University of Southern Queensland

Faculty of Engineering and Surveying

CSG WATER AS A MEDIUM TO GROW MARINE MICROALGAE FOR BIOFUEL PRODUCTION

A dissertation submitted by

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ABSTRACT

Over the next decade, the expanding Coal Seam Gas (CSG) industry in the Bowen and Surat Basins is expected to produce between 50 to 300 GL of CSG water per year as a by-product of its methane extraction processes. CSG water is high in sodium, salts, carbon in the form of bicarbonates and other undesirable substances making it unfit for direct use.

Typically CSG water has been considered as a waste product, and is currently discharged in large evaporation ponds. The QLD Government has recently introduced policy encouraging the beneficial utilization of this water. Due to its high bicarbonate concentration, CSG water has the potential to be used as a medium for growing microalgae for the production of biofuel.

Microalgae derived biofuel is one of the more promising alternate green energy fuel sources to emerge in recent years. This method is superior to traditional crop based biofuels as it requires substantially less water and land area to yield equivalent oil volumes. Furthermore, it has the additional potential of cleansing nutrient rich waste waters.

Hence, the aim of this dissertation was to assess the potential of using CSG water as a medium for growing microalgae to produce biofuel. Additionally, investigation was made of the carbon sequestration and nutrient removal capacity of this process. Three sets of batch experiments were conducted using a 3.5 L batch bio-reactor. In all trials, DO, pH and temperature were monitored in real time, along with daily sampling of carbon, nitrogen and phosphorous to calculate the depletion rates. Furthermore, algal growth was documented by measuring suspended solids concentrations, and by optical density measurement using a spectrophotometer.

A preliminary set of trials were completed to validate the growth and monitoring capacity of the bio-reactor. The trials inoculated microalgae *Chlorella vulgaris* in a controlled MBL media. A florescent light source, compressed air and a CO_2 feed were provided to facilitate algal growth. The pH was set within the range 7.5±0.6. Trial results generally validated monitoring and growth capacity using the installed bio-reactors.

Trials were then conducted using the microalgae *Dunaliella tertiolecta* in a CSG water medium. All trials were run for 5 days. The reactor was filled with 3L CSG water, inoculated with 250ml *Dunaliella tertiolecta*, and 5ml/L of F2 concentrate was added to provide a nutrient source. The bicarbonate level in the CSG water was increased to a mean concentration level (216 C mg/L), through the addition of sodium bicarbonate ($NaHCO_3$). The pH was controlled at a set point 7.6±0.5. A fluorescent light source was provided, and assessment was made of the effect of aeration on algal growth and carbon stripping. Poor growth was recorded for non-aeration and aeration scenarios, with initial growth rates of 0.0292 g SS/L/d and 0.0303 g SS/L/d, respectively. Over the five day trial periods, algal carbon sequestration quantities of up to 90.9mg/L were achieved, and aeration was found to cause carbon stripping of up to 81.52 mg/L. Nitrogen and phosphorous removal rates were 0.818mg N/L/d and 0.362 mg P/L/d for the non-aeration trial, and 1.523 mg N/L/d and 0.381 mg P/L/d for the aeration trial. Nutrient depletion P:N ratios of 1:2 to 1:4 were observed.

Due to the poor growth performance, identification was made of the optimal salinity level for growth of *Dunaliella Tertiolecta* in CSG water (10 mg NaCl/L), trials were then repeated. Results found high growth in the non-aeration and aeration trials, with growth rates of 0.0935 g SS/L/d and 0.0808 g SS/L/d, respectively. Growth performance suggested no overall benefit in adopting aeration for algal growth facilitation. Furthermore, carbon sequestration levels of up to 82.2mg/L were achieved, and carbon aeration was found to cause carbon stripping of up to 72.4 mg/L. Nitrogen and phosphorous removal rates were 2.335 mg N/L/d and 1.156 mg P/L/d for the non-aeration trial, and 2.808 mg N/L/d and 0.959 mg/L/d for the aeration trial. Nutrient depletion P:N ratios of 1:2 to 1:3 were observed. The algal dry mass and total lipid content of the trials were 0.39 g and 24% for the non-aeration, and 0.41 g and 20% for the aeration trial.

The research suggest that the microalgae *Dunaliella Tertiolecta* has the potential to be grown in CSG water, in an open pond settings, for biofuel production purposes. Trials found that the microalgae *Dunaliella Tertiolecta* would grow in the CSG media, however increased salinity levels of about 10 g/L were required to achieve optimal growth. This suggests that if the CSG water is subjected to reverse osmosis treatment, then the resulting brine having a high concentrated salinity could be used as an ideal medium to grow the desired algal strand. Further analysis would be required to determine the economic viability of this process.

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CERTIFICATION

I certify that the ideas, designs and experimental work, results, analyses and conclusions set out in this dissertation are entirely my own effort, except where otherwise indicated and acknowledged.

I further certify that the work is original and has not been previously submitted for assessment in any other course or institution, except where specifically stated.

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INTRODUCTION

The expected increases in world energy usage in the coming years, means that a number of new green renewable energy sources must be developed to meet future demands and to reduce greenhouse gas emissions (Mata et al 2010). In Australia, approximately 75% of transport fuel comes from local oil and gas sources. It is predicted that this market portion could reduce to 45% by 2030 with growing national demand, and without any new discoveries or technological breakthroughs (CSIRO 2011). Alternate methods of fuel production will need to be developed and or expanded in the coming years to limit the potential of future foreign fuel source dependency, and to ensure national fuel security.

Currently, the prominent alternate to fossil fuels is crop based biofuel, accounting for approximately 2% of the domestic fuel market (Australian Treasury 2011). This production method has limitations however, it is not sustainable and it competes with the agricultural industry for farmable land. Microalgae derived biofuels offer an alternative that overcomes these limitations. This fuel production method requires significantly less land area, does not need arable land and can utilize saltwater, brackish water or wastewater for production. Conservative estimates by the CSIRO, based on an algal oil content of 20%, states that a pond area of 10,000km^2 would be required to produce enough microalgae biofuel to meet Australia's current domestic demand needs. Alternately, a farmable area of 1,320,000 km^2 (17% of Australia's land mass) and a fresh water supply would be required to produce the same oil yield using a soy bean crop, which is one of the more popular biofuel crops. This disparity highlights both the limitations of traditional methods and the potential of microalgae biofuels.

The utilization of Coal Seam Gas (CSG) water as a medium for microalgae biofuel production presents one opportunity for the creation of a new carbon neutral fuel source. CSG water, a by-product of the CSG extraction process, is considered as a waste material and is typically disposed of in large evaporation ponds. This water is rich in carbon in the form of bicarbonates.

Optimal algal production, requires a source of carbon, this generally is supplied by a CO_2 feed from industry or power stations. This project differentiates in that the carbon source is pre-

existing in the CSG water. Therefore there is the potential to use existing open pond infrastructure for algal growth, without the need to develop a CO_2 feed method. There are several other potential benefits in using CSG water for biofuel production, including offsetting carbon emissions by replacing fossil fuel products, generating new industry in rural areas and assisting in decentralising fuel production.

With no current published research pertaining to algal growth for biofuel production in a CSG water media, this dissertation was intended as a preliminary investigation to evaluate the feasibility of this process. Assessment will be made by conducting lab scale batch experiments growing a marine algae (*Dunaliella tertiolecta*) in a CSG water media. A critical analysis will be made of the algal growth, carbon sequestration, nutrient removal and oil production capacity of this process.

1.1 AIMS AND OBJECTIVES

The aim of this project is to assess the potential of growing microalgae *Dunaliella tertiolecta* in a Coal Seam Gas water media, for the purpose of producing biofuel. Determination of the carbon sequestration and nutrient removal capacity of this process will also be assessed.

The objectives of the research were to:

- Obtain a CSG water sample and test for nutrient and chemical composition
- Conduct batch experiments with microalgae *Dunaliella tertiolecta* in CSG water and simultaneously measure
    - algal growth
    - pH variation
    - dissolved oxygen variation
    - carbon variation
    - nutrient variation (supplemented nitrogen and phosphorous)
- Identify optimal salinity levels for algal growth
- Identify algal growth, pH and DO patterns
- Measure carbon and nutrient depletion rates
- Harvest the algae and measure total lipid production

1.2 SCOPE OF STUDY

The scope of this study is to identify the potential of undertaking biofuel production, carbon sequestration and nutrient removal, using *Dunaliella tertiolecta* in a CSG water medium.

Limitations of this research were:

- Only one algal strand was tested in CSG water
- All batch experiments were only conducted once
- Assessment could not be made of the quantities and removal rates of trace elements
- While most of the inputs and outputs could be assessed, the chemical and biological reactions within the system could not be described. Essentially the system represented a black box.

1.3 DISSERTATION OUTLINE

Chapter 2 Literature Review

This chapter reviews and summarises the current literature pertaining to beneficial uses of microalgae, culturing techniques and algal growth phases. The properties and issues of CSG water in Queensland are evaluated. And a brief overview of photosynthesis and the resulting DO and pH fluctuations is provided.

Chapter 3 Methodology

This chapter provides an overview of the methodology used to analysis wastewater characteristics, microalgae growth patterns, carbon and nutrient depletion rates and lipid content. It also demonstrates how the experimental equipment was set up, and reviews the equipment used.

Chapter 4 Growth Characteristics of Microalgae Chlorella vulgaris Grown in MBL Media and the effects of CO2 input.

This chapter presents the results relating to the testing of microalgae *Chorella vulgaris* grown in MBL media. It shows growth patterns, pH fluctuations, dissolved oxygen variability and nitrogen removal. This chapter also assesses the effect of CO_2 feed on algal growth.

Chapter 5 *Growth Characteristics of Microalgae Dunaliella tertiolecta Grown in a CSG Water Media and the Effects of Aeration*

This chapter presents the results relating to the testing of microalgae *Dunaliella tertiolecta* grown in a CSG water medium. It shows growth patterns, carbon sequestration, nutrient removal, pH fluctuations and dissolved oxygen variability. This chapter also assesses the effect of aeration on algal growth.

Chapter 6 *Growth Characteristics and Lipid Production of Microalgae Dunaliella tertiolecta Grown in Salinity Modified CSG Water Media and the Effects of Aeration*

This chapter presents the results relating to the testing of microalgae *Dunaliella tertiolecta* grown in a salinity modified CSG water medium. It shows growth patterns, carbon sequestration, nutrient removal, pH fluctuations and dissolved oxygen variability. This chapter also assesses the effect of aeration on algal growth and evaluates lipid extraction results.

Chapter 7 Conclusions and Future Works

This chapter presents the conclusions of the study and reviews future works.

LITERATURE REVIEW

The literature review covers the topic of biofuel, and compares traditional crop methods with microalgae biofuels. Assessment is made of CSG water, and its potential utilisation. In addition, an overview of microalgae *Dunaliella tertiolecta*, algal culturing techniques and reactions involving photosynthesis are provided.

2.1 MICROALGAE AS A FUEL SOURCE

The oil sector accounts for approximately 35% of the global energy market (Lin et al. 2010). With increasing crude oil prices and limitations on future reserves, coupled with a conscious public and political shift towards reducing carbon emissions, huge opportunities are now emerging for greener renewable fuel sources. Biofuels including biodiesel, bioethanol and biomethane represent a viable alternative to petroleum. These fuels are theoretically carbon neutral, renewable, and can generally be applied as a blend or direct substitute for petroleum, with little or no modification to modern vehicle engines (Mata 2010).

It is expected that the global biofuel industry will increase rapidly, to a value of over US $500bn by 2050 (Stern 2007). In Australia biofuel currently accounts for only 2% of the national fuel market. However the federal treasury department predicts that the inclusion of heavy vehicle fuel usage into the impending carbon tax legislation will drive significant investment and development into the biodiesel industry. Modelling by the department suggests that biodiesel usage will become the dominant fuel source for heavy vehicles by 2030, and it will represent over 75% of the market by 2050. The department further states that the transition to a biofuel market will occur regardless of a carbon tax (Federal Treasury Department 2011).

Biofuels are produced predominantly from plant matter. Essentially the production process entails growing plant matter which converts solar energy into chemical energy through photosynthesis. This chemical energy in the form of fats, sugars and oils is then extracted through various processes to create a usable fuel source. Traditional biofuels are produced using

higher order plant crops, with the most popular crops including corn, soy bean, canola and rapeseed (Singh & Gu 2010).

Microalgae derived biofuels have recently emerged as a potential fuel source able to overcome many of the environmental and economic limitations of traditional biofuel methods. Fundamentally this process replaces higher order plant crops with microalgae as the biomass for fuel production. Microalgae have an extractable oil content of between 10% and 80%, with oil contends of 20% to 50% being the most common (Chisti 2007). Oil content is dependent on the algal strand and the set growing conditions.

2.2 MICROALGAE BIOFUELS Vs CROP BASED BIOFUELS

Fuel derived from higher order plants is currently the most common form of biofuel production globally (Schneck etal. 2008). This method is problematic for two main reasons, the limitations of arable land availability and the developing competition with food production industries for feedstock acquisition.

Crop based biofuels could not be considered as a future substitute to petroleum fuels, as the arable land area required to meet fuel demand needs, is at best unsustainable and at worst unachievable. Table 2.1 shows a comparison of some of the more popular biofuel crop types, and their oil yield per hectare ratios. Also shown, are estimates of the land areas required to meet 50% of the USA's current fuel demand, and the equivalent percentage of existing crop area in the USA (Chisti 2007). Results clearly demonstrate the limitations of traditional crop methods. In Australia it is conservatively estimated that 1 million hectares or 0.13% of the nation's total land area would be required to produce enough algae (20% oil content) to meet all of the current domestic fuel demand needs (CSIRO 2011).

An additional concern with traditional crop based biofuel production, is the potential that existing rainforest and ecologically significant areas, particularly in developing countries, could be lost as a result of increased pressures for cultivation of biofuel cash crops (Mata, 2011).

TABLE 2.1 Comparision of some biofuel sources (Chisti 2007)

Crop	Oil yield (L/ha)	Land area to meet 50% of USA fuel demand (million ha)	Equivalent precent of existing USA cropping area (%)
Corn	1540	1540	846
Soybeans	594	594	326
Canola	1190	223	122
Jatropha	1892	140	77
Coconut	2689	99	54
Oil Palm	5950	45	24
Microalgae (70% oil)	136,900	2	1.1
Microaglae (30% oil)	58,700	4.5	2.5

The second major limitation of traditional biofuel methods is that it competes directly with agricultural industries for arable land usage. Over 75% of the cost associated with producing crop based fuels comes from the acquisition of feedstock (Schneck etal. 2008). As traditional biofuel production expands, so does the industry's demand for additional feedstocks, which in turn leads to greater competition between food and biofuel producers for land use, driving up prices for both forms of industry. Crop based bio-fuel production has increased tenfold from 2000 to 2008, and since 2002 a strengthening correlation has been observed between world food prices and biodiesel production (L Lin et al. 2011). Shortages and subsequent increases in prices of food stocks can have, and has had major detrimental effects on a global scale. Recent food price rises have been linked civil unrest in northern and western Africa (L Lin et al. 2011).

Microalgae biofuels have the advantage in that they do not require fertile or productive lands for cultivation and they use much less area and water for equivalent yields of oil (Harun et al 2011). The algae strands used for these fuels typically require water, sunlight, CO_2, O_2, and key nutrients potassium and nitrogen (Schneck etal. 2008). Additionally there is the capacity for algae to be farmed in saltwater or brackish, thereby reducing dependency on limited fresh water supplies (Singh & Gu 2010).

2.3 ADDITIONAL USES

There are a number of products that can be produced from alga. Including food and nutrient supplements, organic fertilisers, livestock feed and fine organic chemicals for pharmaceutical goods (Singh & Gu 2010). Furthermore, as microalgae have a greater capacity for photosynthesis than higher plants, there is much potential to utilize this organism for offsetting CO_2 emissions (Fernandes 2010). Table 2.2 shows the Greenhouse gas emissions over the biofuel life cycle for microalgae and other popular biofuel crops. As a comparison, the rates for gasoline and diesel are 94 kg CO^2/MJ and 83 kg CO^2/MJ, respectively (Groom et al. 2008). The high negative net carbon output rate for microalgae (-183 kg CO^2/MJ) demonstrates the sequestration potential of this process.

TABLE 2.2 Comparison of Greenhouse gas emissions over biofuel life cycle for popular biofuel crops (Groom et al. 2008)

Crop	**GHG emissions (kg CO^2/MJ)**
Corn	81 to 85
Soybeans	49
Canola or Rapeseed	37
Oil Palm	51
Sugar Cane	4 to 12
Native prairie grasses	-88
Microalgae (Biodiesel)	-183

There is also the potential to combine algal biofuel production with water cleaning processes. Nutrient rich industry wastewater can be used as a feedstock for algae biofuel production, algae have the capacity to absorb and remove nutrients from wastewater. The treatment of wastewater can be costly for industries, by combining water treatment with bio-fuel production, there is the capacity to turn a waste cost into a profitable resource (SARDI 2010).

Another production alternately, is to use the biomass waste remaining after lipid extraction to produce biogas through anaerobic digestion. Harun et al (2011) suggest that production cost and carbon emission from biofuel production systems could theoretically be reduced by 33% and 75% respectively through the integration of biodiesel production with biogas production.

2.4 MICROALGAE BIOFUELS AS A VEHICLE FOR DECENTRALISATION AND NATIONAL FUEL SECURITY

The decentralisation of energy distribution is generally recognised as a more efficient, reliable and environmentally friendlier method for delivering energy, compared to the traditional centralised distribution method (Alanne & Saari 2005). Through this process, large energy conversion units are replaced by smaller ones, with the capacity to be located closer to energy consumers. Microalgae biofuel production can be easily applied to a decentralisation model, as there is capacity at most wastewater and carbon emitting facilities to generate this fuel source.

Furthermore, a microalgae biofuel market has the potential to provide a secure national fuel source. In Australia approximately 75% of transport fuel comes from local sources, however with growing demands and source limitations, this market portion could reduce to 45% by 2030 (CSIRO 2011). Therefore alternate fuel sources must be developed to avoid future dependency on foreign fuel importation. Algal derived biofuels have the capacity to be produced year round and can be harvested daily, ensuring a steady oil supply.

2.5 CSG WATER – PROPERTIES, ISSUES AND POTENTIALS

The Coal Seam Gas (CSG) industry has grown substantially in Queensland over the last decade. Encouraged through the State Governments "Queensland Energy Policy – A Cleaner Energy Strategy", the production of CSG increased from 4PJ in 1998-1999 to 125PJ in 2007-2008. In 2010 it comprised over 80% of Queensland's gas market. Despite the significant grow to date it is still considered a new industry with major expansion expected (Kinnon 2010).

The CSG extraction method involves drilling wells into coal seams and pumping the water from these wells to reduce pressure within the matrix that acts to contain the methane gas in the coal. This causes methane to desorb and begin to flow from the coal, gas and water then travel together to the surface where they are then separated (Kinnon 2010). Through this process, large quantities of CSG water is produced. Current estimates for CSG water to be generated from Queensland's Surat and Bowen Basins over the next decade range from 50 to 300GL per year (Pratt et al 2011).

CSG water contains variable levels of sodium, salts, carbon in the form of bicarbonates and several trace elements, making it unfit for direct use. Table 2.3 shows water quality sampling results from various well depths and locations within a single field in the Bowen Basin. Comparison is made with the chemical composition of seawater.

