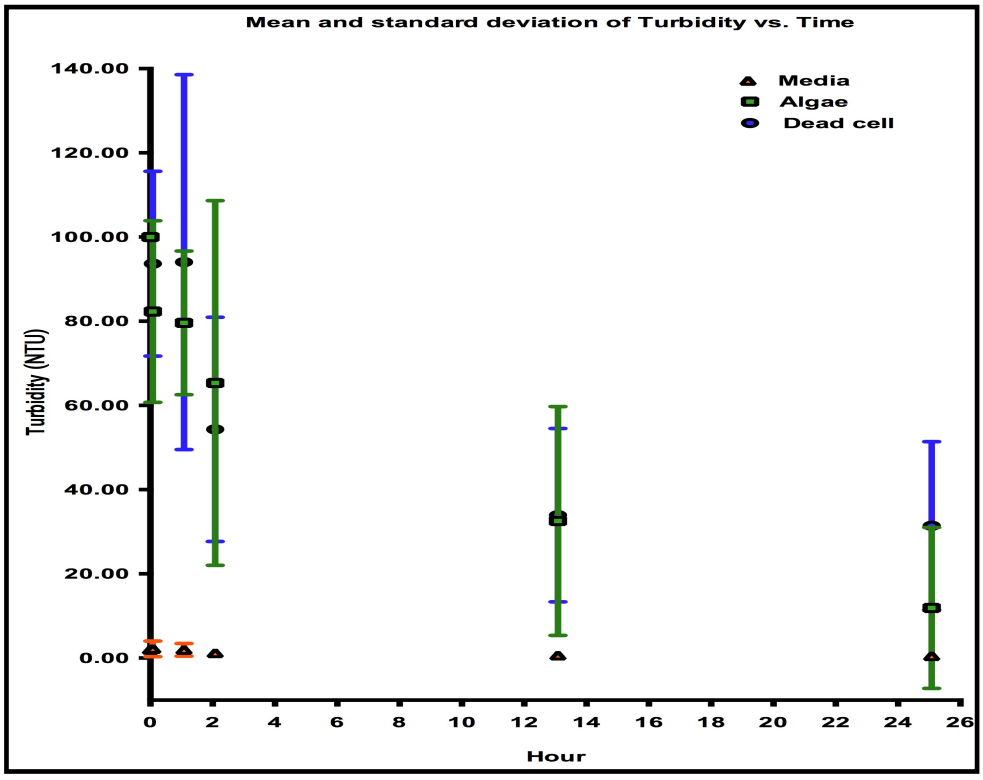


Figure 6-41 The mean and standard deviation of media, living cell cultures and dead cell suspensions without added flocculants. n=19.

2) Internal groups

2.1) Media

The results in **Figure 6-42** show that on the addition of alum, $FeCl_3$, $AlCl_3$, MW chitosan, HW chitosan and crab shell, while there is an increase and variation in turbidity of the media alone this rapidly settles down and consolidates to a very low level. The highest mean on first adding flocculants is 5.70 NTU with the standard deviation of 2.01 when adding 1,000 mg/L of $FeCl_3$. This becomes a mean of 4.74 after stirring for 1 hour. However, after 1 hr. settling, turbidity for all conditions is around 2 NTU or less and less than 1.25 NTU after 24 hours. The results confirm that there is no significant interaction between the flocculants and the media that affects the turbidity and therefore the turbidity readings for algal settlement are a true reflection of what is happening to the cells.

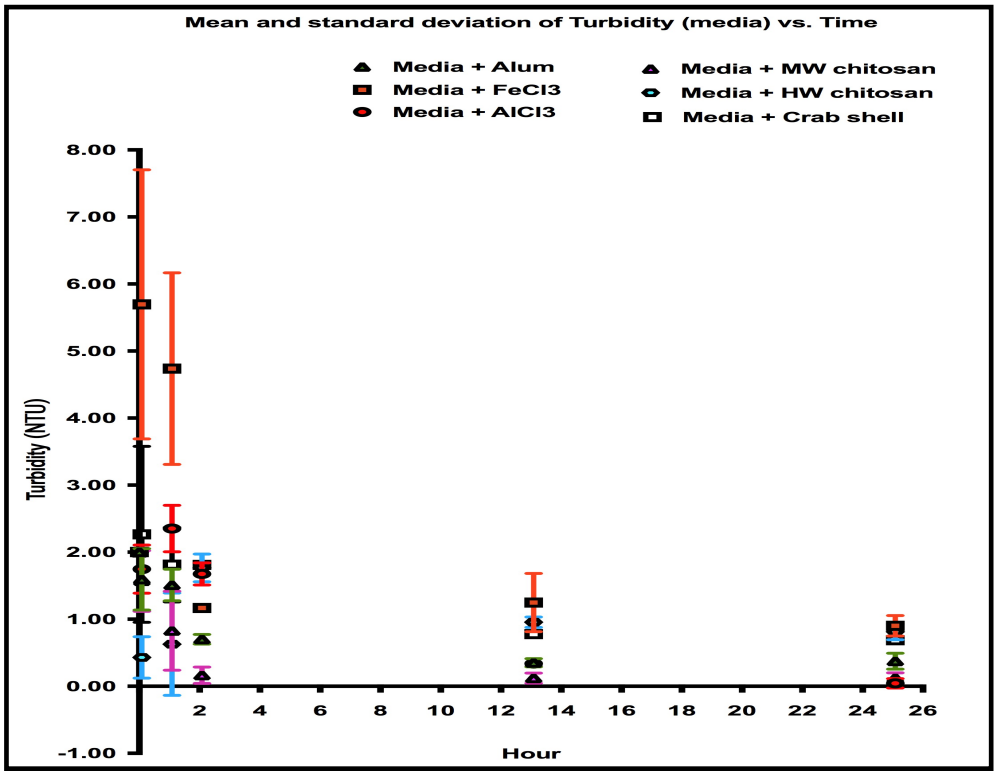


Figure 6-42 The mean and standard deviation of turbidity of the media with added flocculants (note that all the turbidity readings are very low – compare to **Figure 6-43**).

2.2) Living algal cell suspensions

The mean and standard deviations of living algal cell suspensions are shown in **Figure 6-43**. The greatest range of standard deviation is in the first two hours, particularly for additions of $FeCl_3$. At 12 and 24 hours settling the standard deviations of the turbidity measurements are very small, especially for the chitosan/crab shell additions.

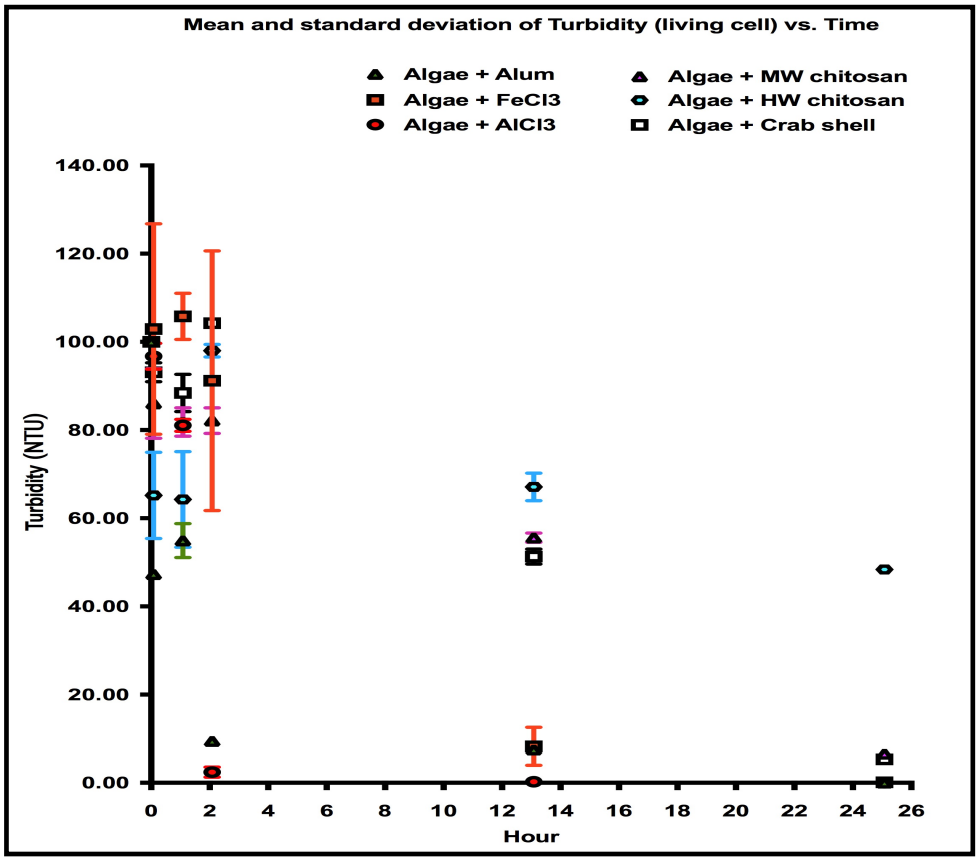


Figure 6-43 The mean and standard deviation of living algal cell suspensions with added flocculants.

2.3) Dead cell suspensions

The dead cell suspensions show generally a greater range of standard deviations within the additions when compared to the living cells. Also, in contrast to the living cell suspensions, the additions of chitosan/crab shell show greater standard deviations than the chemicals such as $FeCl_3$ (**Figure 6-44**).

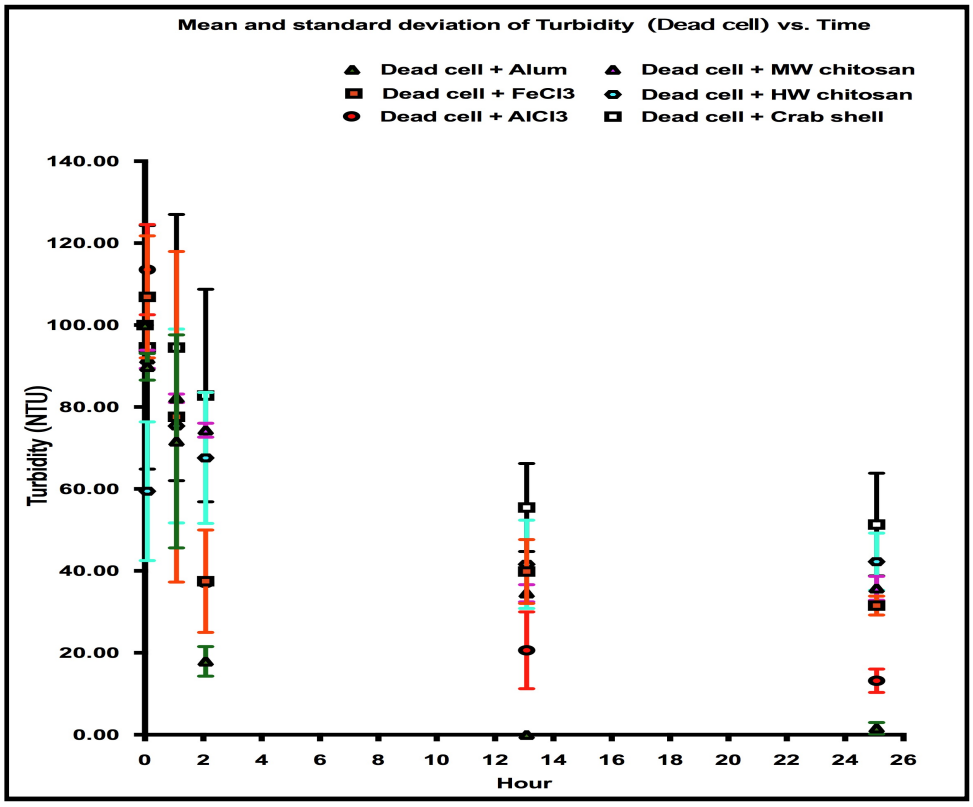


Figure 6-44 The mean and standard deviation of dead cell suspensions with added flocculants.

6.3.3.2 Optical density

The study found that the trends of the mean and standard deviations of results of optical density measurement are similar to the turbidity measurements but with the standard deviations tending to be smaller. The plots (**Figures 6-45** to **6-46**) are given here for completeness (see **Appendix E**)

Figure 6-45 shows that the mean OD_{680} of dead cell suspensions are higher than the live algae suspensions. The standard deviations show a wider range. Possibly, this may be caused by the dead cells having less and more variable weight. The bonding/forces between the dead algae in suspension are not strong. Dead cells float with little bonding between them and they are more likely to follow any liquid flow in the suspension which leads to fluctuating data. Living cells have a stronger bonding force to connect together in a more uniform way that makes the results closer to the mean (see **Figure 6-45** and **Figure 6-46**).

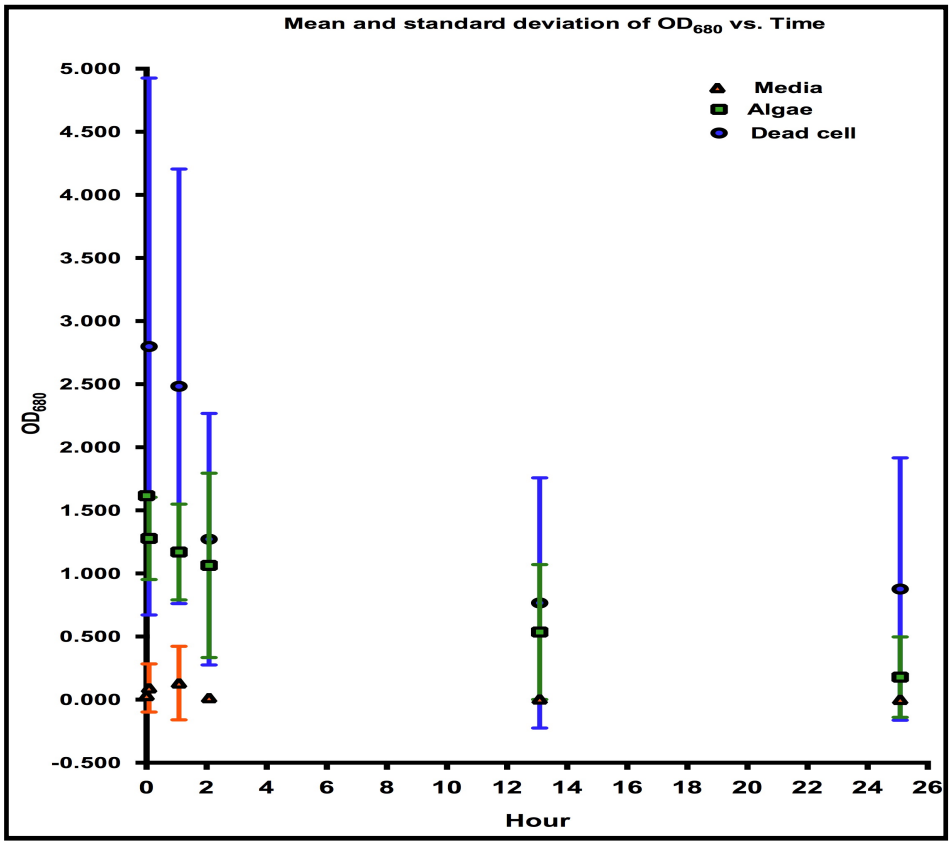


Figure 6-45 The mean and standard deviation of optical density of media, living and dead cell suspensions, n=19.

Figure 6-46 shows the mean and standard deviation of the optical density of the media with added metallic salts and biopolymers. The results are nearly all the same except media with $FeCl_3$ additions which, on addition and after stirring (the first hour) shows different results and large standard deviations. As soon as settling starts this difference disappears. It may be that the $FeCl_3$ produces much looser bonds which are easily disrupted by stirring.

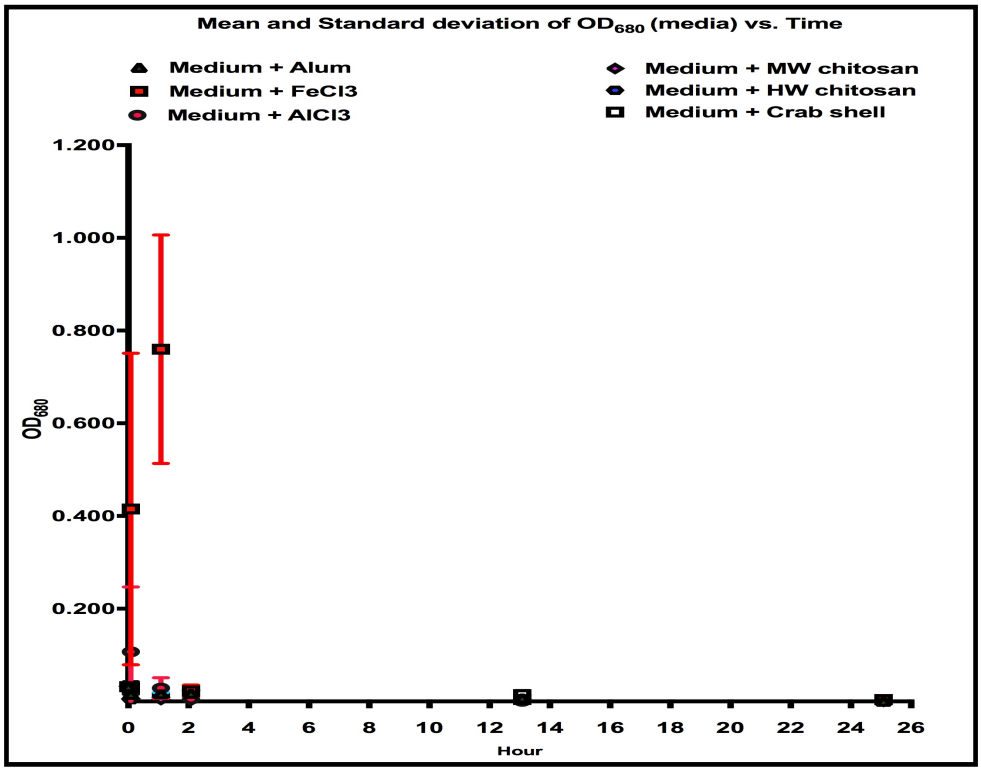


Figure 6-46 The mean and standard deviation of optical density of media with added flocculants.

Living cells in suspension with added flocculants show different values. This is due to the flocculants bonding with living algae in various forms. However, similar to the media alone, living algal suspensions with added $FeCl_3$ show greater variation in the first two hours than the other flocculants. The next most variable results come in the same first two hours with HW chitosan. However, unlike $FeCl_3$, the means of the HW chitosan measurements do not drop to the level of the other flocculants. It is possible that the molecular chain length of HW chitosan is too long and do not network easily, or only loosely, with the algal cells (see **Figure 6-47**).

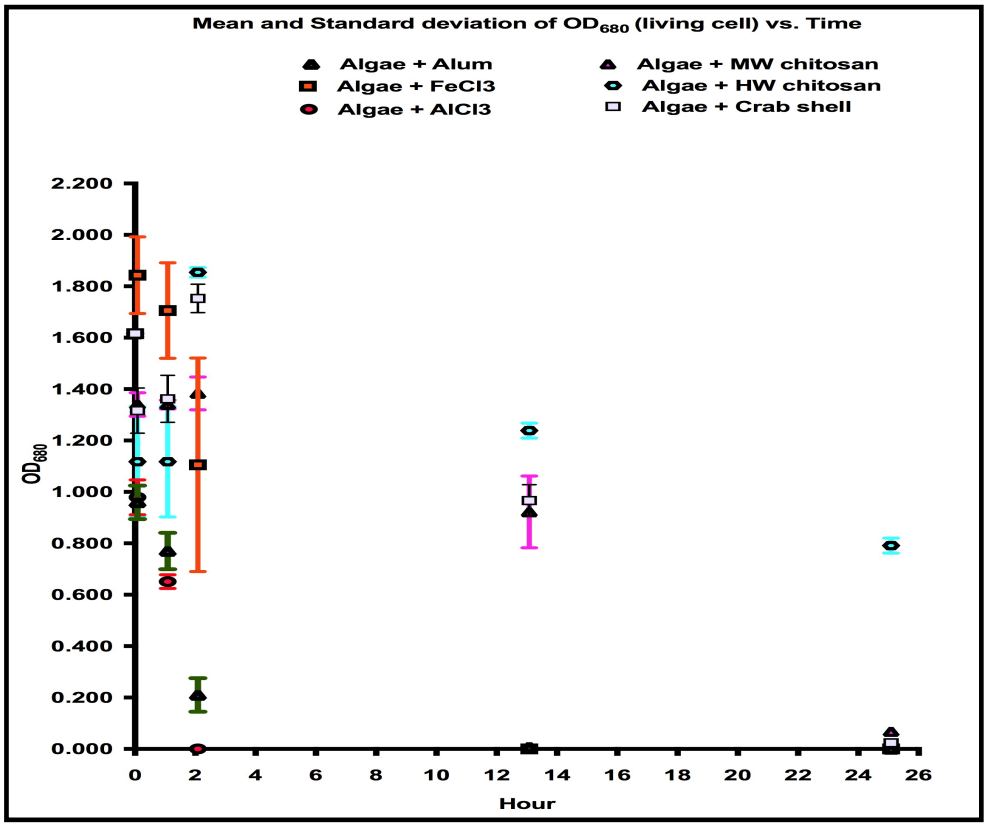


Figure 6-47 The mean and standard deviation of optical density of living algae suspensions with added flocculants.

Dead cells show more diverse results when compared with the living cell suspension results above. Again, $FeCl_3$ shows large standard deviations. The next largest standard deviations come from cell suspensions with added $AlCl_3$. Both show a slight rise, and increase in standard deviation, of OD at 24 hr. when compared to 12 hr. This may indicate the beginning of a breakdown in the bonding that enabled the initial flocculation (**Figure 6-48**).

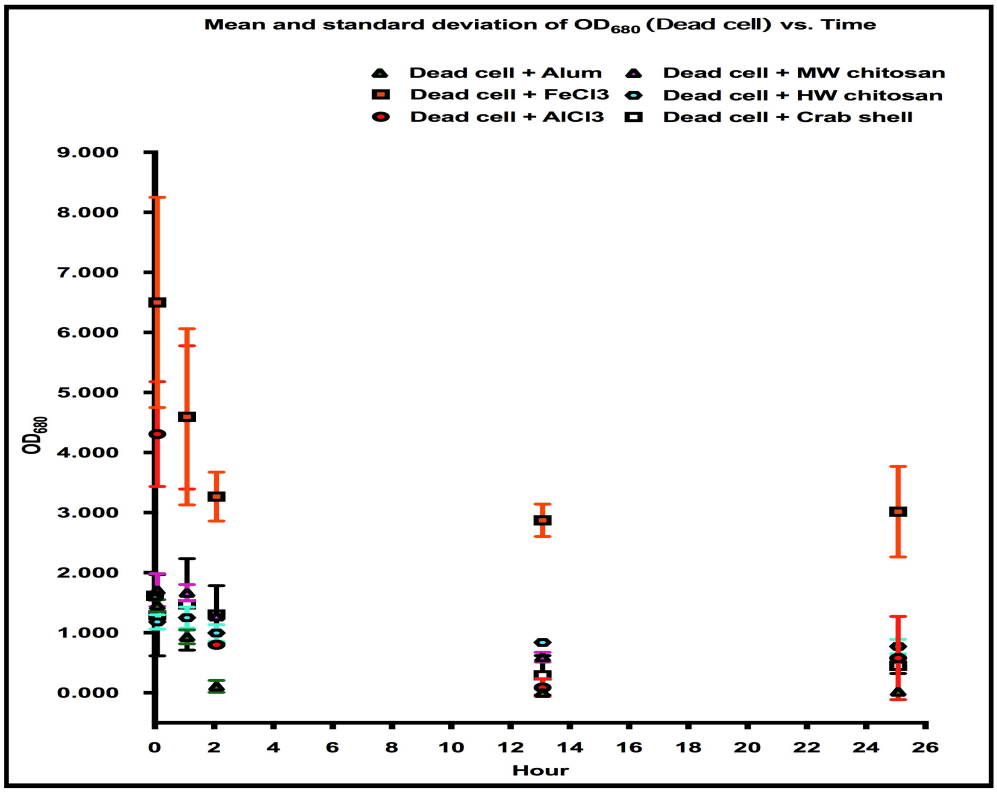


Figure 6-48 Mean and standard deviation of optical density of dead cell suspensions with added flocculants.

6.3.3.3 pH

1) Between groups

Perhaps surprisingly, the standard deviations of the pH of media, living and dead cell suspensions with no added flocculants are large. Overall the mean pH does drop with time (e.g. from 5.9 to 4.99 for dead cell suspensions) but the size of the standard deviation does not allow any definite conclusions to be drawn (**Figure 6-49**).

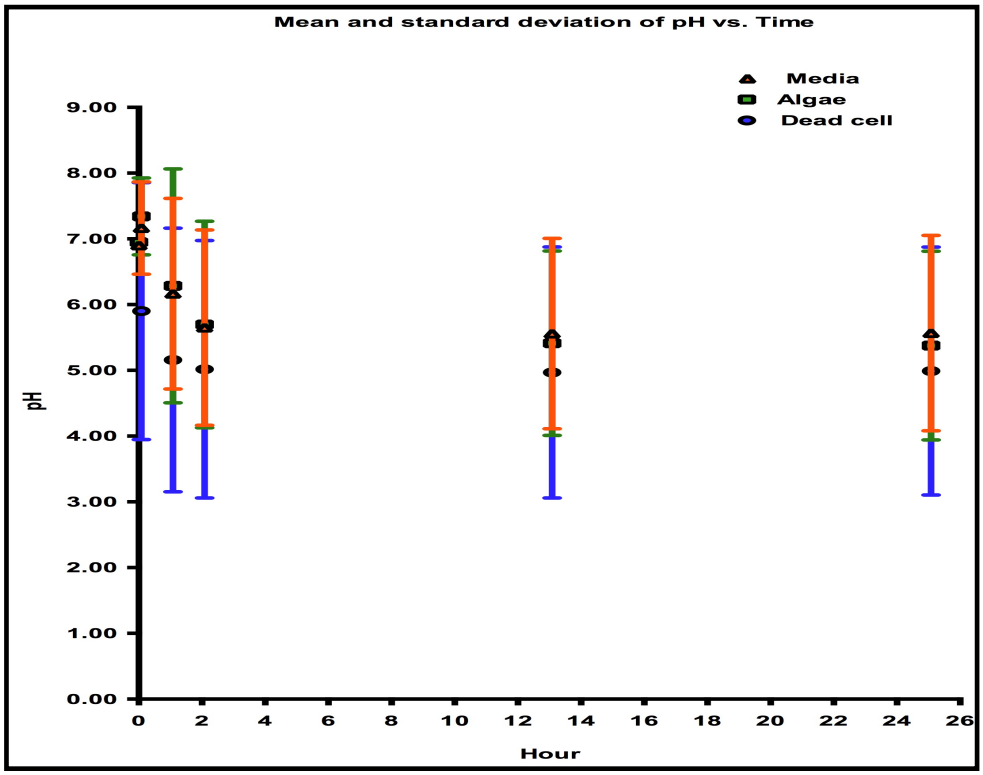


Figure 6-49 Mean and standard deviation of pH of media, living and dead cell suspensions with no added flocculants.

2) Internal groups

2.1) Medium

The addition of the metallic salt flocculants to the medium causes the pH to fall further than in the media with no added flocculants (compare **Figure 6-50** with **Figure 6-51**). In contrast addition of chitosan/crab shell has little effect on the pH (**Figure 6-51**). What is of note is the very small standard deviations of the results when flocculants are added compared to when flocculants are not added (compare **Figure 6-50** and **6-51**).

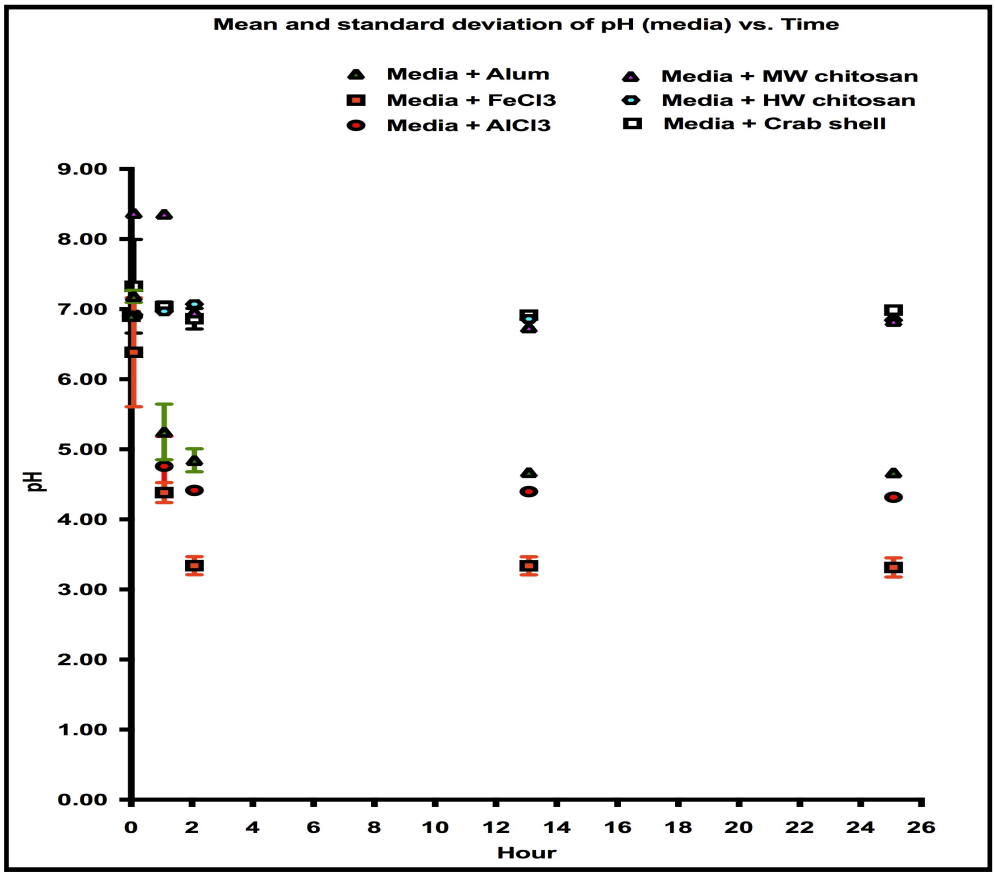


Figure 6-50 The mean and standard deviation of pH of media with added flocculants.

2.2) Living algal cell suspensions

Again, the most remarkable point when looking at the mean and standard deviation of the pH of algal cell suspensions with added flocculants is the small standard deviations, even when all the results of the various additions of flocculants are combined. This indicates that there is no difference in effect on pH of additions between 600, 800 and 1,000 mg/L (**Figure 6-51**).

The study found that algae with added metallic salts results in lower pH values than biopolymers (chitosans/crab shell).

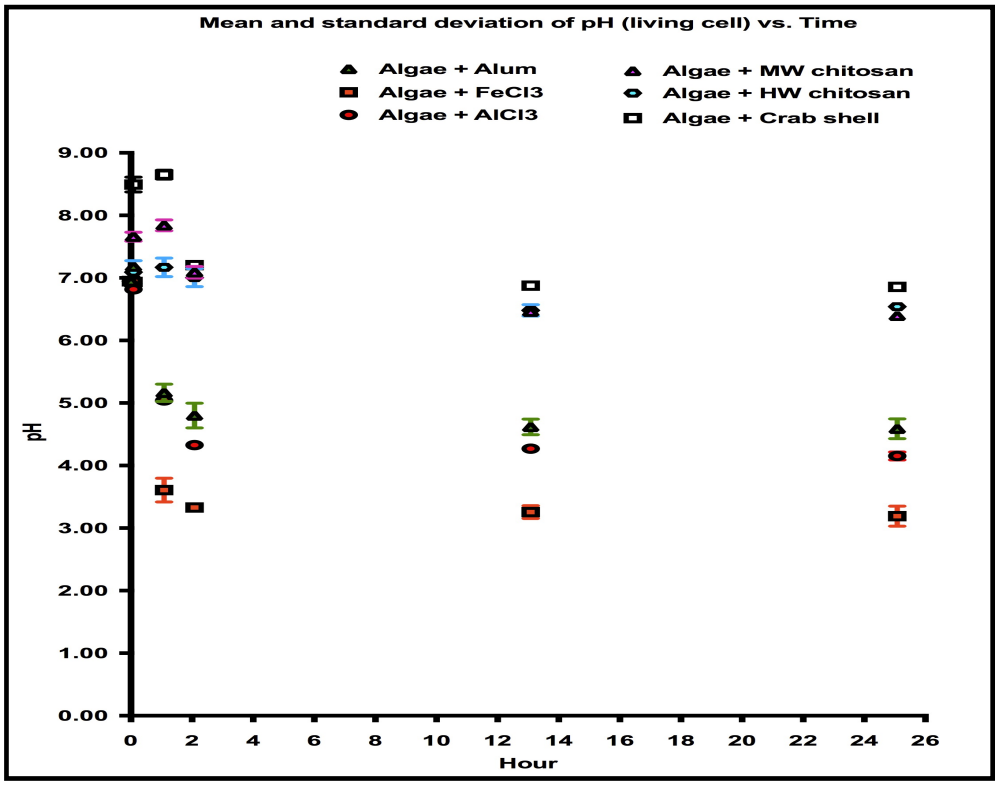


Figure 6-51 The mean and standard deviation of pH of living cell suspensions with added flocculants.

2.3) Dead cell suspensions

While the pH of dead cell suspensions treated with chitosans/crab shell remains fairly steady throughout the experiments, the pH of dead cell suspensions treated with metallic salt flocculants fall further than the pH of live cell suspensions treated similarly. Again the most remarkable result is the very small standard deviation of the results no matter the level of flocculant addition (**Figure 6-52**).

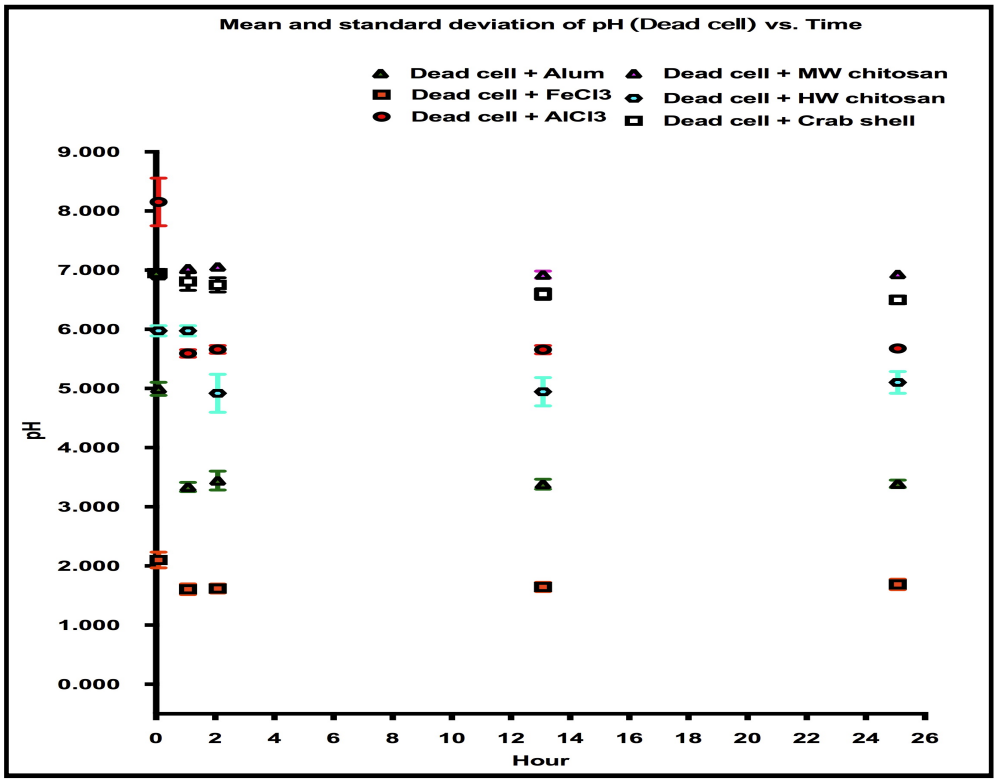


Figure 6-52 The mean and standard deviation of pH of dead cell suspensions with added flocculants.

6.3.4 Determination of percentage removal

6.3.4.1 Turbidity

All flocculants except HW chitosan result in more than 90% removal of live cells after 24 hr. settling. HW chitosan only shows a removal of around 50%. The results of turbidity and optical density look the same.

Some of the metallic salt additions cause the living algae to settling very fast: 5 min after adding alum at 600, 800, 1,000 mg/L, 50% of living cells were already removed. Most of the metal salt additions cause the settlement of over 90% of living cells by 12 hours. Indeed alum and aluminium chloride remove over 90% of the biomass after 1 hour of settlement. Chitosans and crab shell are slower with around 50% removal at 12 hours and taking 24 hours to reach over 90% removal.

After 12 hr. settling the study found that adding $AlCl_3$ can remove 100% of living algal cells at every concentration. Alum results in 91.00-93.00% while $FeCl_3$ is in the range of 86.78-94.72%. MW chitosan will remove between 43-44% of living algal biomass in 12 hours while crab shell is better at close to 50%, and HW chitosan worse at between 29-35%.

After 24 hr. settling untreated living algae auto-settle at around 55% adding alum and $AlCl_3$ gives 100% removal while adding $FeCl_3$ gives more than 99 %. Crab shell gives 94-95%, MW chitosan between 92-94% while, in contrast, HW chitosan only shows 50% removal – less than the untreated algae (**Table 6-2**).

Table 6-2 The effect of added flocculants on the percentage removal of *C.vulgaris* biomass calculated from turbidity data (n=5).

% Removal as estimated by turbidity						
Algal samples	**Before adding chemical**	**Adding chemical**	**Setting time**			
			0	**1**	**12**	**24**
Living algae	0.00	10.00	16.00	20.00	52.00	55.00
Algae + Alum 600 mg/L	0.00	53.25	44.86	90.47	92.99	100.00
Algae + Alum 800 mg/L	0.00	52.94	49.00	90.09	92.51	100.00
Algae + Alum 1,000 mg/L	0.00	51.90	41.29	91.00	91.93	100.00
Algae + $FeCl_3$ 600 mg/L	0.00	-0.20	-1.90	20.53	94.72	99.89
Algae + $FeCl_3$ 800 mg/L	0.00	19.50	-3.73	30.58	93.70	99.90
Algae + $FeCl_3$ 1,000 mg/L	0.00	-28.00	-11.73	-24.68	86.78	99.88
Algae + $AlCl_3$ 600 mg/L	0.00	5.20	17.64	96.77	99.32	100.00
Algae + $AlCl_3$ 800 mg/L	0.00	4.69	18.77	97.11	100.00	100.00
Algae + $AlCl_3$ 1,000 mg/L	0.00	-0.13	20.37	98.92	100.00	100.00
Algae + MW chitosan 600 mg/L	0.00	19.93	19.56	11.54	44.48	92.93
Algae + MW chitosan 800 mg/L	0.00	16.99	19.55	13.50	45.41	93.86
Algae + MW chitosan 1,000 mg/L	0.00	5.03	14.51	15.81	43.30	93.52
Algae + HW chitosan 600 mg/L	0.00	41.72	44.53	3.31	34.62	52.16
Algae + HW chitosan 800 mg/L	0.00	39.12	43.25	0.51	29.28	51.13
Algae + HW chitosan 1,000 mg/L	0.00	23.59	42.93	2.18	34.85	51.54
Algae + Crab shell 600 mg/L	0.00	4.44	16.46	-4.10	46.74	94.31
Algae + Crab shell 800 mg/L	0.00	8.47	9.35	-4.94	50.00	95.01
Algae + Crab shell 1,000 mg/L	0.00	7.77	8.93	-3.68	49.30	94.78
Dead cell	0.00	-11.91	53.82	19.53	31.49	30.15
Dead cell + Alum 600 mg/L	0.00	6.52	-1.49	78.30	100.00	97.19
Dead cell + Alum 800 mg/L	0.00	11.33	40.60	82.39	100	98.10
Dead cell + Alum 1,000 mg/L	0.00	12.75	46.04	85.54	100.00	99.94
Dead cell + $FeCl_3$ 600 mg/L	0.00	-18.51	-23.79	48.18	51.84	69.33
Dead cell + $FeCl_3$ 800 mg/L	0.00	9.91	40.16	68.33	61.31	70.22
Dead cell + $FeCl_3$ 1,000 mg/L	0.00	-12.02	50.82	71.08	67.28	65.81
Dead cell + $AlCl_3$ 600 mg/L	0.00	-1.23	-83.99	63.67	89.96	87.67
Dead cell + $AlCl_3$ 800 mg/L	0.00	-22.50	-82.73	62.06	72.00	89.15
Dead cell + $AlCl_3$ 1,000 mg/L	0.00	-16.83	-70.22	62.58	76.24	83.64
Dead cell + MW chitosan 600 mg/L	0.00	5.80	16.69	25.52	66.93	66.93
Dead cell + MW chitosan 800 mg/L	0.00	9.94	18.68	24.01	66.38	64.71
Dead cell + MW chitosan 1,000 mg/L	0.00	9.22	18.20	27.42	63.04	61.13
Dead cell + HW chitosan 600 mg/L	0.00	57.88	3.02	18.45	52.09	51.66
Dead cell + HW chitosan 800 mg/L	0.00	24.06	20.90	28.96	52.23	56.27
Dead cell + HW chitosan 1,000 mg/L	0.00	39.70	49.93	49.89	63.77	65.36
Dead cell + Crab shell 600 mg/L	0.00	21.68	20.80	29.62	51.50	56.38
Dead cell + Crab shell 800 mg/L	0.00	23.50	27.49	34.63	49.93	55.44
Dead cell + Crab shell 1,000 mg/L	0.00	-28.91	-31.82	-12.61	32.16	34.23

6.4.1.2 Optical density

Although, as might be expected, the results of biomass removal calculated using optical density measurement show similar trends to the results calculated using turbidity measurements there are some interesting differences. These are mainly more definite results (more and earlier 100% removals for example). The study found that 5 min after adding chemicals, the optical density of living algal suspensions with added alum, and $AlCl_3$ show a biomass removal of between 35-45 %, whilst adding $FeCl_3$ appears to increase the biomass (due to an increase in the optical density). MW chitosan removes between 13-19% of algae. Crab shell is in the range of 13-24% while HW chitosan gives a wide range of 15-40 % removal.

At settling time 0 hr. (after stopping stirring), alum and $AlCl_3$ are dominant. They have removed 50-60% of the algal biomass.

After settling for 1 hr. $AlCl_3$ dosed live algae suspensions show 100% removal. Alum-treated cultures show more than 83% removal while the other treatments are very variable.

After 12 hr. settling, live algae suspensions with added $FeCl_3$ and $AlCl_3$ show 100%, removal and alum-treated cultures are close to that percentage. Algae with added MW chitosan show between 44 - 49% and the removal is between 37- 44% with crab shell, but only 21-25% for HW chitosan (while untreated algae show 51.53% removal).

After 24 hr. settling the metallic salt flocculants (Alum, $FeCl_3$, $AlCl_3$) have removed 100% of the algal biomass (compared to 55% removal for non-treated algae). In this same time, MW chitosan and crab shell show more than 95% removal but HW chitosan shows only around 50% removal (i.e. less than untreated live algae).

The dead cell suspensions with added flocculants fluctuate. In several cases, the calculated biomass increases (a negative number in **Table 6-3**) as the added flocculants increase the optical density. In some cases, with $FeCl_3$ additions, this increase in optical density, hence in apparent gain in biomass, persists for the whole 24 hour settling (**Table 6-3**). In most cases the flocculants work better with live algal cultures than with dead cell suspensions. Again this is an important point for industrial applications.

Table 6-3 Percentage biomass removal as measured using optical density (OD_{680}) data.

% Removal as estimated by OD_{680}						
Algal samples	**Before adding chemical**	**Adding chemical**	**Settling time**			
			0	**1**	**12**	**24**
Living Algae	0.00	0.00	13.71	19.72	51.53	54.64
Algae + Alum 600 mg/L	0.00	45.29	50.35	91.58	99.81	100.00
Algae + Alum 800 mg/L	0.00	38.17	52.46	83.92	98.77	100.00
Algae + Alum 1,000 mg/L	0.00	38.43	47.89	85.43	99.94	100.00
Algae + $FeCl_3$ 600 mg/L	0.00	-6.67	5.07	42.50	100.00	100.00
Algae + $FeCl_3$ 800 mg/L	0.00	-11.11	-3.96	50.00	100.00	100.00
Algae + $FeCl_3$ 1,000 mg/L	0.00	-24.44	-17.78	2.22	100.00	100.00
Algae + $AlCl_3$ 600 mg/L	0.00	44.10	61.26	100.00	100.00	100.00
Algae + $AlCl_3$ 800 mg/L	0.00	35.92	59.94	100.00	100.00	100.00
Algae + $AlCl_3$ 1,000 mg/L	0.00	38.21	57.99	100.00	100.00	100.00
Algae + MW chitosan 600 mg/L	0.00	18.59	17.92	9.83	32.95	95.95
Algae + MW chitosan 800 mg/L	0.00	18.79	17.34	16.38	47.21	95.76
Algae + MW chitosan 1,000 mg/L	0.00	13.78	15.90	16.96	48.55	95.57
Algae + HW chitosan 600 mg/L	0.00	37.14	36.87	-15.79	25.44	52.74
Algae + HW chitosan 800 mg/L	0.00	39.86	38.13	-15.02	21.93	49.12
Algae + HW chitosan 1,000 mg/L	0.00	15.57	39.69	-13.49	22.70	51.21
Algae + crab shell 600 mg/L	0.00	24.23	22.22	-12.39	44.44	98.18
Algae + crab shell 800 mg/L	0.00	13.39	12.57	-7.10	36.98	98.68
Algae + crab shell 1,000 mg/L	0.00	17.85	12.30	-6.01	39.16	98.72
Dead cell	0.00	-138.22	-127.45	42.33	67.69	79.65
Dead cell + Alum 600 mg/L	0.00	3.18	34.46	87.55	99.63	100.00
Dead cell + Alum 800 mg/L	0.00	14.42	43.82	92.51	100.00	99.25
Dead cell + Alum 1,000 mg/L	0.00	12.55	48.69	100.00	100.00	97.00
Dead cell + $FeCl_3$ 600 mg/L	0.00	-386.67	-286.67	-120.00	-93.33	-140.00
Dead cell + $FeCl_3$ 800 mg/L	0.00	-180.00	-153.33	-73.33	-80.00	-53.33
Dead cell + $FeCl_3$ 1,000 mg/L	0.00	-340.00	-113.33	-113.35	-60.00	-66.67
Dead cell + $AlCl_3$ 600 mg/L	0.00	-225.15	-260.74	52.15	84.05	87.73
Dead cell + $AlCl_3$ 800 mg/L	0.00	-156.44	-177.30	52.15	100.00	90.18
Dead cell + $AlCl_3$ 1,000 mg/L	0.00	-118.40	-113.50	47.24	100.00	87.73
Dead cell + MW chitosan 600 mg/L	0.00	-9.52	-12.70	23.81	58.73	63.49
Dead cell + MW chitosan 800 mg/L	0.00	-20.63	3.17	20.63	63.49	65.08
Dead cell + MW chitosan 1,000 mg/L	0.00	12.70	0.00	19.05	68.25	58.73
Dead cell + HW chitosan 600 mg/L	0.00	19.94	10.19	28.68	46.72	46.14
Dead cell + HW chitosan 800 mg/L	0.00	34.64	26.35	41.78	52.26	60.55
Dead cell + HW chitosan 1,000 mg/L	0.00	26.35	31.30	44.83	45.41	50.07
Dead cell + Crab shell 600 mg/L	0.00	39.67	38.18	38.92	86.01	97.83
Dead cell + Crab shell 800 mg/L	0.00	48.70	34.16	34.16	100.00	75.25
Dead cell + Crab shell 1,000 mg/L	0.00	-29.21	-45.48	-14.91	60.02	63.24

6.3.5 Effect on carbon measurements (TC, TIC, TOC) after adding chitosan

6.3.5.1 Effects of 150 mg/L MW chitosan on TC, TIC, and TOC

Figure 6-53 shows the results of total carbon (TC), total inorganic carbon (TIC), and total organic carbon (TOC) measurements. TC and TIC decreased after adding MW-Chitosan indicating some adsorption of inorganic carbon onto the chitosan. In contrast, TOC increases indicating some release of organic material from the chitosan. However, the changes are minimal indicating that the chitosan is largely inert (at least not dissolving) when added as a flocculating agent.

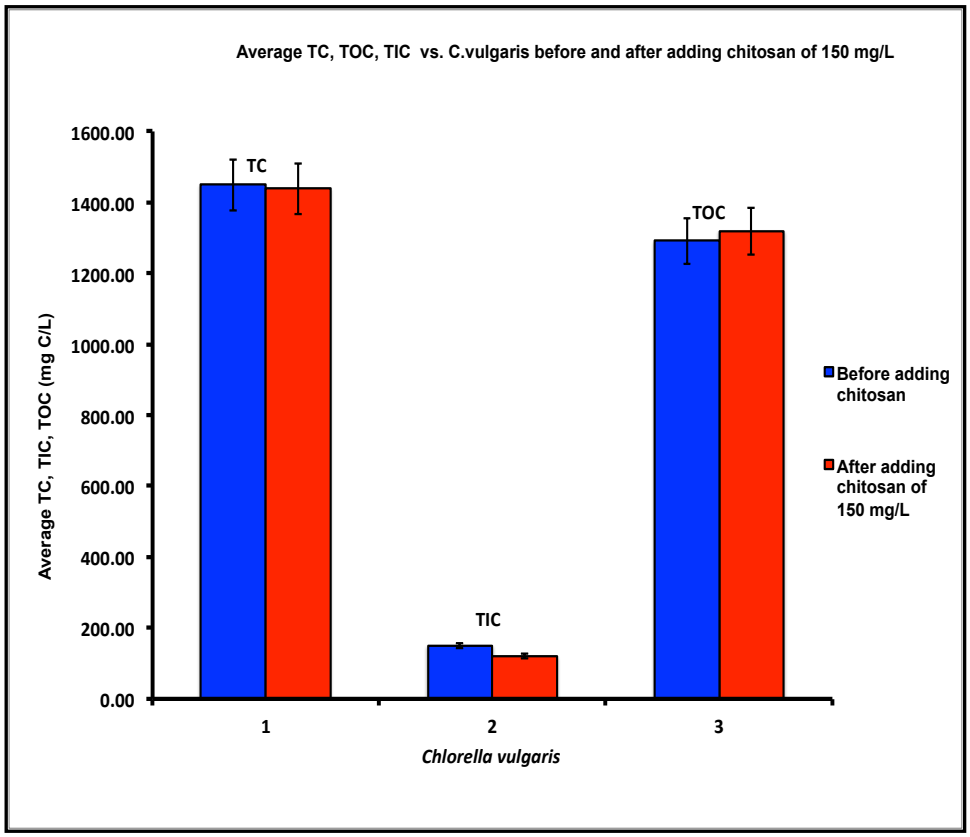
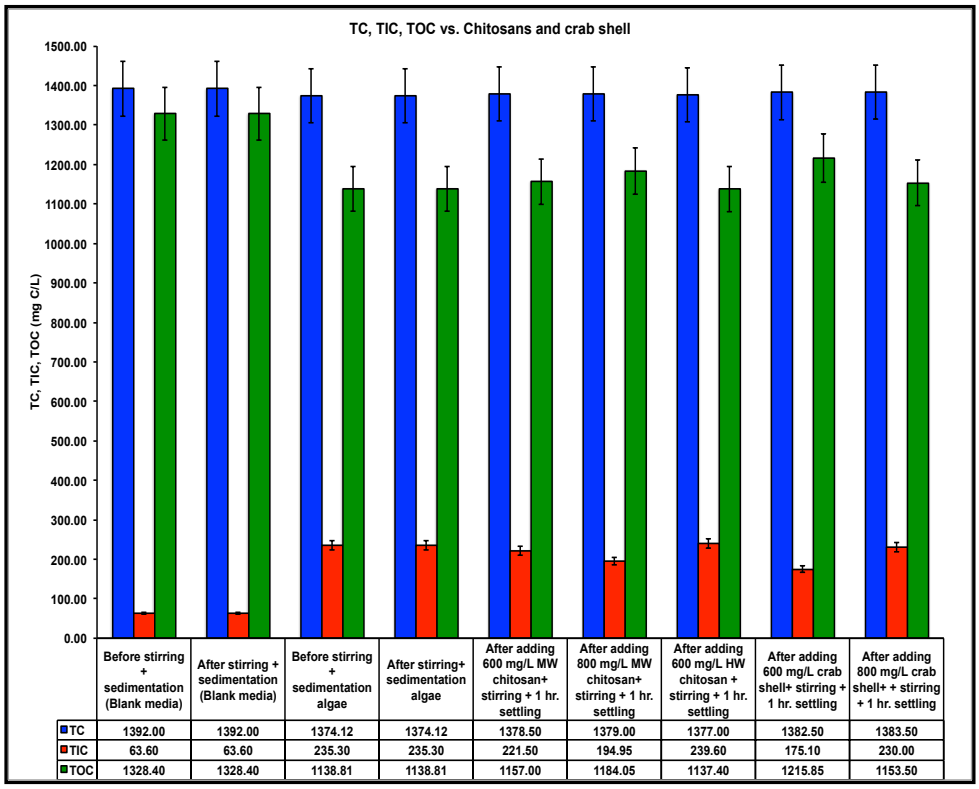


Figure 6-53 Average Total Carbon (TC), Total Inorganic Carbon (TIC) and Total Organic Carbon (TOC) before and after adding 150 mg/L MW chitosan to an algal culture. n=3. S.D. is 5%.

6.3.5.2 Effect of chitosans and crab shell on TC, TIC, and TOC change

After stirring and sedimentation there is very little discernible difference between the amounts of TC, TIC and TOC in algal cultures with added biopolymer and algal cultures without biopolymer **(Figure 6-54)**.

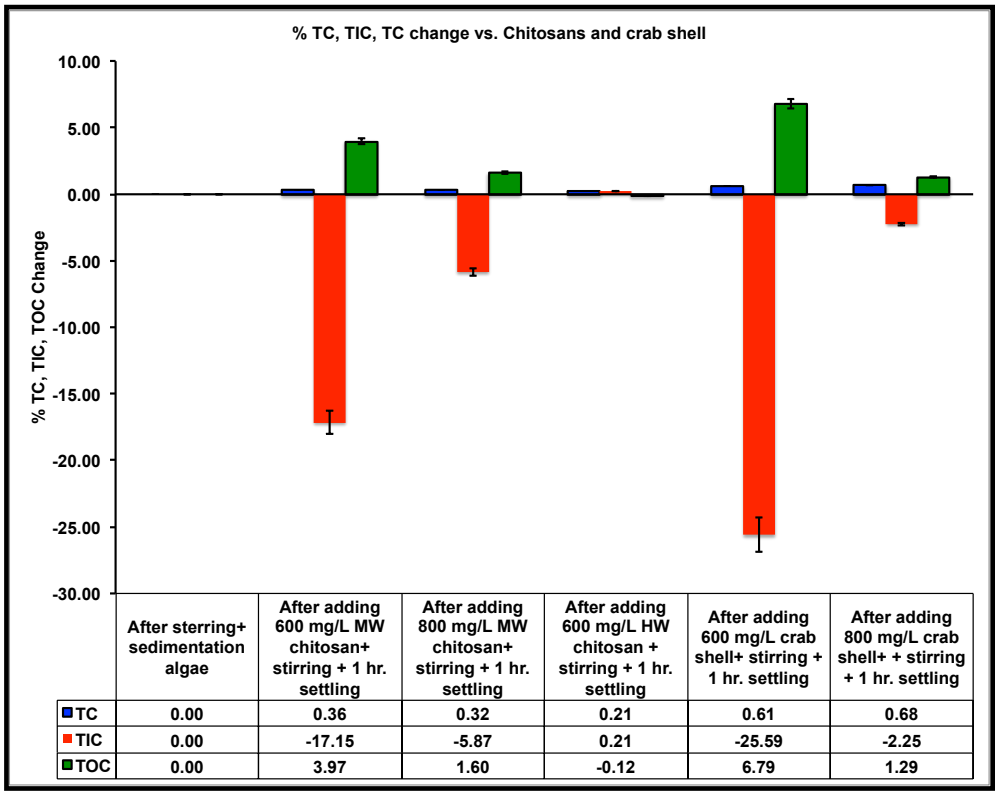
However, looking at the percentage change **(Figure 6-55)** there is virtually no change in any measure of carbon for the HW chitosan (less than 0.25%) while there were definite changes (mostly in removal of TIC – up to 25%) for the other biopolymers.



	Before stirring + sedimentation (Blank media)	After stirring + sedimentation (Blank media)	Before stirring + sedimentation algae	After stirring+ sedimentation algae	After adding 600 mg/L MW chitosan+ stirring + 1 hr. settling	After adding 800 mg/L MW chitosan+ stirring + 1 hr. settling	After adding 600 mg/L HW chitosan + stirring + 1 hr. settling	After adding 600 mg/L crab shell+ stirring + 1 hr. settling	After adding 800 mg/L crab shell+ + stirring + 1 hr. settling
TC	1392.00	1392.00	1374.12	1374.12	1378.50	1379.00	1377.00	1382.50	1383.50
TIC	63.60	63.60	235.30	235.30	221.50	194.95	239.60	175.10	230.00
TOC	1328.40	1328.40	1138.81	1138.81	1157.00	1184.05	1137.40	1215.85	1153.50

Figure 6-54 TC, TIC, TOC levels when chitosan/crab shell are first added, then after 1 hr. of stirring, and 1 hr. of settling. Data for controls are also given. S.D. set at 5% both side cap.

The results in **Figure 6-55** show that TC and TOC slightly increase after adding chitosan and crab shell then stirring for 1 hr., and settling for 1 hr. TIC is more variable. When adding MW chitosan at 600-800 mg/L TIC declines, in contrast, when adding 600 HW chitosan the TIC increases. Addition of crab shell at 600-800 mg/L results in a decline in TIC. This may be because algae are bonding with chitosan/crab shell while the unstabilised networking of the HW chitosan results in uncertain values.



	After sterring+ sedimentation algae	After adding 600 mg/L MW chitosan+ stirring + 1 hr. settling	After adding 800 mg/L MW chitosan+ stirring + 1 hr. settling	After adding 600 mg/L HW chitosan + stirring + 1 hr. settling	After adding 600 mg/L crab shell+ stirring + 1 hr. settling	After adding 800 mg/L crab shell+ + stirring + 1 hr. settling
TC	0.00	0.36	0.32	0.21	0.61	0.68
TIC	0.00	-17.15	-5.87	0.21	-25.59	-2.25
TOC	0.00	3.97	1.60	-0.12	6.79	1.29

Figure 6-55 Percentage change in TC, TIC, and TOC in algal cultures and with added chitosans/crab shell. S.D is 5% upper and lower of the data.

6.4 Discussion

6.4.1 Effect of dose and mixing speed when adding medium molecular weight chitosan

At chitosan doses of less than 200 mg/L, the turbidity does not change. Between 200-250 mg/L there is in rise in turbidity to 380 NTU. At 300-450 mg/L chitosan the turbidity slightly declines.

There is a gradual rise in pH when adding chitosan up to 150 mg/L. The pH remains stable until there is a slight drop when adding chitosan over 350 mg/L. A small chitosan dosage and low speed of circulation are less capable of inducing turbidity and pH changes.

6.4.2 Effect of adding metallic salts and chitosan in the sedimentation process

In industrial algal culture the harvesting process, for example by sedimentation, is very important. It was found that different flocculants provide different settling characteristics due to different interactions between the flocculant and the algae, such as charge and bonding. For example,

aluminium sulphate and chitosan differ in how they interact with water and with the components on the outside of the algal cell. Water interactions are shown in **Figure 6-56**. It seems than ionic bonding of alum occurs closer to the algal cell than it does for chitosan. This may be why the linking chains of chitosan need time to connect with the algal cells and thus the longer time taken for settling.

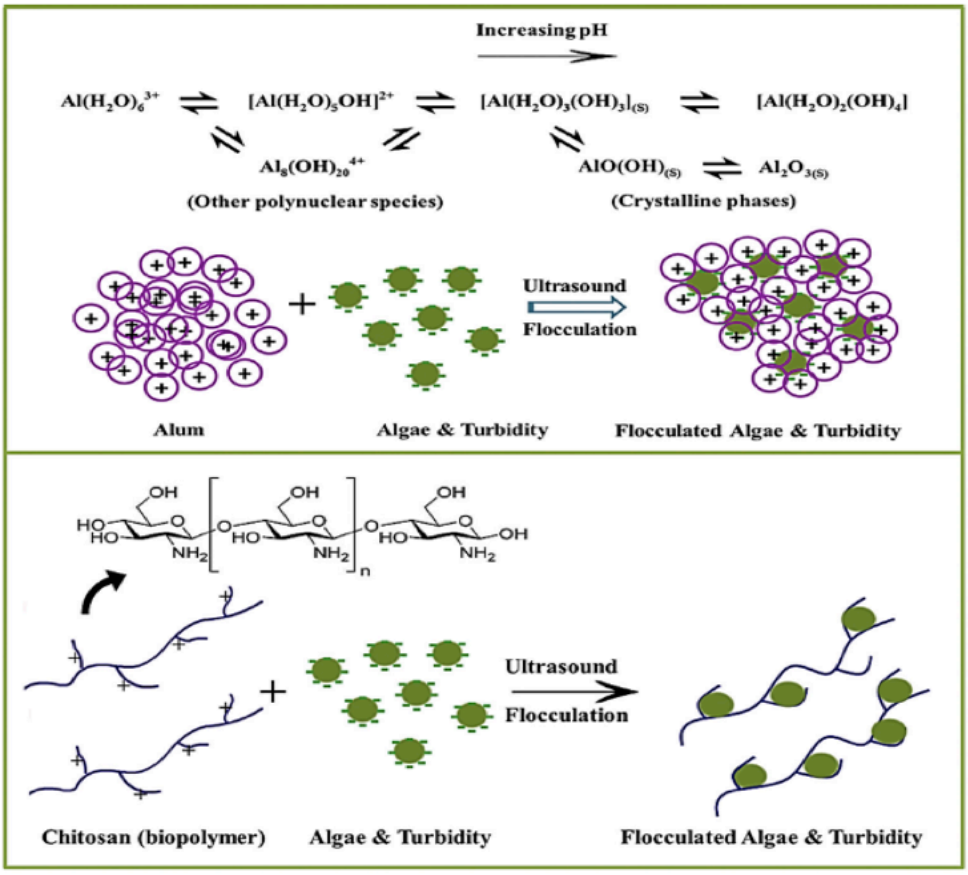


Figure 6-56 Mechanism of alum and chitosan enhanced flocculation processes (Fast *et al.*, 2014)

When adding Alum, ferric chloride, and aluminium chloride to the algal suspensions, the solutions become more acid. Acidification may be an advantage to the operation of these chemicals. Garzon-Sanabria *et al.* (2012) found that the best flocculation condition using aluminium chloride is 0.0016 ng/cell, at pH 5.3 with a removal efficiency of 96%.

Although the metallic salts studied are highly efficient at removing algae from the supernatant, the chemical will have to be removed from the sludge either because it contaminates the desired product or because it will not be allowed in the waste stream. Thus using chemical flocculants will contribute to operational costs and need additional processing.

MW chitosan and crab shell enhance settling of both living and dead algal cells, but the effect is more pronounced with living cells and nearly all the biomass has settled in 24 hours.

A study by Morales *et al.* (1985) found that chitosan can be used to flocculate marine algae (*Skeletonema cotastum, Dunaliella tertiolecta, Thalassiosira nordenskoldii and Chlorella sp.*). On adding 40-60 mg/L. Chitosan and stirring at a rapid speed (1-100 min-rpm) followed by a low speed (4-40 min-rpm) an OD_{678} of 0.80 declined to 0.00 (100% removal) with a pH change from 7.2 to 7.6. The study also found that the highest efficiency occurs when the pH is over 8.0. However, the study lacks information on how long the algae take time to floc and settle.

Divakaran and Pillai (2002) used chitosan to flocculate *Spilulina*, *Oscillatoria*, *Chlorella* and *Synechocystis* in the range of 80-800 mg/m^{-3}. The study found that the maximum flocculation efficiency is at 15 mg/L. They mentioned that re-bonding flocs with polymer takes a long time but are not clear how long these flocs take to settle out. These authors also found that the pH changed from 5 to 8. However, they found the optimal pH to be 7 with a removal of 90%.