TABLE 2.3 CSG water quality statistics within a single field in the Bowen Basin grouped according to seam and position relative to fault (Kinnon et al. 2010)

	Upper seam north of fault	**Upper seam south of fault**	**Upper seam north of fault**	**Lower seam north of fault**	**Seawater**
Depth (m)	273	177	333	228	
pH	7.9	7.9	8	7.8	8.1
Electrical Conductivity at 25°C (S/m)	10,570	7,865	8,645	11,140	
TDS at 180°C (ppm)	5,810	4,488	4,838	5,846	35,000
Hydroxide alkalinity as $CaCO_3$ (mg/L)	<1	<1	<1	<1	
Carbonate alkalinity as $CaCO_3$ (mg/L)	25	4	12	<1	
Bicarbonate alkalinity as $CaCO_3$ (mg/L)	719	667	1,482	550	
Total alkalinity as $CaCO_3$ (mg/L)	727	670	1,494	550	
Sulphate as SO^{2}_{-4} (mg/L)	<1	<1	<1	1	
Cl (mg/L)	3,280	2,330	2,223	3,670	19,700
Ca (mg/L)	40	46	15	52	410
Mg (mg/L)	23	16	9	26	1310
Na (mg/L)	2,423	1,730	2,054	2,464	1,900
K (mg/L)	17	10	8	12	390
Fe (mg/L)	5.46	5.30	5.37	5.14	<0.02
Al (mg/L)	0.06	0.36	0.07	0.04	<0.01
F (mg/L)	1.3	1.8	2.4	1.6	1.4

Typically CSG water has been disposed of in large evaporation ponds, it is essentially considered a waste product. In October 2008 the Queensland Government released the Queensland Coal Seam Gas Water Management Policy, which outlined the strategy for CSG water management. Central to the policy was the discontinuation of evaporation ponds as the primary means of CSG water disposal. A 3 year transitional period was allocated for the remediation of existing open ponds. In June 2010 DERM implemented the Coal Seam Gas Water Management Policy. This policy states that the preferred management options for CSG water utilization was either through augmenting depleted natural aquifers, through direct use methods or through treatment methods for varying application. The purpose of the DERM policy is twofold to ensure that CSG water does not contaminate the environment, and to encourage the beneficial use of CSG water.

The policy further states that, an alternate use approval can be granted where CSG water is converted from a waste to a resource that can be used for beneficial purposes (DERM 2010). This paper examines the potential to utilise this carbon rich CSG water for algae biofuel production purposes. Potential benefits include creating industry in rural areas, aiding to decentralise fuel production sources and create a net CO_2 offset by replacing fossil fuels and other carbon intensive products with alga derived products.

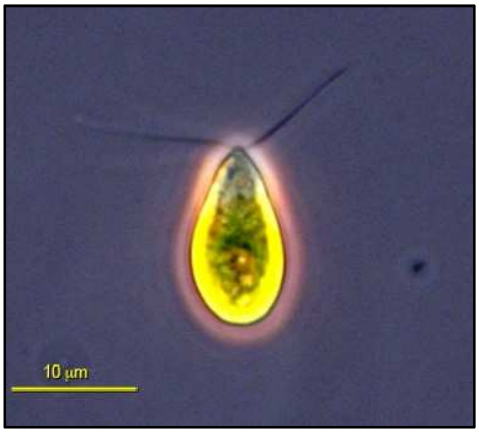
Table 2.4 shows estimates by Pratt et al. (2011), of the production and profit generation potential of algal derived products grown in a CSG water medium with a nutrient waste supplement, also estimated is the carbon capture and offset capacity of this process. Estimates have been based on conceptual 900 ha pond design, managing 10 GL of CSG water per year and incorporating agricultural waste inputs to produce biodiesel, methane and biosolid products.

TABLE 2.4 Input/output estimates of a 900 ha pond managing 10GL of CSG water per year (Pratt et al. 2011)

Product	Volume	Value
Algae	108,000 t	
Water	10 GL	
Biodiesel	24,000 m^3	$24 M
Methane	891, 000 GJ	$ 6.2 M
Biosolids	45,000 t DS	$1.13
CO_2 capture and off-sett		
Capture in algae biomass	206,000 t	
Off-set via biodiesel	58,500 t	$1.17 M
Off-set via methane	54,000 t	$1.08 M

2.6 MICROALGAE STRAND DUNALIELLA TERTIOLECTA

The microalgae *Dunaliella tertiolecta* (Figure 2.1 a & b) is a unicellular green marine alga, 9-11µm in size. This alga has a reported oil yield of 36%-42%. It is simple to cultivate, fast growing, does not clump or form chains, and it can be grown in saltwater, wastewater or brackish water (Chen et al 2011). As such *Dunaliella tertiolecta* is an ideal candidate for application in an untested medium. Should the lab scale experiments using *Dunaliella tertiolecta* prove successful, there is the future potential to use this algal strand in large outdoor pond settings.

a) 

b) 

FIGURE 2.1 Microalgae *Dunaliella tertiolecta*. a) (Encyclopaedia of Life 2011), b) (CSIRO 2011).

2.7 CULTURING TECHNIQUES

The growth of algae is limited by various physical and biotic factors (Moheimani 2005). The following section addresses the main contributors to algae growth.

2.7.1 LIGHT

The most important limiting factor for algae growth is light (Moheimani 2005). For a fixed fluid dynamic and temperature, the growth rate of microalgae is a function of light exposure within a reactor. At low light intensity, net oxygen rates resulting from photosynthesis increase linearly with light intensity (Masojidek, Koblizek & Torzillo 2004). In dense cultures, light penetration can be impeded by self-shading and light absorption (Ferdnandes 2010).

Analysis of the light effects on *Dunaliella tertiolecta* by Tang et al (2010), found a significant increase in cell growth rates, when light intensity was increased from 100μE/(m^2s) to 200μE/(m^2s), while only a slight difference in growth occurred between light intensities of 200μE/(m^2s) and 350μE/(m^2s). They also found that growth was unaffected by varying the light wavelength. Using red LEDs, white LEDs or florescent lights as the main source had an insignificant effect on the algae's growth rate over time.

2.7.2 CARBON SUPPLY – $C0^2$ AND HCO_3^-

Carbon is a key parameter for intensive culturing of unicellular algae (Camiro-Vargas et al. 2004). The CO_2 content in air is 0.03%, algae have the capacity to grow under these conditions, however greater concentrations of carbon are required to achieve high growth rates. Tang et al. (2010) found that CO_2 levels of between 2-6% produced optimal growth rates for *Dunaliella tertiolecta,* with sharp reductions in growth outside of this optimal range. Algae can tolerate up to 12% CO_2 at 35°C (Pulz 2001).

Most species of alga are capable of importing either CO_2 or HCO_3^- through the cell membrane for photosynthesis (Giordano et al. 2005, Chi et al. 2011). However, the utilization of CO_2 or HCO_3^- as the preferred source for photosynthesis has been found to be species dependent (Huertas et al. 2000). Microaglae *Dunaliella tertiolecta* has been shown to have good capacity to extract carbon from CO_2 and HCO_3^- , although uptake for HCO_3^- in pH dependent. Fabergas et

al. (1993) found that *Dunaliella tertiolecta* was unable to uptake carbon in the form of bicarbonates at a pH greater than 8.3.

2.7.3 SALINITY

Marine algae are extremely tolerant to changes in salinity. Salinity variation and tolerance of marine plants is closely associated with intertidal habitat, with evaporation and heavy rain attributing further to salinity variability (Boney 1966). Most marine species grow best in salinity conditions that are slightly lower than their native habitat, with optimal growth generally observed between levels of 20-24 g NaCl/L (Barsanti & Gualitieri 2006).

The optimal salinity level for growth is different for every algal strand. Fazeli et al. (2005) found optimal growth of *Dunaliella tertiolecta* at a NaCl concentration of 0.5 M. The maximum NaCl tolerance range for *Dunaliella tertiolecta* was found to be 0.05 - 3 M (Janke & White 2003).

2.7.4 AERATION

Aeration is often used in algal culturing to provide a convenient method for mixing organisms with nutrients, and to increase light penetration into the media. Air bubbling can also supply a source of CO_2 to the cultures, albeit at low concentrations (Rodriguez-Maroto et al. 2005). Furthermore the energy introduced into the culture by bubbling has been shown to enhance algal productivity. Aguilera et al. (1994) found a linear correlation between uptake of nitrogen and the subsidiary energy introduced into a media in the form of bubbling.

Aeration can also reduce the bicarbonate concentration of a media, through stripping. Rodriguez-Maroto et al. (2005) found that when bubbling was used in a bicarbonate rich solution, aeration did not contribute to bicarbonate uptake by the algae. They demonstrated that there was a loss of CO_2 from the media to the air bubbles, which escaped from the culture vessel, and no transfer of CO_2 from the air to the water occurred. This was explained by carbon moving from a medium of high concentration, in this case the water, to a medium of low carbon concentration, the air supply.

2.7.5 NUTRIENTS

The key nutrients required for algae growth are nitrogen, phosphorous and iron. Nitrogen generally accounts for 7% to 10% of cell dry weight, it is an essential component of structural and functional proteins within an algae cell. Phosphate facilitates cellular metabolic processes by forming a number of structural and functional components needed for the development of microalgae. Iron has an important role in cellular biochemical composition, specifically for its role in photosynthesis, respiration, nitrogen fixation and DNA synthesis (Richmond 2004).

Chen et al (2011) found that *Dunaliella tertiolecta* was able to use either ammonium or nitrate as a nitrogen source. However, high levels of ammonium inhibited growth, while in contrast high levels of nitrate increased cell density. Deprivation of nitrate or iron resulted in a sudden increase in lipid content. They also found that the limiting of phosphate had little effect on growth, due to intra cellular phosphate storage.

Lundquist (2006) suggest that most alga require a substrate in a C:N:P ratio of 50:8:1 for growth. Alternately, the Redfield ratio C:N:P of 106:16:1 has been adopted by some authors (Grobberlaar 2004). Due to the high carbon demand rate, a CO_2 or Bicarbonate source must be provided to achieve high algal growth rates.

2.7.6 TEMPERATURE

All microalgae have an optimal temperature at which maximum growth rates occur. This temperature varies between algae strands. Most algae can tolerate temperatures up to 15°C lower than their optimal, while temperatures exceeding the optimal by 2 - 5°C can result in the death of the culture (Moheimani 2005). Chen et al (2011) found that the optimal growth rate for *Dunaleilla tertiolecta* was 23°C.

2.8 GROWTH PHASES

Figure 1.2 represents the theoretical algal growth curve and the nutrient depletion curve for a batch experiment. There are five distinct growth phases that alga can transition through in this type of experiment. These are the lag phase, exponential phase, linear phase, stationary phase and the death phase (Mata et al. 2010).

In the lag phase no immediate increase in cell numbers or mass is generally observed. In this phase the cells are becoming acclimatised to the media. The duration of the lag phase can be dependent on age of the culture and the difference in chemical composition of the new media type (Prescott et al. 1990). In the exponential phase cells are acclimatised, they grow and divide at a maximum rate. As cell numbers increase nutrient levels and light source availability decrease at an inverse rate, ultimately resulting in the death phase (Prescott et al. 1990).

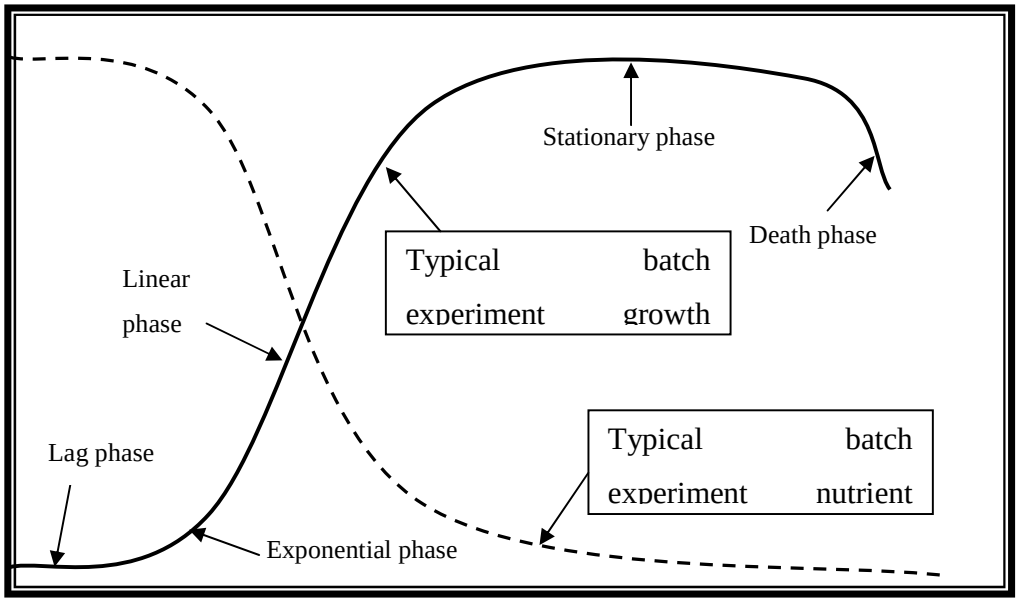


FIGURE 2.2 Theoretical algal growth phases and nutrient depletion rates for batch experiments

2.9 PHOTOSYNTHESIS

Photosynthesis is the main mechanism for capturing carbon, producing O_2, algal growth and pH variability. Algal cells capture carbon through photosynthesis and release CO_2 through respiration. When there is sufficient light, photosynthesis and respiration take place simultaneously, resulting in a net mitigation of carbon and the production of O_2. However in insufficient light conditions, the photosynthesis capacity of alga will be restricted, potentially resulting in a net uptake of oxygen and a release of CO_2 through respiration.

Most algal cells have the capacity to utilize either CO_2 or HCO_3^- as the carbon source. In a bicarbonate rich solution, it is hypothesised that absorbed CO_2 and HCO_3^- first accumulates in the chloroplast stroma of a cell, mainly as HCO_3^-. From there, HCO_3^- is transported to the

thylakoid lumen and converted into CO_2 in the acid lumen. The concentration of CO_2 is then diffused through pyrenoid tubules in the thaylakoid membrane to the pyreniod matrix, where it is fixed by Rubisco (Chi et al. 2011).

According to equilibrium:

H_2O	+	CO_2	« »	H^+	+	HCO_3^-
water		carbon dioxide		hydrogen ion		bicarbonate ion

Thus, H^+ is consumed during the conversion of HCO_3^- to CO_2, and this CO_2 is fixed by Rubisco during photosynthesis. Therefore, when HCO_3^- is used as the carbon source for photosynthesis, a build up of OH- remains in the cell, this build up is neutralised by the uptake of H^+ from the extracellular environment. Consequently, the uptake of H^+, results in an increase in the pH level of a solution (Chi et al. 2011). Therefore photosynthesis using a bicarbonate source leads to increases in pH of a medium.

2.10 ALGAL HARVESTING

There is still no universal method for harvesting algae. The development of an appropriate and economical process is an ongoing active area of research (Mata et al. 2010). Common methods adopted today include sedimentation, centrifugation, filtration and ultra-filtration. Flocculation can be used to facilitate harvesting methods. This process aggregates microalgae, increasing effective particle size, therefore making the collection of cells a simpler process (Mata et al. 2010).

2.11 CHAPTER SUMMARY

This chapter reviewed the literature relevant to microalgae biofuel and the potential benefits of its application. A review was made of the properties of CSG water and its possible utilization. The topics of decentralisation, fuel security, culturing techniques and photosynthesis in a bicarbonate medium were discussed.

CHAPTER 3 METHODOLOGY

The assessment of growth, lipid production, carbon sequestration and nutrient depletion rates of microalgae strands *Dunaliella tertiolecta* in CSG water, and *Chlorella vulgaris* in an MBL medium, consisted of the following steps.

1. Culturing of microalgae
2. Collection and preparation of medium
3. Determination of water characteristics and nutrient/carbon addition
4. Online monitoring of pH, DO and temperature in the bio-reactor
5. Sample collection for carbon, nitrogen, phosphorous, spectroscopy and suspended solids, as well as measurement of medium volumes within the bioreactor
6. Harvesting and freeze drying of algae
7. Lipid extraction and analysis
8. Collation and assessment of data

3.1 CULTURING OF MICROALGAE

3.1.1 ALGAL STRAND DUNALIELLA TERTIOLECTA

A sample (20mL) of microalgae strand *Dunaliella tertiolecta* was ordered from the CSIRO's Australian National Algae Culture Collection, located in Hobart, Tasmania. The strand was first pre-cultured in 250ml flasks in an F/2 medium (1mL/L) (Refer to section 3.3.3). Culturing was initially conducted in two different salinities, with NaCl levels of 3.5g/L (CSG water equivalent), and 35g/L (Seawater equivalent).

3.1.2 ALGAL STRAND CHOLERA VULGARIS

A pre-existing culture of *Cholera vulgarise* was acquired from the USQ (FoES) Environmental Engineering laboratory. The algal stand was re-cultured in 250ml flasks using a prepared MBL media.

3.2 WATER COLLECTION AND PREPERATION

3.2.1 CSG WATER

The CSG water was obtained from the National Centre for Engineering in Agriculture (NCEA), located on the USQ Toowoomba campus. The water was initially acquired by the NCEA from a CSG exploration company. Due to confidentiality restrictions, the original source of the CSG water cannot be identified.

The collected CSG water was filtered using 0.45μm filter paper, with the aid of a vacuum pump system. The water was then stored between 20°C to 25°C in the USQ (FoES) Environmental Engineering laboratory, before being utilized in the bioreactor experiments.

3.2.2 MBL MEDIA

The MBL media was prepared in the USQ (FoES) Environmental Engineering laboratory. Details of the components and preparation method of the solution are provided in section 3.3.2.

3.3 WATER CHARICTERISTICS AND NUTRIENT/BICARBONATE ADDITION

3.3.1 CSG WATER

The obtained CSG water was tested for total carbon (TC) and total nitrogen (TN), using a Total Organic Carbon/Total Nitrogen Analyzer (TOC-VCPH/CPN). Ion composition (F, Cl, NO_2, NO_3 and PO_4) was assessed, using the Ion Chromatography system (IC, Dionex ICS 2000). Results are shown in Table 3.1. Research by Kinnon et al (2010) (Section 1.5) provides a more detailed listing of CSG water compositions, for various sample sites and depths in the Surat basin.

TABLE 3.1 Composition of CSG Water sample

Elements	Concentration (mg/L)
Total Organic Carbon/Total Nitrogen Analyzer (TOC-VCPH/CPN).	
Total Carbon (TC)	23.5 C mg/L
Total Nitrogen (TN)	0.3 N mg/L
Ion Chromatography system (IC, Dionex ICS 2000)	
Fluorine (F)	6.5 F mg/L
Chlorine (Cl)	805.5 mg/L
Nitrite (NO_2)	0.0 mg/L
Nitrate (NO_3)	0.0 mg/L
Phosphate (PO_4)	0.0mg/L

3.3.2 CARBON ADDITION TO CSG WATER

Based on the findings by (Kinnon et al. 2010), concentrations of bicarbonate (expressed as calcium carbonate ($CaCO_3$)) in CSG water, were shown to vary between 300 mg/L to 2,860 mg/L, with an average mean concentration of 901.5 mg/L (216 C mg/L). The low levels of carbon found in the current CSG water samples (24 C mg/L), suggest that the water has previously been treated. To emulate real life CSG water conditions, the bicarbonate level in the water was increased to the mean concentration level (216 C mg/L), through the addition of sodium bicarbonate ($NaHCO_3$).