In 2011, Cheng *et al*. showed that a pH of 8.5 is suitable for settling *C.valibilis NC64A* with chitosan. They studied settlement with chitosan levels of 0-69.6 mg/L. and showed an efficiency of 60% removal was found when more than 60 mg/L chitosan was used (Cheng *et al.*, 2011). In the same year, De Godos *et al*. (2011) showed that ferric chloride at 125-250 mg/L can remove algae (*Chlorella sorokiniana, Scenedemus obliquus, Chlorococcum* sp.) with a bacterial consortium at 66-98% efficiency. Using polymers (Drewfloc 447, Flocudex C5/500, Flocusol CM/78, Chemifloc CV/300 and Chitosan) removal was in the range of 25-50 mg/L. They found the highest removal was 30% using 25 mg/L chitosan.

Other flocculants are possible. A study by Salim *et al.* (2011) shows that bio-flocculation (adding the algae *A. Alcatus*, *S. obliquus* and *Tetraselmis suecica* to induce *C.vulgaris* to flocculate) gave a recovery efficiency of 60% after 8 hr. The study mentioned that bridging and patching mechanisms are involved in polymer induced flocculation.

This study found that adding a small amount of chemical or biopolymer aids flocculation enabling more than 90% of an algal culture to be sedimented. The actual flocculation process takes a shorter time than the sedimentation process. Sedimentation is the sinking of the flocs through the water (media) column under the influence of gravity, flocculation is the mechanism by which the flocs form and become dense enough to sediment faster than the algae alone. The mechanisms of flocculation, including charge nuetralisation, electrostatistic patch, brindging and sweeping flocculation, are rapid. For example, the algal surface electric charge (Zeta potential) which helps determine the degree and speed of flocculation, can be assessed using a zeta meter (Cheng *et al.*, 2011). Zeta potential (Zp) can be calculated by **Equation 6.1**.

$$Z_p = \frac{\eta 4\pi}{D} E_m \quad (6.1)$$

where, E_m is the electrophoretic mobility ($cm^2.V^{-1}.s^{-1}$); η is the viscosity ($g.cm^{-1}.s^{-1}$); and D is the dielectric constant ($C.V^{-1}.cm^{-1}$) (Sukenik and Shelef, 1984).

The time and degree of aggregation depends on chemical dose, pH, particle size, shear stress, zeta potential, and dynamic velocity of the fluid (Apostol *et al.*, 2011; Cheng *et al.*, 2011; Guibai and Gregory, 1991; Roselet *et al.*, 2016; Sukenik *et al.*, 1988) (see **Chapter 2**).

This study investigated the sedimentation process after settling times of 0, 1, 12 and 24 hrs. The results show that the sedimentation process can be achieved in 24 hours or less, considerably quicker than allowing the algae to settle by themselves (autoflocculation) which can take 8-10 days (Sukenik and Shelef, (1984); Granados *et al.* (2012)). Theoretically, sedimentation is by particular mechanisms for example; discrete particle settling, mass fraction settling, zone settling and compressive settling (Guibai and Gregory, 1991). The settling velocity depends on the Stokes' law assuming that velocity is proportional to the square of the radius of the floc and the difference in density between the microalgal cells and the medium as shown in **Equation 6.2**.

$$\text{Setting velocity} = \frac{2}{9} g \frac{r^2}{\eta} (\rho_s - \rho_l) \quad (6.2)$$

where r is cell radius, η is fluid dynamic viscosity and ρ_s and ρ_1 are the solid and liquid density.

This can be defined that why adding flocculant to increase the diameter of floc (algal cell size), also suspension viscosity, media and algal density result in the change of settling rate.

6.4.3 Effect of flocculants on turbidity, optical density, and pH measurements investigating the settlement of living alga and dead cell

The dead cell suspensions with added flocculants are unstable moving in the algal suspension. The flocculants work better with live algal cultures than with dead cell suspensions. The electron charge in dead cells may differ from that of living cells. This is an important point for industrial applications.

6.4.4 Effect on carbon measurements (TC, TIC, TOC) after adding chitosan

The study indicates a difference in "reactivity" between HW chitosan and MW chitosan and crab shell that may explain why HW chitosan is a poor flocculant. HW chitosan does not have the structure to adsorb (or absorb) carbon and therefore has less affinity for the bonding required in flocculation. It seems adding MW chitosan or crab shell at 600 mg/L causes a higher change in TIC and TOC than adding them at 800 mg/L. This may be due to how networks form between the algal cells and the flocculant.

6.5 Conclusion

A chitosan dose of 200-250 NTU causes a rise in turbidity to 380 NTU. There is a pH change when adding chitosan at 150 mg/L or more. A small chitosan dose and low speed of circulation have less effect on turbidity and pH changes.

A number of factors influence algal setting, such as algae and chemical characteristics, bonding force and environmental conditions such as pH, mixing, and mass concentration.

Metallic salts and biopolymers (MW chitosan and crab shell) are able to accelerate or facilitate the removal of algae by sedimentation.

Aluminium chloride, alum, and ferric chloride result in a high percentage of removal in 12 hr. (more than 95%). Crab shell and MW chitosan sediment more than 90% of the algae at 24 hr. In contrast, HW chitosan shows much less effect on algal sedimentation, not being any better that allowing the algae to settle without flocculant addition. Thus the choice of polymer, particularly molecular weight in the case of chitosan and perhaps others, is important. While chitosans have to be extracted and refined, raw crab shell material is a low-cost waste which can be easily sourced and prepared. It does less damage to the cells (by stabilising pH around pH 7) and is less hazardous to the algal products or final algal waste, yet it is as good as refined MW chitosan at encouraging settlement. Improvements in sedimentation require correct choice of flocculant, correct dosing, appropriate mixing, and sufficient settling time.

When adding MW chitosan at 150 mg/L then stirring, the study found that TC and TIC are slightly decreased while TOC is increased. The results indicate that, physically, chitosan is largely inert. There is thus less chance of it affecting the quality of the algae or their products on harvesting and it may be able to be recovered and re-used.

Adding MW chitosan and Crab shell at 600 and 800 mg/L to algal cultures causes TC and TOC to slightly increase, while TIC declines. In contrast, additions of HW chitosan has virtually no effect.

Chapter 7: A theoretical consideration of using CO_2 from flue gas of an electric power plant in Thailand to grow algae

7.1 Introduction

Thailand is investigating bioethanol and biodiesel as alternative energy sources because there are abundant feedstocks, for example; sugarcane, molasses, cassava, palm oil and wastes from these products. In addition, with abundant solar energy and water, growing microalgae is also a distinct economic possibility, particularly when such growth can be enhanced, and this chapter will look at applying the results from the laboratory experiments, described earlier in this thesis, to a theoretical algal production plant based on using the CO_2 from a real Thai electrical power plant.

7.2 Methodology

The study described in this chapter is based on a review of electricity production in Thailand and then the CO_2 production from an example electric power plant in Ratchaburi province, Thailand. The methodology is given in **Table 7-1**.

Table 7-1 Materials and Methodology

Study	Methodology
1) Background	Background to the energy demand and supply, energy policy, electricity generation in Thailand, The power plant in Ratchaburi
2) CO_2 production	The CO_2 production from the Ratchaburi power plant is calculated from the data available and this information used to design the algal cultivation system
3) Plant costs	An estimation of the cost of a photobioreactor system (Norsker *et al.* (2011) taking into account regional variations.
4) Energy requirements	An estimation of the energy required by the photobioreactor system and literature reviews
5) Comparison of the environmental conditions,	The study compares the environmental conditions for example temperature and light, from growing algae in the laboratory in The UK with growing algae in Thailand
6) SWOT Analysis (Strengths, Weakness, Opportunities and Threats)	A SWOT analysis was carried out using site visits, meeting with manager and researchers and employers (focus group) at The Ratchaburi power plant

7.3 Results

7.3.1 Geography of Thailand and Energy Policy

7.3.1.1 Background

Thailand is in the Southeast Asia at latitude 5° 37' - 20° 27'N and longitude 97° 21' - 105° 37'E. It is the 50th largest area of the world (slightly smaller than Yemen and larger than Spain). Thailand consists of 4 regions 1) mountains and forests in the North; 2) the central plains (rice fields); 3) semi-arid farmlands in the Northeast plateau and 4) tropical islands and a long peninsular coastline in the South. The country comprises 77 provinces.

7.3.1.2 Weather

Thailand has a tropical climate. The average temperature is around 32.4 ℃. Solar radiation is influenced by the northeast and the southeast monsoons. The highest intensity, of 20-24 MJ/m^2 per day, is found in April and May. The mean solar energy per year is 18.2 MJ/m^2 per day (Chirarattananon and Limmechokchai, 1996). This high solar radiation is a significant benefit to reducing electricity costs when culturing algae in Thailand.

7.3.1.3 Energy demand

Thailand's economy has reported a slight increase in energy consumption. Between 2005 and 2010 primary energy demand rose by an average of 4.1% per year. This is dependent on the economy; a fall in the economy leads to a fall in energy demand and vice-versa (IEA, 2016). In 2010 the Thai economy grew by 7.8% resulting in an increase in electricity demand of 9.11% (IEA, 2016).

7.3.1.4 Energy situation in Thailand

In 2008, the Thai domestic primary energy production was 61,930 Ktoe (kilo tonnes of oil equivalent) which was increased by 6.3% compared in 2007 (Jaruwongwittaya and Chen, 2010). The total electricity consumption was 11,632 Ktoe. 45% of this is used for commercial and residential air conditioning (Jaruwongwittaya and Chen, 2010).

In 2009, The Energy Policy and Planning Office (EPPO) reported that the total electricity consumption, categorised by economic sector was: residential 32,634 GWh or 24.2% of the total energy demand; industrial 44.1% or 59,402 GWh; agricultural 316 GWh (0.2%). This electricity was provided by: thermal power plants (9,667 MW, 34.8%), combined cycle power plants (12,806 MW, 46.0%), gas turbine and diesel power plants (972 MW, 3.5%), hydropower (3,764, 13.6%) and other renewable power plants (279 MW, 1.0%) (Sawangphol and Pharino, 2011). The ministry of energy reported in 2015 that that the country's peak the power demand reached 27,663.5 MW. Thailand ranks 17th in the use of fossil fuels of Organisation for Economic Co-Operation and Development (OECD) member countries.

7.3.1.5 Energy policy and planning

In 2010, a renewable energy development plan (REDP) was announced. This aims that renewable energy should contribute 30% of the total energy consumption by 2036 (Sawangphol and Pharino, 2011). Biofuel is to be a major contributor, particularly gasohol and biodiesel. For gasohol, the plan is to move from 1.3 to 9.0 million litres per day by 2022. For biodiesel, the goal is to move from 1.9 million litres per day in 2010 to 4.5 million litres per day by 2022.

In 2015, Ministry of Energy (2015) launched an integrated energy plan: "Thailand Integrated Energy Blueprint (TIEB)" running from 2015 to 2036 with the objective to reduce the total energy use by 30% (around 56,142 Ktoe) compared to 2010. The expected energy saving will be around 89,672 GWh in 2036. The plan has 5 sectors:

1) The Power Development Plan (PDP),
2) The Energy Efficiency Plans (EPP),
3) The Alternative Energy Development Plan (AEDP),
4) The Oil Plan 2015 and,
5) The Gas Plan 2015.

7.3.1.6 Electric supply in Thailand

In 1968, Thailand had only one electrical producer (The Electricity Generating Authority of Thailand, (EGAT). In 1994 the government enabled private sector competition resulting in a number of Independent

Power Producers (IPP), Small Power Producers (SPP) and Very Small Power Producers (VSPP).

In 2009, the electricity supply consisted of EGAT (53%), IPP (38%), SPP (7%), and imported and exchange (2%) (Sawangphol and Pharino, 2011). In 2015, the electricity generation was at 192,189 GWh rising by 3.3% compared to 2014. It consists of 67% natural gas. While coal/lignite, electric import, and renewable energy were 18%, 8%, and 5% respectively. 2% was derived from hydropower and 0.5% from oil.

7.3.2 The Electric Power Plant in Ratchaburi province

7.3.2.1 Background

The electric power plant providing the basis of the theoretical application is in Ratchaburi province in the West of Thailand (see **Figure 7-1**). It is an Independent Power Produce (IPP) in the Ratch group. The power plant makes its electricity from 97.53% natural gas, 2.36% fuel oil, and 0.11% diesel. The power plant sells the electricity to EGAT under a 25-year contract (2008 to 2033).

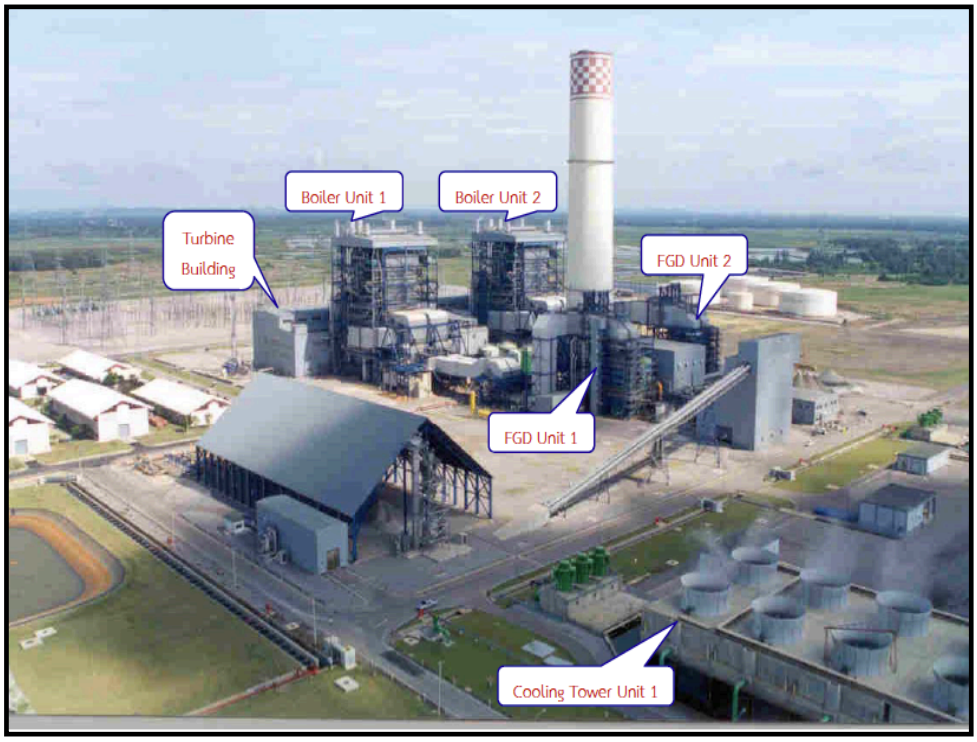


Figure 7-1 The Ratchaburi power plant (Tosomboon, 2013)

Ratchaburi power plant produced 17,109,009.69 million megawatt hours (MWh) in 2016. CO_2 emissions from the power station are 0.4433 tCO_2e/MWh. They have 2 types of power plant. Type 1 uses thermal technology from Mitsubishi and runs on natural gas. It uses fuel oil for reserve generation. The other plant uses a combined cycle gas & Stream turbine technology from GE Frame. The total capacity is 3,645 MW.

Type 1 generators use a Mitsubishi supercritical sliding pressure once through boiler technology with high thermal efficiency and superheat. They can provide high partial load, less thermal stress with a high pressure turbine, fast response and loading demand. They have low NO_x & particulate matter burners with mixed fuel operation. There are 2 units with a capacity of 735 MW each (total 1,470 MW). Each includes a 3 stages turbine, a cooled water generator, and flue gas desulphurization (FGD). The loading/de-loading rate is 7-60 MW. The primary response is 20 MW. For 5 min, it is 137 MW (Tosomboon, 2013). Details are given in **Table 7-2**.

Table 7-2 Type 1 generator specification (Ando *et al.*, 2002)

Equipment		Specification
Plant	Output	735 MW at generator terminal
	Steam condition	22.22 MPa x 538/566 ℃ (at turbine inlet)
	Fuel	Oil & gas (exclusive firing/mixed firing)
	Cooling system	Mechanical draft wet cooling tower
	Condenser vacuum	700/685 mm Hg.
	Feed water heater	8 stages
	Feed water treatment	Combined water treatment (CWT)
Boiler	Type	Supercritical sliding pressure operation once-through boiler
Turbine	Type	3,000 rpm tandem compound quadruple exhaust condensing type reheat and regenerating turbine, Low pressure (LP) end blade length: 35.4 inches
Generator	Type	Total enclosed, stator water cooled, rotor hydrogen cooled, complete with stationary armature and cylindrical rotor, directly coupled to the steam turbine
	Capacity	990 MVA
	Exciter	Thyristor excitation system
Flue gas desulfurisation (FGD)	Type	Wet lime/lime stone gypsum process

The layout of The Ratchaburi Power Plant and equipment are shown in **Figure 7-2** and **Table 7-3**. The turbine, generator and multi stage type 1 condenser are shown in **Figures 7-3 - 7-5**.

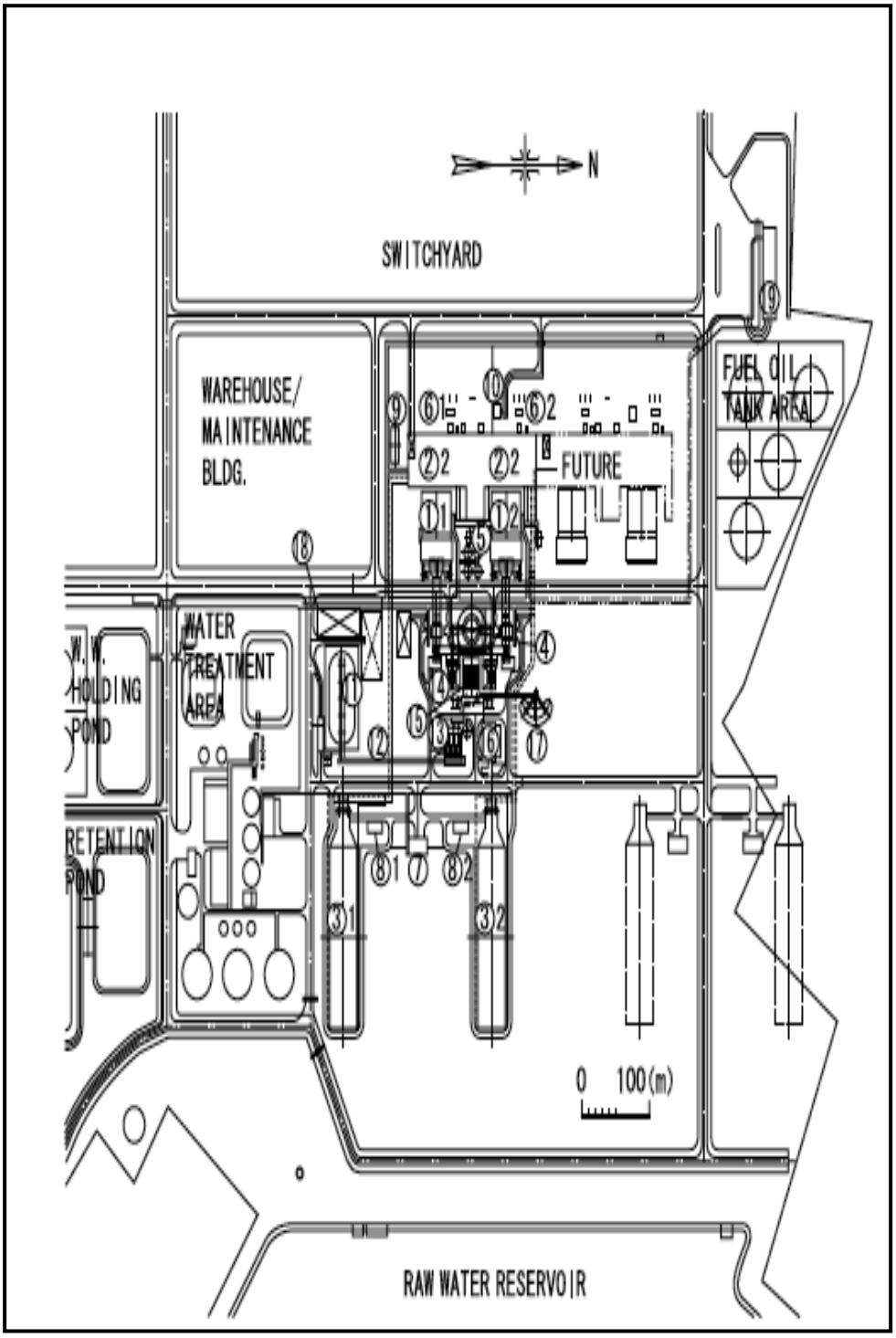


Figure 7-2 The layout of Ratchaburi power plant station and equipment as delivered by MHI (Ando *et al.*, 2002).

Table 7-3 The Power Plant and equipment (Ando *et al.*, 2002).

No.	Description
1	Boiler
2	Turbine & Generator
3	Cooling Tower & Cooling water pump (CWP)
4	Boiler stack
5	DEMI. /COND. Water storage tanks
6	Transformers
7	CHROL INTION Equipment
8	Electrical Building for cooling tower
9	Auxiliary boiler
10	Emergency Generator
11	Limestone Storage Area
12	Limestone Conveyor
13	Limestone Prep. Building
14	Slurry Recycle Building
15	FGD Byproduct Process
16	FGD Byproduct Process
17	Sludge Stock out
18	Flue gas reducing station
19	Flue oil pump house

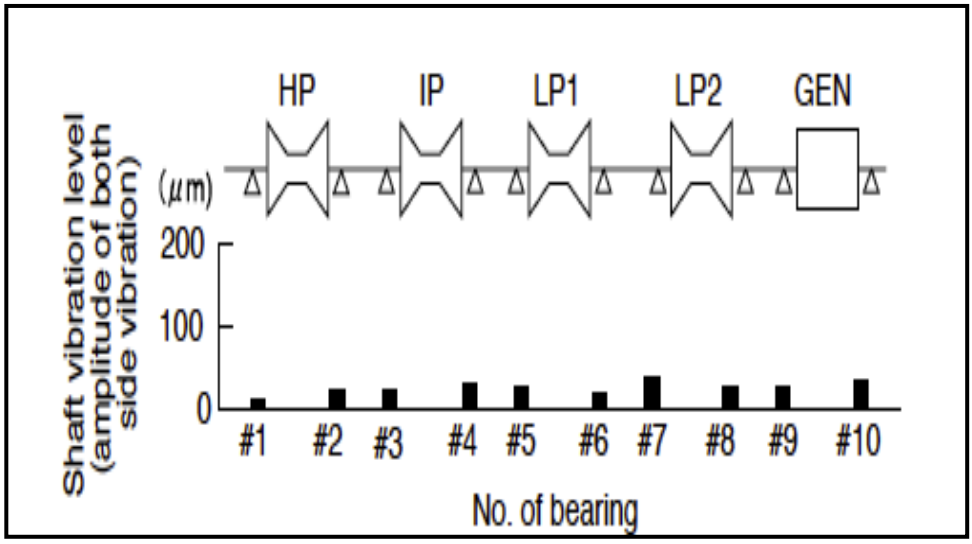


Figure 7-3 Turbine generator for the Ratchaburi unit No.1 (Ando *et al.*, 2002).

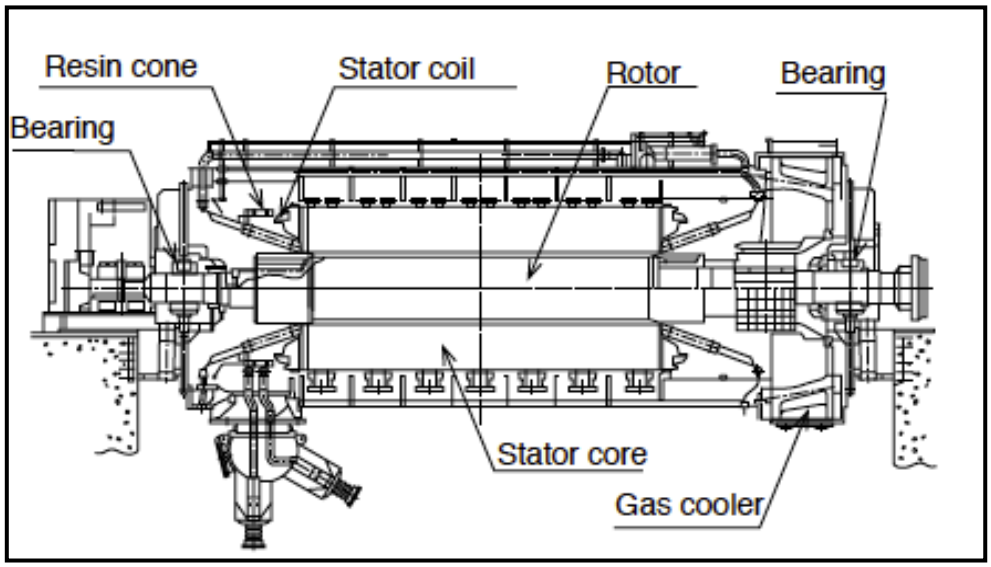


Figure 7-4 Construction of Ratchaburi generator (Ando *et al.*, 2002).

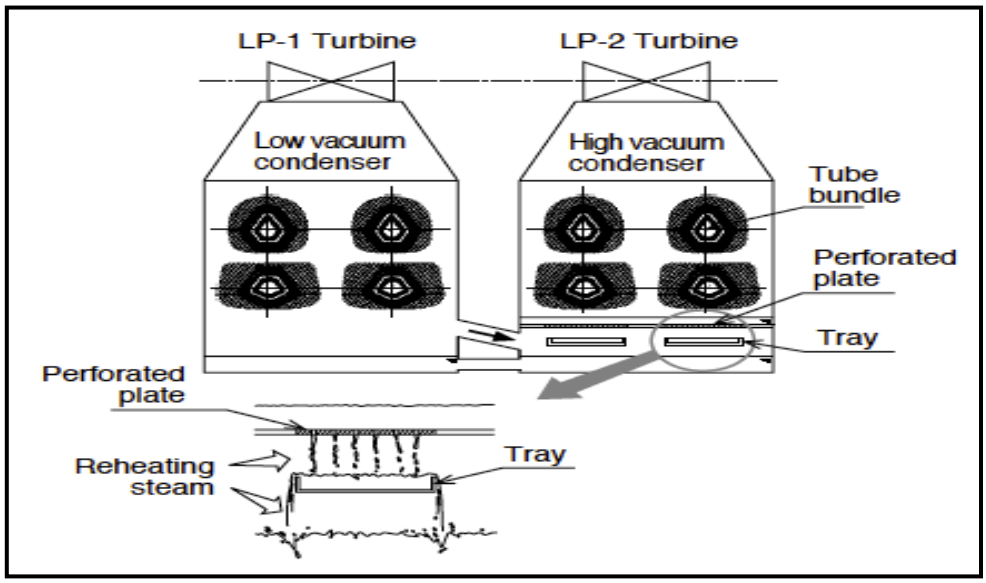


Figure 7-5 Construction of multi-stage pressure type condenser (Ando *et al.*, 2002).

There are three units of the second type of generator. These are natural gas-fired gas turbine combined cycle, GTCC plants of 725 MW capacity each. The total is 2,175 MW. The power generation units comprise 4-M701F gas turbines, 2 steam turbines, 4 heat recovery steam generators (HRSGs) and 6 generators.

The GTCC power plants combine gas and steam turbines to produce electricity in 2 stages utilising high-temperature exhaust gas from the gas

turbine. The high efficiency process enables a reduction in fuel consumption with less emissions (Ikuno, 2005). The GTCC is shown in **Figure 7-6**.

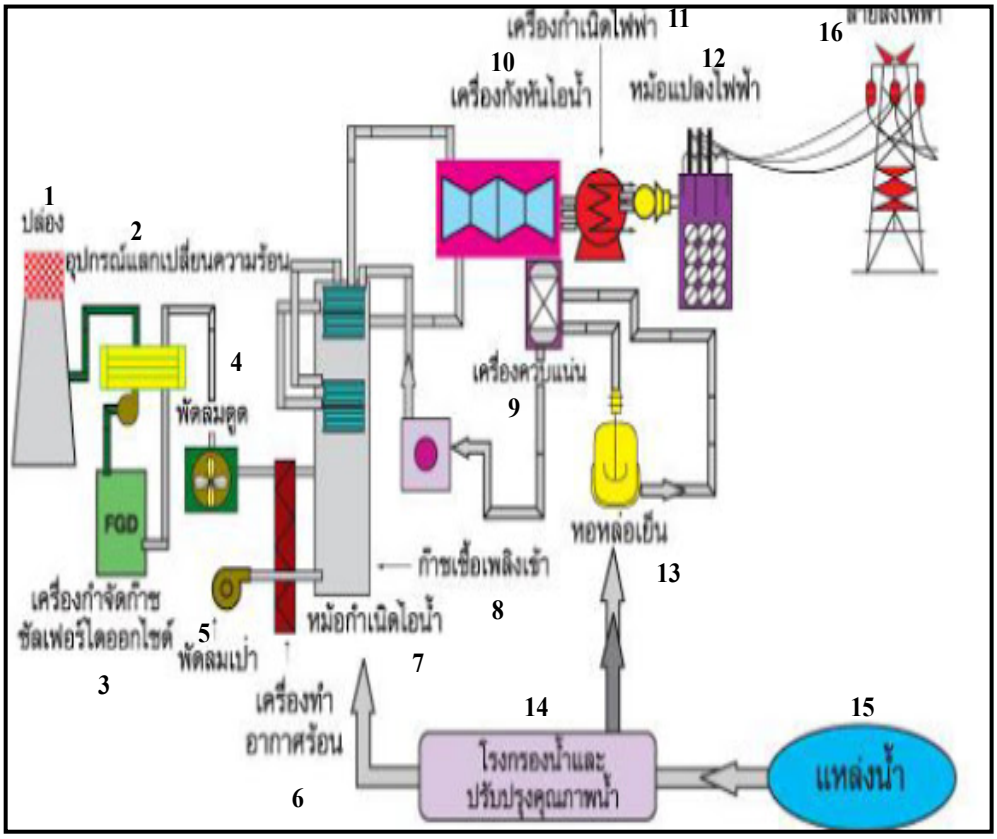


Figure 7-6 Diagram of the GTCC system including; 1) chimney 2) heat exchange equipment 3) FGD 4) a fan blowing air in 5) a fan blowing air out 6) heat recovery stream generator 7) stream generator 8) raw material inlet 9) condensor 10) steam turbine 11) generator 12) transformer 13) cooling tower 14) water quality improvement centre 15) water source 16) power transmission (Chaipak, 2015).

The main equipment of a combined-cycle power plant (Type 2) consists of; 1) gas turbine fuel 2) Heat recovery 3) steam turbine.

1) Gas turbine fuel

The fuel is compressed and heated to a very high temperature. The hot air–fuel mixture moves through the gas turbine blades, mixing it spin. The fast spinning turbine drives a generator to converts to electricity (**Figure 7-7)**

Figure 7-7 Ratchaburi gas-fired gas turbine combined cycle, GTCC (Ikuno, 2005). The figure shows of a typical gas turbine engine. Air is compressed by the fan blades as it enters the engine, and it is mixed and burned fuel with fuel in the combustible section. The hot exhausted gases provide forward thrust and turn the turbine the turbines with drive the compressor fan blades.

2) Heat recovery from steam

A heat recovery steam generator (HRSG) captures exhaust heat from the gas turbine that would otherwise escape through the chimney. HRSGs consist of 4 major components: 1) the economiser, 2) evaporator, 3) super heater, and 4) water preheater. The different components are put together to operation.

The power plant uses once-through steam generator (OTSG) which is a type of HRSG without boiler drums. The feed water follows a continuous path through the economiser, evaporators, and super heater. This provides a high degree of flexibility based on the heat load being received from the gas turbine. The absence of drums allows for quick changes in steam production and fewer variables to control and cycle the base load operation.

3) Steam turbine

This is a rotary heat engine that converts thermal energy contained in the stream to mechanical energy and then to electric energy.

Mitsubishi Electric Corporation built the generators in collaboration with Sino-Thai Engineering & Construction Public Company Limited (STECON) (Ikuno, 2005). The specification is given in **Table 7-4**.

Table 7-4 Type 2 Generator specification.

Ratchaburi Power GTCC Power Plant Thailand	
Latitude	13.608
Longitude	99.8902
Design capacity (Megawatt electrical, MWe)	725 MW
Type of plant	Open cycle gas turbine
Type of fuel	Primary: Natural gas
	Secondary: Light fuel oil/Diesel
Gas supply (Million standard cubic metre per day, MMSCMD)	Required for 90% PLF
Configuration of boiler/ Turbine/Gen	2 blocks of 2+2+1 CCGT
Electric Power grid connection	National grid
Operator	EGAT
NO_x control device type	Low NOx Burner with Over fire air
Unit No.1	Capacity: 230 (MWe), Turbine model: GT-1M701, manufacturer by MHI, Generator manufacturer: Melco, Boiler/HRSG manufacturer: MHI Japan
Unit No. 2	Capacity: 230 (MWe), Turbine model: GT-2M701F manufacturer by MHI, Generator manufacturer: Melco, Boiler/HRSG manufacturer: MHI Japan
Unit No. 3	Capacity:230 (MWe), Turbine model: ST-1, manufacturer by MHI Generator manufacturer: Melco
Unit No. 4	Capacity: 230 (MWe), Turbine manufacturer by MHI model GT-3M701F, Generator manufacturer: Melco, Boiler/HRSG manufacturer: MHI Japan
Unit No.5	Capacity: 230 (MWe), Turbine manufacturer by MHI model: GT-4M701F, Generator manufacturer: Melco, Boiler/HRSG manufacturer: MHI Japan
Unit No 6.	Capacity: 230 (MWe), Turbine manufacturer by MHI model: ST2, Generator manufacturer: Melco
Owner	25% Hong Kong Electric Holding Limited (HEH) 25% Ratchaburi Electricity Generating Holding Public Company Ltd.

(http://globalenergyobservatory.org/geoid/41481)

The burner system is capable of both exclusive firing and mixed firing of oil and natural gas. The power plant uses low NO_x and low SO_x burners and incorporates a high performance FGD system (see **Figure 7-7**).

7.3.2.2 Emission controls

The power plant has emission controls for the exhaust gases from the production process:

1) CO_2

The Ratchaburi Power Plant uses 97.4% natural gas, 2.49% fuel oil, and 0.2% diesel as its fuel. The literature review in **Chapter 2** found that algae grows well at 1-5% CO_2 and the laboratory results of this work, found that *Chorella sp*. can grow best up to 12% CO_2. When the Ratchaburi Power Plant uses a natural gas as a raw material to generate electricity it emits and average of 4.4% CO_2 (v/v) which, as high purity CO_2 gas is not required for algal culture, can be directly fed into a photobioreactor system (University, 1996).

In 2016, the total GHG emissions from this plant in terms of CO_2 equivalent was 7,584,424 tCO_2e calculated from the net actual electrical generation of 17,109,009.69 MWh x 0.4433 tCO_2e/MWh. The GHG emissions are about 0.4433 tCO_2e/MWh. The study assumes that most of CO_2 equivalent is from CO_2 gas. The amount is more than enough to provide for any industrial photobioreactor system (see below).

2) NO_x

Products such as NO_x or SO_x can be used as nutrients for microalgae. Microalgae can tolerate and grow in a medium containing 240 ppm NOx, with pH adjustment. Yoshihara *et al.* (1996) found that marine algae NOA-113 cultured in a modified f/2 seawater medium at pH 6 under 15% CO_2 (v/v) grew best at levels of 100 ppm NO but growth declined at 200-300 ppm NO.

The power plant type 1 burners have low NO_x burners and flue gas re-circulation to control NO_x. While, type 2 (GTCC) plant uses a dry low NO_x burner when using natural gas. If switching to diesel oil, water is

injected into the burning system to control temperature and to reduce oxidation with NO_x. It includes an extractive dilution system (Ratchaburi *et al.*, 2017). In 2016, the average NO_x was between 2.98-89.43 ppm (Ratchaburi *et al.*, 2017) (see **Table 7-5**) depending on the fuel (natural gas/oil). Thus, there is no need to eliminate NO_x when using the flue gas to grow algae.

3) SO_2

SO_2 may have a significant effect on growth rate and health of algae when the concentration reaches 400 ppm due, mainly, to a lowering of the pH (Stepan *et al.*, 2002).

The power plant has 2 units of FGD to reduce SO_2 (at 95.14% efficiency). These meet the Thai Environmental Impact Assessment (EIA) requirements which recommend at least 90% efficiency. Type 1 power plants produce up to 116 mg SO_2/Nm^3 (Nm^3 is normal cubic metre which presents 1 cubic metre at 15 ℃ at 101.325 kPa). Type 2 produces 112 mg SO_2/Nm^3 when burning heavy oil containing 3% sulphur.

The flue gas passes through the FGD and then a cooling tower to circulate and to reduce temperature. Emissions are monitored by an online Continuous Environmental Monitoring system (CEMs) under Ministry of Industry Standards. In 2016, the total SO_2 was 158.7 tonne (about 0.000005 tonne/MWh). An example of the NO_x and SO_2 emissions is given in **Table 7-5**. These emissions will not adversely affect algal growth.

Table 7-5 NO_x and SO_2 emissions from the Ratchaburi power plant (Ratchaburi *et al.*, 2017).

Power plant	No. of units	Average concentrated emissions from Combustion released from the power plant				
		NO_x (ppm)		SO_2 (ppm)		Fuel
		Natural gas	Oil	Natural gas	Oil	
Type1: Ratchaburi Thermal plants	2 units (capacity of each 735 MW), total 1,470 MW	2.98	19.78	0.72	18.87	Fuel oil
Type 2: Ratchaburi Combined-Cycle plants	3 units (capacity of each 725 MW) total 2,175 MW	21.26	89.43	1.40	4.79	Diesel
Total	3,645 MW					
Thai emission Standard		120	180	20	320	-

4) Soot and dust

Soot and ash containing heavy metals may limit algal growth rate. To remove dust, the power plant has a double-contact-flow scrubber (DCFS) that can remove 85% of the total dust at a high velocity gas flow (10 m/s). In 2016, the average of five monitoring stations was 19 μ/m^3 (Ratchaburi *et al.*, 2017). This is unlikely to be detrimental to algal culture.

5) Temperature

The Ratchaburi power plant uses once through cooling tower technology. Parker (1979) states that over to 90% of the heat from flue gas can be removed by cooling towers. However, it is likely that flue gas used for algal growth will have to be captured and directed to the photobioreactors before the cooling towers, thus a heat exchanger system would be needed. The flue gas outlet temperature of Type 1 and Type 2 are in **Table 7-6** and **Table 7-7**.

Table 7-6 Ratchaburi power plant Type 1 combustion (700 MW) data (Mahidol University (1996)).

Parameter	Natural Gas (Pure Methane)	Fuel Oil (2% S) Fuel
Fuel consumption (SCF/h)	6,920,450	-
(Kg/h)	-	165,780
Emissions at 6% O_2		
NO_x (part per million volume dry, ppmvd)**		
NO_x, as NO_2 (g/s)**	175	190
SO_2, uncontrolled (part per media volume dry, ppmvd)	220	253
SO_2, uncontrolled (g/s)	-	994
TSP (g/s)	-	1,840
Unburned hydrocarbon UHC (ppmvd)	5	9
UHC (g/s)	20	18
CO (ppmvd)	9	8
CO (g/s)	42	40
Opacity (%)	20	20
Exhaust gas flow (g/s)*	857,215	873,610
(m^3/s)*	1,070	1,050
Exhaust gas velocity	28	27.3
Exhaust gas temperature (℃)	129	179
Chimney height (m)	150	150
Chimney diameter (m)	7	7

-Value are appropriate for one 700 MW unit

-with 80% SO_2 control efficiency

-Assume that NO_2 emission constitutes 20% of total NO_x emission

*Based on 10% excess air, 15% air heater leakage, and actual conditions

(265 °F, 20 H_2O)

**Based on Low NO_x burners and/or over fired air, no reductions included for flue gas recirculation

Table 7-7 Ratchaburi power plant Type 2 combustion (200 MW turbine: GE-9 FA) data (Mahidol University (1996)).

Parameter	Natural Gas (Pure Methane)	Diesel (0.25% S)
Fuel consumption (SCF/h) (kg/h)	2,230,625	
Emissions at 6% O_2		53,970
NOx (part per million volume dry, ppmvd)	75	75
NOx, as NO_2 (g/s)	76.67	81.86
SO_2, uncontrolled (ppmvd)	--	62.0
SO_2, uncontrolled (g/s)	--	74.81
TSP (g/s)	1.9	3.8
UHC (ppmvd)	7	7
UHC (g/s)	2.3	2.4
CO (ppmvd)	15	20
CO (g/s)	8	11
Opacity (%)	5 +/-	10 +/-
Exhaust gas flow (g/s) at standard condition (60 °F, 14.696 psia)	579,380	595,083
Exhaust gas flow rate (m^3/s)	486.48	496.32
Exhaust gas velocity (m/s) - Combustion Turbine Generator (CTG) (bypass case) -Heat recovery steam generator (HRSG) (normal case)	 54.8 26.88	 57.2 29.20
Exhaust gas temperature (℃) -CTG -HRSG	 607 103	 602 128
Chimney height (m) CT HRSG	35 35	35 35
Chimney Diameter (m) CT HRSG	5.79 5.5	5.79 5.5

6) Wastewater treatment

The Ratchaburi power plant has large volumes of water used for cooling systems, FGD plant, boiler cleaning, ash transport, and demineraliser plant regeneration. It is possible that this water could be used to grow algae, saving costs while at the same time cleaning the water of pollutants (particularly

nitrogen compounds) by the algae. The use of such water would depend on the nature of any contaminants and the use to which the algal biomass is to be put.

In 2016, the total water volume used by the power plant was 24.68 million m^3 (Ratchaburi *et al.*, 2017). *C.vulgaris* will tolerate a degree of both heavy metal contamination and high temperature. While **Table 7-8** gives an idea of the condition of the wastewater, more studies will have to be undertaken before it could be considered for use to grow algae.

Table 7-8 Average effluent water quality from Ratchaburi Power Plant in 2016 (Ratchaburi *et al.*, 2017)

Index	Result	Royal Irrigation Department standard	Ministry of Industrial Standard	Unit
Temperature	31	≤ 33(standards only for Ratchaburi power stations) (Others: up to 40)	≤ 40	℃
pH	8.06	6.5 – 8.5	5.5 -9.0	-
BOD	3.3	≤ 20	≤ 20	mg/L
COD	32.7	≤ 100	≤ 120	mg/L
TDS	577	≤ 1,300	≤ 3,000	mg/L
Conductivity	900	≤ 2,000	Not specified	$\mu S/cm$

7.3.3 Designing an industrial scale algal cultivation system

1) Light

Thailand's solar radiation is about 18-20 MJ/m^2 per day or 5.278-5.556 kWh/m^2 day. This is around 231.5 W/m^2. At 25% efficiency, it is 57.875 W/m^2. Solar radiation captured is around 1 kWh/d or 58 W. This the sun's intensity in Thailand is close to the illumination used in the laboratory. The only difference would be that the light/dark period for natural sunlight in Thailand is 12:12 rather than the 16:8 used in the laboratory. It would be costly and un-necessary to use any additional artificial lighting (https://barani.biz/apps/solar/).

2) pH

Olaizola (2003) recommends a pH of between 7.3-7.8 to grow the algae. Some pH adjustment may be needed depending on the source of the water. For example, if the general waste water is to be used then acid will be needed to balance the pH from 8.06 (of the effluent water) to pH 7.0. This balancing may be possible by mixing various effluent water streams from the power plant rather than using costly (both monetary and environmentally) acid.

3) Temperature

The average temperature in Thailand is between 30-35 ℃. The photobioreactors may need to be shaded from direct sunlight so not to overheat the algae. Apart from this there should be no need for any other temperature control of the photobioreactors. The flue gas itself will be cooled by a flue gas cooler/chiller system and then distributed through the photobioreactors. The cooler system could be used to help maintain a consistent temperature in the photobioreactors through a suitable feedback loop.

Type 1 (thermal power plant) has an outlet temperature between 129-179 ℃ (see **Table 7-6**). The outlet flue gas for algal cultivation needs to be adjusted to 30 ℃ (a 76% decrease of the outlet temperature).

The exhaust temperature of the outlet flue gas on Type 2 is between 103-128 ℃ (see **Table 7-7**) and therefore needs a similar cooling system to bring the temperature to 30 ℃ (about 71 % decrease of the outlet temperature). Such reductions in temperature, while incurring a cost, are not difficult to obtain using a suitable heat exchange system.

4) Tubular photobioreactors

In tubular photobioreactors, algae are circulated in transparent tubes by a centrifugal pump (using the flue gas) and pass through an air sparged vessel where accumulated oxygen is blown off to balance the systems high O_2 concentrations that may reduce algal productivity. The turbulent gas flow, controlled at 0.15 vvm will mix the cells between illuminated and dark zones around the photobioreactor tube. This will avoid both thermal and

light induced stratification. The CO_2 contained in the flue gas is dissolved in the growth medium and consumed by the algae. The process would be optimised by feedback monitoring of the QA and QC system.

5) Harvesting processes

Mature cells will be continuously removed by pumping to a storage and separation system. Chitosan containing material (crustacean shell) can be used to aid the sedimentation process in the separating step.

7.3.4 Photobioreactor system designs

The literature review found that CO_2 from the boilers (Doucha *et al.*, 2005), chimney (Van Den Hende *et al.*, 2012) and FGD system (McGinn *et al.*, 2011; Nakamura *et al.*, 2001) have all been suggested to be used for algal growth. The moisture content of the gas will not be a problem for algal growth, though it may need to be controlled to avoid corrosion in the supply system. However, the gas will need to be reduced in temperature and this will also condense water out. A diagram of the envisaged system feeding the bioreactors is shown in **Figure 7-8**.

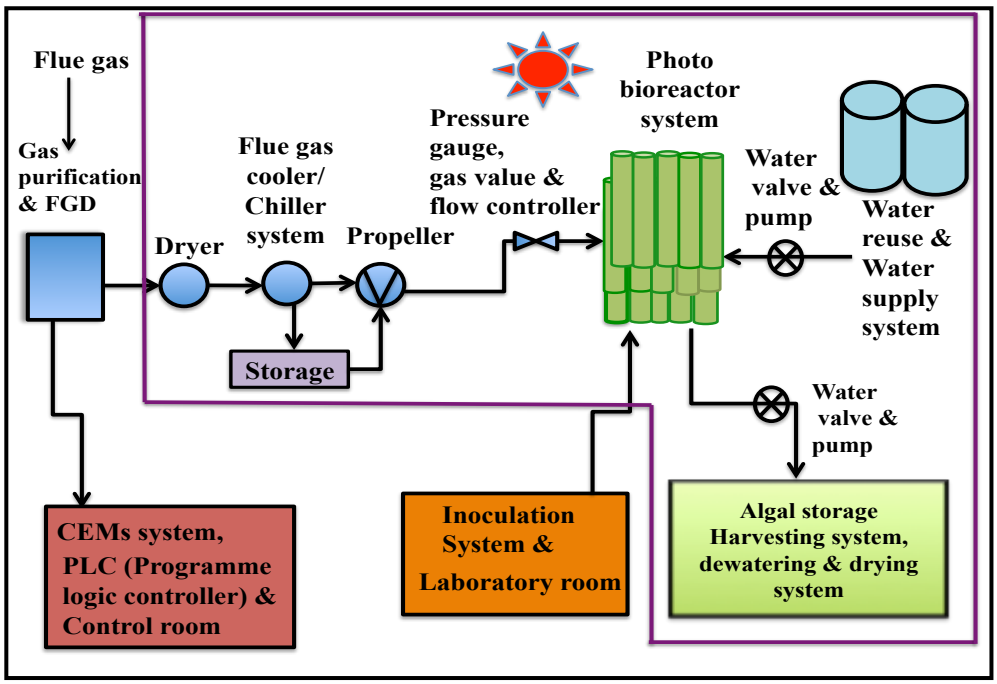


Figure 7-8 Diagram of the design of a commercial scale photobioreactor using flue gas. The required flue gas is diverted downstream of the FGD, cooled and dried to 30 ℃. and passed to the photobioreactors. Some of flue gas may be kept in the storage tank to balance the supply.

7.3.4.1 Estimation of the total CO_2 emissions from flue gas

In 2016, the Ratchaburi power plant operated at 17,109,009.69 MWh per year. The emission factor such a power plant is of 0.4433 tCO_2e/MWh (Krittayakasem *et al.*, 2011; Ratchaburi *et al.*, 2017). The CO_2 emitted from the chimney is thus 7,584,423.99 tCO_2e (0.4433 tCO_2e/MWh x 17,109,009.69 MWh).

1 mol of ideal gas at 0°C at 1 bar pressure (STP) is equal to 22.4 L (44 g CO_2 weight). The study will be at RTP (at 25°C) as this is close to Thailand temperature. 25°C at 1 bar pressure gives 24.47 L. (PV = nRT). At RTP, 24.47 L $CO_{2(g)}$ equals 44 g CO_2. Thus, 1 tonne CO_2 (1,000 kg) is 22,727 mol (from 1,000,000 g/44 g) or 556,130 L or 556.13 m^3 (from 22,727 mol x 24.47 L). The total CO_2 emissions are $4.22x10^{12}$ L per year (7,584,423.99 tCO_2e x 556,130 L). This study assumed that all the CO_2 equivalent come from the CO_2.

At the flow rate of 0.15 vvm. (or $LL^{-1}M^{-1}$) CO_2, 0.15 L can feed 1 L algal solution per min. Therefore, 1 L algal solution will consume 72,576 L CO_2 per year (0.15 L CO_2 x 60 min x 24 hr. x28 days x 12 months). Thus, $4.22x10^{12}$ L CO_2 can feed $5.81 x10^7$ L algal suspension (calculated by CO_2 total $4.22x10^{12}$ L/72,576 L using 1 L algal suspension) in 1 year.

7.3.4.2 Land use estimation

The Ratchaburi power plant has an area of 345 ha. There is space enough to develop a commercial photobioreactor project (around 1 ha of photobioreactors plus space for support buildings etc.) as there is a large area of un-utilised land, both on-site and surrounding the site. Considering the number of photobioreactors per 1 hectare (100 m. wide x 100 m. length) or 10,000 m^2 and 1 m^2 for each photobioreactor tube, the total is 10,000 tubes (**Figure 7-9**).

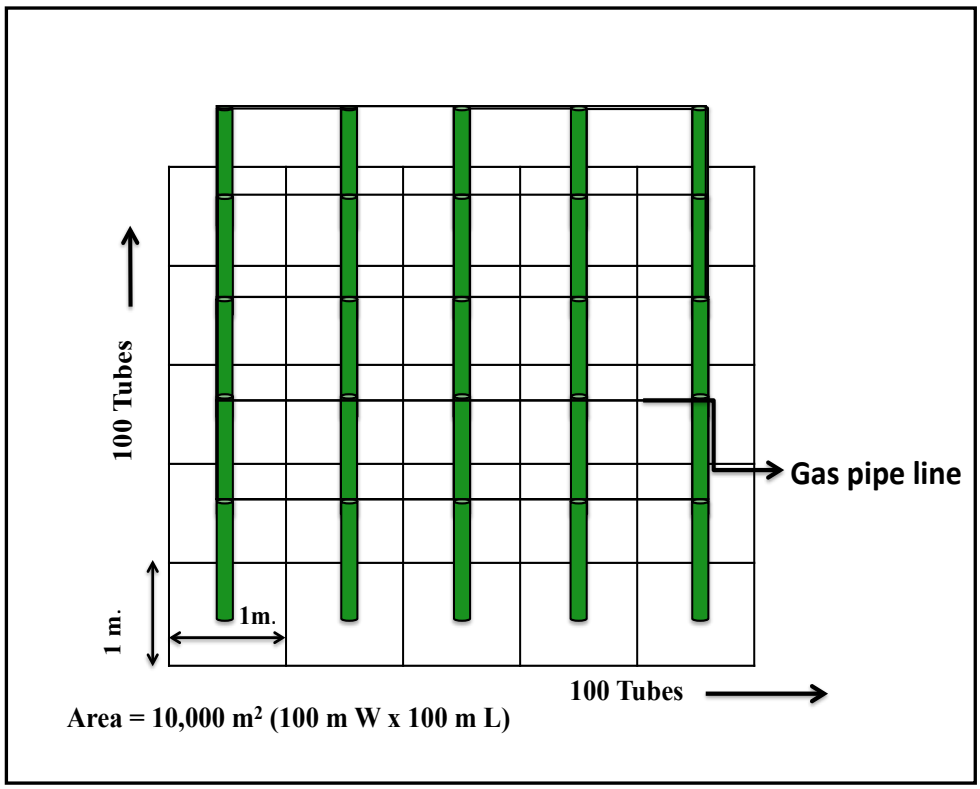


Figure 7-9 Land use for the photobioreactor 10,000 tubes.

7.3.4.3 Estimation of algae suspension volume

Each acrylic photobioreactor tube is 9.00 cm. OD x 8.00 cm. ID, 0.5 cm. wall thickness and 200 cm. in length (**Equation 7.1**).

$$\text{Volume} = \pi r^2 h \qquad (7.1)$$

$= 3.14 \times (4.00\ \text{cm})^2 \times 200\ \text{cm} = 10{,}048\ \text{cm}^3 = 10.048\ \text{L}$ per 1 tube

Consider 10,000 tubes, covering 1 ha, the liquid media (working volume) is 1.05×10^5 L. In 1 month (28 days) with 4 harvests, these will require of 4.20×10^5 L of liquid media (from 1.05×10^5 x 4 crops). For 1 year, this is 5.04×10^6 L yr^{-1} (4.20×10^5 L x 12 months) or 5,040 m^3.

7.3.4.4 Estimation of the total CO_2 consumption using algae

The Ratchaburi power plant emits CO_2 of between 4 and 6 % CO_2 (average 4.4 % v/v (Mahidol University, 1996). Therefore, the flue gas can be fed directly to the photobioreactors without any mixing or dilution but with cooling. Flue gas could be pumped to the photobioreactor system with a flow rate of 0.15 vvm. and a temperature of 30-35 ℃ (vvm, LLM, L/L.m is 0.15 litre CO_2 passing through 1 litre of medium in 1 min, first v = volume of gas, second v = volume of liquid, m = min).

At the conditions of;

1) 1 L algal solution will consume 72,576 L CO_2 per 1 year (**see 6.1**).

2) The total CO_2 emissions are $4.22x10^{12}$ L per year and the flow rate is fixed at 0.15 $vv^{-1}m^{-1}$

3) Algal volume per 1 year is 5.04 $x10^6$ L

Therefore the estimated CO_2 use for algae growth is:

1) At 4 % CO_2 (v/v and a flow rate 0.15 $vv^{-1}m^{-1}$. For 1 year, CO_2 use will be 3.66 $x10^{11}$ L (5.04 $x10^6$ L yr^{-1}algal solution x 72,576 L CO_2/1 L algal solution) or 8.67 % of the total emitted CO_2 (calculated from 3.66 $x10^{11}$L x100/$4.22x10^{12}$ L) i.e. around 6.58 $x10^5$ tyr^{-1} ($3.66x10^{11}$ L/556, 130 L CO_2 t^{-1}, 1 t CO_2 = 556,130 L).

2) At 6% CO_2, (the highest potential output) the CO_2 volume supplied will be $5.49x10^{11}$ $L.yr^{-1}$ (3.66 $x10^{11}$ algal volume x 6%/4 %) or 13.01 % of the total exhaust CO_2 ($5.49x10^{11}$ $L.yr^{-1}$ x100/$4.22x10^{12}$) or about $9.87x10^5$ t yr^{-1} ($5.49x10^{11}$ $L.yr^{-1}$/556,130).

To sum up, at 4-6% CO_2 around 6.58 $x10^5$ tyr^{-1}- $9.87x10^5$ t yr^{-1} CO_2 is consumed. This is around 10% of the CO_2 produced by the power station.

7.3.4.5 Estimate of the dry algal mass produced

An estimate of the dry mass of algae can be made assuming that, at between 4 and 6% CO_2, the conversion factor does not change and thus the data from the laboratory studies in this work for 6% CO_2 can be used. The data is then used for algae grown in 10,000 photobioreactor tubes or 5.04 $x10^6$ L yr^{-1}.

From the laboratory studies, algae dry mass is 18.2 g/L per month (28 days) or 0.65 $g.L^{-1}d^{-1}$. Thus, the dry mass for 1 year would be 1.10 $x10^9$ g (5.04 $x10^6$ L yr^{-1} x 0.65 $g.L^{-1}d^{-1}$ x 28 days x 12 months) or 1.10 x 10^6 kg yr^{-1} (1.1 $x10^3$ t yr^{-1}).

However, the efficiency of the harvesting process can cause losses. The literature gives a range of figures for losses on harvesting of between 0.5 and 27% (Milledge and Heaven, 2013). Such a wide range indicates that the harvesting procedure is critical. In this case, the study uses crustacean/ crab shell to improve the sedimentation of the algae. A conservative estimate would be a loss of 10% or around 100 tyr^{-1}. The harvested dry algal mass would therefore be 1,000 t yr^{-1}.

7.3.4.6 Estimate of algal extraction process and biodiesel from microalgal oil

The lipid content of algal cells (*C.vulgaris*) is 14-22% of dry mass (Demirbaş, 2008). Some reports go up to 50%, however for this study it will be assumed to be 30% or 300 t yr^{-1} (1,000 tyr^{-1} x 30%) following the study of Chisti (2007) and Demirbas and Demirbas (2011) who give a 30% oil yield.

The extracted oil fatty acid (raw material to produce methyl ester or 100% biodiesel or B100) from algae is around 50 %. This depends on extraction method/technology (Mercer and Armenta, 2011). Thus the biodiesel yield would be 150 t yr^{-1} per hectre (300 t yr^{-1} x 50%).

To sum up the total CO_2 volume is 7,584,423.99 $t.yr^{-1}$ or $4.22x10^{12}$ L yr^{-1}. This could yield an algal suspension of 5.81 $x10^{7}$ Lyr^{-1}. One hectre of photobioreactors would use 3.66 $x10^{11}$ L - $5.49x10^{11}$ L CO_2 (for 4-6%) to grow 5.04 $x10^{6}$ L of algal suspension. Dry algal biomass is 1,100 t yr^{-1} or 1,000 t yr^{-1} after harvesting. Lipid yield would be 300 t yr^{-1}, producing 150 t yr^{-1} of biodiesel (B100). A summary of the design criteria is given in **Table 7-9**.

Table 7-9 Summary design criteria for a commercial scale photobioreactor (it should be noted that the summary given is for 10,000 photobioreactor tubes occupying 1 ha (plus and additional 1 ha. for equipment giving 2 ha, overall). It is likely that, prior to such a commercial operation, a considerably smaller "pilot scale" system would need to be built and run to prove the concept.

Co-operation partner in the algal project	-Ratchaburi Power plant -University Research Centre -Biodiesel company, Ethanol company -Algal oil company
Financial sources/ support	- Joint venture company (international) - CDM, CO_2 trading fund - IEA - World Bank - WTO - Thai Government
Location of the project	- Ratchaburi Power plant Thailand
Project period	-10 years (1 June 2019 – 30 May 2029)
Contraction period/ Commissioning test	-1 year (1 June 2019 -30 May 2020)
Operation start up	-1 June 2020
Maintaining period	- After Year 1
Photobioreactor size	- 9.00 cm. OD x 8.00 cm. ID, 0.5 cm. wall thickness and 200 cm. in length
Total photoreactor	-10,000 tubes
Area	- 1 hectare (100 m. x 100 m (WxL) or 10,000 m^2 1 photoreactor/1 m^2
Temperature maintenance	30-35 ℃
CO_2 concentration	4 -6% v/v
CO_2 consumption	$3.66x10^{11}$ L -$5.49x10^{11}$ L yr^{-1} or 6.58 $x10^5$ tyr^{-1}- $9.87x10^5$ t yr-1
Algal volume	5.04 $x10^6$ L yr^{-1}
Harvesting method	Estimate Loss at 10% of algal volume
Biomass productivity (Wet algae 1 ha)	1,000 t yr^{-1}
	Estimate
Oil yield (fatty acid, crude oil)	300 t yr^{-1} (estimate at 30% of dry mass)
Vary extraction processes and transesterification processes	Estimated extracted fatty acid at 50 %
Biodiesel from algal oil, methyl ester or B100)	150 t yr^{-1}

7.3.5 Costs and benefits estimation

Commercial algal biodiesel production will not occur unless the economics are favourable. The details are explored below.

7.3.5.1 Benefit analysis

There are advantages when using CO_2 to grow algae.

1) CO_2 reduction costs

CO_2 fixation by algae produces a new biofuel resource. However Carbon capture or sequestration costs and benefits may become more expensive/valuable than the production of electricity or than the value of the algae (Kunjapur and Eldridge, 2010).

Nguyen *et al.* (2007) reported that GHG abatement from cassava ethanol production in Thailand is worth about $99 per tonne of CO_2 (or 4,257 baht t^{-1}). Therefore a value can be put on the CO_2 removed in this study;

1.1) If 100% CO_2 was removed from the power plant flue gas (4.22 $x10^{12}$ L or 7,584,423.99 tCO_2e or 7.6 million t yr^{-1}), this could be worth $ 750 million yr^{-1} based on the $99 per tonne figure of Nguyen *et al.* (2007) This is £537 million yr^{-1}

1.2) The algae projected to be grown in this study use between 6.58 $x10^5$ - $9.87x10^5$ t $CO_2 yr^{-1}$ (3.66 $x10^{11}$ - $5.49x10^{11}$ L) or an average of 4.5 $x10^5$ t yr^{-1}. The benefit will be $ 44.55 million yr^{-1} (4.5 $x10^5$ x $99). This is around £ 32 million yr^{-1}. Note that: 1 t CO_2 = 556,130 L

2) Dry mass

The study found that algal dry mass retail price is $104.64 per kg (£64)(https://www.ebay.com/itm/Chlorella-Chlorella-Vulgaris-Organic-1kg-/112267708757?hash=item1a23ad4955) or 2,760 baht kg^{-1}. The dry algal biomass yield for this study is 1,000 tonnes or 1 million kg yr^{-1}. Therefore, the retail value of the algae produced would be £64 million per year ($2.76x10^9$ baht yr^{-1}) if it could be sold this way.

3) Crude algal oil sale (fatty acid)

Chisti (2008) states that crude bioalgal oil is sold at $ 100 per barrel (1 barrel =159 L). This is $1.59 L^{-1} (£1.2 or 52 baht L^{-1}). For 300 tonnes would be £ 360,000 or 15.5 million baht yr^{-1}.

The 150 tonnes algal biodiesel (B100) that can be derived from 300 tonnes of algal crude oil has a retail price about the same as fossil oil

derived diesel. This is currently around £1.25 L^{-1} in the UK. There is a small premium above this for biodiesel and there may be Government subsidies in different countries. Allowing for a slight premium and therefore a price of £1.50 L^{-1} then 150 tonnes of algal biodiesel would be worth £225,000.

There is a large difference between the values so far given and this explains the lack of development of algal production facilities. On one hand, the retail price of dry algal biomass plus the potential value of the sequestered carbon dioxide is around £96 million per hectare per year while on the other hand, the value of algal oil production with no CO_2 sequestration value is between £225,000 and £360,000. As the "value" of CO_2 removed cannot be guaranteed and will be highly variable, then it would not be wise to rely on any such payments. It could be considered that the retail value of the dry algal biomass is good, even if the wholesale price was considered to be one tenth of the retail price (therefore £6.4 m yr^{-1}). However, there is a limited market for dry algal biomass and, unless other value added products can be derived from the algae, this price is likely to drop due to overproduction.

4) Value added by-products

Li *et al.* (2008) found that high-value bio-products such as phycobiliproteins and carotenoids can be extracted from microalgae. They are used for pigments, cosmetics and pro-vitamins. Brennan and Owende (2010) give prices between 215–1,790 €. kg^{-1} (8,385-69,810 baht.kg^{-1} or £195-1,623 kg^{-1}) for native pigments and up to 10,700 €.kg^{-1} (417,300 baht or £ 9,700. kg^{-1}) for refined cross-linked pigments. Polyunsaturated fatty acids (PUFAs) can also be obtained and are used as food additives and nutraceuticals (Brennan and Owende, 2010; Li *et al.*, 2008; Spolaore *et al.*, 2006). However, there will be significant processing costs associated with such products.

7.3.5.2 Cost analysis

Due to the experimental nature of algae production and the commercial sensitivity of the few companies who are trying to develop algal biomass, there are few cost estimations for algal production available. Norsker *et al.* (2011) made a study of the average costs of algal biomass production in the Netherlands for raceway, tubular and flat panel photobioreactor

based algal culture systems and found these to be € 4.95, 4.15 and 5.96 kg^{-1} respectively (£ 4-6 or 157-226 baht kg^{-1}). Chisti (2007) estimated costs to be about \$2.95 kg^{-1} dry mass for a raceway (£2.26 or 97 baht kg^{-1}).