3.3.3 F/2 CONCENTRATE

An F/2 concentration (Algaboost), was ordered from AusAqua Pty Ltd. The F/2 concentrate was used to provide key nutrients such as phosphate and nitrogen and other vital vitamins, to facilitate the growth of *Dunaliella tertiolecta* in a CSG medium. The composition of F/2 was not supplied by the manufacturer, therefore initial testing was undertaken to assess nutrient quantities. Adopting an initial dosage rate of 1ml F/2 to 1 L CSG water, the following nutrient concentrations results were obtained (Table 3.2).

TABLE 3.2 Composition of CSG Water sample (plus 1mg/L of F2 concentrate)

Elements	Concentration (mg/L)
Total Organic Carbon/Total Nitrogen Analyzer (TOC-VCPH/CPN).	
Total Carbon (TC)	26.5 C mg/L
Total Nitrogen (TN)	13.1 N mg/L
Ion Chromatography system (IC, Dionex ICS 2000)	
Fluorine (F)	6.2 F mg/L
Chlorine (Cl)	802.5 mg/L
Nitrite (NO_2)	0.0 mg/L
Nitrate (NO_3)	93.5 NO_3^- mg/L (21.0 N mg/L)
Phosphate (PO_4)	5.5 PO_4 mg/L (1.7 P mg/L)

Results show recorded nitrogen levels from the two machines varying between 13.1 mg/L and 21.0 mg/L, and a phosphorous reading of 1.7 mg/L. F/2 media composition does not indicate the presence of ammonia or organic nitrogen that could have shown increased TN concentration measured via TOC machine. Assuming an average nitrogen reading of 17 mg/L, this gave a N:P ratio of approximately 10:1.

For the *Dunaliella tertiolecta* trials, the F/2 dosing rate was increased to 5ml concentrate per 1 L of CSG water. The intention of this increased dosage was to avoid phosphate concentrations in the trial periods dropping below the measuring range capacity of the Ion Chromatography system (IC, Dionex ICS 2000) indicating the possibility of P limiting the growth of algae. This increase in dosage resulted in a final C:N:P ratio of 192:85:8.5. Although this adjustment meant that the ratio of carbon was below theoretically optimal levels, it was predicted that due to the short period of the *Dunaliella tertiolceta* trials (5 days), carbon availability would not become an issue.

3.3.4 MBL MEDIA

The MBL media was prepared as shown in Table 3.3 (Nichols 1973).

Stock Solutions	Per Litre Distilled/milliQ water	
$CaCl_2.2H_2O$	36.76g	
$MgSO_4.7H_2O$	36.97 g	
$NaHCO_3$	12.60 g	
K_2HPO_4	8.71 g	
$NaNO_3$	85.01 g	
$Na_2SiO_3.9H_2O$	28.42 g	
Na_2EDTA	4.36 g	
$FeCl_3.6H_2O$	3.15 g	
Metal Mix		
$CuSO_4.5H_2O$	0.01 g	Add each component separately to 750 mL, fully dissolved between additions and then increase volume to 1 L with distilled water
$ZnSO_4.7H_2O$	0.022 g	
$CoCl_2.6H_2O$	0.01 g	
$MnCl_2.4H_2O$	0.18 g	
$Na_2MoO_4.2H_2O$	0.006 g	
Vitamin Stock		
Cyanocobalamin (Vitamin B12)	0.0005 g/L	
Thiamine HCl (Vitamin B1)	0.10 g/L	
Biotin	0.0005 g/L	
Tris stock	250.00 g/L	

TABLE 3.3 Composition of MBL media

3.4 BIO-REACTOR DESIGN

The algae based titrimetric bio-reactor (Figure 3.1 & 3.2) was located in the USQ (FoES) Environmental Engineering laboratory. The bio-reactor enabled the collection of real time data, relating to the growth of microalgae strands *Dunaliella tertiolecta* and *Chlorella vulgaris* in

conducted batch experiments. All batch experiments were carried out in a single reactor tank with a 4L capacity.

3.4.1 INPUT VARIABLES

The utilization of compressed air and CO_2 inputs was subject to the specific parameters of each experiment, refer to Chapters 4, 5 & 6 for further details. In applicable experiments, compressed air was supplied continuously into the bio-reactor at a rate of 250ml/min. CO_2 input was used in the experiments with *Chlorella vulgaris* in MBL media, to provide a carbon source. The timing and input of CO_2 addition was controlled by the Labview program. When utilized, CO_2 was administered continually at a steady rate of 5 ml/min, and in concentrated (60ml/min) 4 second burst, once every 2 hours.

No CO_2 input was provided for the first 2 days of the *Chlorella vulgaris* experiments. Initial trials and previous experimental works found the buffer capacity of the solution was poor in the early stages of these experiments, resulting in unacceptable pH volatility with the addition of CO_2. This poor capacity generally coincided with the lag growth phase, when algal were generally inactive.

A light source was provided using three florescent tubes (2000 lux each). The tubes were positioned on three sides of the tank, at a distance of 5 cm away. Light was supplied for 12 hours per day, from 6am to 6pm. A stirrer was also used at a constant rate for mixing purposes. Figure 3.1 shows the schematic of the bio-reactor.

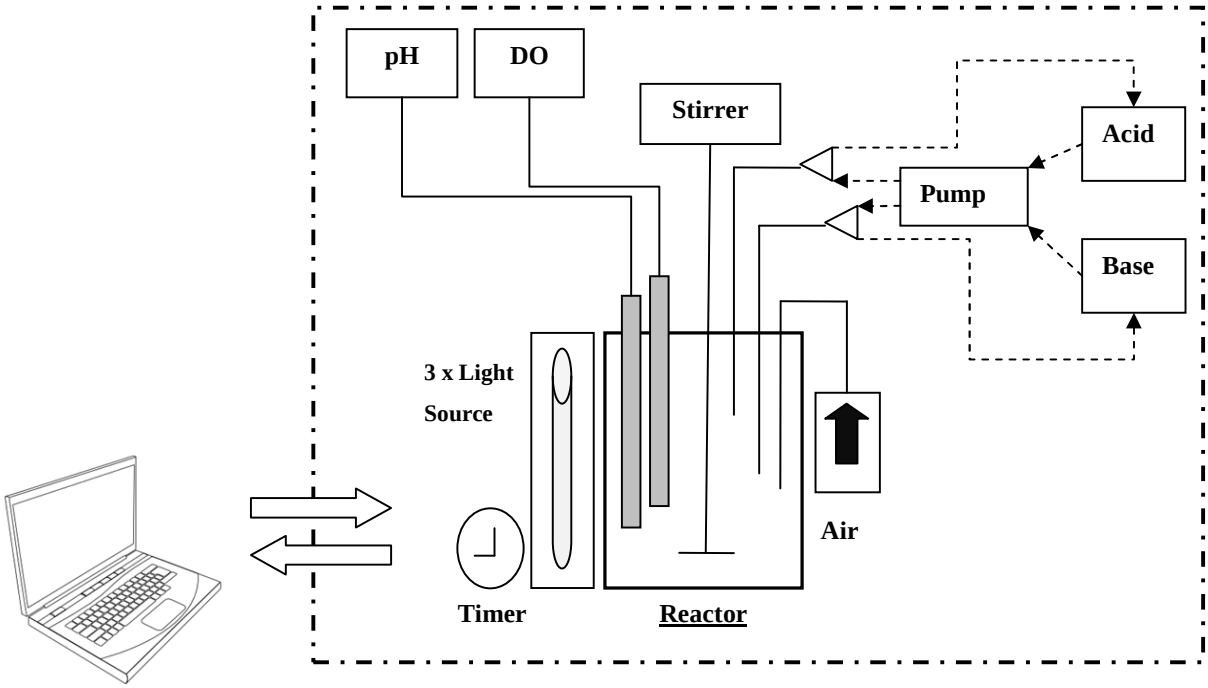


FIGURE 3.1 Schematic diagram of the Bio-reactor

3.4.2 pH MONITORING AND CONTROLLING

An Ionode pH electrode was positioned within the 4 L bio-reactor tank and was connected to a pH transmitter (TSP Mini Chem), which provides real time pH measurements. Readings from the pH transmitter were also linked to the integrated laptop. The Labview program logged pH levels, and allowed for the set-limit of a pH range. The pH level within the tank was controlled by acid (0.1 N H_2SO_4) and base (0.1 N NaOH) addition. Acid and base were continuously pumped around a tube loop network with a peristaltic pump, this enabled a constant pressure to be kept and allowed accurate dose rates (10ml) to be administered to the tank through a 3-way solenoid valves.

3.4.3 DO AND TEMPERATURE MONITORING

A dissolved oxygen electrode (YSI) was positioned within the bioreactor, it connected to a dissolved oxygen meter (TPS 90-D), which provided real time DO and temperature readings. The DO meter was linked to the integrated laptop, onto which all results were logged.

The temperature of the lab was regulated at 25°C using the building's air-conditioning system. However results found temperature fluctuations between 19°C and 24°C. Temperature correction was made to the obtained DO readings to a base level of 20°C.

3.4.4 LABVIEW PACKAGE

The Labview software monitored pH, DO and temperature serial outputs from the pH transmitter and the DO meter, with high frequency. The 0-1 volt signals from the transmitter and meter where logged in the integrated laptop with the Labview software, and a combined A/D I/O card (National Instruments, PCI-6013). All recorded data was saved in Microsoft Excel spreadsheet format. The user could set experiment parameters on the front panel of the program, with tolerance set-point limits. Figure 3.2 shows the bio-reactor set up.

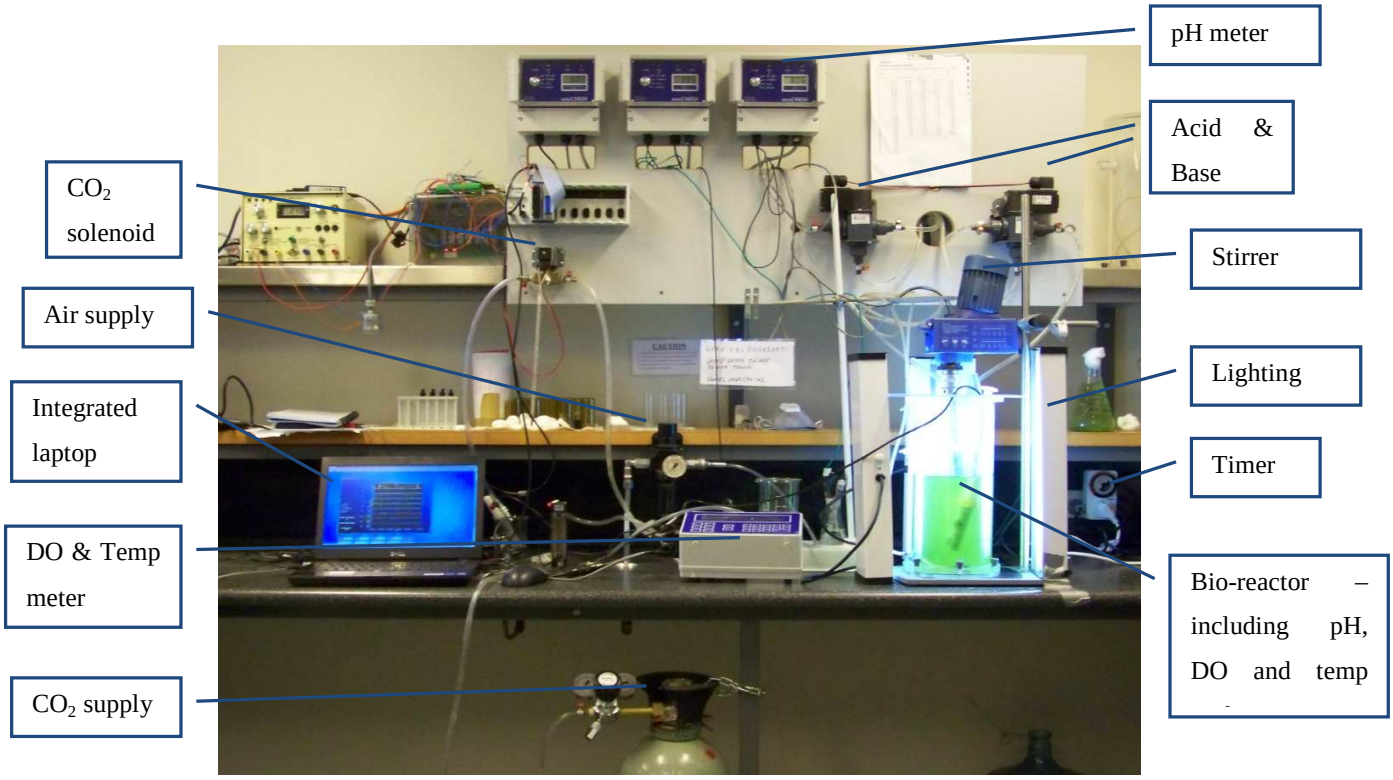


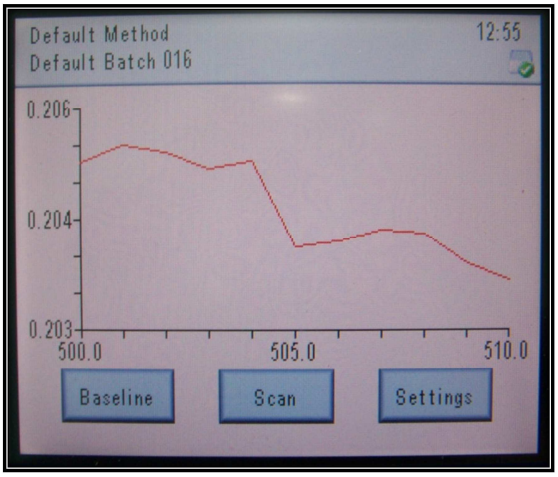
FIGURE 3.2 The Bio-reactor setup.

3.5 EXPERIMENTAL MEASUREMENTS

3.5.1 MESUREMENT OF ALGAE GROWTH

Algal growth was initially quantified (*Chlorella vulgaris* experiments) through spectroscopy measurements (Figure 3.3 a) and Suspended Solids (SS) (Figure 3.3 b). Suspended Solids were measured using Standard Methods (APHA 1995), with 10ml samples. Spectroscopy (Jenway 6705 UV/Vis. Spectrometer) quantified the cell density. A wavelength of 505 nm was adopted for spectroscopy sampling, and a baseline was established with MBL media for *Chlorella vulgaris* trials, and CSG water for the *Dunaliella tertiolectra* trails.

Measurements for both methods were undertaken daily, for the full length of the trials. Following completion of the *Chlorella vulgaris* trials, assessment established a high correlation (89.4%) between the two growth measurement procedures. Based on this finding, only the spectroscopy methodology was adopted for the *Dunaliella tertiolectra* trails. Daily photos of the bio-reactor tank were also taken to document algal growth.

a) 

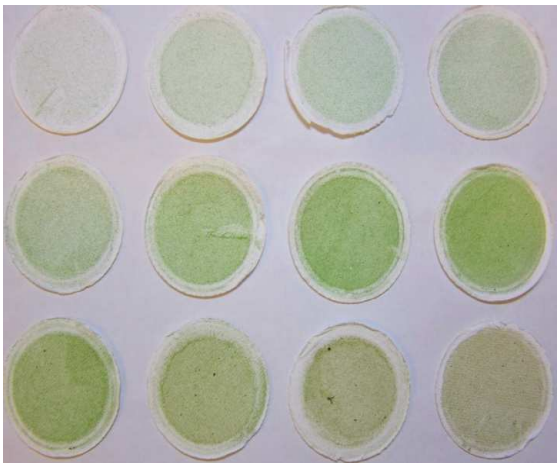
b) 

FIGURE 3.3 a) & b) a) Jenway 6705 UV/Vis. Spectrometer output and b) filter paper samples for a 12 day period.

3.5.2 MEASUREMENT OF NUTRIENT DEPLETION & CARBON SEQUESTRATION LEVELS

Concentrations of carbon, nitrogen and phosphorous were measured using a Nitrogen Analyzer (TOC-VCPH/CPN) and an Ion Chromatography system (IC, Dionex ICS 2000). The Nitrogen

Analyzer measured carbon (TOC, IC & TC) and nitrogen (TN) levels, the Chromatography system was used to measure phosphorous (PO_4) and also nitrogen (NO_2 & NO_3) levels. Daily samples (15ml Nitrogen Analyzer, 5ml Chromatography system), were taken throughout the trial periods. Prior to sampling, algae were put into suspension by temporarily accelerating the rotational speed of the stirrer. Before sampling, the volume of the media was recorded, concentration adjustment was subsequently made to all results at the data analysis stage (Refer to section 3.8). Note that only nitrogen levels were assessed in the *Chlorella vulgari*s trials.

Two daily samples were taken from each analysis for the *Dunaliella tertiolecta* trials, average readings were derived from the results. To reduce volume losses in the bioreactor, samples were diluted with distilled water at a ratio of 1:4 (media:distilled water).

3.5.3 MEASUREMENT OF DO AND pH DATA

The pH and DO probes were calibrated before the commencement of each trial, to maintain accuracy. Readings from the monitoring equipment were logged at 1 minute intervals, into the Labview software (Figure 3.4). The continual real-time monitoring allowed the researcher the capacity to intervene if data monitoring faults or growth behaviour issues arose. The pH, DO and temperature monitoring components of the bio-reactor are described in Section 3.4.

Logged readings allowed for observational and quantifiable assessment. Documented sudden drops in pH levels were typically associated with acid or CO_2 addition, conversely sudden spikes in pH represented base addition. Algal growth behaviour was identifiable by steady variations in pH levels. DO readings allowed for the identification of photosynthesis and respiration behaviours of the algae. It also allowed for the calculation of oxygen production quantities of the algae. Refer to section 2.7 for further details regarding algal growth behaviour and its influence on pH and DO levels.

At the completion of a trial, all data was collated in Excel format. Temperature adjustment was made to DO readings, to maintain a 20°C base temperature. DO and pH results were then graphed using the computer program MATLAB. Graphed results allowed for the identification and adjustment of outliers.

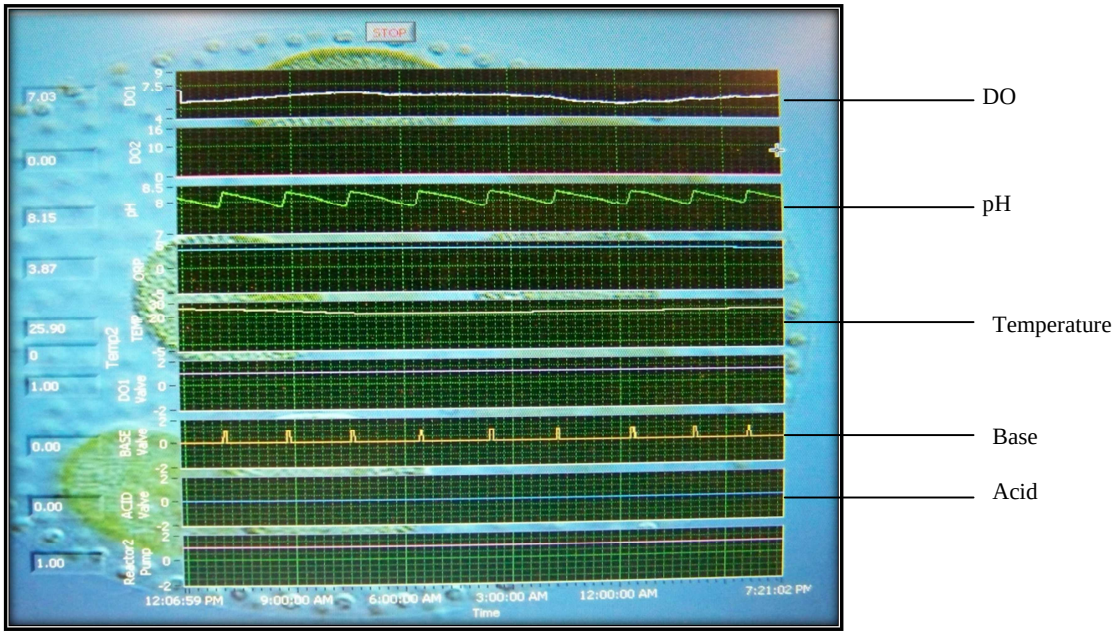


FIGURE 3.4 Labview software output logging DO, pH, temperature and acid/base addition in real time.