For separation, MW chitosan or crab shell would be used at 600 and 800 mg/L respectively (600 kg yr^{-1} and 800 kg yr^{-1}). Thailand produces high volumes of chitosan (around £22 per kg.) and crab shell (£0.71-2.14 per kg) depending on the market. This would be a cost of £13,300 yr-1 for chitosan or around £1,600 yr-1 for crab shell (http://www.chitosanthai.com/inter/product-inter.html,https://classifieds.thaivisa.com/ad/7MT6XI9V/1-kg-chitosan-powder-s-a-p-foodgrade-200-mesh-for-sale-new,https://www.alibaba.com/premium/chitosan_powder.htm).

There is thus a wide range of production costs and it is difficult to separate capital and running costs from the figures. However, if an estimate overall algal production cost is taken to be £5 kg^{-1} then the 1,000 tonnes (1,000,000 kg) of dry algal biomass would cost £5 million to produce. Even taking the lowest cost given (£2.26 kg^{-1}, from Chisti, 2007) and assuming that a photobioreactor system in Thailand could be more efficient and yet have the same costs as a raceway, then the costs would be £2.26 m per year.

Therefore, the cost of producing, crude oil from the dry biomass would be £5 m for 300 tonnes at £5 kg^{-1} dry biomass cost or £2.26m at £2.26 kg^{-1}. This is between £7.5 and £17 L^{-1} (324-730 baht L^{-1}). This is 6 - 15 times the value of the oil as given by Chisti (2008).

Sawaengsak *et al.* (2014) carried out a life cycle analysis (LCA) for the cost of biodiesel production from microalgae in Thailand. They found that, the cost of producing biodiesel from raceways and photobioreactors was between £1.58 and £5.21 (68 and 224 baht L^{-1}). This is considerably less than the costs based on Chisti (2008) and Norsker *et al.* (2011) given above which would give £15 to £34 L^{-1}. Again, these large differences reflect the uncertainties in the costings and differences in costs between countries. Even on the figures given by Sawaengsak et al. (2014) only their lower estimate (£1.58 L^{-1}) gets near to breaking even. However, they do

suggest that other value-added products, such as omega-3 fatty acids could be extracted from the oil.

Further large variations are given by other authors. Huntley and Redalje (2007) found that biofuel production from algae would be between $39-127 per barrel (1 barrel =159 L) or 8 – 27 baht L^{-1} or £ 0.2 - 0.6 L^{-1}, while Rosenberg *et al.* (2011) state that the total cost of algal biodiesel would be at least $ 30 per gallon (£ 6.65 or 286 baht L^{-1}). Again there is a 10 to 20 times difference is cost estimates.

While the literature on the cost of producing algal biomass or algal oil/biodiesel varies widely, it can be seen that the economics of algal production depends on the value assigned to the carbon dioxide removed from the flue gas and the production of value added products rather than the value of the oil or biodiesel that could be produced.

On the figures available there is no feasibility of financial viability relying on the crude oil or biodiesel production and sale alone. However, the large range of potential value of the biomass, between £ 225,000 for crude oil to £64m for retail dry biomass indicates that there is potential if suitable value added products can be produced from the algae.

7.3.6 SWOT analysis

A SWOT analysis (Strength, Weakness, Opportunity, and Threats) was carried out at The Ratchaburi power plant. The power plant did have a pilot plant scale (raceway) to grow algae. The aim was to continue on to commercial production if there was the budget to support it. However, the company has a 25 year contract commitment to produce electricity for EGAT in line with government policy. Algae and it's by-products are not the company's main business and they could not see the financial benefit after the preliminary project, particularly the initial capital costs. After the project closed, expertise was lost. A SWOT analysis (**Table 7-10**) was carried out.

Table 7-10 The SWOT analysis of an algal commercial production at The power plant in Ratchaburi province.

1. Strengths	2. Opportunities
1) The power plant has a large area of land (2,158 rai or around 345 ha), thus there are no costs or issues around the land required 2) The project would have CO_2, treated water, and sunlight on site. 3) The project could claim funds from an international CO_2 reduction fund. 4) There will be a commercial value to the algae produced. 5) The algae could be used to clean up wastewater and will remove other components of the flue gas 6) The power plant still has most of the pilot facilities to support a raceway pond system and bag culture. Infrastructure, laboratory etc. This may reduce some initial costs.	1) Algal oil fuel may be an alternate to build up energy security in Thailand and could balance or challenge biodiesel from palm oil if harvest and environmental conditions. 2) Algal fuel is a second generation biofuel. It is a good opportunity to set up a new business and to create new products (by-product). 3) The staff at the plant will learn a new technology and this will enable some develop their career path bioenergy skills. 4) The researchers would have an opportunity to study and run the system at a real power plant. The staff would have to respond to problem immediately. This results in awareness and desire to solve problems as they arise. 5) A photobioreactor system can be compared to the data already available on the raceway design. This may enhance the knowledge gains and bring creative ideas for the best yield.
3.Weaknesses	**4. Threats**
1) The raceway pilot plant closed as the management decided that the production of algae had a high investment cost and could not be competitive with diesel and other biofuels in the present market. The management would not be sympathetic to a new scheme. 2) Algal biomass is not the main business of the company running the power plant. This results in a lack of commitment to financial support. 3) Most of the knowledge base from the pilot raceway project has been lost. Researchers have left. 4) This is a new and challenging market with many uncertainties in the products and their value.	1) There are technical and cost problems, particularly being sure of a return on investment 2) The lesson from the last study was that the algal raceway, though simple in concept had high manpower requirements for little benefit. 3) New staff with suitable knowledge would have to be recruited .

7.4 Discussion

7.4.1 CO_2 sequestration

Air quality affects public health and welfare. Increasing fuel consumption supports human life but emits CO_2 and other GHGs into the atmosphere. Emissions relate to an increasing population, GDP and hence energy demand. The Thai Ministry of Energy (MOE) reported that CO_2 emissions per capita in Thailand increased from 1.85 to 3.06 metric tonnes between 1993 and 2008. The electrical consumption per head of population rose

from 965 KWh to 2,129 KWh. Concerns about these figures has resulted in an energy and environmental policy with the target to reduce GHGs by 20% from 2005 levels by 2030 (Misila *et al.*, 2017). Sawangpho and Pharino (2011) suggested that Thailand should promote the use of renewable energy derived from the agricultural products and residues both to reduce GHGs and the reliance on imported fossil fuels.

Douskova *et al.* (2009) report flue gas emissions of between 9.1-34.9 m^3 h^{-1}. The wide variation is explained by the fuel being used. Olaizola *et al.* (2004) report that flue gases have different compositions according to the fuels used.

An "emission factor" is widely used to estimate emissions per unit of power production. In principle, CO_2 emission can be calculated by **Equation 7.2**.

$$CO_2 \text{ Emission} = \sum(EF_{Fuel} \text{ x } FC_{Fuel}) \qquad (7.2)$$

Where: CO_2 Emission is the amount of CO_2 from energy consumption; EF_{fuel} is the CO_2 emission factor or emission coefficient which differ for each fuel type; FC_{fuel} is the amount of utilisation of each fuel type.

Emissions depend on the technology and raw materials used. Thermal (stream turbine; lignite, fuel oil, co-firing fuel oil), gas turbine (natural gas) and combined cycle (natural gas) result in a different CO_2 volumes and, thus, different conversion factors (Krittayakasem *et al.* (2011)).

Carbon capture is one way to reduce GHG emissions to the atmosphere and using plants to remove CO_2 from flue gas is an environmentally friendly and relatively low cost method for this removal. Of all the plants available, single celled algae are the most productive. However, they can only ever be a small part of the solution to GHG control.

7.4.2 Algal cultivation

To scale up to a commercial plant, a project must consider:

- Algal cell cultures. Assess to and characteristics of the algal strains (lipid, protein, phylogenetic analysis, gene sequencing etc.);
- Suitable growth media;

- Highest possible rate of CO_2 uptake obtainable;
- Flue gas supply, quality, temperature and monitoring;
- Other nutrient requirements;
- Optimisation of the design and construction of the algal bioreactor system;
- Operation and maintenance;
- monitoring performance;
- Optimisation of the processing technologies;
- Recovering and processing algae for products;
- Mass and energy balance and economic feasibility.

7.4.3 Biomass production

Commercial projects of non CO_2 supplemented algal growth have reported productivities of 250 - 300 t ha^{-1} yr^{-1}(Packer, 2009; Singh *et al.*, 2011). From the laboratory results and the Thai power plant information, it is calculated that the dry algal mass production would be around 1,000 t yr^{-1} with an oil yield around 300 t. which could produce 150 $t.yr^{-1}$ of algal biodiesel (B100). This is a high potential yield due to the extra CO_2 supplied to the algae, the use of photobioreactor tubes and the favourable environmental conditions in Thailand.

Oil production from the algae depends on the oil accumulation in the algal cell, the algal strain, harvesting methodology and extraction technique. In addition, the yield of biodiesel from this oil depends on the efficiency of the trans-esterification process. However, this chapter shows that biodiesel production is not currently economically feasible on its own and other products will have to be derived from the algae, either together with, or in place of, biodiesel production.

The main competition for biodiesel production in Thailand is palm oil. The current price (2017) of crude palm oil is around £500 $tonne^{-1}$ (22,000 baht) on the open market. This is £ 0.5 L^{-1} (21.50 baht) and is far lower than most of the production cost estimates for algal oil given in the literature (apart from Schenk *et al.* (2008) and Huntley and Redalje (2007) which give a similar price to palm oil). There is thus a huge variation and uncertainty in the costings of any algal biomass production unit. If the plant

is on land already available, adjacent to a "free" supply of CO_2 and there is no need for additional light or temperature energy input then the costs, or at least the recurring costs, are going to be much less than some given in the literature. Each country has a different conditions of temperature, light, manpower and infrastructure costs. Thus it is difficult to be sure of costings based on reports in the literature from other parts of the world.

When looking at the detail, this study found that, apart from potential value-added products, the main benefit comes from CO_2 reduction.

However, the actual cost of CO_2 reduction the local market (Thailand) is uncertain. It does thus seem that the higher value added pharma and other products from algal biomass will be the tipping point in determining algal photobioreactor ecomomics.

There are advantages and disadvantages to producing algal biomass in Thailand. Beneficially, algae can reduce CO_2 from the emission source and produce an algal oil to blend with diesel and, possibly, higher value-added pharma products. This aids Thailand's biofuel market and aspirations, reduces crude oil imports and CO_2 emissions and helps energy security, economic and social sustainability in the future. For disadvantages, Thailand lacks a stable project base to operate suitable pilot scale tests over a sufficient time period. Funding is limited and expertise in short supply.

More detailed feasibility studies, life cycle analysis (LCA) and environmental impact assessments (EIA) plus pilot plant studies will need to be made to determine the financial and environmental implications of alga biomass production in a photobioreactor system running on CO_2 from a Thai power station.

7.5. Conclusions

In a commercial scale up, with photobioreactors of 9 cm OD x 8 cm. ID, 0.5 cm. wall thickness and 200 cm. height each occupying 1 m^2. land giving 10,000 photobioreactor tubes in 1 hectare, these would produce a total dry algal biomass of 1,000 $tyr^{-1}ha^{-1}$. This would give 300 tyr^{-1} ha^{-1}algal oil and around 150 $tyr^{-1}ha^{-1}$ biodiesel.

Algae grow well in Thailand and could provide a high volume of biodiesel if the overall costs could be made competitive with fossil fuels.

Some advantages, such as the environmental advantages of CO_2 capture, are difficult to put a value on but might attract support and funding through an international level agreement. Green energy promotion and national subsidy policy may also act as motivation.

As the commercial market for biofuels is still very unstable, due to the high variability in the price of crude oil and uncertainties in the costs of production, Thailand should look for a sustainable market to sell the products of algal biomass, looking perhaps to base the production and costing around producing high value-added pharma products with algal oil as a by-product.

Chapter 8: Overall discussion, conclusions and future work

8.1 Overall discussion

While the most used source of energy is still fossil fuels and is likely to be so for some time, "renewable" resources are increasing in importance with many countries committing themselves to reach a certain percentage of their energy requirements to be met by renewables by set dates.

There is a wide range of renewable energy sources, the main ones being hydroelectricity, solar and wind power. Of energy derived from plants, direct combustion of wood and wastes are long established as is ethanol from sugar cane and other crops. However, crop derived energy has problems of efficiency and competition with food supplies. Plants that can be grown on non-agricultural or marginal land are now being investigated. Of these, single-celled algae, with their high productivity and ability to grow in a wide variety of water conditions show much promise. Not only can they produce an easily used biofuel directly (biodiesel from the oils produced by the algae) but also their residual biomass, if not contaminated, can be used to produce methane by anaerobic digestion and finally provide a useful soil enhancer.

This thesis looks at two important areas of growing microalgae with the ultimate aim of its use in biofuel production. These are the possible enhancement of biomass production by increasing the amount of CO_2 provided to the algae and methods of separating the algae from the liquid medium, particularly avoiding adding and any contaminants which may hamper downstream processing.

As algae rely on CO_2 as part of their photosynthetic conversion of light energy then it is natural to assume that the more CO_2 that can be provided, the more biomass will be produced. This has a particular context as the ideal source of such CO_2 would be warm CO_2 laden gases from industrial processes, thus incorporating the CO_2 back into a beneficial, rather than a detrimental, cycle.

In order to utilise the biomass, the algae have to be concentrated (separated from the bulk water phase). There are methods where certain

components of the algae can be extracted without such concentration but there would still be left a large bulk of "waste" material to be disposed of. Being able to concentrate the biomass enables more efficient downstream processing and use of the material produced. It also enables easier treatment of the separated water phase. There are a range of methods for concentrating algal biomass which range from expensive (such as centrifugation) to low cost (settling ponds). Enhanced settling is a desirable and low cost method of separation. However, the enhancement is often achieved by adding chemicals. While, as shown in this thesis, such chemicals can be very efficient, they may be detrimental to the desired products and can cause problems in the use or disposal of any residual waste. Thus simple, benign alternative additives for enhanced settlement are required and two types of chitosan (and, ultimately, crab shell) were investigated in this work.

8.1.1 Algal strains, cell selection and screening

A strain of *Chlorella vulgaris* from Thailand was chosen as the main alga to use in this work. *Chlorella vulgaris* is known to be able to provide a high oil yield, grow rapidly and is easily accessed from pure cultures (Brennan and Owende, 2010). The particular strain used in this work was accessed from the Thai culture collection as it is the potential for commercial development in Thailand that this study aims to underpin.

However, while this particular strain, was already isolated, the study reported in **Chapter 4** also showed that there are simple ways of separating and screening for other algae taken direct from the environment. Repeated simple filtration and separation can result in very good cultures and there should be more screening of natural sites in different countries to find more local algal strains with suitable characteristics for commercial culture.

Assessing the colour of cultures by eye can also provide a method to assess growth. This would be difficult to use quantitatively but as a qualitative guide to healthy algal growth is would be highly valuable in commercial culturing. Knowing that a culture is growing well by a quick visual examination can be very useful for spotting problems even before any measurement is made. A strong dark green colour indicated healthy calls and good photosynthetic reactions, light green, yellow or shades towards brown or black indicate poor culture conditions (for example in this study,

too much CO_2). Such colours can indicate both too rapid growth and too little growth as well as problems of contamination or culture collapse caused by other reasons (excessive nitrogen, phosphorous, potassium or dead cells).

8.1.2 Photobioreactor design

Some commercial microalgal cultivations are carried out on open pond systems. The aim is to reduce costs (such operation and maintainable cost) as much as possible and maximise the use of natural, solar, light energy. However, the performance of open ponds is usually poor due to difficulties in controlling the culture conditions such as UV radiation, day/night cycles, temperature and contamination.

The advantages of a photobioreactor system are better control of all the factors. However, the aim must still be to use natural light energy direct from the sun as opposed to artificial light which may be using energy sourced from fossil fuels. Certain parts of the world are suitable for combining the control advantages of bioreactors with the ability to use direct (or filtered) sunlight, suitable temperatures and a fairly standard day/night cycle.

Thailand is one such country and has initiated several algal cultivation projects. As part of enhancing R&D, the private sector, universities, and government bodies are involved in the development of such projects. Generally, most of the research so far reported is of laboratory studies (Apostol *et al.*, 2011; Brennan and Owende, 2010; Carroll *et al.*, 1991; Chauhan *et al.*, 2010; Chen and Durbin, 1994; Kiatsiriroat and Vorayos, 2011; Lee *et al.*, 2011; Lee, 1997; Lv *et al.*, 2010; Maneeruttanarungroj *et al.*, 2010; Molina *et al.*, 2001; Sari *et al.*, 2016; Soetaert and Vandamme, 2009; Vitova *et al.*, 2015) and Ruangsomboon (2012) report investigations into algae strains and environmental conditions. However, any scale up to the commercial application is still rare. There is only *Spirulina* production for the health food industry by Siam Algae Co., Neotech food Co.Ltd. and Boonsom's farm (in Chiang Mai -the dominant seller) (Lee, 1997). This is due to lack of investment and subsidy. Another reason is that many projects involve intellectual property belonging to companies which are not reported in the open literature.

Both open pond (Boonsom algal farm) and photobioreactors, for example airlift bioreactors (Poonkum *et al.*, 2015; Sirikulrat and Koonaphapdeelert,

2013), bubble column (Monkonsit *et al.*, 2011), vertical photobioreactor (Kiatsiriroat and Vorayos, 2011) have been studied. To develop algae to produce biofuel in Thailand in the future, The Department of alternative energy development and efficiency (DEDE) of the Ministry of Energy has a renewable energy and development plan (REDP) that covers both short and long term goals. There are some reviews of costs by LCA assessment (Sawaengsak *et al.*, 2014) and a feasibility study (Kiatsiriroat and Vorayos, 2011). The work reported in this thesis will add to this knowledge base.

On the commercial scale, the giant oil seller companies such as the Public Company limited (PTT) and The Bangchak Petroleum Public Co. Ltd. considerably influence the attitudes towards alternative energy development in Thailand. Other factors such as initial investment and maintenance costs, internal rate return (IRR), benefit-cost analysis and risk assessment need more consideration.

8.1.3 Nutrient and algal growth rates

Growth rates (biomass production) of algal vary considerably depending on a variety of factors such as nutrients, temperature, light quality etc. This study found that BBM medium can be used to grow this strain of *C.vulgaris* very well and agrees with the findings of Bhola *et al.* (2011) who found that BBM was the media best suited to biomass production (out of: BBM, Bristol-$NaCl_3$, AF6, BG-11). Vitova *et al.* (2015) emphasised that N, S, P are important elements to growing algae. Lack of essential nutrient may cause algal dead or less reproduction. This study found that pond water from The Crookes Valley Park is less enriched in nutrients and thus limits algal growth. Unwanted viruses or bacteria may also inhibit the number of algae.

This work found a growth rate of around 1 $g.L^{-1}d^{-1}$ (0.98-1.25) in cultures supplied with aeration enhanced with CO_2 at 6-12%. The data show that algae grown in 6% have a wider range of possible harvesting times than those grown in the other levels of CO_2 (v/v). They give a good yield on both Day 4 and Day 7. While algae grow in 12% (v/v) and 24% (v/v) should be harvested every 4 days (7 times/month). The life cycle of the algal culture is shorter at the higher CO_2 levels than at 6% CO_2 (v/v) and algae cannot survive for long under high CO_2 concentrations.

The extended study showed that algae cultured in 6% CO_2 give a wide range of good harvesting periods (Day 4-7). The optimal is to harvest on Day 6 (5 times/month). Algae grow better in this situation than any other condition and the stability indicated in this work would be an important factor in commercial culture. However, in the future a significant aim of commercial culture could be to remove the greatest amount of CO_2 from a particular source and then the understanding of the behavior of the algae, and need for shorter harvesting times, becomes important.

Reported yields in the literature vary considerably e.g. *C. vulgaris* is reported to yield 1.2-1.3 $gL^{-1}d^{-1}$ (Anjos *et al.*, 2013). The study of Fang-Fang *et al.* (2011) used aeration rate of 0.05-0.5 $vv^{-1}m^{-1}$. in 100 L airlift to culture *S.obliquus WUST4* using 6-18% CO_2 v/v and found that at 12% CO_2, the removal rate of CO_2 is 67%. Molina *et al.* (2001) cultured *Phaeodactylum tricornutum* UTEX 640 in a 20 L- airlift bubble column, the loop had an internal diameter of 0.06 m. and was 80 m. long connected to a 4 m. tall airlift with an artificial light at 2000 $\mu Em^{-2}s^{-1}$ by halogen lamps. Liquid flow velocity was 0.5 m/s. The study found that biomass gives a high yield (1.9 $gL^{-1}d^{-1}$).

There are various factors resulting in different yields. These not only include the media but factors such as the algal strain, types of an algal cultivation (open pond/photobioreactor) engineering designs, also environmental conditions such as light intensity, aeration, flow rate, dissolve O_2 and gas in media solution. The mass balance should be optimised between mass input and output in the culture system. O_2 and gas transfer in suspension is the main factor which needs most concern when designing and maintaining the system (see **Chapter 2**).

As discussed below, there are many variables controlling algal growth and biomass production and there is little merit in trying to directly compare growth rates reported from one set of experiments with growth rates reported for very different culture conditions. The literature can thus only be used as a guide and the most valid results are comparing the changes brought about by one variable within otherwise identical culture conditions.

However, it is not just the absolute biomass that is important but the products of the algae. For example, Liang *et al.* (2009) cited by Chen *et al.* (2011)

reported *C. vulgaris 259* (University of Texas) to be able to produce up to 38% lipid (of DCW – dry carbon weight) with optimal growth at 2 g/L. Other reports give growth rates similar to the present work, for example 1.83 $g.L^{-1}d^{-1}$ reported Naderi *et al.* (2015).

Certainly, the addition of a suitable amount of CO_2 in the aeration of cultures of *Chlorella vulgaris* is beneficial to their growth and biomass production. In this work, the optimum CO_2 addition of 6% (v/v) gave a growth rate on Day 7 measured by optical density (OD_{680}) of 9.705, considerably greater than that found with no CO_2 addition (1.924).

Bhola (2011) grew algae sparging the media with air and air enhanced with CO_2 at 0.1-4.0% (v/v) for 15 days. They found that 4% CO_2 gives the best yield at 1,222 mg/L on Day 15. This may indicate that a lower CO_2 level gives a high yield over a longer time period than the higher concentrations over shorter time periods. However, the life cycle of the algal culture is shorter when adding a higher concentration of CO_2 and, commercially, either for biomass or efficient removal of CO_2 a higher CO_2 level with shorter harvesting times, is likely to be of advantage. It is important to remember that the species of algae, and even the strain of a species, will show different growth patterns and reactions to different levels of CO_2. In addition, environmental conditions such as nutrients and temperature may give different growth rates and yield and CO_2 solubility and availability depends on factors including temperature and pH, all of which may have effects on algae growth (Carroll *et al.*, 1991). Sutherland *et al.* (2015) have shown that even the time of day has an effect on physical factors and hence algal growth. Using experiments varying the frequency of CO_2 additions (15 min, 30 min, 60 min, 90 min), and controlling the pH at 8 they found that a dissolved inorganic carbon (DIC) measurement of 500 mg/L in the morning (08.00) declined to 300 mg/L in the afternoon (18.00) when adding CO_2 every 60-min and 90-min. There was less difference between morning and afternoon when adding CO_2 every 15-min. However, if there is no CO_2 added (a controlled blank) the study found that a DIC of 300 mg/L in the morning declines close to zero in the afternoon.

While the results of the present study support previous findings (Bhola, 2011; Chinnasamy, 2009) that some added CO_2 enhances algal

growth, and while the results at 6% fall in line with the results by these authors (4 and 5% respectively), what is surprising, and potentially very important commercially, is the growth rates, albeit over a shorter period, at higher CO_2 levels. Growing and harvesting several cultures in a set time period using higher CO_2 levels can produce as good or better results as growing and harvesting fewer cultures at lower CO_2 levels in the same time period. This could be important in enabling the culture and harvesting conditions to be adjusted to the level of CO_2 content of a natural industrial source – in other words, within limits, any level of CO_2 in an off gas can be used and the culture cycle adjusted to suit (While high levels of CO_2 in an off gas can easily be diluted, concentrating CO_2 upwards is energy intensive and, commercially, would be avoided if at all possible).

8.1.4. Cell number

The results show that the highest total cumulative cell number for algae grown in 6% CO_2 (v/v) and harvested every 7 days (for 28 days) is $2.15x10^7$cell/mL. For 12% CO_2 (v/v), harvesting every 4 days (for 28 days), the highest results are $2.79x10^7$ cell/mL (see **Figure 5-16**). Scragg *et al.* (2002), found that the cell number is around 40-50 $x10^6$ cell/mL in the first 5 days of a 35 day culture when growing algae in a nitrate concentration of around 50 mg/mL. These authors also found that the cell number declines to nearly zero when the nitrate level was raised to 600 mg/L.

8.1.5 Specific growth rate

The study found that algae grown at 6%, 12%, 24% CO_2 (v/v) provide the highest specific growth rate (SGR), as estimated by turbidity, on Day 2 (1.04, 2.21, 1.25 (d^{-1})). The results are as similar when estimated by optical density (1.247,1.599, 2.092). This indicates how fast the cells are dividing in a culture and indicate that the algae can adapt themselves to the environment within 2 days (see **Table 5-2** and **Table 5-3**). Algae grown at 0% and 50% CO_2 are very slow to grow, the algae cannot tolerate the high CO_2 environment, or are naturally slower growing in a low CO_2 concentration.

Yoo *et al.* (2010) found the highest biomass for *C.vulgaris* was when cultured in media supplied with 10% CO_2 for 14 days. The biomass productivity was 0.104 g dw $L^{-1}d^{-1}$. Chinnasamy *et al.* (2009) found that *C.vulgaris ARC 1* grown in media supplied with air to which 20% CO_2 (v/v)

had been added gave the highest specific growth rate (μ of 0.222 d^{-1}) with 6% CO_2, which is similar to this work.

8.1.6 Dry weight

The study found that algae grown at 6% CO_2 (v/v) show the highest daily dry weight on Day 2,4,6,7 at 0.81, 0.87, 0.98, 0.86 $gL^{-1}d^{-1}$ respectively. At 12% v/v, the highest dry weight is on Day 2 (1.25 $gL^{-1}d^{-1}$) and Day 3 (0.85 $gL^{-1}d^{-1}$) which is similar to algae grown with 24% CO_2 (Day 2 at 0.96 $gL^{-1}d^{-1}$, and Day 3 at 1.28 $gL^{-1}d^{-1}$). Algae grown in air alone and 50% CO_2 show a lower dry mass except for 50% CO_2 on Day 6 (1.30 $gL^{-1}d^{-1}$) (**Table 5-6**). However, by visual inspection, algae grown in 50% CO_2 are light green/yellow in colour, rapidly decline in yield, and quickly die. Calculating cumulative dry weight (g/L) this study found that algae cultured in 6% CO_2 (v/v) should be harvested at Day 7 with 4.55 g/L. At 12% CO_2 they can be harvested on Day 5 at 3.51 g/L (see **Table 5-7**).

Rodolfi *et al.* (2009), who only added CO_2 to control the pH between 7.5 and 8.0 found that the freshwater algae; *Chlorella sp. F&M 48*, *C. vulgaris CCAP 211/11b* and *C.vulgaris F&M-M49* gave a biomass yield of only 0.23, 0.20, and 0.17 $g.L^{-1}.d^{-1}$ (they used BG 11 medium with continuous illumination in an orbital shaker at 25 ℃ and an air flow rate of 0.3 $LL^{-1}m^{-1}$).

Stressed conditions, such as the higher CO_2 concentration levels of 24% and 50%, not only give shorter life cycles but the stress can be seen both in the colour of the cultures (light green, yellow, brown) and under the microscope where weak algae cells can sometimes be seen budding a small size offshoot then rapidly die. The growth rates given in section 5.3.3 show that CO_2 at lower concentrations (6 and 12%) provide a longer life cycle than the higher levels of 24% and 50% CO_2.

It is, however, only by considering cumulative biomass yield that true biomass for relatively high CO_2 levels be judged. Algae grown under 6% CO_2 (v/v) should be harvested on Day 7 (4 times/month) to give a biomass yield of 18.20 g/L. At 12% CO_2 harvesting should be on Day 6, (5 times/ month at 21.06 g/L). There is some difference depending on the method of measurement (see **Table 5-8**) for example calculating using growth rate still gives a 7 day harvesting cycle for 6% CO_2 but at 12% CO_2

the algal can be collected on Day 4 or Day 5 (**Figure 5-10**, **Figure 5-13**, and **Figure 5-15**). It must also be noted that some of these calculations using several harvests over a set time period are very close and that two set periods have had to be used (28 days and 30 days) and this should be taken into account.

8.1.7 Light

The study in **Chapter 4** found that algae grown in the dark have, as would be expected, a very limited growth rate. This is due to algae lacking light energy to activate their photosynthesis process. The colour of the algal culture is light green. Algae change their colour to dark green when given enough light.

When grown under light conditions of 60 μEinsteins $m^{-2}s^{-1}$ (or $\mu mol.m^{-2}s^{-1}$) using 11W G3 cool white fluorescent tubes algae can grow well. Around 500 μEinsteins $m^{-2}s^{-1}$ is obtained from two-40 W fluorescent lamps in the photobioreactor.

Bhola *et al.* (2011) reported that *C.vulgaris* can grow at light intensities between the range of 150-350 $\mu mol.m^{-2}s^{-1}$. The further study tested on light intensity of 369.33 $\mu mol.m^{-2}s^{-1}$ found that growth rate is limitation. Lee *et al.* (2011) tested light intensities of 39.19, 72.79,105.41,116.22,135.14 and 175.68 $\mu mol/m^2/s$ while adding 0.07,1.4, 3.0 and 5.0% CO_2 (v/v). The results show that the highest yield (1.93 $gL^{-1}d^{-1}$ on Day 11) is at 105.11 $\mu mol/m^2/s$ with 3% CO_2 (v/v).

8.1.8 pH

Chen and Durbin (1994) found that carbon uptake rates rise when the pH is less than 8.3. The study showed that high pH may limit algal growth because CO_2 is in the form of carbonates. Alkaline pH possibly prevents the flexibility of the cell wall of mother cells in budding and inhibits autospore formation. This may result in an increase in triglyceride accumulation. In contrast, a decrease in membrane-associated polar lipids is found leading to cell cycle inhibition.

8.1.9 Temperature

While temperature was not specifically investigated in this study (a laboratory control of 22-25 ℃ was used) it is known that most algae grow within the temperature range 15-40℃ (Barghbani *et al.*, 2012; Blinová *et al.*, 2015; Chinnasamy *et al.*, 2009; Juneja *et al.*, 2013). The study of Chinnasamy *et al.* (2009) reported on the growth of the algae *C. vulgaris ARC 1* at temperatures of 30, 40 and 50 ℃ and found that the best yield was at 30 ℃. In a country such a Thailand, photobioreactors using natural sunlight will have no problems with low temperatures, however, it may be that they will need to be cooled or shaded at certain times to avoid too high temperatures.

8.1.10 Aeration and carbon dioxide enhancement

Aeration is essential to the rapid growth of algal cultures. Not only does it provide the required CO_2 and O_2 but it also aids mixing in the reactor. Aeration rates of 0.17 L/min (0.085 $vv^{-1}m^{-1}$, air volume flow per unit of working volume per minute) and 0.30 L/min (or 0.15 $vv^{-1}m^{-1}$) were found to be suitable in this work.

8.1.11 Effect of $NaHCO_3$ on pH change and algal growth

Adding $NaHCO_3$ may assist CO_2 use in terms of it being a solid form of fertiliser parallel with CO_2 (gas). Borowitzka (2005) (cited in Barghbani *et al.* (2012)) reported that algae cannot tolerate $NaHCO_3$ at concentrations above 0.2 M. However, Akin *et al.* (1993) reported in Ozkan *et al.* (2012) found that adding up to 0.24 M $NaCHO_3$ to cultures of *Botryococcus braunii* provided with 10% CO_2 enriched air gave good results.

The study of Wong *et al.* (2014) found that maximum growth rate (0.782 and 0.820 day^{-1}) was at a bicarbonate concentration of 5 g/L, a nitrate concentration of 1 g/L and 4500 Lux with an average value of 0.801 day^{-1}. However, in this work (**Chapter 5**) it is shown that the addition of $NaHCO_3$ at levels of above 0.001 M are detrimental to algal growth and even adding $NaHCO_3$ at 0.001 M, while doing no harm, did not enhance growth over the controls.

8.1.12 Productivity

The productivity of *C.vulgaris* from this work is compared with reports from the literature in **Appendix F**. The various conditions and aims/objectives of the experiments are different as are the methodologies and basis of measurements. Thus the results are difficult to compare directly. However, the results of this work give higher productivity than many other reports.

8.1.13 Algal separation by enhanced sedimentation

The effect of various chemical additions, along with chitosan and crab-shell, on the sedimentation rates of *C. vulgaris* were studied. The study found that metal salts are very effective enhancers of sedimentation of the algae and, while this data provides a baseline against which to measure other additives, metallic salts are toxic to the algae and will contaminate the products and increase the cost of waste disposal. A natural biopolymer, such as chitosan and crab shell, is non-hazardous and is shown to increase the particle size of flocs and hence increases the sinking rate of the algae. The types of binding forces and bonding of the interactions of the chitosan with algae are discussed in **Chapter 2**.

The study found that there is a difference according to the type of chitosan used. Medium molecular weight chitosan was found to remove algae most efficiently (up to 90%), while high molecular weight chitosan was relatively poor in enhancing algal sedimentation (51-52 %) This level of sedimentation is no better than the control algal suspensions with no added flocculant. This is a most interesting result which will require further study.

Of course, purified chitosan is expensive and thus low cost sources of this material were explored. Crab-shell is easily available and is the raw source of chitosan. It was found to be a very good enhancer of algal sedimentation. The removal is close to 90%.

When using chitosan as a flocculant to aid algal settlement, crab shell is as good or better than MW chitosan while HW chitosan gives poorer results, in fact no better than allowing the algae to settle without chitosan. This is an important result as it shows that there is a significance in the chitosan chain length and selecting the wrong chitosan will not aid

settlement. While this is an interesting point in terms of what might be on the mechanisms involved, the fact that crude crab shell is the best chitosan containing material is of great practical importance to industrial applications. In addition, pH change may also affect algal settling. The pH of HW chitosan being more variable than the others.

Dead cell suspensions show much more variability and lower biomass removal. The greatest variability can be shown in the results for alum additions (**Table 6-2**).

After settling for 1 hour, living cell suspensions treated with 600-1,000 mg/L of alum show more than 90% removal. In contrast, living cell suspensions without added alum give less than 52% removal.

After 12 hours of settling, living cell suspensions with additions Of 600-800 mg/L alum show 92% removal. Dead cell suspensions treated with 800 mg/L alum show a 100% removal.

After 24 hours, living cell suspensions treated with 600,800 and 1,000 mg/L show 100% removal. Dead cells treated with 600,800 or 1,000 mg/L show more than 97% removal.

Riaño *et al.* (2012) found that chitosan has a high efficiency to flocculate *C.vulgaris* and *Microcystis sp*. at 92% and to a lesser amount *Acutodemus obliquss* at a concentration of 214 mg/L when measured by optical density (OD_{550}). Apostol *et al.* (2011) state that efficiency and mechanism of the coagulation-flocculation process depends on several factors, the most relevant being initial turbidity, pH, reagent (coagulant) dose, and type, system hydrodynamics in the flocculation stage, temperature and alkalinity. Although the study found that metallic salts can flocculate algae more rapidly than biopolymers algal cells can become contaminated by the chemicals and quickly die before harvesting (Apostol *et al.*, 2011). Like other aspects of algal culture, especially on an industrial scale, the type and dosing level of a flocculant have to be tailored to the algae and other parameters of the culture.

Ahmad *et al.* (2006) found that chitosan addition at 0.5 g/L (500 mg/L) is the optimal point to coagulation of residue oil and suspended solid in palm oil mill effluent. The condition is at contact time: 15 min, mixing rate 100 rpm, sedimentation time 20 min pH 4. While alum and polyaluminium chloride (PAC) the optimal dose are 0.8 g/L (800 mg/L) and 0.6 g/L (600 mg/L) by mixing time at 100 rpm, settling time 50 and 60 min respectively. pH

is 4.5. While the study of Ahmad *et al*. looks at the effects on a totally different system there are similarities in terms of oil and suspended solids to an algal culture rich in cells containing (and leaking) lipids. This study using chitosan and crab shell at 600 mg/L (0.6 g/L) to give an efficiency of 90% is close to Ahmad's study but the optimal pH in this study is nearly 7, whilst Ahmad's is 4.0-4.5.

Most reported studies look only at the flocculation process, however, the study reported in this thesis also looked at the whole sedimentation process. The flocculation time is shorter than the whole settling process. The settling time depends on various externality factors such as 1) electronegativity charge of the chemical elements when bonding with algae, 2) environmental conditions (pH, flow rate) 3) algal biomass concentration. This data may be useful when using chitosan or crab shell on a commercial scale. It can help to manage time and economic investment in the harvesting process.

8.1.14 Effect of additions to enhance settlement on pH

Although the addition of metallic salts results in shorter settlement times than using biopolymers metal salts cause a greater change in pH making the environment more acidic. This may lead to cell death. The study found that the optimal pH for MW chitosan is at pH 7.00-7.90 and there is less difference between living and dead cells after settling time of 1 hr. than for HW chitosan which shows greater pH variation. Using HW chitosan living cells give a pH of 6.84-7.10, and dead cells of a pH 4.46 – 5.17. Algal suspensions (both living and dead cells) to which crab shell has been added are in the range of pH 6.47-6.97. Studies by Divakaran and Pillai (2002) found that pH 7 was best for flocculating algae using chitosan. They also showed that pH influences algal sedimentation (see section 5.3.2.3). Xu *et al.* (2013) found that pH 6 is the best condition for *C.sorokiniana (UTEX 1230).*

8.1.15 Effects of chitosan on carbon content of the cultures

This study found that, on adding the biopolymer (MW chitosan and Crab shell), Total Inorganic Carbon (TIC) levels fell slightly, indicating that the chitosan adsorbs carbon either itself or as part of a complex with algae. Total Carbon (TC) and Total Organic Carbon (TOC) increase.

The increase in TOC could be due to organic carbon coming from the chitosan. However, the chitosan has been extracted and refined and is unlikely to have any carbon that will readily dissolve in the media (the total C in chitosan is 6-8% and this is strongly bound in the structure). Therefore, it may be that the binding of the chitosan with the algae results in more organic carbon compounds dissolving in the media and increasing the dissolved TOC.

In contrast, for HW chitosan the TC and TIC shows a small increase while TOC shows a slight fall. As the HW chitosan does not aid flocculation, the changes seen for MW chitosan and crab shell may not be expected to be seen and this reinforces the above theory.

8.1.16 Scale up algal cultivation using CO_2 from a power plant in Thailand

The study estimates the emitted CO_2 of The Ratchaburi power plant is 7,584,423.99 tCO_2e or around $4.22x10^{12}$ L per year. The study found that an algal (*C.vulgaris*) volume of 5.04 $x10^6$ L yr^{-1} could be produced in 1 ha and would use 6.58 $x10^5$ tyr^{-1}- $9.87x10^5$ t CO_2yr^{-1}. The biomass productivity is 1,000 $t.yr^{-1}$. Total algal oil is 300 $t.yr^{-1}$.This compares very favourably to reports of, for example sunflower oil production of 380-687 kg per ha per year (Zheljazkov *et al.*, 2011) (5 to 10 times greater productivity over sunflower oil). Biodiesel produced from the algae should be at least 150 $t.yr^{-1}$.

In a comparison between biodiesel from algal oil and palm oil, it was found that, at present and without any carbon dioxide removal premium, biodiesel from palm oil is far cheaper (21 baht $^{-1}$ or around £ 0.49 L^{-1}) than algal biodiesel (£7.5 L^{-1}). Added value products, such as pharma and cosmetics as well as incentives to reduce carbon dioxide emissions would be needed to commercialise algal production.

8.2 Conclusions

This study found that adding CO_2 to the aeration system enhanced algal biomass production. Additions of up to 24% can be used in short-term cultivation (4 days or less) Measurements of biomass by cumulative turbidity, optical density and cell count, for 24% CO_2 over a 28 day period

(7 harvests) are 1,552.04 NTU, 45.19 and 2.94x10^7 cell/mL respectively. The cumulative dry weight is 21.84 g/L.

For additions of 12% and 4 days cultivation, measurements of biomass by cumulative turbidity, optical density and cell count, over a 28 day period (7 harvests) for 12% CO_2 are 1,852.34 NTU, 41.82, and 2.80 x10^7 cell/mL respectively. The cumulative dry weight is 20.16 g/L.

6% CO_2 produces good enhancement of growth over 7-10 days. The cumulative turbidity is 2,146 NTU. for 4 harvests over 28 days. Optical density and cell number give 41.97 and 2.15 x10^7 cell/mL respectively. The cumulative dry weight is 18.20 g/L.

The study found that algae grown in high CO_2 concentrations (50%) results in far less biomass production, while algae grown in media sparged with air alone takes much longer to grow and cannot produce much biomass within the time scale studied (10 days).

Adding $NaHCO_3$ to control pH is not useful. Only levels of 0.001 M or less are not detrimental to algal growth and even using these, growth is not enhanced over the controls. However, these results could be useful if pH needs to be controlled for other reasons (to enhance a particular metabolic product for example).

While adding chemicals to enhance sedimentation is efficient, it may be detrimental to the algae and/or their products and lead to disposal problems. The use of biopolymers, such as chitosan, are able to aid flocculation and sedimentation but it is shown that the molecular weight of the chitosan is an important factor. A low-cost version of crude chitosan is crab shell and the results show that this could be used commercially, particularly in a country, such as Thailand, that has a large shellfish industry.

Algal production using CO_2 from an electricity power plant will cut GHGs. In addition, it can provide biodiesel from waste by changing waste to energy. The green product also supports eco-friendly energy.

Commercial scale up, with photobioreactors of 9 cm OD x 8 cm ID, 0.5 cm wall thickness and 200 cm height each occupying 1 m^2 land giving 10,000 photobioreactor tubes in 1 hectare would produce a total dry algal biomass of 1000 tyr^{-1} ha^{-1}. This would provide 300 tyr^{-1} algal oil or around

150 tyr^{-1} biodiesel. Crab shell is cheaper than refined chitosan. Both of them can be applied to use in the harvesting process.

The study found that algal biofuel may not compete with biodiesel from palm or other oils. This is due to a high investment cost and a high technological production. This is suggested that algal biomass production should not be only focused on biodiesel production (and the removal of carbon dioxide from the power station, but look to produce other value added products such as pharma and cosmetic products.

8.3 Future work

The suggestions for future work are:

Having determined the range of CO_2 addition which enhances algal growth, and that there is a relationship between the level of CO_2, growth rate and the length of time such growth rate can be maintained, further studies should carry out more investigation as to the biomass production efficiency and commercial potential of a short (4 days at higher CO_2) in comparison to longer (7-10 days) at lower CO_2 levels.

Future work should also look at "real" sources of CO_2. That is to investigate using actual off-gases from industry. While these may have advantages of being warm and being a low-cost source of CO_2, it will be important to understand the effects of other components in such gasses which could either enhance growth further or be detrimental to the algae.

Optimisation of the use of crab-shell to aid settlement should be investigated. Other crustaceans with similar shell compositions should also be investigated (e.g. shrimp) There may be further simple treatments (with acids or alkalis for example) either of the crab-shell or creating a "pH shift" (or other effect in the media) (Fast *et al.*, 2014). Other natural flocculants have been studied and enhanced in various ways (Chauhan *et al.*, 2010; Gutierrez *et al.*, 2015; Sari *et al.*, 2016), and such enhancements can be tried on crab-shell.

Further work on the extraction of useful products from the algae will be required, as well as the culture conditions that will enhance such production (e.g. of lipids). Value-added products such as pharma products,

(Younes and Rinaudo, 2015), supplements, and cosmetics should be investigated.

The main algae studied in this work was a strain of *Chlorella vulgaris* originally collected in Thailand. This was a deliberate choice as the aim of the work has been to lay the foundations for future studies in Thailand. One study that is recommended is to look at low cost large scale cultivation using sunlight (rather than artificial light in the laboratory), using (and possibly protecting from) natural temperatures and developing low cost bioreactors and combination reactors (Brennan and Owende, 2010). Such studies will need to be carried out at a suitable site in Thailand.

As part of such future studies, life cycle analysis (LCA) and CO_2 modelling of an integrated system should be undertaken including processes such as the production of biomethane from the residual biomass after extraction of useful products (Schenk *et al.*, 2008; Stucki *et al.*, 2009). Such an LCA on an integrated system will provide economic information that is vital to any scale up to commercial production and allow optimisation of, for example, energy use.

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Appendices

Appendix A: Advantages and disadvantages of a photobioreactor system

Advantages and disadvantages of the photobioreactor systems are shown in **Table A-1.**

Table A-1 Advantages and disadvantages of the photobioreactor system (Yadala and Cremaschi, 2014).

Closed systems	Advantages	Disadvantages
TPBR	1. Large illuminating surface area and suitable for outdoor cultures 2. Good biomass productivity 3. Inexpensive	1.Requires a large area 2.Photo-inhibition is common 3.Poor mass transfer 4.Gradients of pH, DO and CO_2 along the tubes, fouling
Vertical-Column PBR	1. High mass transfer, photosynthetic efficiency, Good mixing with low shear stress 2. Low energy consumption 3. High potentials for scalability 4. Effortless sterilisation 5. Good temperature control 6. Immobilisation 7. Good light path 8. Low O_2 build up 9. Large illuminative surface area 10. Suitable for outdoor cultures 11. Fairly good biomass productivity 12. Reduce photo-inhibition and photo-oxidation 13. Low cost, compact, and easy to operate 14. Greater gas holds up 15. Least land use 16. Promising large scale cultivation	1. Small illuminative area 2. Low surface area, and volume ratio 3.The construction requires more 4. More a shear stress 5.Expensive compared to open ponds 6. Decrease of illuminating area may occur on scaling-up
FP-PBR	1. Large illuminative surface area to volume ratios 2. Suitable for outdoor cultures 3. Immobilisation 4. Good light path 5. Inexpensive 6. Readily tempered 7. Low O_2 build-up 8. High biomass productivity 9. Uniform distribution of light 10. Inexpensive 11. Easy construction and maintenance, and cleanability 12. High photosynthetic efficiency	1.Difficult to scale-up 2.Algae may adhere to walls 3.Low photosynthetic efficiency 4.Low potentials for scalability 5. Possibility of hydrodynamic stress to some algal strain
Plastic bag PBR	1. Low cost and good sterilise 2. Good control temperature	1. Disposal plastic bag cost 2. Difficult to scale up

Appendix B: 3N BBM media preparation

1. Chemicals and method

The following volumes of the appropriate stock solutions are based on preparing 1L of BBM media, and are added in numerical order, as given in **Table B-1**, to a conical graduated flask containing distilled water. When all chemicals have been added distilled water is added to adjust to 1 L of total media.

-10 mL of each stock solution 1-6

-1 mL of each stock solution 7-9

- 0.1 mL of each stock solution 10-14

B_1 and B_{12} were added 1 mL/L media. pH is adjusted to 6.7± 0.3. Every batch of media is autoclaved at 121℃ for 15 min.

Table B-1 Stock solutions

No.	Chemical name	Formula	Weight (g)	Distilled water (mL)	Assay	CAS No.	Cost (£)
1	Di-potassium hydrogen orthophosphate	K_2HPO_4	1.875	250	>/=98.0%	7758-11-4	29.20
2	Potassium di-hydrogen orthophosphate	KH_2PO_4	4.375	250	>/=99.0%	7778-77-0	12.90
3	Magnesium sulphate	$MgSO_4.7H_2O$	1.875	250	>/=98.0%	10034-99-8	25.50
4	Sodium Nitrate	$NaNO_3$	6.250	250	>/=99.0%	7631-99-4	24.10
5	Calcium chloride	$CaCl_2.2H_2O$	0.625	250	>/=99.9%	017-013-00-2	44.50
6	Sodium chloride	NaCl	0.625	250	>/=99.5%	7647-14-5	15.40
7	EDTA tetrasodium salt	EDTA-Na_4	5.000	100	>/=99.9%	15708-41-5	29.40
8	Potassium hydroxide	KOH	3.100		>/=99.9%	1310-58-3	69.30
9	Ferrous sulphate	$FeSO_4.7H_2O$	0.498	100	>/=99%	7782-63-0	9.50
10	Sulphuric acid conc (wt per mL =1.84g)	H_2SO_4	0.1mL		>/=95-98%	7664-93-9	18.30
11	Boric acid	H_3BO_3	1.142	100	>/=99.5%	10043-35-3	10.20
12	Zinc Sulphate	$ZnSO_4.7H_2O$	0.353	25	for cell	7446-20-0	16.70
13	Manganese chloride	$MnCl_2.4H_2O$	0.058	25	>/=99.99 %	13446-34-9	27.80
14	Cupric sulphate	$CuSO_4.5H_2O$	0.063	25	>/=98%	7758-99-8	27.80
15	Cobalt (II) nitrate hexahydrate	$Co(NO_3)_2.6H_2O$	0.020	25	>/=98%	10026-22-9	15.00
16	Sodium molybdate dihydrate	$Na_2MoO_4.2H_2O$	0.048	25	>/=99.0%	10102-40-6	29.30
17	Vitamin B1 (Thiamine hydrochlorid)	Thiamine hydrochloride	0.120	100		67-03-8	23.40
18	Vitamin B12		0.100	100	>/=98%	68-19-9	13.40
19	Hydrochloric acid	0.1 N HCl		adj. pH		7647-01-0	10.80
20	Sodiumhydroxide	0.1 N NaOH		adj. pH			10.30

Note that: Chemicals order from SIGMA-ALDRICH

2. Storage conditions and expiry date check of stock solution

Stock solutions No. 1-6 can be stored up to 3 months, while solutions No.7-14 may be stored up to 12 months if autoclaved. All solutions can be stored at room temperature in the dark. The prepared and autoclaved media solutions can be stored in a refrigerator or in the laboratory.

Borosilicate glassware, ¼ inch silicone tube, and 1 ml micropipette tip were autoclaved to ensure sterility, as was all glassware. Aseptic technique has been used to perform the entire laboratory work.

Appendix C: Quality control (QC) and Calibration

All reagents, equipment, and glassware used are verified to be contamination free. To ensure the accuracy and precision of data in the study it is necessary to:

- Prepare blank controls
- Using sterile and aseptic techniques for glassware and telflon tubes etc.
- Recheck (laboratory duplicates), and at least triple reading values
- Using Standard Reference Material (SRM), and standard procedures to control laboratory work.
- Perform calibration procedures where appropriate (See in calibration from no. C-1-C-3.

Calibration Form No. C-1

1. Details of equipment

Name of equipment:……4- digital VWR..Microbalance…….

Equipment manufacturer & brand:……..…VWR……...….

Model & S/N :…LA 214, S/N IT1303262 …Range…Max:..220 g…….

2. Detail of equipment calibration

…....Laboratory room No...A03, Hadfield Building, The University of Sheffield…….

3. Standard/ Methodology

The balance must be level. Firstly, adjust the feet so that the level indicators (spirit level) show the balance to be level. Secondly, the VWR microbalance must be calibrated using a 220g calibration weight made from anti-magnetic, polished stainless steel, which is suitable for medium resolution precision electronic balances. The calibration weight must be placed at the middle of the weighing pan. The shield should always be closed before reading the result. Zero point is adjusted by pressing the tare key and waiting until there is a stable display. The result is recorded as below (**Figure A-1**).

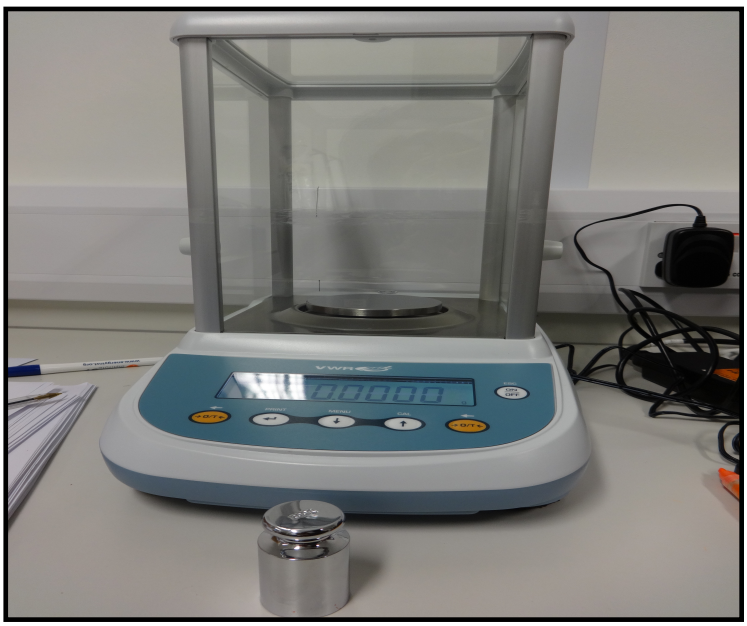

Figure A-1 Microbalance with weight calibration standard 220 g

4. Results

The results in Table C1 below show the difference between the actual reading value compared to the known weight standard at three calibrations. There is small different between actual reading values and the calibration standard value. (see **Table C-1** and **Figure A-2**). Each time (for these examples and other calibrations) the equipment was adjusted to the standard weight.

Table C-1 Microbalance calibration results (n=3)

Date	Temp (°C)	Expected data	Actual Reading			Average	Adjustment
			1	2	3		
10/10/14	20	220.0000	220.0196	220.0187	220.0200	220.0194	220.0000
9/10/15	22	220.0000	220.0197	220.0205	220.0196	220.0199	220.0000
7/10/16	20	220.0000	220.0206	220.0189	220.0189	220.0195	220.0000

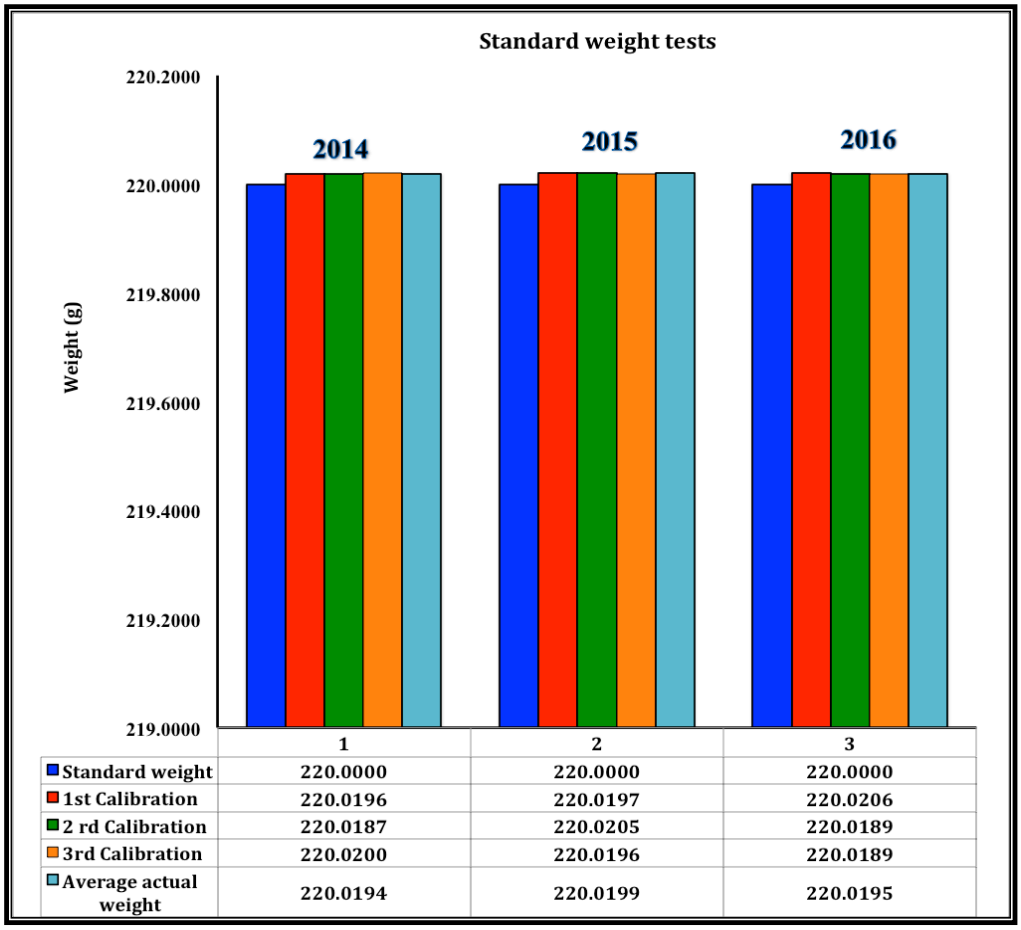

Figure A-2 Bar chart standard weight tests

Calibration Form No. C-2

1. Details of equipment

Name of equipment:……pH Meter……...

Equipment manufacturer & brand:… … HANNA Instruments……...

Model & S/N :… … HI 2211 ORP ……Range……-2.00 to 16.00…….

2. Detail of equipment calibration

Laboratory room No.:..A03, Sir Hadfield Building The University of Sheffield……..

3. Standard/ Methodology

An accurate pH measurement can be obtained by calibrating a series of a reference pH buffers following the operation manual. Before calibration, proper cleaning of the electrode is essential to ensure that it is uncontaminated by bacteria and any other contaminants. The sensor and the buffer should be at the same temperature. Then, the meter should be turned on to allow adequate time to warm up. HANNA Instruments pH 7.01 (HI7007L pH 7.01) and 4.01(HI7004L pH 40.1) buffers were used as standard calibration buffer because they are close to the range of expected samples. The electrode is placed in the first buffer and allowed to equliberate until stabilised data has been repeated 3 times over several minutes. A record of the temperature is taken. Then, the electrode is rinsed with distilled water, dried and placed in the next buffer and the stabilization repeated. (see **Figure A-3**). The results were recorded as shown in **Table C-2**. Actual data were adjusted to standard values.

Figure A-3 pH calibration test

Table C-2 Calibration results.

Date	Temp (℃)	Expected data	Actual data			Average	Adjustment
			1	2	3		
10/10/14	20	7.01	7.01	7.01	7.00	7.01	7.01
		4.01	4.01	4.00	4.01	4.01	4.01
9/10/15	21	7.01	7.00	7.01	7.00	7.00	7.01
		4.01	4.01	4.00	4.01	4.01	4.01
7/10/16	20	7.01	6.89	6.99	7.00	6.99	7.01
		4.01	4.03	4.02	4.01	4.02	4.01

Calibration Form No. C-3

1. Details of equipment

Name of equipment:…….Turbidity meter……....

Equipment manufacturer & brand:……....

Model & S/N :……Range……..0-20 NTU……...

2. Detail of equipment calibration

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3. Turbidity standard preparation

To check zero point by measuring distilled water and adjust the zero point to 0. The results are shown in **Table C-3**.

4. Calibration

Calibration is to ensure the zero point adjustment against distilled water.

Table C-3 Zero measurement results and adjustment.

Date	Temp (℃)	Expected value	Actual reading			Adjustment
20/04/2514	20	0.00	0.02	0.00	0.01	0.00
26/03/2015	22	0.00	0.01	0.00	0.02	0.00
23/04/2516	22	0.00	0.03	0.00	0.01	0.00

Appendix D: Effect of dose and mixing of chitosan and metallic salts to aid algal sedimentation

1. Effect of dose and mixing speed

The effect of dose and mixing speed when adding medium molecular weight chitosan are shown by the data in **Table D-1** and **D-2**.

Table D-1 Dosage and mixing results.

Chitosaan concentration	sterring: 1-120, 30-45 (min-rpm.)		sterring: 5-120, 30-45 (min-rpm.)		sterring: 5-150, 30-45 (min-rpm.)		sterring: 5-200, 30-45 (min-rpm.)	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
0	303.56	3.42	309.94	4.58	310.39	3.07	314.44	4.38
25	320.39	13.74	318.06	16.49	312.67	19.78	302.72	11.67
50	309.61	17.45	330.28	10.82	316.61	12.13	328.78	17.93
100	300.61	14.53	300.72	16.86	301.11	17.22	311.89	10.70
150	306.28	7.78	318.06	19.37	317.94	7.16	322.11	26.09
200	344.00	15.71	360.00	11.19	317.94	7.16	380.50	7.52
250	337.11	3.88	351.06	7.00	334.61	18.01	380.50	7.52
300	299.39	16.55	310.06	14.21	318.44	21.25	320.28	15.21
350	290.72	6.49	289.44	1.79	304.61	1.88	318.89	3.83
400	287.17	14.48	288.50	12.36	295.61	8.44	302.67	6.84
450	280.44	7.05	294.67	5.57	311.00	2.40	313.17	3.01

Table D-2 pH measurement

Chitosaan concentration	sterring: 1-120, 30-45 (min-rpm.)		sterring: 5-120, 30-45 (min-rpm.)		sterring: 5-150, 30-45 (min-rpm.)		sterring: 5-200, 30-45 (min-rpm.)	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
0	7.81	0.007	7.82	0.008	7.82	0.006	7.84	0.000
25	7.86	0.000	7.96	0.011	7.99	0.000	7.95	0.005
50	7.96	0.000	7.93	0.008	7.88	0.000	7.89	0.006
100	7.96	0.013	8.06	0.010	8.07	0.000	8.10	0.006
150	8.06	0.000	8.11	0.016	8.11	0.010	8.13	0.006
200	7.96	0.003	7.99	0.003	8.11	0.009	8.05	0.010
250	8.06	0.008	8.05	0.000	8.10	0.017	8.05	0.017
300	7.98	0.000	7.99	0.020	8.06	0.000	8.05	0.010
350	8.02	0.005	8.04	0.005	8.11	0.007	8.14	0.008
400	7.97	0.006	7.95	0.000	7.95	0.006	7.97	0.000
450	8.02	0.015	8.03	0.005	8.03	0.000	8.03	0.007

2. A comparison of metallic salts and biopolymer addition on algal settlement.

2.1) Turbidity measurement

There are 360 samples (5 replications x 12 sub groups x 6 testing points) for each chemical added. There are 6 chemicals. Thus, a total is 2,160 samples (360 samples x 6 chemicals). Turbidity, Optical density (OD_{680}) and pH were measured. Therefore, there are a total of 6,480 measurements (2,160 samples x 3 parameters; Turbidity, OD and pH) (see in **Table D-3-D-20**).

Table D-3 Measurements of Turbidity when adding Alum

Time	hr.	Media + Alum							
		Media		Media + 600 mg/L		Media + 800 mg/L		Media + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0.000	6.90	0.010	6.90	0.010	6.90	0.010	6.90	0.010
After adding chemical	0.083	6.87	0.011	7.13	0.007	7.13	0.004	7.28	0.013
Settling time at 0 hr	1.083	6.88	0.013	5.18	0.004	4.89	0.007	5.68	0.011
Settling time at 1 hr	2.083	6.89	0.007	5.00	0.005	4.85	0.004	4.68	0.009
Settling time at 12 hr	13.083	6.89	0.004	4.71	0.005	4.68	0.004	4.62	0.008
Settling time at 24 hr	25.083	6.88	0.005	4.71	0.005	4.67	0.004	4.62	0.005
Time	hr.	Living algae + Media + Alum							
		Living algae		Algae + 600 mg/L		Algae + 800 mg/L		Algae + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0.000	6.95	0.011	6.95	0.011	6.95	0.011	6.95	0.011
After adding chemical	0.083	6.95	0.005	7.20	0.007	7.20	0.005	7.14	0.007
Settling time at 0 hr	1.083	6.98	0.007	5.32	0.005	5.09	0.005	5.08	0.007
Settling time at 1 hr	2.083	6.98	0.004	5.01	0.008	4.77	0.005	4.61	0.005
Settling time at 12 hr	13.083	6.98	0.007	4.76	0.005	4.58	0.007	4.51	0.005
Settling time at 24 hr	25.083	6.98	0.008	4.76	0.004	4.55	0.007	4.45	0.015
Time	hr.	Dead cell +Media+ Alum							
		Dead cell		Dead cell + 600 mg/L		Dead cell + 800 mg/L		Dead cell + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0.000	6.95	0.005	6.95	0.005	6.95	0.005	6.95	0.005
After adding chemical	0.083	6.87	0.007	4.87	0.009	5.05	0.008	5.06	0.022
Settling time at 0 hr	1.083	6.95	0.005	3.42	0.005	3.30	0.022	3.28	0.011
Settling time at 1 hr	2.083	6.95	0.005	3.62	0.016	3.41	0.000	3.30	0.008
Settling time at 12 hr	13.083	6.95	0.005	3.47	0.000	3.37	0.000	3.30	0.004
Settling time at 24 hr	25.083	6.95	0.005	3.45	0.000	3.39	0.004	3.32	0.004

Table D-4 Measurements of Turbidity when adding $FeCl_3$.