3.6 MICROALGAE HARVEST

Alga was separated from the water using a two step centrifugation process. Initially algae underwent centrifugation at 8000rpm for 10 minutes, with a 3 minute cool down period (Beckman Avanti CentrifugeJ-25 I) (Figure 3.5 a). Excess water was then removed, and the algae were washed with distilled water, before undergoing a second stage of centrifugation at 4000rpm for 10 minutes (Eppendorf Centrifuge 5810 R) (Figure 3.5 b). Once separation was complete, the algae pellets were frozen for 24 hours and then freeze dried at -56°C and 30torr (VirTis 2KBTES-55)(Figure 3.5c). They were then stored at room temperature in a desiccators, for later lipid measurements. The timing and the growth phase at which the algae were harvested was specific to each experiment.

a) 

c) 

b) 

FIGURE 3.5 a), b) & c) a) Beckman Avanti CentrifugeJ-25 I, b) Eppendorf Centrifuge 5810 R and c) VirTis 2KBTES-55 Freeze Dryer.

3.6.1 MEASUREMENT OF ALGAL DRY WEIGHT

To obtain the algal dry weight, the freeze dry jar was first weighed before algae were added. After freeze drying the jar with the algae was re-weighed. The dry weight of the algae was equal to the difference between two measurements, as shown in equation 3.1.

3.7 LIPID EXTRACTION AND ANALYSIS

The lipid content of the freeze dried algae was extracted using a modified version of the Folch method (Folch et al 1957). Modification was required, due to the small sample sizes. The following steps were taken.

1. Place sample in 5 ml McConkey bottle.
2. Add 1.6 ml of $CHCl_3$ and 0.8 ml of MoOH (ratio 2:1)
3. Parafilm under lid, shake by hand for 20min
4. Transfer to 10 ml centrifuge tube, wash with 1.0 ml of $CHCl_3$
5. Spin at 3000rpm for 10 minutes
6. Transfer upper phase to new centrifuge, use distilled water to transfer
7. Spin again at 3000rpm for 10 minutes
8. Siphon off top layer and discard.
9. Transfer bottom layer to new 5 ml McConkey. Use $CHCl_3$ to mix, use no more than 1.5 ml. Open lid evaporate in hood up to 2 days.

Once all steps are completed, samples are then dried and weighed to determine lipid content.

3.8 DATA ANALYSIS

3.8.1 CARBON AND NUTRIENT UTILIZATION CALCULATIONS

Reductions of the media volume within bio-reactor, resulting from evaporation and sampling, altered the carbon and nutrient concentrations. To account for this concentration variability, daily recorded tank volumes, time lapse periods between samples and carbon/nutrient sampling results, were entered into an excel spreadsheet, for correction.

Correction methodology differentiated between the *Chlorella vulgari*s and *Dunaliella tertiolecta* trials. In the *Chlorella vulgari*s trials, MBL media was initially used to top up the tank volume, consequently this nutrient addition had to be accounted for. In the *Dunaliella tertiolecta* trials, only distilled water was used for toping up, therefore no additional nutrients were added to the media after commencement of the trial.

3.8.2 NITRONGEN CONCENTRATION ADJUSTMENT OF CHORELLA VULGARIS TRIALS

To establish the nitrogen utilization for the total trial period, determination of the final theoretical nitrogen concentrations, resulting from MBL topping up (excluding nitrogen utilization) was first calculated. To do this, the combined tank volume was summed for each trial day, as shown in Equations 3.2.

$$Combined\ Volume\ (L) = Reactor\ Volume\ (L) + Added\ MBL\ media\ (L) \qquad (Eqn.\ 3.2)$$

Adjusted nitrogen concentrations were then determined using Equation 3.3. By following all calculations through to the completion of the trail, determination could be made of the final theoretical nitrogen concentration.

$$Adjusted\ tank\ concentration\ \left(\frac{mg}{L}\right) = \frac{previous\ adjusted\ tank\ concentration\ (mg/L)\ \times\ previous\ combined\ volume\ (L)}{current\ combination\ volume\ (L)} + \frac{initial\ tank\ concentration\ \left(\frac{mg}{L}\right) \times current\ added\ MBL\ (L)}{current\ combined\ volume\ (L)} \qquad (Eqn.\ 3.3)$$

The cumulative consumed nitrogen was then found for each day by subtracting the recorded concentrations for each sample from the calculated adjusted concentrations from the same day (Equation 3.4).

$$Daily\ consumed\ \left(\frac{mg}{L}\right) = adjusted\ tank\ concentration\left(\frac{mg}{L}\right) - recorded\ tank\ concentration\left(\frac{mg}{L}\right) \qquad (Eqn.\ 3.4)$$

The consumed concentration for each day is then subtracted from the final theoretical nitrogen concentration, in an iterative process, to determine the rate of nitrogen consumption for the total duration of the trail.

3.8.3 CONCENTRATION ADJUSTMENT FOR DUNALIELLA TERTIOLECTA TRIALS

Unconsumed levels were determined by first multiplying the recorded tank volumes (L) for each day, by the initial sample reading (mg/L), this gave the initial nutrient/carbon values (mg). These values were then divided by current water levels to determine unconsumed levels (Equation 3.5).

$$Unconsumed\ nutrients\ \left(\frac{mg}{L}\right) = \frac{initial\ nutrients\ (mg)}{current\ water\ volume\ (L)} \qquad (Eqn.\ 3.5)$$

Daily consumed/lost levels of nutrients and carbon was then determined by subtracting sample readings from derived unconsumed nutrient values. The consumed/lost concentration for each day is then subtracted from the unconsumed concentration in an iterative process. This adjustment method was used for nutrients, carbon and growth measurements.

3.8.4 SPECIFIC GROWTH RATE CALCULATIONS

The specific growth rate was calculated for nutrient depletion and oxygen release. Nutrient depletion was found by dividing the slope of the nutrient change over a time period by the slope of the optical density reading over the same time period (Eqn. 3.7). Oxygen production during the day time, was equal to area under DO graphs for a 24 hour period divided by the light period (hr) and multiplied by the wastewater volume in the bioreactor (Eqn. 3.8). The DO area under the graph was established by setting night DO levels as the baseline.

$$Specific\ growth\ rate = \frac{Slope\ \frac{nutrient}{time}}{Slope\ \frac{optical\ density}{time}} \qquad (Eqn.\ 3.7)$$

$$Oxygen\ produced\ during\ light\ period\ (mg) = \frac{area\ under\ DO\ graph}{light\ period\ \times}\ wastewater\ volume \qquad (Eqn\ 3.8)$$

3.8.5 DATA MANIPULATION

In the batch experiments, Lab View continually logs all online data into an Excel spreadsheet. Error readings for DO, pH and temperature were corrected manually through linear approximations. Due to the large amount of raw data produced, Matlab was utilised for all graphical presentation purposes. Dissolved oxygen readings were converted to 20°C equivalent values, through the use of automated lookup tables. Trial time readings were logged in excel, in the form of text (24 hour period, serial numbers), in hours, minutes and seconds, e.g. 083200 represented 8:32am. For use in Matlab time readings were converted to decimal days. Logged Excel trial time data was first converted to a dd:hh:mm:ss format to include cumulative day values, cell values were then converted to decimal day values. For example 3:35pm on Day 5, was first changed to 05:15:35:00, and then to 5.634722.

A line used to indicate light and dark periods was included into the graphical results. The illustrative line was developed by inserting another column into the excel results and manually entering two alternating values corresponding to light and dark periods, i.e. inputting 5 for the light period and 3 for the dark period.

3.9 RISK MANAGEMENT

Before commencing work within the USQ laboratories, the researcher was required to undergo a safety induction and complete a Safe Work Procedures form, to assists in identifying potential safety issues that may arise during the project period. The following safety concerns were identified.

3.9.1 WORKING WITH HAZARDS CHEMICALS

The project required the use of hazardous chemicals including concentrated acid and base solutions. To avoid potential harm, PPE including gloves, face marks, eye protection and closed shoes were worn as required. All diluting of concentrated acid/base solutions will be undertaken within the venting chamber.

3.9.2 DISEASE PREVENTION

When using wastewater, there was a potential of bacterial contamination. To prevent contamination, gloves were worn when appropriate, and a disinfectant hand wash was used after any handling of waste products.

3.9.3 DISPOSAL METHOD OF BIOHAZARD MATERIALS

Because the bio-hazardous materials contained vast amounts of microalgae, they could not be discharged directly down the sink, as there is a high risk of algae growth in the drains causing clogging. As such when disposing of large amount of algae, it was first autoclaved and then disposed of.

3.9.4 RISKS BEYOND COMPLETION OF THE PROJECT

Following completion of this project, all waste materials were correctly disposed of, all chemicals were stored away, and the bioreactor was unplugged, cleaned and put aside until future use. Therefore no safety risk posed beyond the completion of the project by the materials used or microalgae growth mediums created.

3.10 CHAPTER SUMMARY

This chapter discussed the procedure for collection and preparation of algae and media, methods for determining CSG water characteristics, as well as adopted methods for calculating required levels of nutrient and bicarbonate addition. The bio-reactor set up was described and an overview was provided of the monitoring and logging processes for DO, pH and temperature readings. Adopted methodology for measurement of algal growth and nutrient depletion was examined. Methods for algal harvesting, freeze drying and lipid extraction were reviewed.

CHAPTER 4 GROWTH CHARICTERISTICS OF MICROALGAE CHLORELLA VULGARIS GROWN IN MBL MEDIA AND THE EFFECTS OF CO2 INPUT.

This chapter presents the results and discussions on the growth behaviour and nutrient removal of *Chlorella vulgaris* in MBL media, using batch experiments. The discussions further extend to interpret the observations on real-time monitoring using the dissolved oxygen and pH probes during the growth period.

4.1 BATCH EXPERIMENTS

Two batch experiments were performed using MBL media. Trials were differentiated by the inclusion of a CO_2 gas input in Experiment 1. The purpose of the experiments was twofold, to determine the effects of CO_2 input on algal growth, and to assess the effectiveness of on-line measurement systems as indicators of the real-time algal growth.

4.1.1 EXPERIMENT 1 (MBL MEDIA – CO_2 ADDITION)

Experiment 1 was intended as an assessment of the bioreactors capacity to facilitate and measure algal growth. At the commencement of the trial, 2 litres of MBL media was inoculated with 250 ml of *Chlorella vulgaris* culture (6.95mg of biomass). Monitoring of growth and nutrient removal was conducted for the entire length of the trial. In order to mimic the real world scenario, photo-energy required for the algal growth was provided by means of a light source. The light source was implemented on a 12 hour on (6am to 6pm)/off cycle.

4.1.2 EXPERIMENT 2 (MBL MEDIA – NO CO_2 ADDITION)

The only variable changed for Experiments 2 was the removal of CO_2 input. It was predicted that growth and subsequent lipid production and nutrient removal would be reduced with the absence of CO_2 feed. This experiment was essentially conducted to check for a placebo effect.

4.2 ALGAL GROWTH

Algal growth was quantified using daily Suspended Solids (SS) and Spectrometer measurements. Photo documentation was also used to capture the transition of algal growth through the various phases.

4.2.1 EXPERIMENT 1: GROWTH CHARICTERISTICS

Figure 4.1 shows the recorded growth trends of *Chlorella vulgaris* in Experiment 1, represented through optical density and suspended solids measurements. The added trend lines are based on a 3rd order polynomial. Results suggest a lag phase occurring within the first day of the experiment. Lag phase normally occurs when an inoculums is transferred from its ideal media into an alternate media with different compositions. When introduced, algae first take some time to get acclimatized in a new environment. In this study however, the lag period was reduced due to there being no change in media type from culturing to inoculation, as such transfer shock was minimal.

The exponential growth period appears to take place between days 1 and 3, with a linear growth phase from day 3 to 7, and a stationary phase extending from day 7 through to the completion of the trial. In order to get the maximum lipid extraction, the trial was terminated just before the expected death phase.

Log records showed that approximately 100ml of media in the bioreactor was lost per day as a result of evaporation and sampling. For the first two weeks of the trial, MBL media was used to replace any losses within the bioreactor. The continual addition of a nutrient rich media was adopted with the intention of prolonging the stationary growth phase. It was thought that by prolonging this steady phase consistent algae growth and nutrient depletion measurements could

be achieved. Obtained results generally confirmed this theory. From the start of the third week, the MBL media was substituted with distilled water for topping up.

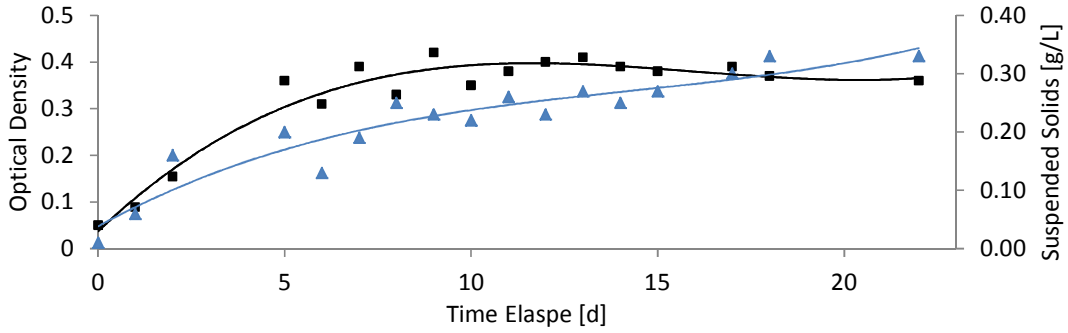


FIGURE 4.1 Experiment 1 – Algal growth in MBL media with CO_2 addition measured with Optical Density (■) at 505 nm and Suspended Solids (▲)

Figures 4.2 show the relationships between suspended solids and optical density measurements for Experiment 1. This relationship can be expressed as shown in Equation 4.1. The result suggests a moderate correlation (68.8%) between the two algal growth measurement procedures.

Suspended Solids = 0.06367 *Optical Density* + 0.0098 (R2 = 68.8%) (Eqn. 4.1)

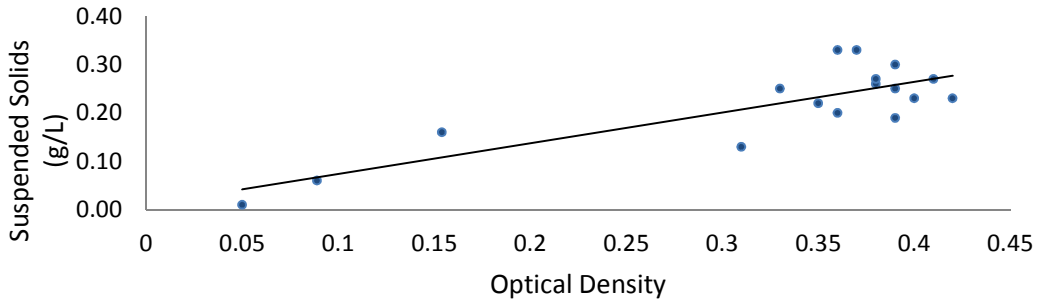


FIGURE 4.2 Experiment 1 - Relationship between Suspended Solids and Optical Density at 505nm.

4.2.2 EXPERIMENT 2: GROWTH CHARICTERISTICS

Figure 4.3 shows the recorded growth characteristics of *Chlorella vulgaris* without CO_2 input. The trend line was again derived using a 3rd order polynomial. The data suggests a lag phase occurring within day 1 of the experiment, with an exponential growth period between days 1 and 3. The linear growth trend looks to have extended from days 3 through to 8, followed by a brief

stationary phase, approximately 1 to 2 days, and then a death phase from day 10 through to the end of the trial.

MBL media was only used in the first week for topping up of the bioreactor, after which it was replaced with distilled water. Subsequently results showed a shorter stationary growth period. The trial was allowed to proceed to the death phase, so that all cycles of growth could be captured. The overall resulting plot encompasses all five of the growth phases, and it is in keeping with the theoretical growth pattern.

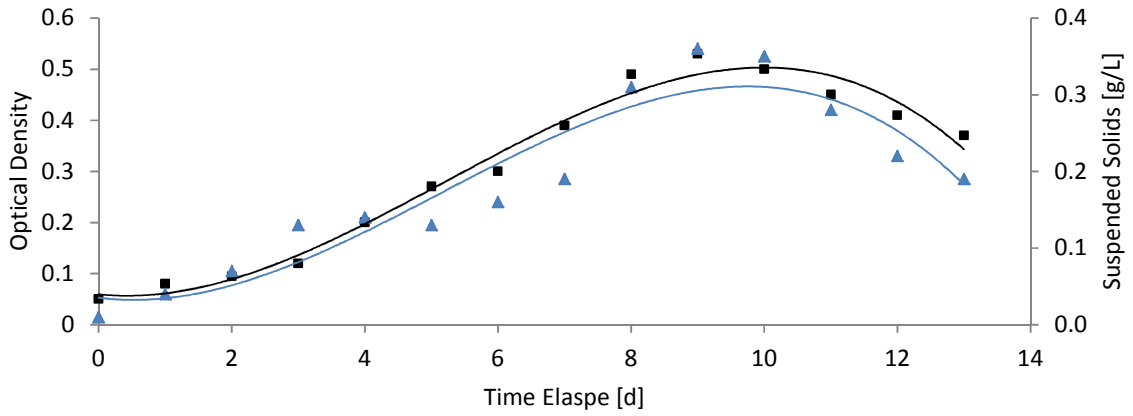


FIGURE 4.3 Experiment 2 - Algal growth in MBL media without CO_2 addition measured with Optical Density (■) at 505 nm and Suspended Solids (▲).

Figure 4.4 shows the relationships between suspended solids and optical density measurements for Experiment 2. This relationship can be expressed as shown in Equation 4.2. The high correlation (89.4%) suggests that either the suspended solids or optical density method could be adopted independently to accurately measure algal growth. For this reason only the spectrometer method was implemented in future trials.

$$\textit{Suspended Solids} = 0.06058\ \textit{Optical Density} + 0.0017\ (R^2 = 89.4\%) \qquad \text{(Eqn. 4.2)}$$

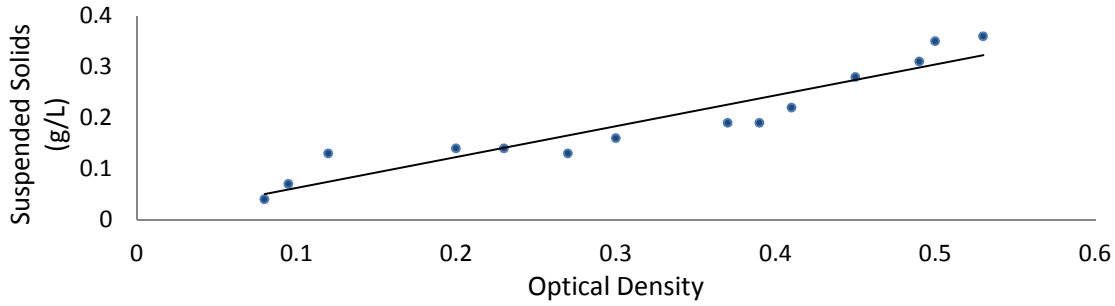
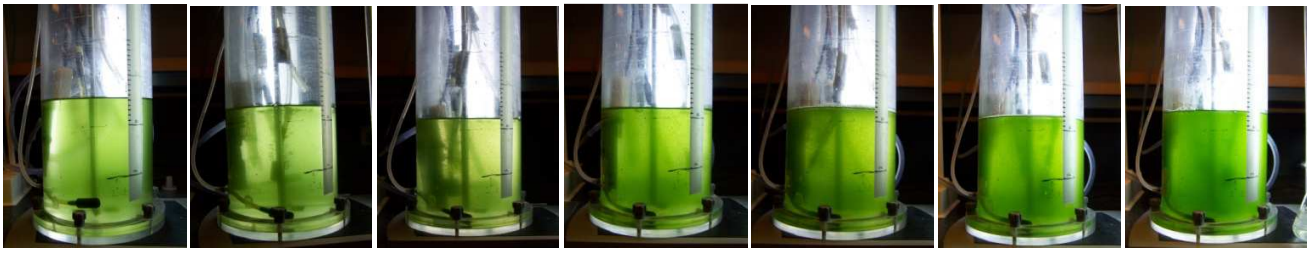
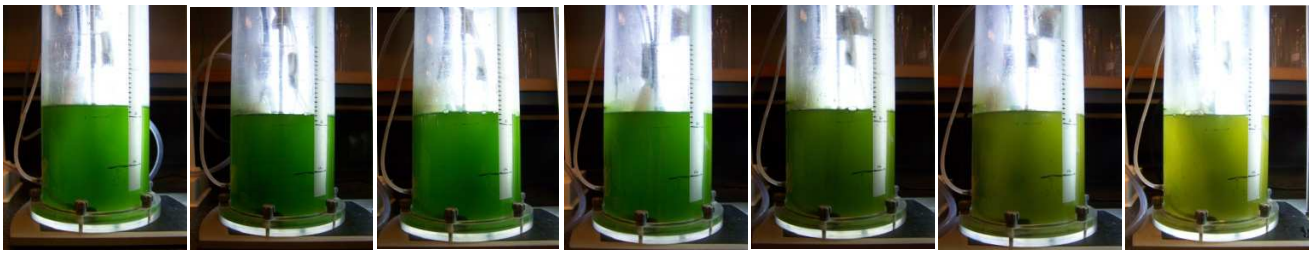


FIGURE 4.4 Experiment 2 - Relationship between Suspended Solids and Optical Density at 505NM.

Photographic documentation of the *Chlorella vulgaris* growth in the bioreactor, for Experiment 2 is shown in Figure 4.5. A photo was taken for each of the 14 days of the experiment, recordings show the algae's transition through all of the growth phases, approximate labelling of each phase is provided.



|‹- Day 1 (Lag)- -›|‹- - -- - - - Day 1-3 (Exponential) - - -›|‹- - - - - - -- - - - - - - - - - - - Day 4 -8 (Linear) -›|



|‹- - - Linear - - -›|‹- - - - Day 9 -10 (Stationary)- - - - -›|‹- Day 10 -14 (Death) - - - - - - - - - -- - - - - - - - - - ›|

FIGURE 4.5 Daily photos of algae growth in the Bioreactor for Experiment 2, documenting all 5 stages of growth.

4.2.3 ALGAL GROWTH SUMMARY

A comparison of the growth characteristics between the two trials is shown in Figure 4.6. As would be expected, the trial with CO_2 input experiences faster initial growth. Interestingly however, the non-CO_2 input experiment recorded the highest Optical Density and Suspended Solids readings. For oil production purposes, emphasis would be placed on increasing biomass to high concentrations within the shorts period of time. As such, current results suggest merit in CO_2 gas addition.

There is significant evidence in research literature to show that CO_2 is a key factor in facilitating algae growth (Watanabe 2005, Brune et al. 2009, Mata et al. 2010). A possible explanation for the high growth recorded in the non-CO_2 input experiment could be that, the algae is extracting enough CO_2 from the atmosphere for growth requirements. The shallow depth, minimal media volume and large exposed surface area of the bioreactor would have assisted with atmospheric CO_2 extraction.

Alternately the concentration of CO_2 in the media may have been too high, inhibiting growth. Tang el al. (2010) found that algal growth was highly sensitive to the concentration levels of CO_2, with inadequate or excessive CO_2 input resulting in poor growth. Further testing with a focus on CO_2 input volumes would be required to determine the cause of the obtained results.

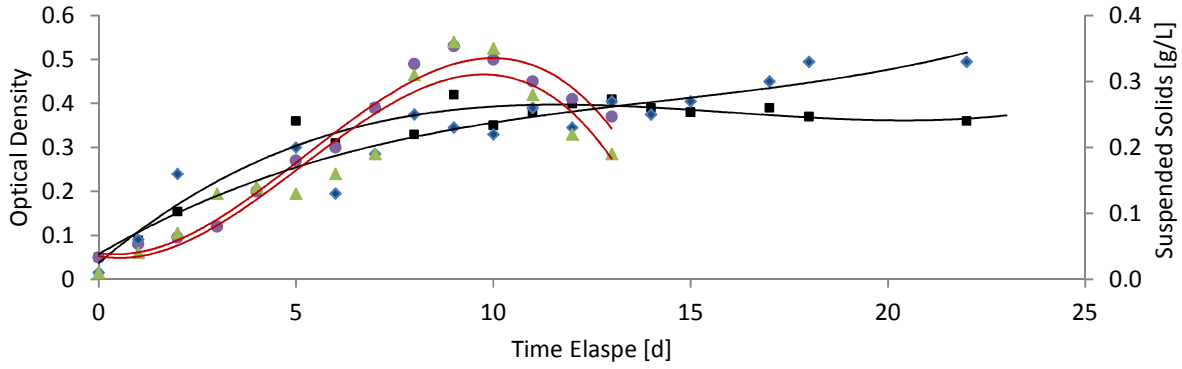


FIGURE 4.6 Experiment 1 & 2 – Comparison of algal growth in MBL media with and without CO_2 addition measured with Optical Density (Experiment 1 = ■, Experiment 2 = ●) at 505 nm and Suspended Solids (Experiment 1 = ◆, Experiment 2 = ▲)

4.3 pH VARIATION

The pH level within the bioreactor was recorded in real-time, measurements were logged once per minute. pH levels are influenced predominantly by two main factors, the input of CO_2, and light source availability. Addition of CO_2 acidifies the medium reducing the pH value (Watanabe 2005). This factor was only applicable to Experiment 1. Light source availability within the reactor determines the growth state of the algae. During light periods, algae extracts CO_2 for photosynthesis, pH levels will rise when the rate of inorganic carbon uptake by microalgae exceeds the rate of CO_2 supply (Harrison & Berges 2005).

4.3.1 EXPERIMENT 1: pH VARIATION

Figure 4.5 shows the measured pH fluctuations of Experiment 1. The CO_2 was supplied in two forms; continuously at a rate of 5ml/min, and in periodic bursts at a rate of 60ml/min, injected once every 2hrs for 4 seconds. The periodic CO_2 input only occurred during the light period i.e. between 6am and 6pm, and was observable by sharp drops in pH every 2 hours.

Overall, the results show volatile readings for the first 2 days, coinciding with the indentified lag and exponential phases. No CO_2 was added during the expected lag and exponential phases, previous batch experiments conducted in the USQ lab found that the media had insufficient buffering capacity to accommodate the periodic CO_2 addition during these initial stages (Pufelski 2010).

From days 2 to 5, higher stable readings were observed. This time period, relates to the identified linear growth phase. From day 5 onwards pH levels reduced whilst remaining stable, they then increased gradually for the remainder of the experiment. The added red line indicates light and dark periods of the experiment, peaks (light) and troughs (dark).

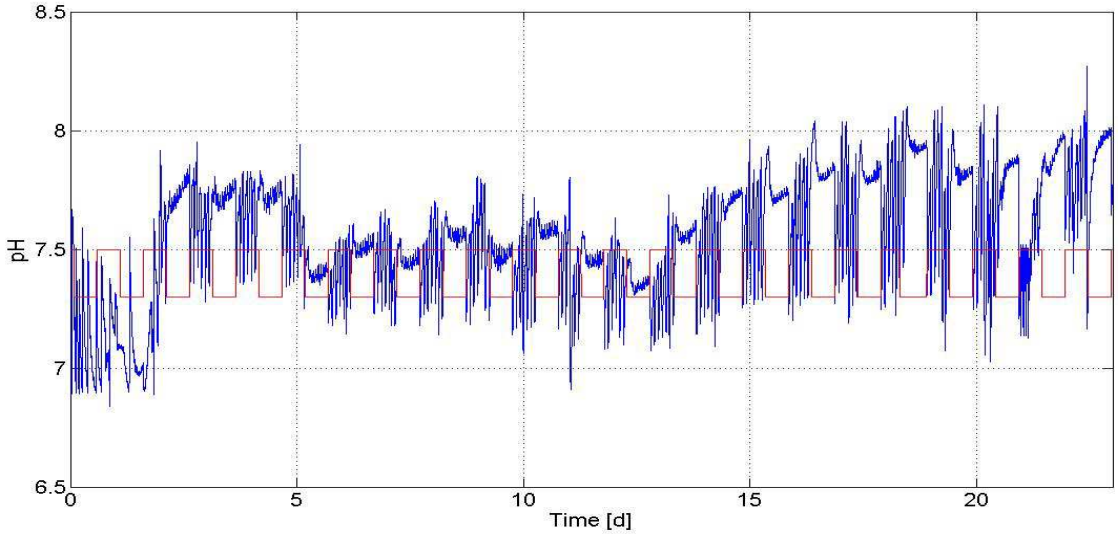


FIGURE 4.7 Experiment 1 – Bioreactor pH readings for the 23 day trial.

Figure 4.8 shows the pH readings for days 1, 3 and 15 of Experiment 1, over a 24 hour period. Based on observations from the growth behaviour shown in Figure 4.2, these days correspond respectively with the identified lag, linear and stationary growth phases.

All readings start at midnight, splitting the dark period into two 6 hour periods. Day 1, the identified lag phase, showed pH levels steadily decreasing for both the day and night periods. The noted sudden increases in pH levels were caused by the automated control system of the bioreactor adding base solution when levels drop below the set limit (≤6.9 pH). The readings for the exponential phase were omitted, as results were erratic. During the lag phase, the growth did not occur, therefore no notable increase in pH was noted.

Days 3 (linear), and 15 (stationary) produced characteristically similar results. In the light period increases in pH levels from photosynthesis, were followed by sudden drops in levels every 2 hours as a result of the CO_2 addition. The pH values on day 15 generally peaked higher than on day 3, this may have been the result of there being larger quantities of active biomass in the latter stages, leading to more photosynthetic reactions. As was expected, dark period readings were relatively flat, indicating no photosynthesis taking place in the night.

The pH increases observed in the light periods for both days 3 and 15 consisted typically of two distinct linear gradients. Immediately after CO_2 addition the pH level increased at a steep rate, approximately 0.37 ph unit/h for day 3 and 0.55 ph unit/h for day 15, after a varying period of

time this rate reduced to approximately 0.17 ph unit/h and 0.34 ph unit/h for days 3 and 15 respectively. This second gradient is more apparent in the day 15 readings. The steepness of the slope within the two hour period represents different rates of growth, with faster growth initially upon the addition of carbon dioxide, followed by relatively sluggish growth after a certain time.

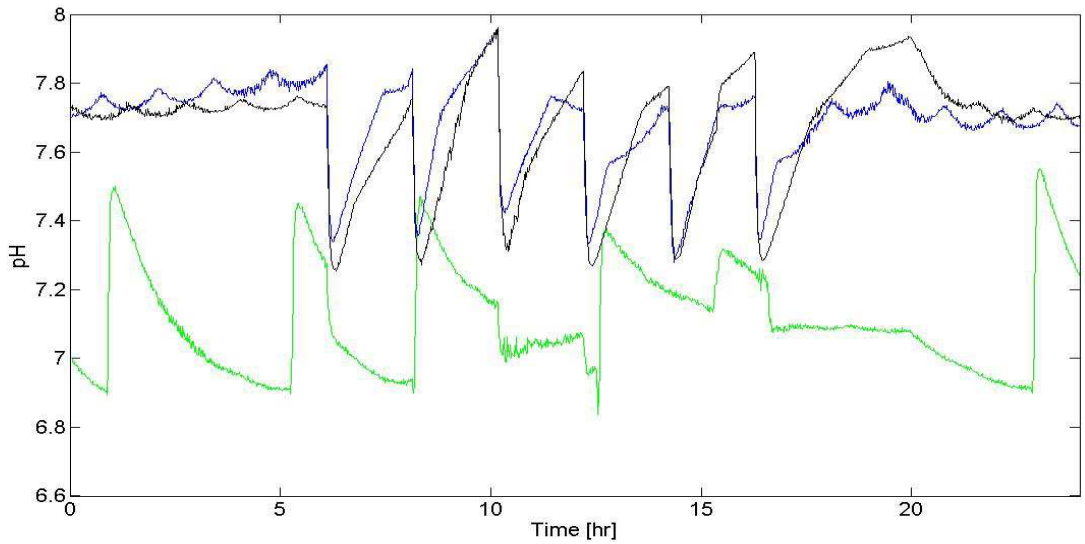


FIGURE 4.8 Experiment 1 – Bioreactor pH readings over a 24 hr period for Day 1 (—), Day 3 (—) and Day 15 (—).

4.3.2 EXPERIMENT 2: pH VARIATION

The pH levels for Experiment 2 are presented in Figure 4.9, results show large fluctuation over the first 2 days, which again coincides with the identified lag and exponential growth phases. After day 2, the average pH level stabilises and gradually increases until day 5 before declining for the remainder of the trial. From day 10, the identified commencement of the death phase, there is a noticeable deterioration in the pH pattern.

The red line shows dark (trough) and light (peak) periods. It is observed that a steady rise in pH readings occurs during the light periods followed by a steady decline in the dark periods, generally resulting in a peak pH value at the end of each light period.

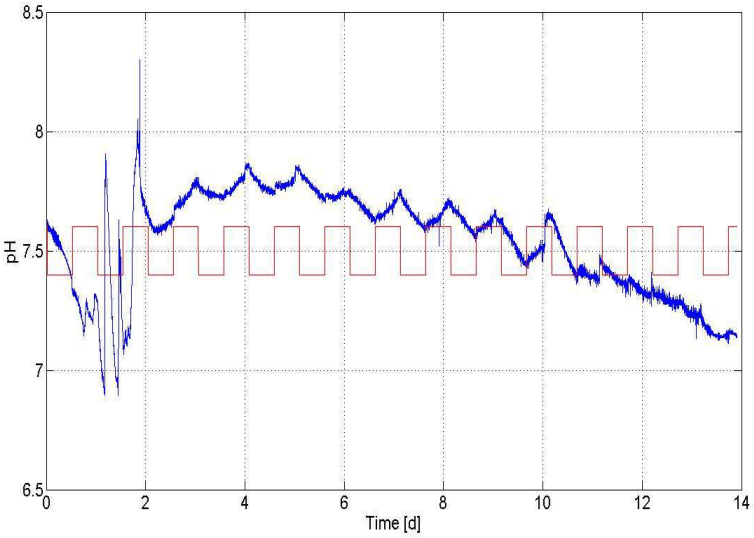


FIGURE 4.9 Experiment 2 – Bioreactor pH readings for the 14 day trial.

Figure 4.10 shows the pH results over a 24 hour period for days 7, 9 and 13, relating to the indentified linear, stationary and death phases respectively. Without CO_2 addition the results are relatively stable. For days 7 and 9 the gradual decline in pH values in the dark period and the increase during the light periods is well demonstrated. Day 13 values are characterised by a steady decline in both light and dark periods.

The pH readings on day 9 (stable) are higher than on day 7 (linear), this could again be attributed to the larger mass of active alga in the latter stage of the experiment, resulting in more photosynthetic reactions occurring. The death phase gives expectedly lower readings, as there are fewer live algae for photosynthesis.

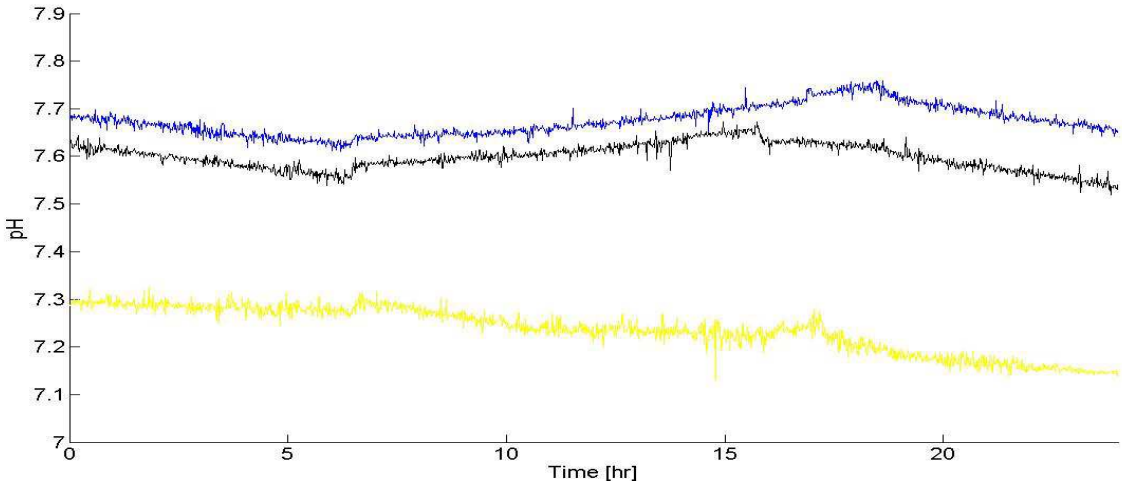


FIGURE 4.10 Experiment 1 – Bioreactor pH readings over 24 hr period for different growth phases; Linear Phase (—), Stationary Phase (—), Death Phase (—).

4.4 DISSOLVED OXYGEN VARIATION

Dissolved oxygen (DO) readings were recorded in real-time, measurements were logged once per minute. The DO level is influenced predominantly by light source intensity. During light periods algae will release O_2 through photosynthesis and absorb O_2 through respiration. If light intensity is sufficient, the net result is O_2 production. In dark periods, only respiration occurs, leading to a net O_2 uptake through respiration. At low light intensity net oxygen rates increase linearly with light intensity (Masojidek, Koblizek & Torzillo 2004).

4.4.1 EXPERIMENT 1: DO VARIATION

Figure 4.11 shows the DO concentrations for Experiment 1. A light and dark phase line has been included. As expected higher DO values were recorded during the light periods. Minimal fluctuations were observed for the first 5 days of the experiment, after which a greater separation was seen between average dark and light period readings. These larger fluctuations generally occurred during the identified linear and stationary growth phases.