Time	hr.	Media + $FeCl_3$							
		Media		Media + 600 mg/L		Media + 800 mg/L		Media + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0	6.90	0.004	6.90	0.004	6.90	0.004	6.90	0.004
After adding chemical	0.083	6.90	0.011	6.86	0.012	6.81	0.008	5.49	0.011
Settling time at 0 hr	1.083	6.90	0.004	4.49	0.007	4.44	0.000	4.22	0.000
Settling time at 1 hr	2.083	6.90	0.004	3.48	0.004	3.30	0.004	3.23	0.004
Settling time at 12 hr	13.08	6.90	0.004	3.48	0.022	3.30	0.007	3.23	0.013
Settling time at 24 hr	25.08	6.90	0.004	3.47	0.000	3.26	0.015	3.21	0.020
Time	**hr.**	**Living algae + Media + $FeCl_3$**							
		Living algae		Algae + 600 mg/L		Algae + 800 mg/L		Algae + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0	6.95	0.011	6.95	0.011	6.95	0.011	6.95	0.011
After adding chemical	0.083	6.95	0.011	6.94	0.000	6.92	0.000	6.95	0.000
Settling time at 0 hr	1.083	6.95	0.011	3.80	0.014	3.61	0.011	3.42	0.000
Settling time at 1 hr	2.083	6.95	0.011	3.35	0.036	3.29	0.005	3.35	0.004
Settling time at 12 hr	13.08	6.95	0.011	3.35	0.005	3.28	0.009	3.14	0.005
Settling time at 24 hr	25.08	6.95	0.011	3.35	0.011	3.19	0.005	3.03	0.023
Time	**hr.**	**Dead cell +Media+ $FeCl_3$**							
		Dead cell		Dead cell + 600 mg/L		Dead cell + 800 mg/L		Dead cell + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0	6.95	0.000	6.95	0.000	6.95	0.000	6.95	0.000
After adding chemical	0.083	6.95	0.000	2.20	0.000	2.15	0.004	1.95	0.000
Settling time at 0 hr	1.083	6.98	0.005	1.70	0.005	1.59	0.000	1.52	0.005
Settling time at 1 hr	2.083	6.98	0.000	1.70	0.007	1.60	0.000	1.55	0.004
Settling time at 12 hr	13.08	6.98	0.005	1.73	0.007	1.63	0.004	1.57	0.005
Settling time at 24 hr	25.08	6.98	0.000	1.78	0.000	1.68	0.004	1.61	0.009

Table D-5 Measurements of turbidity when adding $AlCl_3$.

Time	hr.	Media + $AlCl_3$							
		Media		Media + 600 mg/L		Media + 800 mg/L		Media + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0	6.90	0.000	6.90	0.004	6.90	0.004	6.90	0.004
After adding chemical	0.083	6.90	0.004	6.90	0.000	6.90	0.000	6.90	0.000
Settling time at 0 hr	1.083	6.90	0.000	5.25	0.000	4.54	0.015	4.48	0.030
Settling time at 1 hr	2.083	6.90	0.004	4.43	0.015	4.38	0.026	4.43	0.017
Settling time at 12 hr	13.08	6.90	0.004	4.41	0.000	4.35	0.000	4.43	0.000
Settling time at 24 hr	25.08	6.90	0.004	4.34	0.000	4.32	0.004	4.29	0.000
Time	**hr.**	**Living algae + Media + $AlCl_3$**							
		Algae		Algae + 600 mg/L		Algae + 800 mg/L		Algae + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0	6.95	0.008	6.95	0.008	6.95	0.008	6.95	0.008
After adding chemical	0.083	6.64	0.009	6.87	0.000	6.82	0.000	6.76	0.000
Settling time at 0 hr	1.083	6.88	0.008	5.04	0.009	5.04	0.000	5.04	0.000
Settling time at 1 hr	2.083	6.98	0.008	4.36	0.008	4.34	0.054	4.28	0.013
Settling time at 12 hr	13.08	6.98	0.009	4.24	0.029	4.28	0.050	4.29	0.010
Settling time at 24 hr	25.08	6.98	0.009	4.22	0.005	4.14	0.005	4.10	0.010
Time	**hr.**	**Dead cell +Media+ $AlCl_3$**							
		Dead cell		Dead cell + 600 mg/L		Dead cell + 800 mg/L		Dead cell + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0	6.95	0.000	6.95	0.000	6.95	0.000	6.95	0.000
After adding chemical	0.083	6.96	0.043	8.57	0.008	7.76	0.000	8.13	0.005
Settling time at 0 hr	1.083	6.94	0.007	5.62	0.035	5.52	0.010	5.64	0.030
Settling time at 1 hr	2.083	6.89	0.000	5.73	0.000	5.60	0.004	5.65	0.000
Settling time at 12 hr	13.08	6.96	0.005	5.58	0.008	5.66	0.005	5.72	0.000
Settling time at 24 hr	25.08	6.95	0.004	5.63	0.013	5.68	0.008	5.72	0.012

Table D-6 Measurements of Turbidity when adding MW chitosan.

Time	hr.	Media + MW Chitosan							
		Media		Media + 600 mg/L		Media + 800 mg/L		Media + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0	6.90	0.000	6.90	0.000	6.90	0.000	6.90	0.000
After adding chemical	0.083	6.39	0.000	8.38	0.007	8.38	0.004	8.35	0.007
Settling time at 0 hr	1.083	6.90	0.011	8.37	0.007	8.34	0.004	8.37	0.004
Settling time at 1 hr	2.083	6.90	0.000	6.94	0.004	6.95	0.000	6.96	0.000
Settling time at 12 hr	13.08	6.89	0.000	6.69	0.004	6.75	0.000	6.77	0.000
Settling time at 24 hr	25.08	6.88	0.000	6.82	0.004	6.83	0.011	6.83	0.015
Time	**hr.**	**Living algae + Media + MW Chitosan**							
		Living algae		Algae + 600 mg/L		Algae + 800 mg/L		Algae + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0	6.95	0.000	6.95	0.000	6.95	0.000	6.95	0.000
After adding chemical	0.083	6.95	0.004	7.64	0.000	7.60	0.000	7.74	0.000
Settling time at 0 hr	1.083	6.88	0.004	7.74	0.000	7.90	0.000	7.88	0.000
Settling time at 1 hr	2.083	6.88	0.000	7.00	0.005	7.09	0.030	7.18	0.018
Settling time at 12 hr	13.08	6.98	0.000	6.46	0.021	6.45	0.011	6.46	0.013
Settling time at 24 hr	25.08	6.98	0.000	6.34	0.008	6.41	0.008	6.43	0.011
Time	**hr.**	**Dead cell +Media+ MW Chitosan**							
		Dead cell		Dead cell + 600 mg/L		Dead cell + 800 mg/L		Dead cell + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0	6.95	0.000	6.95	0.000	6.95	0.000	6.95	0.000
After adding chemical	0.083	6.93	0.009	6.92	0.009	6.88	0.000	6.93	0.004
Settling time at 0 hr	1.083	6.88	0.004	7.06	0.024	7.00	0.000	7.04	0.011
Settling time at 1 hr	2.083	6.98	0.007	7.08	0.016	7.05	0.016	7.06	0.013
Settling time at 12 hr	13.08	6.98	0.005	6.92	0.005	6.86	0.004	6.98	0.009
Settling time at 24 hr	25.08	6.98	0.007	6.89	0.013	6.91	0.004	7.00	0.011

Table D-7 Measurements of Turbidity when adding HW Chitosan.

Time	hr.	Media + HW Chitosan							
		Media		Media + 600 mg/L		Media + 800 mg/L		Media + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0	6.90	0.010	6.90	0.010	6.90	0.010	6.90	0.010
After adding chemical	0.083	6.87	0.008	6.90	0.008	6.90	0.008	6.97	0.007
Settling time at 0 hr	1.083	6.90	0.010	6.97	0.004	6.97	0.000	6.97	0.000
Settling time at 1 hr	2.083	6.89	0.010	7.10	0.065	7.03	0.044	7.08	0.129
Settling time at 12 hr	13.08	6.89	0.010	6.82	0.027	6.86	0.025	6.91	0.037
Settling time at 24 hr	25.08	6.88	0.010	6.85	0.004	6.81	0.292	6.87	0.040
Time	**hr.**	**Living algae + Media + HW Chitosan**							
		Living algae		Algae + 600 mg/L		Algae + 800 mg/L		Algae + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0	6.95	0.022	6.95	0.022	6.95	0.022	6.95	0.022
After adding chemical	0.083	6.95	0.022	7.30	0.022	7.03	0.022	6.95	0.022
Settling time at 0 hr	1.083	6.88	0.022	7.01	0.016	7.31	0.358	7.18	0.016
Settling time at 1 hr	2.083	6.98	0.022	6.84	0.089	7.09	0.026	7.09	0.033
Settling time at 12 hr	13.08	6.98	0.022	6.38	0.013	6.50	0.004	6.56	0.100
Settling time at 24 hr	25.08	6.98	0.022	6.48	0.069	6.56	0.009	6.58	0.042
Time	**hr.**	**Dead cell +Media+ HW Chitosan**							
		Dead cell		Dead cell + 600 mg/L		Dead cell + 800 mg/L		Dead cell + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0	6.95	0.004	6.95	0.004	6.95	0.004	6.95	0.004
After adding chemical	0.083	6.95	0.011	5.97	0.011	5.89	0.020	6.06	0.014
Settling time at 0 hr	1.083	6.95	0.014	5.97	0.011	5.89	0.020	6.06	0.014
Settling time at 1 hr	2.083	6.95	0.015	4.56	0.004	5.02	0.016	5.18	0.013
Settling time at 12 hr	13.08	6.98	0.013	4.70	0.263	4.96	0.058	5.18	0.052
Settling time at 24 hr	25.08	6.98	0.013	4.89	0.022	5.19	0.016	5.23	0.000

Table D-8 Measurements of Turbidity when adding Crab shell

Time	hr.	Media + Crab shell							
		Media		Media + 600 mg/L		Media + 800 mg/L		Media + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0	6.90	0.000	6.90	0.000	6.90	0.000	6.90	0.000
After adding chemical	0.083	6.87	0.004	6.91	0.007	6.97	0.004	8.10	0.004
Settling time at 0 hr	1.083	6.89	0.000	6.97	0.007	7.03	0.004	7.10	0.005
Settling time at 1 hr	2.083	6.89	0.000	6.91	0.023	6.70	0.019	6.98	0.020
Settling time at 12 hr	13.08	6.89	0.000	6.89	0.016	6.92	0.023	6.93	0.028
Settling time at 24 hr	25.08	6.88	0.000	6.97	0.050	6.99	0.059	7.00	0.019
Time	**hr.**	**Living algae + Media + Crab shell**							
		Living algae		Algae + 600 mg/L		Algae + 800 mg/L		Algae + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0	6.95	0.028	6.95	0.028	6.95	0.028	6.95	0.028
After adding chemical	0.083	6.95	0.005	8.40	0.033	8.46	0.033	8.63	0.033
Settling time at 0 hr	1.083	6.88	0.005	8.57	0.036	8.67	0.038	8.71	0.038
Settling time at 1 hr	2.083	6.88	0.028	7.14	0.010	7.23	0.021	7.25	0.032
Settling time at 12 hr	13.08	6.98	0.028	6.84	0.011	6.88	0.038	6.91	0.012
Settling time at 24 hr	25.08	6.98	0.028	6.80	0.048	6.87	0.034	6.90	0.029
Time	**hr.**	**Dead cell +Media+ Crab shell**							
		Dead cell		Dead cell + 600 mg/L		Dead cell + 800 mg/L		Dead cell + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0	6.95	0.004	6.95	0.004	6.95	0.004	6.95	0.004
After adding chemical	0.083	6.95	0.004	6.95	0.018	6.95	0.000	6.95	0.005
Settling time at 0 hr	1.083	6.88	0.004	6.64	0.059	6.90	0.020	6.89	0.009
Settling time at 1 hr	2.083	6.76	0.004	6.62	0.018	6.86	0.008	6.77	0.019
Settling time at 12 hr	13.08	6.98	0.004	6.55	0.004	6.70	0.004	6.54	0.000
Settling time at 24 hr	25.08	6.98	0.004	6.47	0.022	6.57	0.004	6.45	0.027

2.2) Optical density measurements

The results by OD_{680} measurement are shown in **Tables D9-D14**.

Table D-9 Measurements of OD_{680} when adding Alum

Time	hr.	Media + Alum							
		Media		Media + 600 mg/L		Media + 800 mg/L		Media + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0.000	0.032	0.001	0.032	0.001	0.032	0.001	0.032	0.001
After adding chemical	0.083	0.026	0.001	0.015	0.001	0.015	0.001	0.015	0.002
Settling time at 0 hr	1.083	0.023	0.001	0.016	0.004	0.015	0.001	0.017	0.002
Settling time at 1 hr	2.083	0.014	0.001	0.017	0.001	0.021	0.001	0.013	0.002
Settling time at 12 hr	13.083	0.007	0.000	0.009	0.001	0.012	0.001	0.000	0.000
Settling time at 24 hr	25.083	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Time	**hr.**	**Living algae + Media + Alum**							
		Living algae		Algae + 600 mg/L		Algae + 800 mg/L		Algae + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0.000	1.616	0.007	1.616	0.007	1.616	0.007	1.616	0.007
After adding chemical	0.083	1.616	0.007	0.884	0.004	0.999	0.004	0.995	0.004
Settling time at 0 hr	1.083	1.357	0.001	0.700	0.015	0.768	0.004	0.842	0.000
Settling time at 1 hr	2.083	1.293	0.000	0.136	0.017	0.260	0.046	0.235	0.000
Settling time at 12 hr	13.083	0.776	0.000	0.003	0.001	0.020	0.013	0.001	0.000
Settling time at 24 hr	25.083	0.727	0.001	0.000	0.000	0.000	0.000	0.000	0.000
Time	**hr.**	**Dead cell +Media+ Alum**							
		Dead cell		Dead cell + 600 mg/L		Dead cell + 800 mg/L		Dead cell + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0.000	1.616	0.007	1.616	0.007	1.616	0.007	1.616	0.007
After adding chemical	0.083	3.529	0.002	1.565	0.096	1.383	0.064	1.413	0.075
Settling time at 0 hr	1.083	4.135	0.007	1.059	0.044	0.908	0.089	0.829	0.025
Settling time at 1 hr	2.083	0.853	0.004	0.201	0.144	0.121	0.147	0.000	0.001
Settling time at 12 hr	13.083	0.317	0.002	0.006	0.013	0.000	0.000	0.000	0.000
Settling time at 24 hr	25.083	0.119	0.004	0.000	0.000	0.012	0.027	0.048	0.069

Table D-10 Measurements of OD_{680} when adding $FeCl_3$

Time	hr.	Media + $FeCl_3$							
		Media		Media + 600 mg/L		Media + 800 mg/L		Media + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0.000	0.032	0.004	0.032	0.004	0.032	0.004	0.032	0.004
After adding chemical	0.083	0.029	0.003	0.032	0.004	0.660	0.042	0.554	0.086
Settling time at 0 hr	1.083	0.028	0.003	0.536	0.085	1.024	0.091	0.720	0.114
Settling time at 1 hr	2.083	0.016	0.003	0.028	0.005	0.032	0.034	0.008	0.011
Settling time at 12 hr	13.083	0.008	0.002	0.004	0.005	0.008	0.010	0.000	0.000
Settling time at 24 hr	25.083	0.000	0.000	0.004	0.004	0.008	0.005	0.000	0.001
Time	hr.	Living algae + Media + $FeCl_3$							
		Living algae		Algae + 600 mg/L		Algae + 800 mg/L		Algae + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0.000	1.616	0.139	1.616	0.139	1.616	0.139	1.616	0.139
After adding chemical	0.083	1.995	1.610	1.723	0.148	1.795	0.154	2.010	0.173
Settling time at 0 hr	1.083	1.490	0.073	1.534	0.169	1.680	0.113	1.903	0.164
Settling time at 1 hr	2.083	1.270	0.126	0.929	0.048	0.808	0.074	1.580	0.034
Settling time at 12 hr	13.083	0.776	0.067	0.000	0.001	0.000	0.000	0.000	0.001
Settling time at 24 hr	25.083	0.727	0.063	0.000	0.000	0.000	0.000	0.000	0.001
Time	hr.	Dead cell +Media+ $FeCl_3$							
		Dead cell		Dead cell + 600 mg/L		Dead cell + 800 mg/L		Dead cell + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0.000	1.616	0.349	1.616	0.349	1.616	0.349	1.616	0.349
After adding chemical	0.083	3.529	0.076	7.863	0.132	4.524	0.381	7.109	0.201
Settling time at 0 hr	1.083	4.134	0.278	6.247	0.076	4.093	0.132	3.447	0.108
Settling time at 1 hr	2.083	0.852	0.107	3.554	0.381	2.801	0.341	3.447	0.108
Settling time at 12 hr	13.083	0.317	0.075	3.124	0.437	2.908	0.170	2.585	0.132
Settling time at 24 hr	25.083	0.119	0.084	3.878	0.488	2.477	0.076	2.693	1.688

Table D-11 Measurements of OD_{680} when adding $AlCl_3$

Time	hr.	Media + $AlCl_3$							
		Media		Media + 600 mg/L		Media + 800 mg/L		Media + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0.000	0.032	0.027	0.032	0.027	0.032	0.027	0.032	0.027
After adding chemical	0.083	0.032	0.003	0.037	0.006	0.268	0.004	0.016	0.004
Settling time at 0 hr	1.083	0.025	0.005	0.003	0.003	0.039	0.004	0.044	0.007
Settling time at 1 hr	2.083	0.014	0.012	0.011	0.004	0.004	0.003	0.000	0.000
Settling time at 12 hr	13.083	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000
Settling time at 24 hr	25.083	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Time	hr.	Living algae + Media + $AlCl_3$							
		Living algae		Algae + 600 mg/L		Algae + 800 mg/L		Algae + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0.000	1.616	0.210	1.616	0.210	1.616	0.210	1.616	0.210
After adding chemical	0.083	1.612	0.005	0.903	0.166	1.035	0.080	0.998	0.122
Settling time at 0 hr	1.083	1.357	0.003	0.626	0.081	0.647	0.084	0.679	0.088
Settling time at 1 hr	2.083	1.272	0.165	0.000	0.000	0.000	0.000	0.000	0.000
Settling time at 12 hr	13.083	0.820	0.106	0.000	0.000	0.000	0.000	0.000	0.000
Settling time at 24 hr	25.083	0.748	0.097	0.000	0.000	0.000	0.000	0.000	0.000
Time	hr.	Dead cell +Media+ $AlCl_3$							
		Dead cell		Dead cell + 600 mg/L		Dead cell + 800 mg/L		Dead cell + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0	1.616	0.028	1.616	0.028	1.616	0.028	1.616	0.028
After adding chemical	0.083	5.420	0.362	5.253	0.000	4.143	0.129	3.529	0.089
Settling time at 0 hr	1.083	1.378	0.059	5.828	0.405	4.480	1.009	3.449	0.779
Settling time at 1 hr	2.083	1.328	0.157	0.773	0.620	0.773	0.012	0.852	0.180
Settling time at 12 hr	13.083	1.546	0.103	0.258	0.022	0.000	0.001	0.000	0.000
Settling time at 24 hr	25.083	1.378	0.124	1.378	0.124	0.159	0.056	0.198	0.198

Table D-12 Measurements of OD_{680} when adding MW Chitosan.

Time	hr.	Media + MW chitosan							
		Media		Media + 600 mg/L		Media + 800 mg/L		Media + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0.000	0.033	0.045	0.033	0.045	0.033	0.045	0.033	0.045
After adding chemical	0.083	0.032	0.008	0.002	0.001	0.006	0.002	0.009	0.003
Settling time at 0 hr	1.083	0.016	0.001	0.004	0.003	0.002	0.001	0.006	0.004
Settling time at 1 hr	2.083	0.014	0.004	0.008	0.007	0.001	0.001	0.000	0.000
Settling time at 12 hr	13.083	0.007	0.010	0.004	0.006	0.000	0.000	0.000	0.000
Settling time at 24 hr	25.083	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Time	hr.	Living algae + Media + MW Chitosan							
		Living algae		Algae + 600 mg/L		Algae + 800 mg/L		Algae + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0.000	1.616	0.127	1.616	0.127	1.616	0.127	1.616	0.127
After adding chemical	0.083	1.612	0.001	1.315	0.017	1.312	0.009	1.393	0.006
Settling time at 0 hr	1.083	1.585	0.004	1.326	0.028	1.335	0.013	1.359	0.055
Settling time at 1 hr	2.083	2.179	0.165	1.457	0.046	1.351	0.071	1.342	0.118
Settling time at 12 hr	13.083	0.802	0.063	1.083	0.212	0.853	0.172	0.831	0.135
Settling time at 24 hr	25.083	0.748	0.059	0.065	0.013	0.068	0.047	0.072	0.018
Time	hr.	Dead cell +Media+ MW Chitosan							
		Dead cell		Dead cell + 600 mg/L		Dead cell + 800 mg/L		Dead cell + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0.000	1.605	0.034	1.605	0.034	1.605	0.034	1.605	0.034
After adding chemical	0.083	3.529	0.048	1.770	0.145	1.949	0.031	1.411	0.040
Settling time at 0 hr	1.083	4.134	0.018	1.821	0.031	1.565	0.172	1.616	0.094
Settling time at 1 hr	2.083	0.852	0.041	1.231	0.026	1.283	0.026	1.308	0.120
Settling time at 12 hr	13.083	0.317	0.042	0.677	0.059	0.590	0.031	0.513	0.193
Settling time at 24 hr	25.083	0.119	0.041	0.590	0.194	0.564	0.051	0.667	0.026

Table D-13 Measurements of OD_{680} when adding HW Chitosan

Time	hr.	Media + HW Chitosan							
		Media		Media + 600 mg/L		Media + 800 mg/L		Media + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0.000	0.032	0.005	0.032	0.005	0.032	0.005	0.032	0.005
After adding chemical	0.083	0.033	0.006	0.006	0.003	0.004	0.003	0.001	0.001
Settling time at 0 hr	1.083	0.020	0.014	0.006	0.003	0.021	0.003	0.000	0.000
Settling time at 1 hr	2.083	0.015	0.001	0.026	0.005	0.026	0.020	0.019	0.014
Settling time at 12 hr	13.083	0.008	0.001	0.002	0.003	0.002	0.002	0.000	0.000
Settling time at 24 hr	25.083	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Time	hr.	Living algae + Media + HW Chitosan							
		Living algae		Algae + 600 mg/L		Algae + 800 mg/L		Algae + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0.000	1.616	0.033	1.616	0.033	1.616	0.033	1.616	0.033
After adding chemical	0.083	1.354	0.000	1.016	0.021	0.972	0.020	1.364	0.028
Settling time at 0 hr	1.083	1.354	0.005	1.016	0.000	0.972	0.000	1.364	0.001
Settling time at 1 hr	2.083	1.293	0.000	1.871	0.231	1.858	0.078	1.834	0.091
Settling time at 12 hr	13.08	0.776	0.002	1.205	0.029	1.261	0.109	1.249	0.118
Settling time at 24 hr	25.08	0.727	0.001	0.764	0.025	0.822	0.028	0.788	0.055
Time	hr.	Dead cell +Media+ HW Chitosan							
		Dead cell		Dead cell + 600 mg/L		Dead cell + 800 mg/L		Dead cell + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0.000	1.616	0.010	1.616	0.010	1.616	0.010	1.616	0.010
After adding chemical	0.083	3.529	0.003	1.294	0.094	1.056	0.005	1.190	0.059
Settling time at 0 hr	1.083	4.135	0.001	1.451	0.075	1.190	0.047	1.110	0.070
Settling time at 1 hr	2.083	0.861	0.001	1.153	0.053	0.941	0.030	0.892	0.067
Settling time at 12 hr	13.083	0.317	0.004	0.861	0.072	0.772	0.108	0.882	0.035
Settling time at 24 hr	25.083	0.119	0.002	0.870	0.040	0.637	0.052	0.807	0.077

Table D-14 Measurements of OD_{680} when adding Crab shell

Time	hr.	Media + Crab shell							
		Media		Media + 600 mg/L		Media + 800 mg/L		Media + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0.000	0.032	0.015	0.032	0.015	0.032	0.015	0.032	0.015
After adding chemical	0.083	0.028	0.005	0.014	0.001	0.032	0.003	0.041	0.003
Settling time at 0 hr	1.083	0.014	0.002	0.006	0.002	0.011	0.003	0.009	0.002
Settling time at 1 hr	2.083	0.013	0.007	0.026	0.007	0.019	0.008	0.021	0.006
Settling time at 12 hr	13.08	0.008	0.004	0.023	0.002	0.006	0.002	0.015	0.003
Settling time at 24 hr	25.08	0.000	0.000	0.004	0.004	0.004	0.004	0.004	0.001
Time	**hr.**	**Living algae + Media + Crab shell**							
		Living algae		Algae + 600 mg/L		Algae + 800 mg/L		Algae + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0.000	1.616	0.078	1.616	0.078	1.616	0.078	1.616	0.078
After adding chemical	0.083	1.612	0.074	1.224	0.002	1.399	0.011	1.327	0.027
Settling time at 0 hr	1.083	1.354	0.001	1.257	0.025	1.413	0.025	1.417	0.004
Settling time at 1 hr	2.083	1.293	0.030	1.816	0.130	1.730	0.186	1.713	0.050
Settling time at 12 hr	13.083	0.795	0.049	0.898	0.091	1.018	0.159	0.983	0.206
Settling time at 24 hr	25.083	0.742	0.046	0.029	0.031	0.021	0.018	0.021	0.013
Time	**hr.**	**Dead cell +Media+ Crab shell**							
		Dead cell		Dead cell + 600 mg/L		Dead cell + 800 mg/L		Dead cell + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0.000	1.616	0.108	1.616	0.108	1.616	0.108	1.616	0.108
After adding chemical	0.083	3.529	0.004	0.975	0.032	0.829	0.111	2.068	0.044
Settling time at 0 hr	1.083	4.135	0.006	0.999	0.508	1.064	0.076	2.351	0.245
Settling time at 1 hr	2.083	0.853	0.017	0.987	0.026	1.064	0.150	1.857	0.177
Settling time at 12 hr	13.083	0.317	0.005	0.226	0.266	0.000	0.001	0.646	0.029
Settling time at 24 hr	25.083	0.119	0.011	0.353	0.227	0.400	0.324	0.594	0.292

2.3) pH

pH is measured as in **Table D-15-D20**.

Table D-15 Measurements of pH when adding Alum

Time	hr.	Media + Alum							
		Media		Media + 600 mg/L		Media + 800 mg/L		Media + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0.000	6.90	0.010	6.90	0.010	6.90	0.010	6.90	0.010
After adding chemical	0.083	6.87	0.011	7.13	0.007	7.13	0.004	7.28	0.013
Settling time at 0 hr	1.083	6.88	0.013	5.18	0.004	4.89	0.007	5.68	0.011
Settling time at 1 hr	2.083	6.89	0.007	5.00	0.005	4.85	0.004	4.68	0.009
Settling time at 12 hr	13.083	6.89	0.004	4.71	0.005	4.68	0.004	4.62	0.008
Settling time at 24 hr	25.083	6.88	0.005	4.71	0.005	4.67	0.004	4.62	0.005
Time	**hr.**	**Living algae + Media + Alum**							
		Living algae		Algae + 600 mg/L		Algae + 800 mg/L		Algae + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0.000	6.95	0.011	6.95	0.011	6.95	0.011	6.95	0.011
After adding chemical	0.083	6.95	0.005	7.20	0.007	7.20	0.005	7.14	0.007
Settling time at 0 hr	1.083	6.98	0.007	5.32	0.005	5.09	0.005	5.08	0.007
Settling time at 1 hr	2.083	6.98	0.004	5.01	0.008	4.77	0.005	4.61	0.005
Settling time at 12 hr	13.083	6.98	0.007	4.76	0.005	4.58	0.007	4.51	0.005
Settling time at 24 hr	25.083	6.98	0.008	4.76	0.004	4.55	0.007	4.45	0.015
Time	**hr.**	**Dead cell +Media+ Alum**							
		Dead cell		Dead cell + 600 mg/L		Dead cell + 800 mg/L		Dead cell + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0.000	6.95	0.005	6.95	0.005	6.95	0.005	6.95	0.005
After adding chemical	0.083	6.87	0.007	4.87	0.009	5.05	0.008	5.06	0.022
Settling time at 0 hr	1.083	6.95	0.005	3.42	0.005	3.30	0.022	3.28	0.011
Settling time at 1 hr	2.083	6.95	0.005	3.62	0.016	3.41	0.000	3.30	0.008
Settling time at 12 hr	13.083	6.95	0.005	3.47	0.000	3.37	0.000	3.30	0.004
Settling time at 24 hr	25.083	6.95	0.005	3.45	0.000	3.39	0.004	3.32	0.004

Table D-16 Measurements of pH when adding $FeCl_3$

Time	hr.	Media + $FeCl_3$							
		Media		Media + 600 mg/L		Media + 800 mg/L		Media + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0	6.90	0.004	6.90	0.004	6.90	0.004	6.90	0.004
After adding chemical	0.083	6.90	0.011	6.86	0.012	6.81	0.008	5.49	0.011
Settling time at 0 hr	1.083	6.90	0.004	4.49	0.007	4.44	0.000	4.22	0.000
Settling time at 1 hr	2.083	6.90	0.004	3.48	0.004	3.30	0.004	3.23	0.004
Settling time at 12 hr	13.08	6.90	0.004	3.48	0.022	3.30	0.007	3.23	0.013
Settling time at 24 hr	25.08	6.90	0.004	3.47	0.000	3.26	0.015	3.21	0.020
Time	**hr.**	**Living algae + Media + $FeCl_3$**							
		Living algae		Algae + 600 mg/L		Algae + 800 mg/L		Algae + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0	6.95	0.011	6.95	0.011	6.95	0.011	6.95	0.011
After adding chemical	0.083	6.95	0.011	6.94	0.000	6.92	0.000	6.95	0.000
Settling time at 0 hr	1.083	6.95	0.011	3.80	0.014	3.61	0.011	3.42	0.000
Settling time at 1 hr	2.083	6.95	0.011	3.35	0.036	3.29	0.005	3.35	0.004
Settling time at 12 hr	13.08	6.95	0.011	3.35	0.005	3.28	0.009	3.14	0.005
Settling time at 24 hr	25.08	6.95	0.011	3.35	0.011	3.19	0.005	3.03	0.023
Time	**hr.**	**Dead cell +Media+ $FeCl_3$**							
		Dead cell		Dead cell + 600 mg/L		Dead cell + 800 mg/L		Dead cell + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0	6.95	0.000	6.95	0.000	6.95	0.000	6.95	0.000
After adding chemical	0.083	6.95	0.000	2.20	0.000	2.15	0.004	1.95	0.000
Settling time at 0 hr	1.083	6.98	0.005	1.70	0.005	1.59	0.000	1.52	0.005
Settling time at 1 hr	2.083	6.98	0.000	1.70	0.007	1.60	0.000	1.55	0.004
Settling time at 12 hr	13.08	6.98	0.005	1.73	0.007	1.63	0.004	1.57	0.005
Settling time at 24 hr	25.08	6.98	0.000	1.78	0.000	1.68	0.004	1.61	0.009