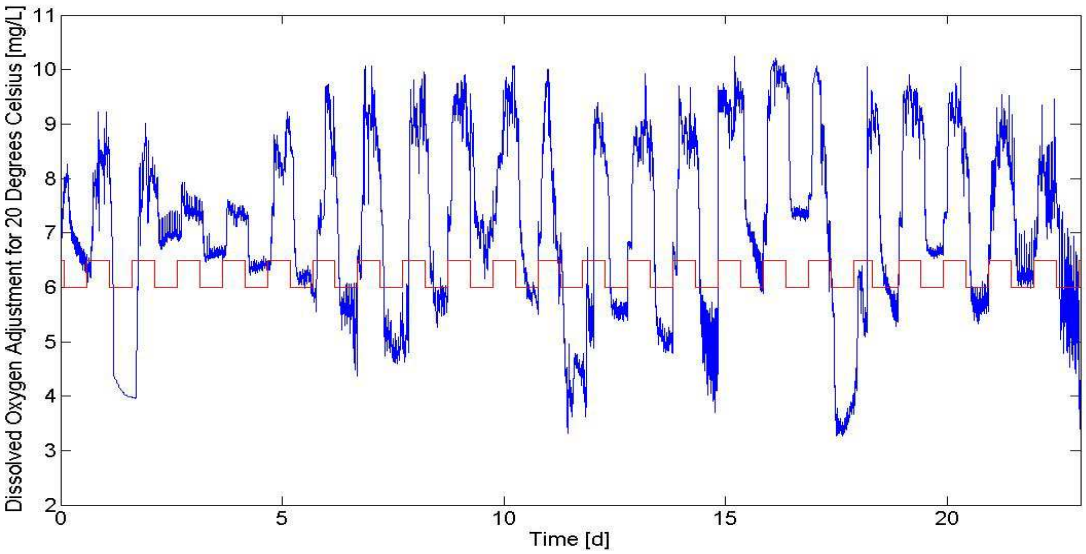


FIGURE 4.11 Experiment 1 – Bioreactor DO readings for the 23 day trial.

Figure 4.12 compares average DO readings during the light period with average DO readings in the dark period for Experiment 1. The relative difference between these two sets of values provides the net oxygen produced during each of the light periods. This level of DO production is indicated with the dashed trend line, which is derived from a 3rd order polynomial. The saturation value of DO at 20°C is 9.17 mg/L, this is also indicated on the graph, as is the measured optical density readings for this trial.

The highest recorded DO production reading was 4.6 mg /L, on day 18. Dissolved oxygen production levels generally increased until the end of day 5 (linear), and remained steady for the remainder of the trial. This increase in DO production is likely the result of a growing volume of active biomass during the lag, exponential and linear phases.

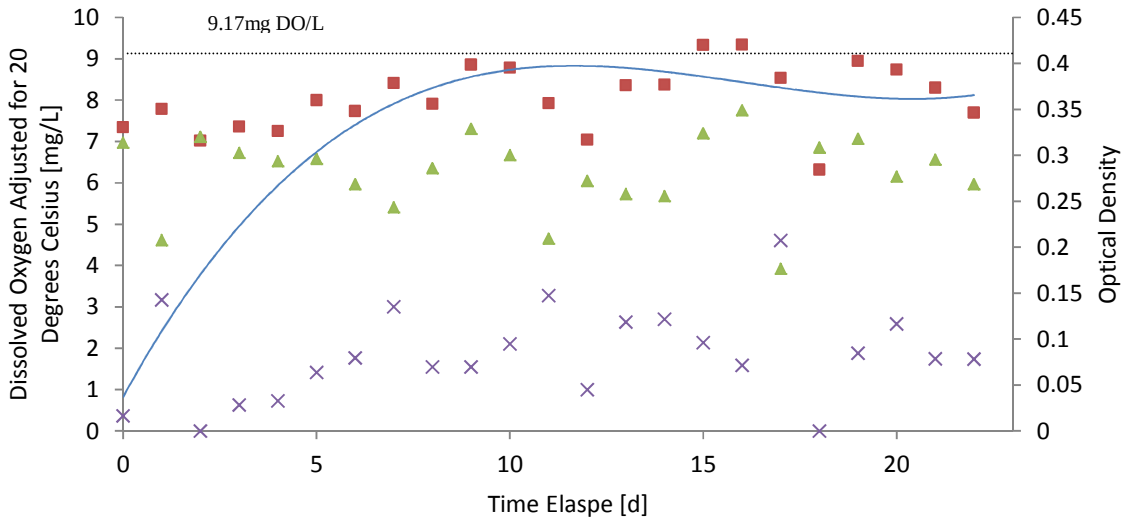


FIGURE 4.12 Experiment 1 –Average maximum DO readings during the light period (■), average minimum DO readings during the dark period (▲), net oxygen produced during the light period (X) and optical density reading (—).

Figure 4.13 shows the estimate oxygen production quantities in the light periods, for each day of Experiment 1. Quantities were derived using equation 3.8. Results show a peak oxygen production of 2.09 mg, produced on day 18.

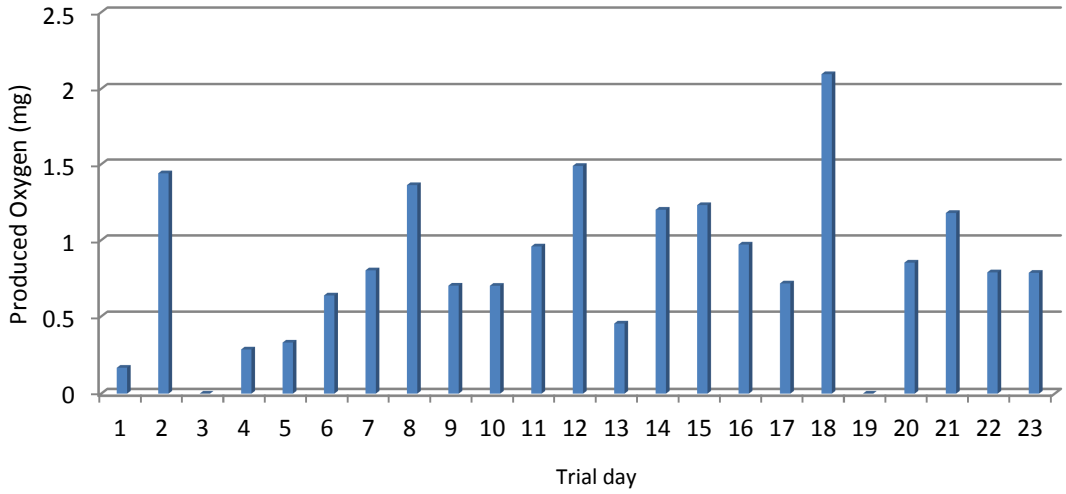


FIGURE 4.13 Experiment 1 – Daily oxygen quantities produced during the light period

4.4.2 EXPERIMENT 2: DO VARIATION

Figure 4.14 shows the DO dynamics for Experiment 2, a line indicating light and dark periods has been included. The results show volatile DO readings extending from the start of the trial until day 6, after which stabilisation occurs. The overall pattern generally reflects the observed growth behaviour (Figure 4.3) with an increase in average levels until day 9 followed by a clear decline in readings for the identified death phase.

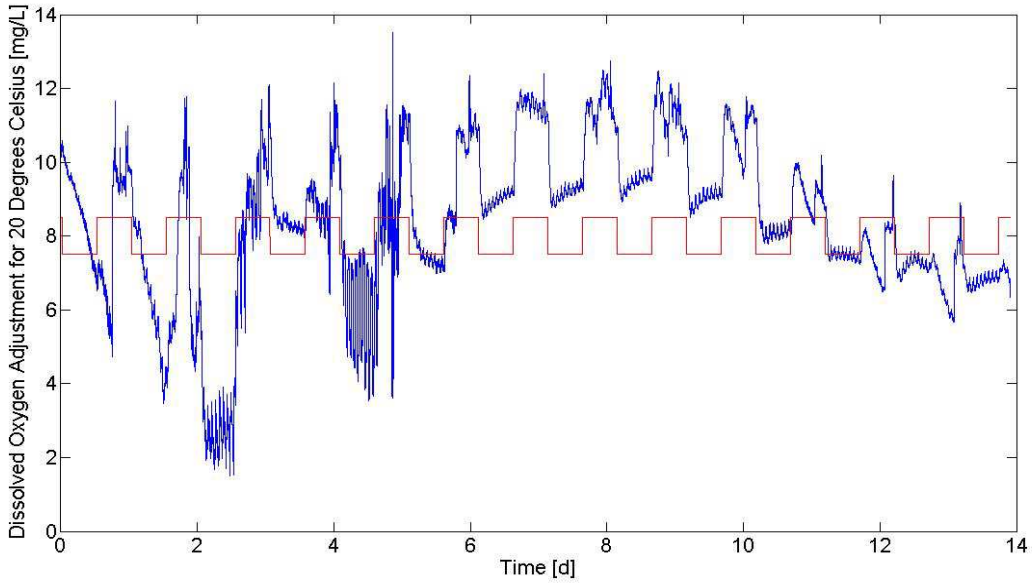


FIGURE 4.14 Experiment 2 – Bioreactor DO readings for the 14 day trial.

Figure 4.15 shows the average light and dark period readings for Experiment 1, also included is the relative DO production during the light period. Obtained results generally show constant levels of DO production from the start of the trail until day 10, after which the difference begins to reduce to zero. Higher average dark and light period readings are achieved in the identified linear and stationary phases. A maximum DO reading of 4.03 mg /L was recorded on day 3.

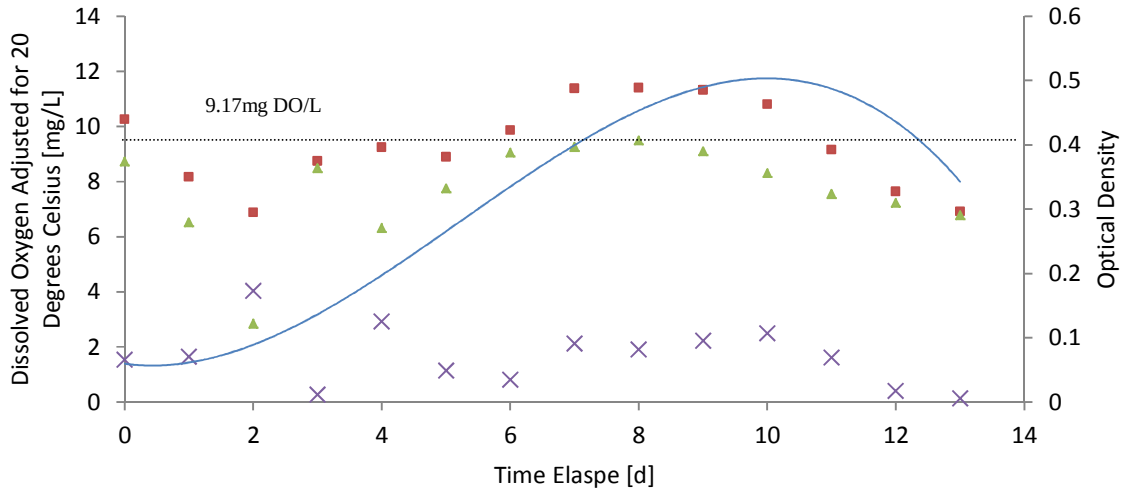


FIGURE 4.15 Experiment 2 –Average maximum DO readings during the light period (■), average minimum DO readings during the dark period (▲), net oxygen produced during the light period (X) and optical density reading (—).

Figure 4.16 shows the estimate oxygen production quantities in the light periods, for each day of Experiment 2. Quantities were derived using equation 3.8. Results show a peak oxygen production of 1.83 mg, produced on day 3.

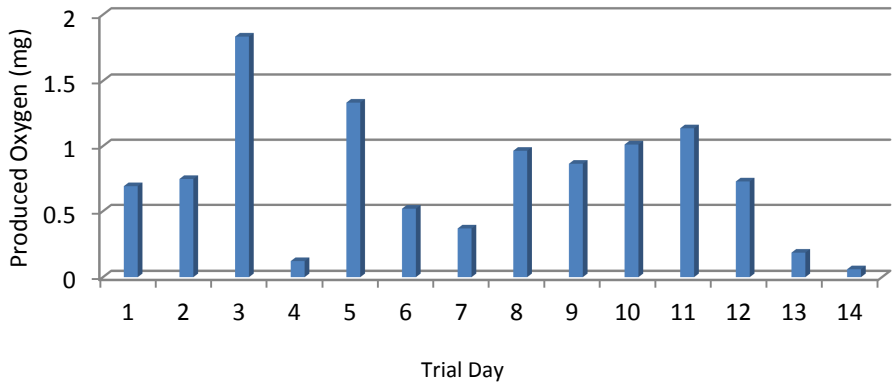


FIGURE 4.16 Experiment 2 – Daily oxygen quantities produced during the light period

4.5 NUTRIENT REMOVAL

Nutrient depletion was assessed for Experiment 1, by measuring daily total nitrogen levels in the bioreactor. Results are shown in Figure 4.17, also included is the optical density readings for algal growth. The graph captures the relationship between nutrient removal and algae growth,

results generally reflect the theoretical relationship as described in section 2.8. As MBL media was used in the first two weeks for topping up the bioreactor, adjustment has been made to the recorded nutrient values.

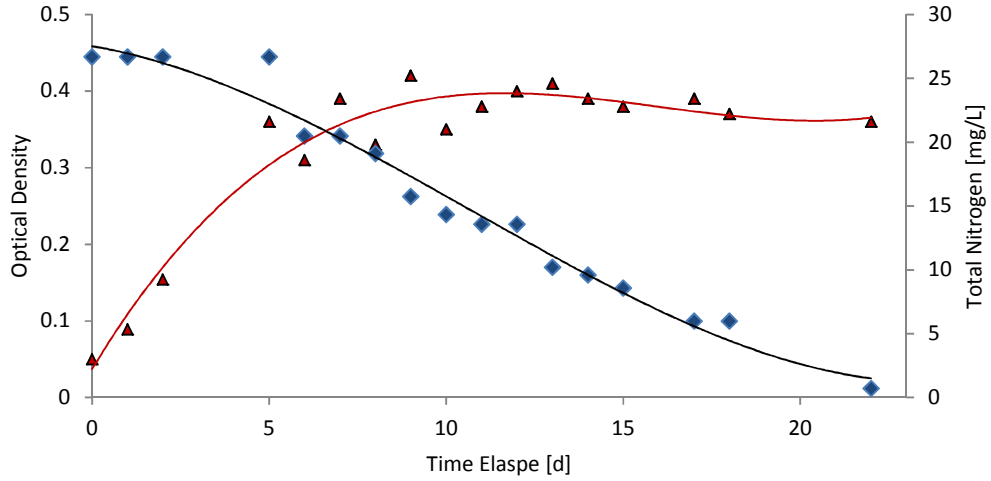


FIGURE 4.17 Experiment 1 – Nutrition removal rate measured through nitrogen depletion (◆), and algal growth in Optical Density (▲).

Figure 4.18 shows the nitrogen removal rate 1.167 mg TN/L/d for the first 9 days of the trials. The corresponding suspended solids rate for this period was 21.0 mg SS/L/d, giving a specific nutrient removal rate of 0.056mg TN/SS.

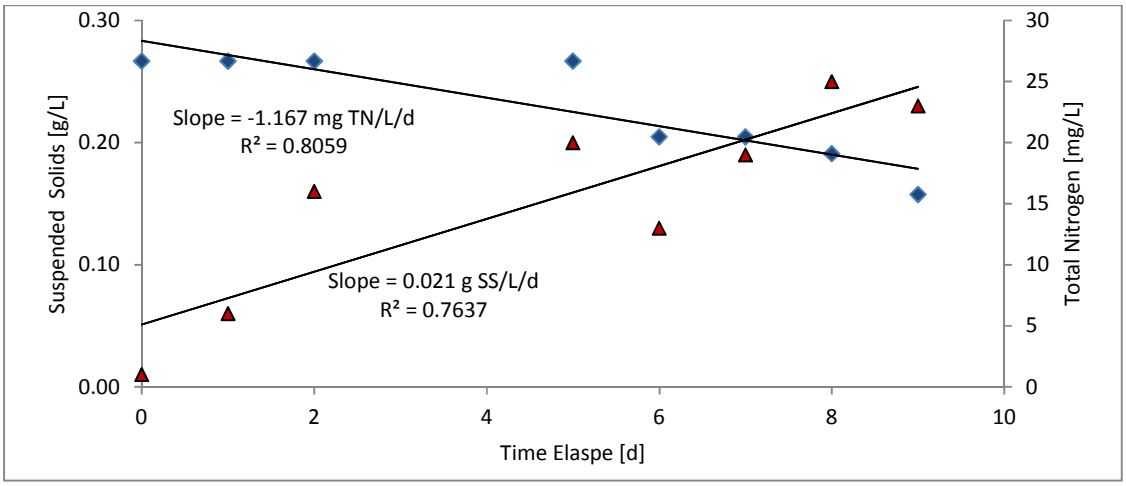


FIGURE 4.18 Experiment 1 – Nutrition removal rate measured through nitrogen depletion (◆), and algal growth in Suspended Solids (▲).

4.6 ALGAE DRY MASS AND LIPID CONTENT

Algal dry mass quantities were, 0.45 g and 0.43 g for experiments 1 and 2 respectively. Results suggest no distinct benefit in either process for total algal production. Lipid extraction processes were not undertaken.

4.7 ENCOUNTED PROCEDURAL ISSUES

4.7.1 pH VOLATILITY

Initially it was intended that no pH buffer (Tris) would be added to the media, so as not to inhibit the pH sensitivity of the solution. However shortly after commencing each of the two trials, pH fluctuations were observed regularly exceeding the set pH limits (pH 7.5 ± 0.6), resulting in excessive acid and base addition by the bioreactor automotive system. To address this issue, a small amount of Tris (125mg/L) was added to the media. The limited Tris addition meant that future pH movements were still observable and were generally within the set limits.

4.7.2 ALGAL SETTLEMENT AND CLUMPING

Early in the trial it was found that the algae was settling at the base of the tank. To address this, the speed of the stirrer was gradually increased over a several day period. Care was taken in this process as there was concern that if the stirring speed was too high it would result in algae death through shearing.

It was also found that algae would clump onto the DO (Figure 4.19) and pH probes, resulting in inaccurate readings. This was particularly problematic for the DO reading, as it would reduce recorded values by between 2 to 4ppm. The alga was removed by gently tapping the probes. Clumping was reduced by placing the probes within the areas of high turbulence created by the stirrer.

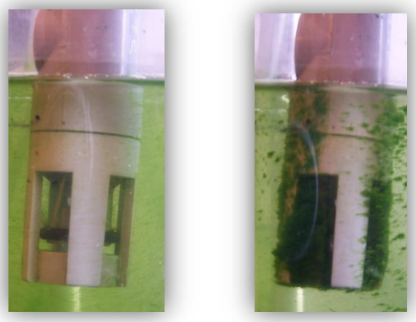


FIGURE 4.19 Algal clumping on the DO probe

4.8 CONCLUSIONS

Research has provided insight into the growth characteristics of microalgae *Chlorella vulgaris* in a MBL medium. The algae grew well in both Experiments 1 and 2, with suggested benefit in CO_2 addition to promote rapid algal growth. An acceptable correlation was found between the suspended solid and spectrometer readings, suggesting that future trials could be measured using only the spectrometer procedure. Furthermore, assessments were made of the pH and DO variability, algal dry mass quantities and nitrogen depletion rates.

CHAPTER 5 GROWTH CHARICTERISTICS AND LIPID PRODUCTION OF MICROALGAE DUNALLIELLA TERTIOLECTA GROWN IN A CSG WATER MEDIA AND THE EFFECTS OF AERATION.

This chapter presents the results and discussions on the growth behaviour, carbon sequestration and nutrient removal of *Dunaliella Tertiolecta* in CSG water using batch experiments. The discussion further extends to evaluate the algal growth facilitation capacity and carbon stripping properties of aeration. This chapter ends with the interpretation of the real-time monitoring results of dissolved oxygen and pH probes during the growth period.

5.1 BATCH EXPERIMENTS

Two batch experiments were performed using microalgae *Dunaliella Tertiolecta* in a CSG water media. Trials were differentiated by the inclusion of a compressed air input in Experiment 2. The purpose of this distinction was twofold, to determine the effects of a compressed input on algal growth, and to test for a carbon stripping effect through aeration.

5.1.1 EXPERIMENT 1 (CSG WATER MEDIA – NON-AERATION)

Experiment 1 was conducted to assess the growth capacity of microalgae *Dunaliella Tertiolecta* in a CSG water medium, without aeration. The experiment was intended as a lab scale assessment, to evaluate the algae's capacity to be grown in an open pond setting. Monitoring of growth and nutrient removal was conducted for the entirety of the trial. Photo-energy required for the algal growth was provided by means of a light source, implemented on a 12 hour on (6am to 6pm)/off cycle .

At the commencement of the trial, 3 litres of filtered CSG water was inoculated with 250 ml of *Dunaliella Tertiolecta* culture (6.95mg of biomass) and 5 ml/L of F2 concentrate. Sodium Bicarbonate ($NaHCO_3$) was added to the media (1344mg/L) to increase the bicarbonate levels to the average CSG water conditions. The C:N:P ratio was 192:85:8.5. Refer to Section 3.3 for further details.

5.1.2 EXPERIMENT 2 (CSG WATER MEDIA – AERATION)

The only variable changed for Experiments 2, was the addition of aeration, fed into the reactor at a rate of 100 ml/min. It was predicted that growth and subsequent lipid production and nutrient removal would increase with an aeration feed. Furthermore, it was hypothesised that aeration would significantly reduce bicarbonate levels within the media through stripping of carbon. Refer to Section 1.7.4 for details of aeration properties.

At the commencement of the trial, 3 litres of filtered CSG water was inoculated with 250 ml of *Dunaliella Tertiolecta* culture (7.12 mg of biomass) and 5 ml/L of F2 concentrate. Sodium Bicarbonate ($NaHCO_3$) was added to the media (1344mg/L) to increase carbon levels to the average CSG water conditions. The C:N:P ratio was 192:85:8.5. Refer to Section 3.3 for further details.

5.2 ALGAL GROWTH

Algal growth was quantified using daily Spectrometer measurements. Photos were taken to document the phase transition of algal growth.

5.2.1 EXPERIMENT 1: GROWTH CHARICTERISTICS

Figure 5.1 shows the recorded growth trends of *Dunaliella Tertiolecta* in Experiment 1, represented through optical density measurements. The added trend line is based on a 3rd order polynomial. Results suggest a lag phase occurring within the first day of the experiment. The exponential growth period appears to take place between days 1 and 2, with a linear growth phase within days 2 and 3. The stationary phase was observed within day 4, with a transition to the death phase from day 4.

Approximately 100ml of media in the bioreactor was lost per day as a result of evaporation and sampling. Once the total volume of the media reduced to 3L, distilled water was used to replace any further losses within the bioreactor.

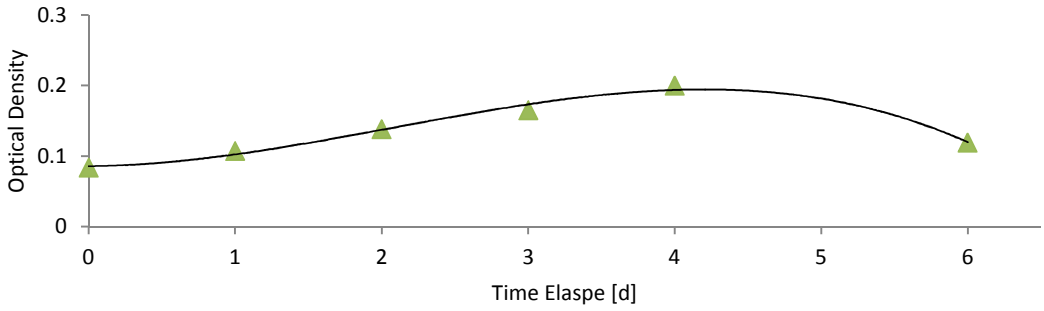


FIGURE 5.1 Experiment 1 – Algal growth in CSG water media without compressed air addition measured with Optical Density (▲) at 505 nm.

Figure 5.2 shows the estimated algae growth rate in terms of suspended solids for the first 4 days of Experiment 1 (0.029 g SS/L/d). The initial suspended solids reading was obtained at the commencement of the trial, through the process of measuring biomass inoculation quantities. All other values were derived from optical density readings, based on an assumed equal correlation between measurement methods. Refer to section 5.4, for application of the algal growth rate to determine nutrient removal rates.

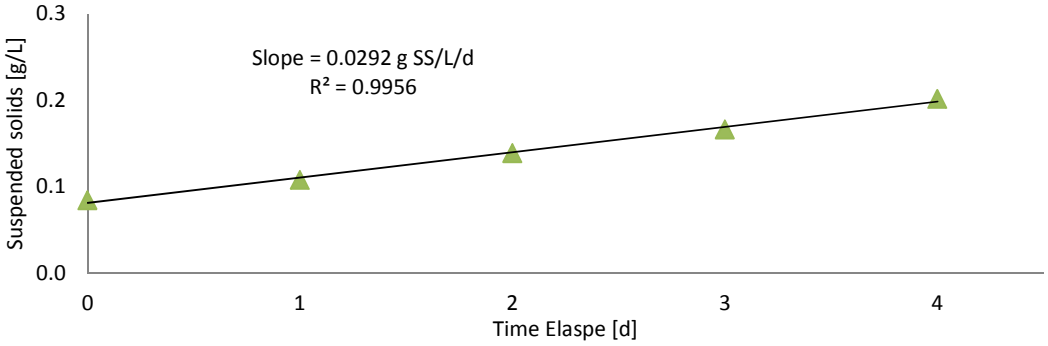


FIGURE 5.2 Experiment 1 – Algal growth in CSG water media without aeration measured with derived Suspended Solids readings (▲) for first 4 days of the trial

5.2.2 EXPERIMENT 2: GROWTH CHARICTERISTICS

Figure 5.3 shows the recorded growth characteristics of *Dunaliella tertiolecta* with aeration. The trend line was again derived using a 3rd order polynomial. The data suggests a possible exponential growth phase occurring within day 1 of the experiment, and a linear growth trend on day 2, followed by a brief stationary phase from days 3 to 4, and then a death phase from day 4 through to the end of the trial. Note that the absence of a documented lag phase, may be due to the spacing (approx. 24hr) between sampling times, more frequent sampling within the first day may have revealed a lag period. Distilled water was again used for topping up of the media once a total volume of 3L was reached.

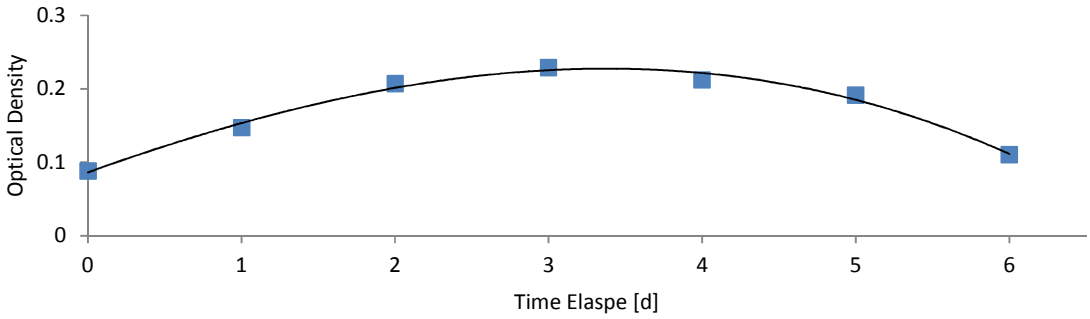


FIGURE 5.3 Experiment 2 - Algal growth in CSG water media with aeration addition measured with Optical Density (■) at 505 nm.

Figure 5.4 shows the estimated algae growth rate in terms of suspended solids for the first 4 days of Experiment 2 (0.030 g SS/L/d). Refer to section 5.4, for application of the algal growth rate to determine nutrient removal rates.

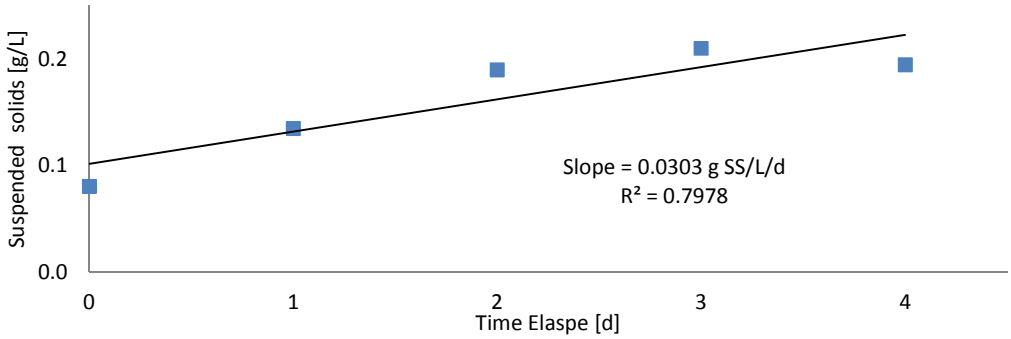


FIGURE 5.4 Experiment 2 – Algal growth in CSG water media with aeration addition measured with derived Suspended Solids readings (■) for first 4 days of trial

5.2.3 ALGAL GROWTH SUMMARY

A comparison of the growth characteristics between the two trials is shown in Figure 5.5. As was expected, the trial with aeration input experienced faster initial growth, and recorded the highest optical density readings (0.229), however difference in growth between trials was not significant. Furthermore, neither trial achieved high levels of growth, with both trials failing within 6 days.

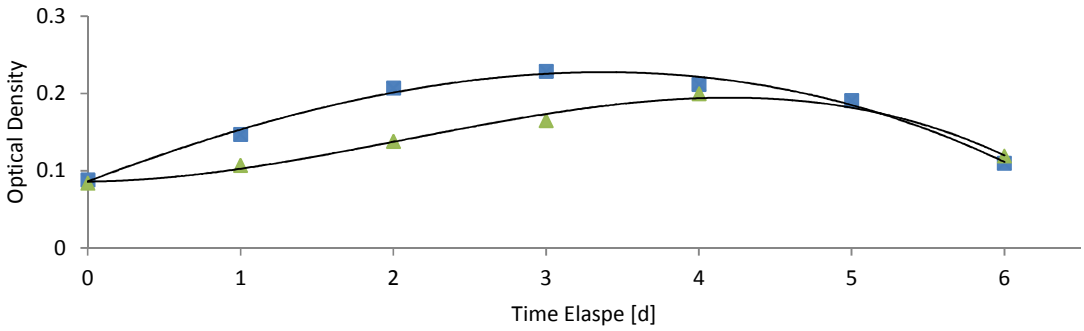
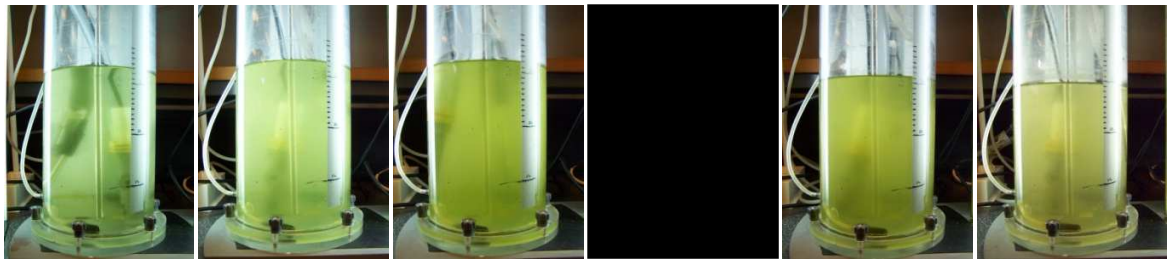


FIGURE 5.5 Experiment 1 & 2 – Comparison of algal growth in CSG water media with and without aeration measured with Optical Density (Experiment 1 = ▲, Experiment 2 = ■) at 505 nm

Due to the poor performance of the trials, assessment was made to identify optimal salinity levels for *Dunaliella tertiolecta* growth in a CSG media. Experiments 1 and 2 were then retested using this identified optimal salinity, refer to Chapter 6 for results.

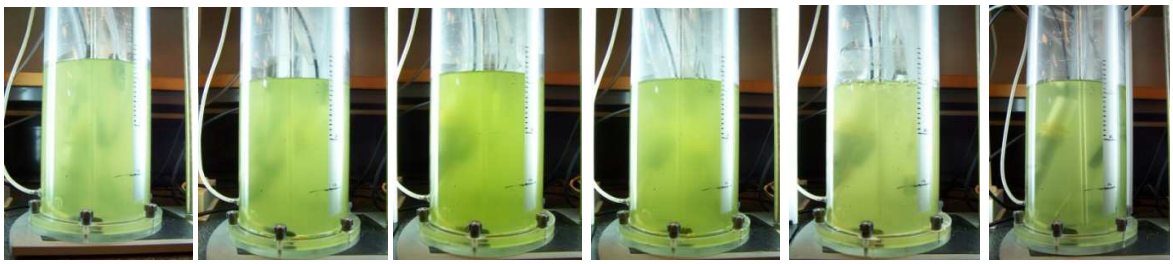
A comparison of the photographic documentation of *Dunaliella tertiolecta* growth in the bioreactor for Experiments 1 and 2 is shown in Figure 5.6. Photos illustrate a relatively poor growth in both experiments, with a transition to the death phase observable in each experiment by day 5.

Experiment 1 (No Aeration)



Day 1 Day 2 Day 3 Day 4 (not recorded) Day 5 Day 6

Experiment 2 (Aeration)



Day 1 Day 2 Day 3 Day 4 Day 5 Day 6

FIGURE 5.6 Photographic comparison of algae growth in the Bioreactor for Experiments 1 and 2 over a 6 day trial period.

5.3 CARBON SEQUESTRATION

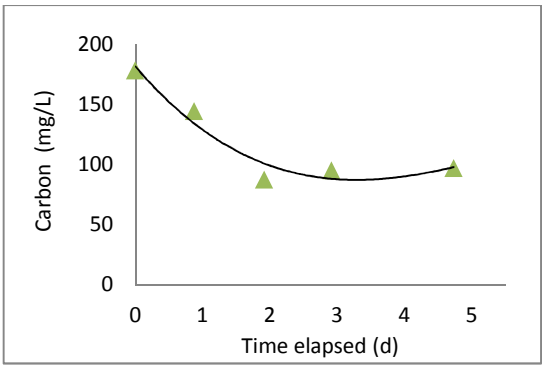
Carbon levels within the media were assessed through daily measurements of Total Carbon (TC), using the Nitrogen Analyser (TOC-VCPH/CPN). Results for Experiments 1 & 2 are shown in Figures 5.7 a) and 5.5 b) respectively. Readings have been adjusted to accommodate tank volume variability, during sampling.

5.3.1 EXPERIMENTS 1 & 2: CARBON LEVELS

Recorded carbon levels in Experiment 1 dropped significantly over the first two days (90.7 mg/L reduction), coinciding with the identified lag, exponential and linear growth phases. Levels then stabilise at approximately 95 mg/L for the remainder of the trail, suggesting no further carbon uptake by the algae. The carbon removal rate for first two days of the trial was 47.36 mg C/L/d.

In Experiment 2, results show a steep decline in carbon levels over the first two days (135.8 mg/L reduction) coinciding with the identified exponential and linear growth phases. The rate of reduction then changes to a shallower grade for the remainder of the trial, a final reading of 15.66 mg/L was recorded. The carbon removal rate was 74.07 mg C/L/d for first two days of the trial, and 11.99 mg C/L/d for the remainder of the trial.

a)



b)

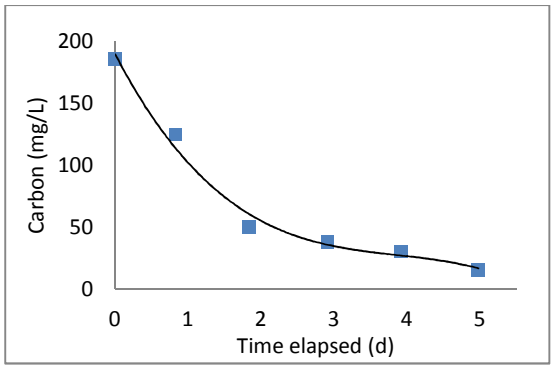


FIGURE 5.7 a) & b) Total Carbon (TC) readings for (a) Experiment 1 (▲) and (b) Experiment 2 (■)

5.3.2 CONTROL TRIAL: CARBON LOSS

A control trial was conducted to identify the rate at which carbon was lost from the CSG media (within the bio-reactor tank), through processes other than aeration stripping or algal absorption. The control trial replicated the parameters of Experiment 1, except for the omission of nutrient addition and algal inoculation. The media was stirred at a constant rate during the trial period, and no aeration was used.

Results found minimal losses (cumulative loss of 12 mg/L) over the duration of a 6 day trial (refer to Section 5.3.3), suggesting that the majority of the media's carbon loss within the bio-reactor results from aeration or algal absorption for experiments 1 and 2.

5.3.3 CARBON SEQUESTION ASSESSMENT

Figure 5.8 shows the TC rates for Experiments 1, Experiment 2, and the control trial. As nutrient addition and algal inoculation are the only differing factors between Experiment 1 and the control trial, the resulting difference in carbon levels between the trials (90.9 mg/L) is likely the result of carbon sequestration by the algae. Similarly, as there was no significant difference in

the growth rate between Experiments 1 and 2, it is likely that the aeration process is the main contributor to the observed difference in carbon reduction between these trials. The final difference in carbon levels between Experiment 1 and Experiment 2 was 81.52 mg/L.

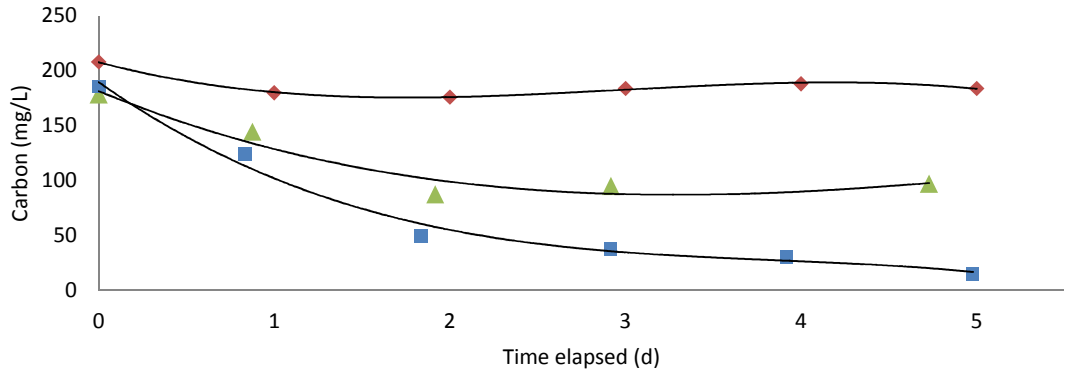


FIGURE 5.8 Carbon depletion rates for Experiment 1 = ▲, Experiment 2 = ■ and the Carbon Loss Control Trial = ◆.