Table D-17 Measurements of pH when adding $AlCl_3$

Time	hr.	Media + $AlCl_3$							
		Media		Media + 600 mg/L		Media + 800 mg/L		Media + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0	6.90	0.000	6.90	0.004	6.90	0.004	6.90	0.004
After adding chemical	0.083	6.90	0.004	6.90	0.000	6.90	0.000	6.90	0.000
Settling time at 0 hr	1.083	6.90	0.000	5.25	0.000	4.54	0.015	4.48	0.030
Settling time at 1 hr	2.083	6.90	0.004	4.43	0.015	4.38	0.026	4.43	0.017
Settling time at 12 hr	13.08	6.90	0.004	4.41	0.000	4.35	0.000	4.43	0.000
Settling time at 24 hr	25.08	6.90	0.004	4.34	0.000	4.32	0.004	4.29	0.000
Time	**hr.**	**Living algae + Media + $AlCl_3$**							
		Living algae		Algae + 600 mg/L		Algae + 800 mg/L		Algae + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0	6.95	0.008	6.95	0.008	6.95	0.008	6.95	0.008
After adding chemical	0.083	6.64	0.009	6.87	0.000	6.82	0.000	6.76	0.000
Settling time at 0 hr	1.083	6.88	0.008	5.04	0.009	5.04	0.000	5.04	0.000
Settling time at 1 hr	2.083	6.98	0.008	4.36	0.008	4.34	0.054	4.28	0.013
Settling time at 12 hr	13.08	6.98	0.009	4.24	0.029	4.28	0.050	4.29	0.010
Settling time at 24 hr	25.08	6.98	0.009	4.22	0.005	4.14	0.005	4.10	0.010
Time	**hr.**	**Dead cell +Media+ $AlCl_3$**							
		Dead cell		Dead cell + 600 mg/L		Dead cell + 800 mg/L		Dead cell + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0	6.95	0.000	6.95	0.000	6.95	0.000	6.95	0.000
After adding chemical	0.083	6.96	0.043	8.57	0.008	7.76	0.000	8.13	0.005
Settling time at 0 hr	1.083	6.94	0.007	5.62	0.035	5.52	0.010	5.64	0.030
Settling time at 1 hr	2.083	6.89	0.000	5.73	0.000	5.60	0.004	5.65	0.000
Settling time at 12 hr	13.08	6.96	0.005	5.58	0.008	5.66	0.005	5.72	0.000
Settling time at 24 hr	25.08	6.95	0.004	5.63	0.013	5.68	0.008	5.72	0.012

Table D-18 Measurements of pH when adding MW Chitosan

Time	hr.	Media + MW Chitosan							
		Media		Media + 600 mg/L		Media + 800 mg/L		Media + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0	6.90	0.000	6.90	0.000	6.90	0.000	6.90	0.000
After adding chemical	0.083	6.39	0.000	8.38	0.007	8.38	0.004	8.35	0.007
Settling time at 0 hr	1.083	6.90	0.011	8.37	0.007	8.34	0.004	8.37	0.004
Settling time at 1 hr	2.083	6.90	0.000	6.94	0.004	6.95	0.000	6.96	0.000
Settling time at 12 hr	13.08	6.89	0.000	6.69	0.004	6.75	0.000	6.77	0.000
Settling time at 24 hr	25.08	6.88	0.000	6.82	0.004	6.83	0.011	6.83	0.015
Time	**hr.**	**Living algae + Media + MW Chitosan**							
		Living algae		Algae + 600 mg/L		Algae + 800 mg/L		Algae + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0	6.95	0.000	6.95	0.000	6.95	0.000	6.95	0.000
After adding chemical	0.083	6.95	0.004	7.64	0.000	7.60	0.000	7.74	0.000
Settling time at 0 hr	1.083	6.88	0.004	7.74	0.000	7.90	0.000	7.88	0.000
Settling time at 1 hr	2.083	6.88	0.000	7.00	0.005	7.09	0.030	7.18	0.018
Settling time at 12 hr	13.08	6.98	0.000	6.46	0.021	6.45	0.011	6.46	0.013
Settling time at 24 hr	25.08	6.98	0.000	6.34	0.008	6.41	0.008	6.43	0.011
Time	**hr.**	**Dead cell +Media+ MW Chitosan**							
		Dead cell		Dead cell + 600 mg/L		Dead cell + 800 mg/L		Dead cell + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0	6.95	0.000	6.95	0.000	6.95	0.000	6.95	0.000
After adding chemical	0.083	6.93	0.009	6.92	0.009	6.88	0.000	6.93	0.004
Settling time at 0 hr	1.083	6.88	0.004	7.06	0.024	7.00	0.000	7.04	0.011
Settling time at 1 hr	2.083	6.98	0.007	7.08	0.016	7.05	0.016	7.06	0.013
Settling time at 12 hr	13.08	6.98	0.005	6.92	0.005	6.86	0.004	6.98	0.009
Settling time at 24 hr	25.08	6.98	0.007	6.89	0.013	6.91	0.004	7.00	0.011

Table D-19 Measurements of pH when adding HW Chitosan

Time	hr.	Media + HW Chitosan							
		Media		Media + 600 mg/L		Media + 800 mg/L		Media + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0	6.90	0.010	6.90	0.010	6.90	0.010	6.90	0.010
After adding chemical	0.083	6.87	0.008	6.90	0.008	6.90	0.008	6.97	0.007
Settling time at 0 hr	1.083	6.90	0.010	6.97	0.004	6.97	0.000	6.97	0.000
Settling time at 1 hr	2.083	6.89	0.010	7.10	0.065	7.03	0.044	7.08	0.129
Settling time at 12 hr	13.08	6.89	0.010	6.82	0.027	6.86	0.025	6.91	0.037
Settling time at 24 hr	25.08	6.88	0.010	6.85	0.004	6.81	0.292	6.87	0.040
Time	**hr.**	**Living algae + Media + HW Chitosan**							
		Living algae		Algae + 600 mg/L		Algae + 800 mg/L		Algae + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0	6.95	0.022	6.95	0.022	6.95	0.022	6.95	0.022
After adding chemical	0.083	6.95	0.022	7.30	0.022	7.03	0.022	6.95	0.022
Settling time at 0 hr	1.083	6.88	0.022	7.01	0.016	7.31	0.358	7.18	0.016
Settling time at 1 hr	2.083	6.98	0.022	6.84	0.089	7.09	0.026	7.09	0.033
Settling time at 12 hr	13.08	6.98	0.022	6.38	0.013	6.50	0.004	6.56	0.100
Settling time at 24 hr	25.08	6.98	0.022	6.48	0.069	6.56	0.009	6.58	0.042
Time	**hr.**	**Dead cell +Media+ HW Chitosan**							
		Dead cell		Dead cell + 600 mg/L		Dead cell + 800 mg/L		Dead cell + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0	6.95	0.004	6.95	0.004	6.95	0.004	6.95	0.004
After adding chemical	0.083	6.95	0.011	5.97	0.011	5.89	0.020	6.06	0.014
Settling time at 0 hr	1.083	6.95	0.014	5.97	0.011	5.89	0.020	6.06	0.014
Settling time at 1 hr	2.083	6.95	0.015	4.56	0.004	5.02	0.016	5.18	0.013
Settling time at 12 hr	13.08	6.98	0.013	4.70	0.263	4.96	0.058	5.18	0.052
Settling time at 24 hr	25.08	6.98	0.013	4.89	0.022	5.19	0.016	5.23	0.000

Table D-20 Measurements of pH when adding Crab shell

Time	hr.	Media + Crab shell							
		Media		Media + 600 mg/L		Media + 800 mg/L		Media + 1,000 mg/L	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0	6.90	0.000	6.90	0.000	6.90	0.000	6.90	0.000
After adding chemical	0.083	6.87	0.004	6.91	0.007	6.97	0.004	8.10	0.004
Settling time at 0 hr	1.083	6.89	0.000	6.97	0.007	7.03	0.004	7.10	0.005
Settling time at 1 hr	2.083	6.89	0.000	6.91	0.023	6.70	0.019	6.98	0.020
Settling time at 12 hr	13.08	6.89	0.000	6.89	0.016	6.92	0.023	6.93	0.028
Settling time at 24 hr	25.08	6.88	0.000	6.97	0.050	6.99	0.059	7.00	0.019
Time	**hr.**	**Living algae + Media + Crab shell**							
		Living algae		**Algae + 600 mg/L**		**Algae + 800 mg/L**		**Algae + 1,000 mg/L**	
		Mean	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**
Start up (before adding chemical)	0	6.95	0.028	6.95	0.028	6.95	0.028	6.95	0.028
After adding chemical	0.083	6.95	0.005	8.40	0.033	8.46	0.033	8.63	0.033
Settling time at 0 hr	1.083	6.88	0.005	8.57	0.036	8.67	0.038	8.71	0.038
Settling time at 1 hr	2.083	6.88	0.028	7.14	0.010	7.23	0.021	7.25	0.032
Settling time at 12 hr	13.08	6.98	0.028	6.84	0.011	6.88	0.038	6.91	0.012
Settling time at 24 hr	25.08	6.98	0.028	6.80	0.048	6.87	0.034	6.90	0.029
Time	**hr.**	**Dead cell +Media+ Crab shell**							
		Dead cell		**Dead cell + 600 mg/L**		**Dead cell + 800 mg/L**		**Dead cell + 1,000 mg/L**	
		Mean	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**
Start up (before adding chemical)	0	6.95	0.004	6.95	0.004	6.95	0.004	6.95	0.004
After adding chemical	0.083	6.95	0.004	6.95	0.018	6.95	0.000	6.95	0.005
Settling time at 0 hr	1.083	6.88	0.004	6.64	0.059	6.90	0.020	6.89	0.009
Settling time at 1 hr	2.083	6.76	0.004	6.62	0.018	6.86	0.008	6.77	0.019
Settling time at 12 hr	13.08	6.98	0.004	6.55	0.004	6.70	0.004	6.54	0.000
Settling time at 24 hr	25.08	6.98	0.004	6.47	0.022	6.57	0.004	6.45	0.027

Appendix E: Group determination and comparison of turbidity, optical density, and pH

A group determination and comparison of the turbidity, optical density and pH is shown in **Tables E-1-E-3**. Sampling replications are 3 (n=3).

Table E-1 Turbidity measurements between groups (Untreated media, living and dead cell suspensions) and within groups; media, living algal cell suspensions, and dead cell suspensions.

Time	hr.	Media					
		Media		Algae		Dead cell	
		Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0.000	2.00	0.00	100.00	0.00	100.00	0.00
After adding chemical	0.083	2.20	1.85	82.31	21.58	93.66	21.95
Settling time at 0 hr	1.083	1.95	1.50	79.62	17.07	94.05	44.52
Settling time at 1 hr	2.083	1.17	0.64	65.34	43.32	54.31	26.63
Settling time at 12 hr	13.083	0.61	0.43	32.53	27.20	33.94	20.59
Settling time at 24 hr	25.083	0.48	0.35	11.90	19.11	31.40	20.00
Time	hr.	Media					
		Media + Alum		Media + $FeCl_3$		Media + $AlCl_3$	
		Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0.000	2.00	0.000	2.00	0.000	2.00	0.000
After adding chemical	0.083	1.59	0.457	5.70	2.007	1.75	0.360
Settling time at 0 hr	1.083	1.51	0.236	4.74	1.430	2.35	0.347
Settling time at 1 hr	2.083	0.70	0.071	1.17	0.026	1.68	0.166
Settling time at 12 hr	13.083	0.35	0.062	1.25	0.435	0.34	0.052
Settling time at 24 hr	25.083	0.37	0.118	0.90	0.152	0.04	0.071
Time	hr.	Media					
		Media + MW Chitosan		Media + HW Chitosan		Media + Crab shell	
		Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0.000	2.00	0.001	2.00	0.000	2.00	0.000
After adding chemical	0.083	1.57	0.452	0.43	0.309	2.27	1.313
Settling time at 0 hr	1.083	0.83	0.589	0.63	0.763	1.81	0.556
Settling time at 1 hr	2.083	0.16	0.122	1.77	0.207	1.81	0.001
Settling time at 12 hr	13.083	0.12	0.076	0.96	0.077	0.78	0.007
Settling time at 24 hr	25.083	0.12	0.078	0.81	0.119	0.69	0.013
Time	hr.	Living algal cell					
		Algae + Alum		Algae + $FeCl_3$		Algae + $AlCl_3$	
		Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0.000	100.00	0.000	100.00	0.000	100.00	0.00
After adding chemical	0.083	47.30	0.706	102.90	23.865	96.75	2.94
Settling time at 0 hr	1.083	54.94	3.863	105.79	5.231	81.07	1.37
Settling time at 1 hr	2.083	9.48	0.455	91.19	29.435	2.40	1.15
Settling time at 12 hr	13.083	7.53	0.528	8.27	4.318	0.23	0.39
Settling time at 24 hr	25.083	0.00	0.000	0.11	0.013	0.00	0.00
Time	hr.	Living algal cell					
		Algae + MW Chitosan		Algae + HW Chitosan		Algae + Crabshell	
		Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0.000	100.00	0.000	100.00	0.000	100.00	0.00
After adding chemical	0.083	86.02	7.888	65.19	9.801	93.10	2.15
Settling time at 0 hr	1.083	81.81	3.227	64.25	10.868	88.42	4.23
Settling time at 1 hr	2.083	82.13	2.912	98.00	1.411	104.24	0.64
Settling time at 12 hr	13.083	55.61	1.055	67.08	3.150	51.32	1.72
Settling time at 24 hr	25.083	6.56	0.471	48.39	0.518	5.30	0.36
Time	hr.	Dead cell					
		Dead + Alum		Dead + $FeCl_3$		Dead cell + $AlCl_3$	
		Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0.000	100.00	0.000	100.00	0.000	100.00	0.000
After adding chemical	0.083	89.80	3.264	106.88	14.892	113.52	11.015
Settling time at 0 hr	1.083	71.62	26.015	77.60	40.355	178.98	7.612
Settling time at 1 hr	2.083	17.92	3.630	37.47	12.502	37.06	0.630
Settling time at 12 hr	13.083	0.00	0.000	39.86	7.794	20.60	9.387
Settling time at 24 hr	25.083	1.59	1.403	31.55	2.326	13.18	2.848
Time	hr.	Dead cell					
		Dead + MW Chitosan		Dead + HW Chitosan		Dead cell + Crab shell	
		Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0.000	100.00	0.000	100.00	0.000	100.00	0.000
After adding chemical	0.083	91.67	2.218	59.45	16.926	94.58	29.747
Settling time at 0 hr	1.083	82.14	1.038	75.38	23.676	94.51	32.484
Settling time at 1 hr	2.083	74.35	1.710	67.57	16.005	82.79	25.949
Settling time at 12 hr	13.083	34.55	2.105	41.61	10.787	55.47	10.740
Settling time at 24 hr	25.083	35.74	2.926	42.24	6.972	51.32	12.526

Table E-2 Optical density measurements between groups (Untreated media, living and dead cell suspensions) and within groups; media, living algal cell suspensions, and dead cell suspensions.

Time	hr.	Media					
		Media		Algae		Dead cell	
		Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0.000	0.032	0.000	1.616	0.000	1.614	0.004
After adding chemical	0.083	0.092	0.191	1.278	0.326	2.799	2.127
Settling time at 0 hr	1.083	0.132	0.292	1.170	0.380	2.483	1.722
Settling time at 1 hr	2.083	0.015	0.010	1.064	0.731	1.272	0.997
Settling time at 12 hr	13.083	0.005	0.006	0.536	0.535	0.767	0.992
Settling time at 24 hr	25.083	0.001	0.002	0.178	0.319	0.877	1.040
Time	**hr.**	**Media**					
		Media + Alum		**Media + $FeCl_3$**		**Media + $AlCl_3$**	
		Mean	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**
Start up (before adding chemical)	0.000	0.032	0.000	0.032	0.000	0.032	0.000
After adding chemical	0.083	0.015	0.000	0.415	0.336	0.107	0.140
Settling time at 0 hr	1.083	0.016	0.001	0.760	0.247	0.029	0.022
Settling time at 1 hr	2.083	0.017	0.004	0.023	0.013	0.005	0.005
Settling time at 12 hr	13.083	0.007	0.006	0.004	0.004	0.000	0.000
Settling time at 24 hr	25.083	0.000	0.000	0.004	0.004	0.000	0.000
Time	**hr.**	**Media**					
		Media + MW Chitosan		**Media + HW Chitosa**		**Media + Crab shell**	
		Mean	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**
Start up (before adding chemical)	0.000	0.032	0.000	0.032	0.000	0.032	0.000
After adding chemical	0.083	0.006	0.004	0.004	0.003	0.029	0.014
Settling time at 0 hr	1.083	0.004	0.002	0.009	0.011	0.009	0.003
Settling time at 1 hr	2.083	0.003	0.004	0.024	0.004	0.022	0.003
Settling time at 12 hr	13.083	0.001	0.002	0.002	0.001	0.015	0.009
Settling time at 24 hr	25.083	0.000	0.000	0.000	0.000	0.004	0.000
Time	**hr.**	**Living algal cell**					
		Algae + Alum		**Algae + $FeCl_3$**		**Algae + $AlCl_3$**	
		Mean	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**
Start up (before adding chemical)	0.000	1.616	0.000	1.616	0.000	1.616	0.000
After adding chemical	0.083	0.959	0.065	1.843	0.149	0.979	0.068
Settling time at 0 hr	1.083	0.770	0.071	1.705	0.186	0.651	0.027
Settling time at 1 hr	2.083	0.210	0.066	1.106	0.415	0.000	0.000
Settling time at 12 hr	13.083	0.008	0.010	0.000	0.000	0.000	0.000
Settling time at 24 hr	25.083	0.000	0.000	0.000	0.000	0.000	0.000
Time	**hr.**	**Living algal cell**					
		Algae + MW Chitosan		**Algae + HW Chitosa**		**Algae + Crabshell**	
		Mean	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**
Start up (before adding chemical)	0.000	1.616	0.000	1.616	0.000	1.616	0.000
After adding chemical	0.083	1.340	0.046	1.117	0.215	1.317	0.088
Settling time at 0 hr	1.083	1.340	0.017	1.117	0.215	1.362	0.091
Settling time at 1 hr	2.083	1.383	0.064	1.854	0.019	1.753	0.055
Settling time at 12 hr	13.083	0.922	0.140	1.239	0.029	0.966	0.062
Settling time at 24 hr	25.083	0.068	0.004	0.791	0.029	0.024	0.005
Time	**hr.**	**Dead cell**					
		Dead + Alum		**Dead + $FeCl_3$**		**Dead cell + $AlCl_3$**	
		Mean	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**
Start up (before adding chemical)	0.000	1.616	0.000	1.616	0.000	1.616	0.000
After adding chemical	0.083	1.454	0.098	6.499	1.751	4.309	0.874
Settling time at 0 hr	1.083	0.932	0.117	4.596	1.466	4.586	1.193
Settling time at 1 hr	2.083	0.107	0.101	3.267	0.407	0.799	0.046
Settling time at 12 hr	13.083	0.002	0.003	2.872	0.271	0.086	0.149
Settling time at 24 hr	25.083	0.020	0.025	3.016	0.754	0.578	0.693
Time	**hr.**	**Dead cell**					
		Dead + MW Chitosan		**Dead + HW Chitosa**		**Dead cell + Crab shel**	
		Mean	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**
Start up (before adding chemical)	0.000	1.616	0.000	1.616	0.000	1.616	0.000
After adding chemical	0.083	1.710	0.274	1.180	0.119	1.291	0.677
Settling time at 0 hr	1.083	1.667	0.136	1.250	0.178	1.471	0.762
Settling time at 1 hr	2.083	1.274	0.039	0.995	0.139	1.303	0.482
Settling time at 12 hr	13.083	0.593	0.082	0.838	0.058	0.291	0.328
Settling time at 24 hr	25.083	0.607	0.054	0.771	0.121	0.449	0.128

Table E-3 pH measurements between groups (Untreated media, living and dead cell suspensions) and within groups; media, living algal cell suspensions, and dead cell suspensions

Time	hr.	Media					
		Media		Algae		Dead cell	
		Mean	S.D.	Mean	S.D.	Mean	S.D.
Start up (before adding chemical)	0.000	6.90	0.001	6.95	0.002	6.95	0.002
After adding chemical	0.083	7.16	0.701	7.34	0.584	5.90	1.954
Settling time at 0 hr	1.083	6.16	1.451	6.28	1.779	5.16	2.005
Settling time at 1 hr	2.083	5.65	1.487	5.70	1.571	5.02	1.958
Settling time at 12 hr	13.083	5.56	1.449	5.41	1.404	4.97	1.909
Settling time at 24 hr	25.083	5.57	1.487	5.38	1.436	4.99	1.885
Time	**hr.**	**Media**					
		Media + Alum		**Media + $FeCl_3$**		**Media + $AlCl_3$**	
		Mean	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**
Start up (before adding chemical)	0.000	6.90	0.000	6.90	0.000	6.90	0.000
After adding chemical	0.083	7.18	0.087	6.38	0.779	6.90	0.000
Settling time at 0 hr	1.083	5.25	0.398	4.38	0.144	4.76	0.427
Settling time at 1 hr	2.083	4.84	0.164	3.34	0.129	4.41	0.025
Settling time at 12 hr	13.083	4.67	0.049	3.34	0.129	4.40	0.042
Settling time at 24 hr	25.083	4.67	0.049	3.31	0.137	4.32	0.025
Time	**hr.**	**Media**					
		Media + MW Chitosan		**Media + HW Chitosan**		**Media + Crab shell**	
		Mean	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**
Start up (before adding chemical)	0.000	6.90	0.000	6.90	0.000	6.90	0.000
After adding chemical	0.083	8.37	0.017	6.92	0.042	7.33	0.669
Settling time at 0 hr	1.083	8.36	0.018	6.97	0.001	7.04	0.067
Settling time at 1 hr	2.083	6.95	0.009	7.07	0.039	6.86	0.147
Settling time at 12 hr	13.083	6.74	0.043	6.86	0.045	6.91	0.023
Settling time at 24 hr	25.083	6.83	0.006	6.84	0.031	6.99	0.015
Time	**hr.**	**Living algal cell**					
		Algae + Alum		**Algae + $FeCl_3$**		**Algae + $AlCl_3$**	
		Mean	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**
Start up (before adding chemical)	0.000	6.95	0.000	6.95	0.000	6.95	0.000
After adding chemical	0.083	7.18	0.036	6.94	0.015	6.82	0.055
Settling time at 0 hr	1.083	5.16	0.139	3.61	0.190	5.04	0.002
Settling time at 1 hr	2.083	4.80	0.198	3.33	0.037	4.33	0.040
Settling time at 12 hr	13.083	4.62	0.125	3.26	0.103	4.27	0.025
Settling time at 24 hr	25.083	4.59	0.158	3.19	0.161	4.15	0.064
Time	**hr.**	**Living algal cell**					
		Algae + MW Chitosan		**Algae + HW Chitosan**		**Algae + Crabshell**	
		Mean	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**
Start up (before adding chemical)	0.000	6.95	0.000	6.95	0.000	6.95	0.000
After adding chemical	0.083	7.66	0.072	7.09	0.183	8.49	0.120
Settling time at 0 hr	1.083	7.84	0.087	7.17	0.149	8.65	0.072
Settling time at 1 hr	2.083	7.09	0.094	7.00	0.144	7.21	0.058
Settling time at 12 hr	13.083	6.46	0.004	6.48	0.092	6.88	0.038
Settling time at 24 hr	25.083	6.39	0.047	6.54	0.055	6.86	0.048
Time	**hr.**	**Dead cell**					
		Dead + Alum		**Dead + $FeCl_3$**		**Dead cell + $AlCl_3$**	
		Mean	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**
Start up (before adding chemical)	0.000	6.95	0.000	6.95	0.000	6.95	0.000
After adding chemical	0.083	7.18	0.036	6.94	0.015	6.82	0.055
Settling time at 0 hr	1.083	5.16	0.139	3.61	0.190	5.04	0.002
Settling time at 1 hr	2.083	4.80	0.198	3.33	0.037	4.33	0.040
Settling time at 12 hr	13.083	4.62	0.125	3.26	0.103	4.27	0.025
Settling time at 24 hr	25.083	4.59	0.158	3.19	0.161	4.15	0.064
Time	**hr.**	**Dead cell**					
		Dead + MW Chitosan		**Dead + HW Chitosan**		**Dead cell + Crab shel**	
		Mean	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**
Start up (before adding chemical)	0.000	6.95	0.000	6.95	0.000	6.95	0.000
After adding chemical	0.083	7.66	0.072	7.09	0.183	8.49	0.120
Settling time at 0 hr	1.083	7.84	0.087	7.17	0.149	8.65	0.072
Settling time at 1 hr	2.083	7.09	0.094	7.00	0.144	7.21	0.058
Settling time at 12 hr	13.083	6.46	0.004	6.48	0.092	6.88	0.038
Settling time at 24 hr	25.083	6.39	0.047	6.54	0.055	6.86	0.048

Appendix F: Example of the productivity of *C.vulgaris* grown in various conditions

An example of the productivity of *C.vulgaris* grown in various conditions are shown in **Table F-1**.

Table F-1 Examples of the productivity of *C.vulgaris* grown in various environmental conditions

Algal strain	OD	Cell no. (cell/mL)	Specifc growth rate (μ_{max}, d^{-1})	Biomass productivity : Dry weight (g/L/d)	Environmental conditions						Ref.
					Nutrient	Air/CO_2	Light (μmol. m^{-2} s^{-1})	pH	T (°C)	Cultured period (d)	
C.vulgaris Bejierinck (CCAP211/11B)	N/A	5.7 x10	0.4	0.04 g/L/d	Watanabe's media	Air 5 L/M	40 W, 130, 24 hr.	6.0	25	36	(Scragg *et al.*, 2002)
C.vulgaris Bejierinck (C CAP 211/11B)	N/A	$7.0x10^6$	0.69/0.12	0.024 g/L/d	Low N media	Air 0.3 L/M	40 W, 130	6.0	25	36	(Scragg *et al.*, 2002)
C.vulgaris ARC1	N/A	N/A	0.222	N/A	BG11	6% CO_2 (v/v)	47	N/A	30	10	(Chinnasamy, 2009)
C.vulgaris	N/A	N/A	N/A	Around 0.7, 0.3 dw g/L when giving KNO_3 of 0.1 mM, and 0.2 mM. and around 0.9, 1.1 dw g/L when giving KNO_3 3.0 mM KNO_3 and 5.0 mM	100 g KNO_3, 10g KH_2PO_4, 10 g Na_2EDTA, 2.5 g $FeSO_4.7H_2O$, 0.25 g $MnSO_4$, 0.006g B_1, 0.00005 g B_{12}	1.2 L/M, 0.5,1.0,12. 0% CO_2 (v/v). The highest biomass at 1% CO_2 (v/v)	Vary light intensity at 24,60,120. The high yield is at 60 μmol. m^{-2} s^{-1} giving a biomass at 0.75 g/L	N/A	25	80 hr.	(Lv *et al.*, 2010)
C.vulgaris LEB-104	N/A	N/A	0.29	0.251 g/L/d	Artificial sea water and Modified Bristol media	Air enriched with 5% CO_2	8-32 w cool white, 3500 Lux D/L:12:12	7.2 ± 0.2	25	15	(Sydney *et al.*, 2010)
C.vulgaris	1.805	N/A	N/A	1.22 g/L	BG 11 with varyNO_3 (0,1,1.5,2,5 and 10 g/L). The highest is 0.5 g/L and PO_4 (0,0.01, 0.04,0.08 and 0.1 g/L).The highest is 0.04 g/L	Air, (0.3-15% CO_2). The highest at 4%. At 6-15% CO_2, the growth decline.	80, D/L 16:8. Vary light intensity between 200-600 μmol. m^{-2} s^{-1}. The best yield is 150-350 μmol. m^{-2} s^{-1}	7.5	25± 1	15	(Bhola *et al.*, 2011)
C.vulgaris	N/A	N/A	N/A	Around 0.2 g/L/d	Swine wastewater M4N	Vary CO_2 of 0.07,1.4, 3.0 and 5%. The highest biomass is 3% CO_2. The flow rate is 0.5 L/m	Vary light 39.19,72.9 7,105.41,1 16.22,135. 14, 175.68 The maximum biomass is at 105.41 for 12 hr.		25± 1	11	(Lee *et al.*, 2011)
C.vulgaris	N/A	$2.74x10^6$ (LC Oligo)	0.84	N/A	Vary media LC Oligo Media, Chu 10 and WC media. LC Oligo is the best choice	N/A	150, D/L; 16:8	7.0	20± 2	12	(Chia *et al.*, 2013)
C.vulgaris P12	N/A	N/A	N/A	1.3 g/L/d	N/A	vary aeration rate of 0.1,0.4,07 v/v/m and vary 2%, 6%,10% CO_2 The best yield is 6% CO_2 by 0.4 $vv^{-1}m^{-1}$	70	N/A	30	Vary 7.9,7.7 7.6 d. The best yield is 7.7 d by 6% CO_2)	(Anjos *et al.*, 2013)
C.vulgaris CCAP 211	N/A	N/A	0.289± 0.027 d^{-1}	0.4 g/L	BBM (vary 0,0.2,0.4, and 0.8 g/L $NaHCO_3$ at 0.2 g/L is the best)	CO_2 + Air 350 L/h	8-36 W, 60 276 Blue (475 nm) /Red (650 nm)	N/A	20± 0.5	15 day flask, fed batch 30 days.	(Frumento *et al.*, 2013)
C.vulgaris, CCAP 211/118B	N/A	N/A	The highest is 1.36±0.06 (5% CO_2)	The highest is 0.164 ± 0.010 (7% CO_2) 0.065	Growth Media	Vary air, 3,5,7,9,10% CO_2	120	7	23.4 ±2.1	7	(Gonçalves *et al.*, 2016)
C.vulgaris	9.70 (Day 7, 6%CO_2 (Day 5, 12%CO_2) **(Figure 5-5)**	$5.56x10^6$ (Day 7, 6%CO_2) $5.36x10^6$ (on Day 6, 12% CO_2) **(Figure 5-6)**	1.04 (Day 2, 6% CO_2) 2.21 (Day 2 at 12% CO_2) **(Table 5-2)**	0.98 (Day 6, 6% CO_2) 1.25 (Day 2, 12% CO_2, on Day 4 is 0.63) **(Table 5-6)**	BBM	0.15 vvm for 2L woking volume Photobio reactor	2-40 W Fluorescene 500	6.7±0.3	22-25	7	This study

Appendix G: Poster, Oral presentation and Paper publications

1. Biofuel in Thailand, Available online: https://www.youtube.com/watch?v=9gAhftlNfRM&index=4&list=PL5D211CA901E27B52&t=64s

2. Microalgae for biofuel production, Ennis Room, Halifax Hall Conference Centre, The Endcliffe Village, Endcliffe Vale Road, Sheffield S10 3ER. 20 th September 2012.

3. Enhancing growth using carbon dioxide and improve efficiency of sedimentation process by using chitosan for *Chlorella vulgaris* in a photobioreactor, Challenges to a sustainable Energy Future, Halifax Hall Conference Centre, The Endcliffe Village, Endcliffe Vale Road, Sheffield S10 3ER, 16-17 th September 2013 (Poster presentation).

4. Algal growth in various nutrients, PGR Conference CBE, The University of Sheffield, 28th May 2014, (Poster presentation) Available online: https://www.researchgate.net/profile/Supatchalee_Sophonthammaphat/publication/262706018_Algae_growth_in_various_nutrients/links/02e7e538882b993606000000/Algae-growth-in-various-nutrients.pdf.

5. CO_2 utilisation and investigation of algae growth rate by using various nutrients. The USE 2014-The University of Sheffield Engineering Symposium, Octagon, The University of Sheffield, 24 th June 2014 (Poster presentation).

6. Algal photobioreactor, E-Futures Conference, The University of Sheffield 22 th September 2014 (Poster presentation).

7. CO_2 utilisation and investigation of algae growth rate by using various nutrients. The CO_2 forum International Sustainable CO_2 Chemical and Biochemical Utilization (Poster and Paper document in conference book: Large Volume CO_2 Utilisation: Enable Technologies for Energy and Resource Efficiency), page 114, CPE Lyon, France, 25- 26 th September 2014 (poster and the conference book).

8. Carbon Dioxide Utilisation Seminar by Katy Armstrong CO_2 Chemical Network Manager, PLB-01, Palm Liversidge Building, The University of Sheffield, 30 th April 2015 (Co-ordinator seminar).

9. Algal sedimentation process using metallic polymers & Chitosan, PGR Conference, CBE, The University of Sheffield, 10th June 2015 (Oral Presentation).

10. Flocculation *Chlorella vulgaris* using chitosan: Fundamentals of renewable energy & application August 2015 volume 5, Issue 5 ISSN:2090-4541 page 148 in Processing of International Congress and Expo on Biofuel & Bioenergy. 25-27 th August 2015, Valencia Spain, Available online: http//dx doi.org/104172/ 2019-4541.S1.003 (Paper document).

11. Algal sedimentation process using metallic polymers & chitosan, 15th September 2015, E-Future conference, The University of Sheffield (Oral presentation).

12. Harvesting algae using flocculation and sedimentation process, The 6 th International Conference on Algal biomass, Biofuel and Bioproduct 26-29th, June 2016 San Diego California, USA. (Poster presentation).