5.4 NUTRIENT REMOVAL

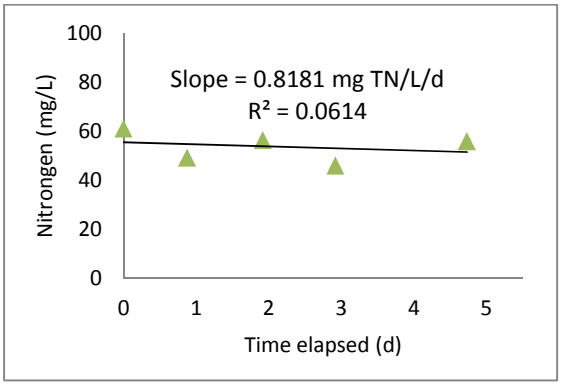
Nutrient depletion was assessed by measuring daily total nitrogen and phosphorous levels in the bioreactor tank. Figures 5.9 a) & b) show the recorded nitrogen levels for Experiment 1 and Experiment 2, respectively. Results have been adjusted to accommodate volume variability within the bioreactor tank, when sampling.

5.4.1 NITROGEN REMOVAL

Results from Experiment 1, document only a slight uniform reduction in nitrogen levels, over the duration of the trial. The nitrogen removal rate for the total trial was 0.818 mg TN/L/d. The algae growth rate in terms of suspended solids from the start of the trial to day 4 was 29.2mg SS/L/d. This gives a specific nutrient removal rate of 0.028 mg TN/SS.

The nitrogen removal rate for Experiment 2 was 1.523 mg TN/L/d. The algal growth rate, from the start of trial to day 4, in terms of suspended solid increase (derived from optical density readings) was 30.1 mg SS/L/d, giving a specific nutrient removal rate of 0.051mg TN/SS.

a)



b)

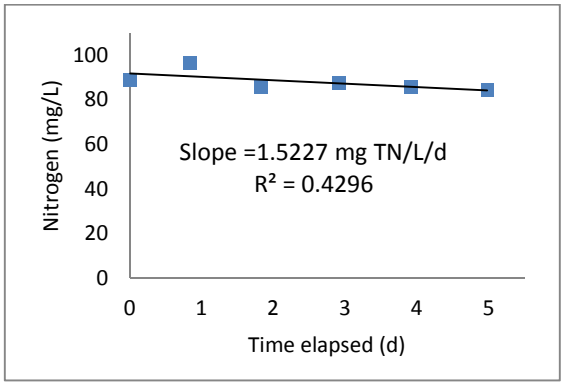


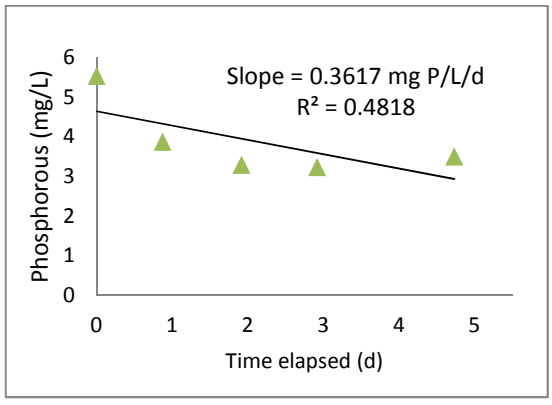
FIGURE 5.9 a) & b) Nitrogen readings for (a) Experiment 1 (▲) and (b) Experiment 2 (■)

5.4.2 PHOSPHOROUS REMOVAL

Figures 5.10 a) & b) show the recorded phosphorous levels for Experiment 1 and Experiment 2, respectively. The phosphorous depletion rate for Experiment 1 was 0.362 mg/L/d giving a specific growth rate of 0.012 P/SS. The ratio of phosphorous to nitrogen depletion was 362:818. The phosphorous depletion rate of Experiment 2 was 0.381mg/L/d, with a specific growth rate of 0.013 P/SS, giving a P:N depletion rate of 381:1523.

The theoretical depletion rate of P:N is between 1:8 and 1:16 (Grobbellar 2004). Current results range from 1:2 to 1:4, this is possibly due to poor correlation coefficient (r^2) in fitting the graphs, indicating that the consumption of N and P did not occur linearly throughout the experiment.

a)



b)

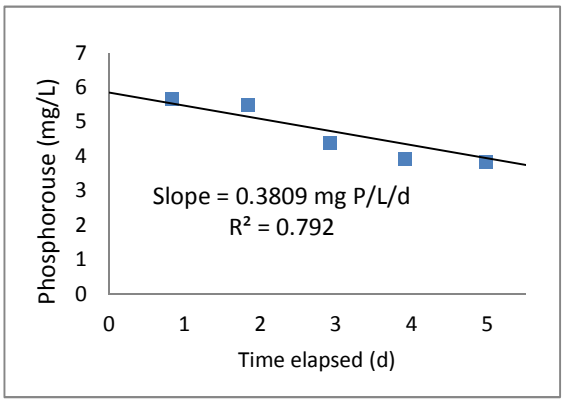


FIGURE 5.10 a) & b) Phosphate readings for (a) Experiment 1 (▲) and (b) Experiment 2 (■)

5.5 pH VARIATION

No CO_2 addition was provided in either of the experiments. Therefore pH variability within the bio-reactor, resulting from algal behaviour, was influenced predominantly by light source availability. The light source was set on a 12hr on/off cycle. The controlled pH range was set at 7.7, with a tolerance of ±0.6, allowing a maximum pH value of 8.3. Fabergas et al. (1993) suggests that algal strands can sequestrate bicarbonate (HCO_3^-) from a media up to a maximum pH of 8.3, with optimal sequestration at 7.5.

5.5.1 EXPERIMENT 1: pH VARIATION

The pH fluctuations for Experiment 1 are shown in Figure 5.11. pH levels rose steadily at all identified growth phases. Dark (troughs) and light (peaks) periods are shown by the added red line. Levels increased in dark and light phases, with predictably sharper rates of increase observed during light periods. Within the lab setting, limited light source availability still existed during the dark periods. The obtained results suggest that the algae *Dunaliella tertiolecta* still had photosynthesis capacity under these limited light source conditions.

Figure 5.9 also suggest, a gradual reduction in the pH buffering capacity of the media. Observable sudden drops in pH, resulted from the bioreactors automated system adding acid when pH levels exceeded 8.3. As the trial progressed, the regulated acid input (10mL) generally caused larger reductions in pH levels. Bicarbonate acts as a pH buffer, the higher the bicarbonate concentration the higher the pH buffering capacity of a solution (Reference). Therefore the observable loss of pH buffering capacity with time, suggests a reduction of the media's bicarbonate concentration.

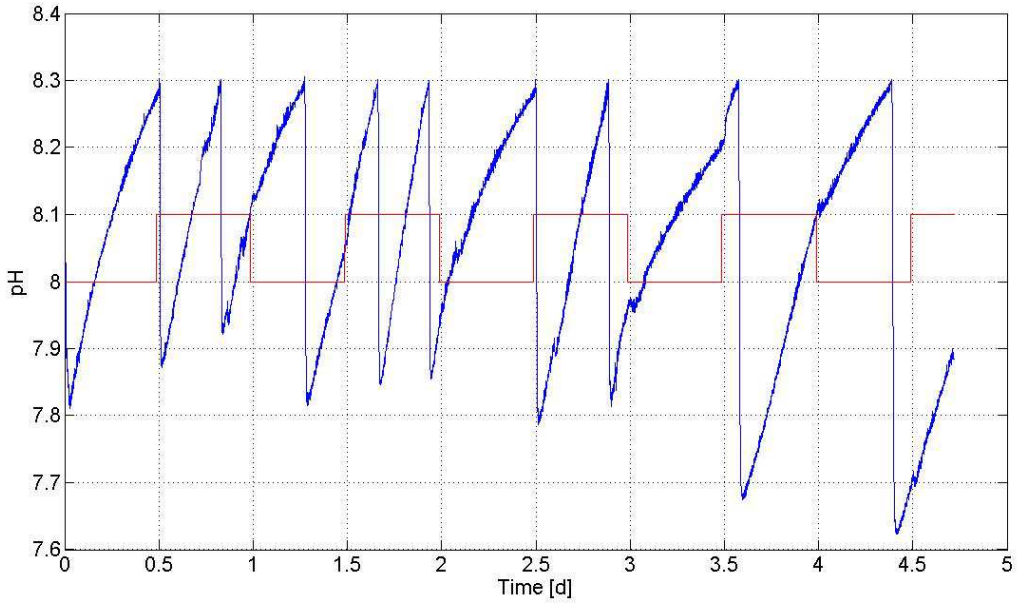


FIGURE 5.11 Experiment 1 – Bioreactor pH readings for the 5 day trial.

Figure 5.12 shows the pH readings for days 1, 2, 3 & 4 of Experiment 1, over a 24 hour period. Based on observations from the growth behaviour shown in Figure 5.1, these days correspond respectively with the identified lag/exponential, linear, stationary and stationary/death growth phases. All 24 hour readings start at midnight, splitting the dark period into two 6 hour sections.

The pattern of pH variability was characteristically similar for all growth phases of the experiment, with steady rises in pH levels in the dark and light periods. For all readings, a slight reduction in the pH gradient was generally observed when pH levels approached 8.3.

In the light periods, the sharpest increase in pH levels was observed on day 2 (linear), approximately 0.073 pH unit/h. The shallowest light period gradient occurred on day 4 (stationary/death), approximately 0.036 pH unit/h. In the dark period, the steepest pH gradient occurred at the end of day 2 (linear), approximately 0.047 pH unit/h, and the slowest pH increase occurred at the end of day 4 (stationary/death), approximately 0.021 pH unit/h.

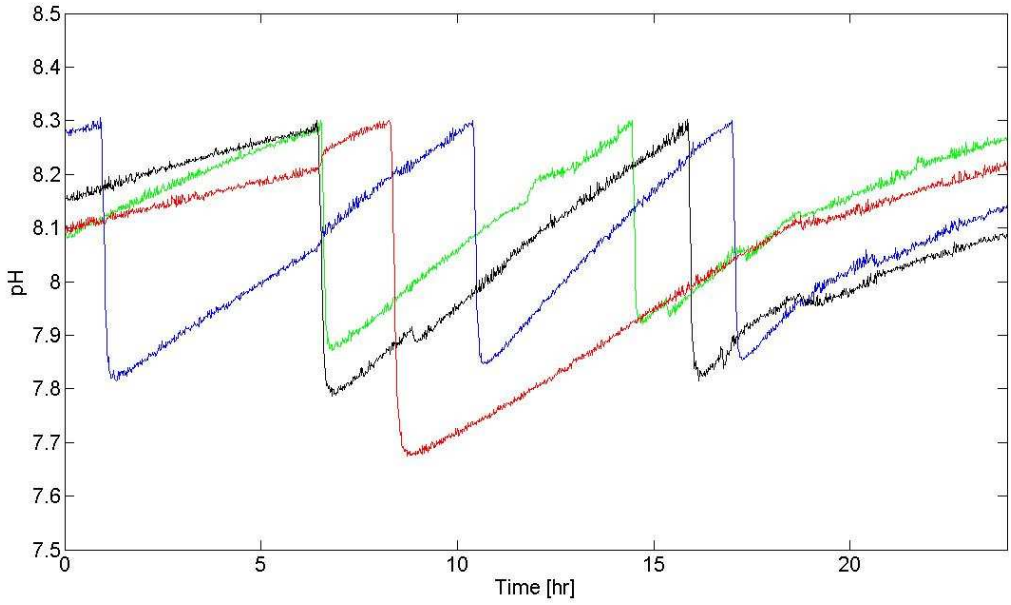


FIGURE 5.12 Experiment 1 – Bioreactor pH readings over a 24 hour period for Day 1 (—), Day 2 (—), Day 3 (—) and Day 4 (—).

5.5.2 EXPERIMENT 2: pH VARIATION

The pH levels for Experiment 2 are presented in Figure 5.13. Sharp steady increases in pH were observed for the first 3 days of the experiment, after which results became more erratic. Dark and light periods are again indicated by the added red line. The pH increased during both periods, with slightly steeper gradients observable during light periods.

The constant supply of compressed air may have attributed to the steep pH gradients observed during both dark and light periods. If the concentration of one of the ions on the right side of Equation 5.1 is increased, the reaction is driven to the left, forming the gases H_20 and CO_2. Bicarbonate and carbonate ions in the water will form carbon dioxide, which can be removed by aeration (GE Power and Water 2011).

H_2O	+	CO_2	« »	H^+	+	HCO_3^-	Eqn. 5.1
water		carbon dioxide		hydrogen ion		bicarbonate ion	

In this process, H^+ will be removed (with HCO_3^-), and the removal of H^+ will increase the pH of the solution. This reduction in HCO_3^- is also supported by the TC measurements for Experiments 1 and 2. The timing within the trial of the sharper rates of loss of carbon from the media (Section 5.3.1) generally coincides with observed steeper pH gradients.

Assessing figure 5.13, the first 3 days coincide with the lag, exponential and linear growth periods, as identified in Figure 5.3. Results for this period clearly demonstrated a steady reduction in the buffering capacity as the trial progresses, suggesting a constant decline in the bicarbonate concentration of the media. By day 4, the buffer capacity of the solution had reduced to a level such that, the 10ml acid addition caused the pH to drop below the lower range limit of 7.1, which led to the automated base addition. The acid/base input quantity was subsequently adjusted to 6ml, steady results were achieved for the remainder of day 4. Minimal pH variability was observed on day 5 (death phase).

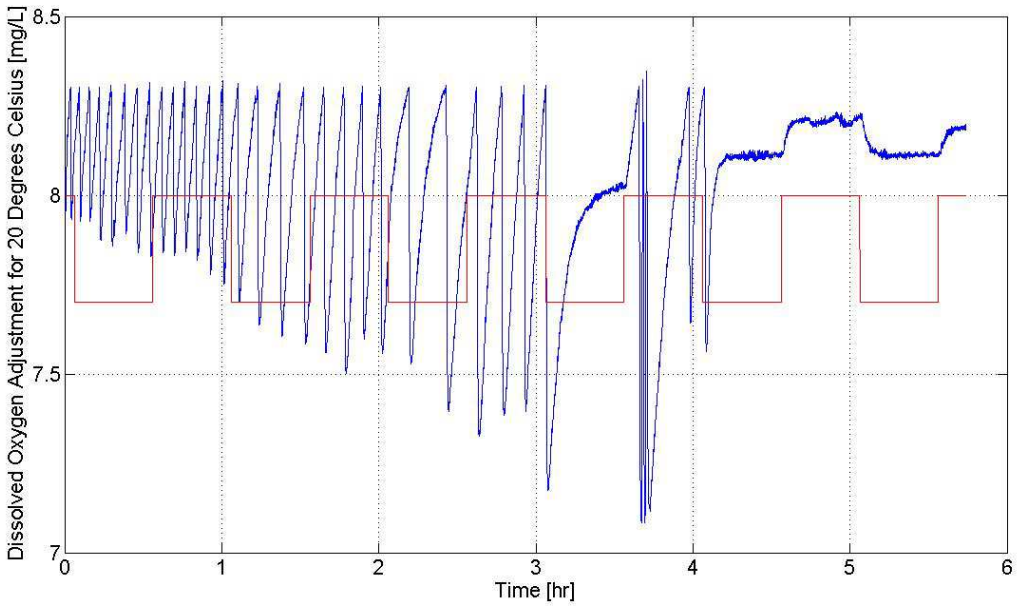


FIGURE 5.13 Experiment 2 – Bioreactor pH readings for the 6 day trial.

Figure 5.14 shows the pH results over a 24 hour period for days 1, 2, 3 and 4, corresponding with the identified lag/exponential, linear, stationary and stationary/death phases respectively. All 24 hour readings start at midnight, splitting the dark period into two 6 hour sections. Results show relatively consistent pH patterns for all days, with the exception of day 4. A distinct change

(reduction) in the gradient, above a pH reading of 8 was frequently observable on the first 3 days of the trial.

Significant increases in pH were observed in both dark and light periods. In the light periods the steepest average pH gradients were observed on Day 1 (lag/exponential), approximately 0.331 pH unit/h. The shallowest average gradients occurred on Day 4 (stationary/death), approximately 0.195 pH unit/h. In the dark period the steepest average pH gradient was recorded at the start of day 1 (lag/exponential), approximately 0.109 pH unit/h. A negative pH gradient was observed at the end of day 4 (stationary/death), approximately -0.042 pH unit/h.

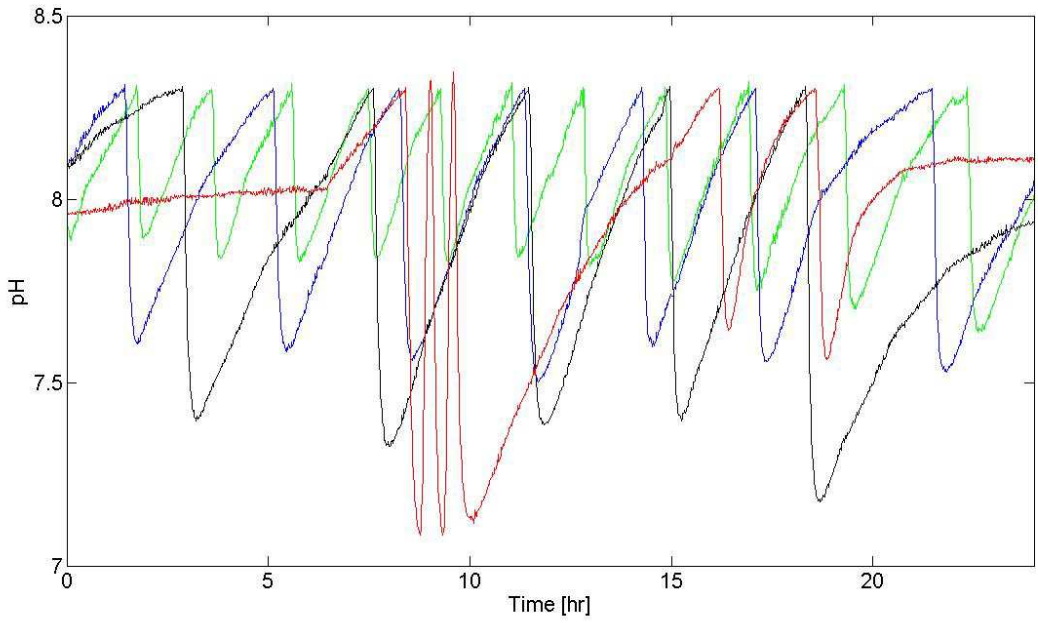


FIGURE 5.14 Experiment 1 – Bioreactor pH readings over 24 hour period for Day 1 (—), Day 2 (—), Day 3 (—) and Day 4 (—).

5.5.3 pH VARIATION SUMMARY

The pH results of the 2 trials show significantly sharper increases in pH levels in Experiment 2. The cause of this steeper gradient is likely due to a combination of algal growth and the removal of H^+ from the solution through carbon stripping.

The sharper increases in pH, may prove to be problematic as regular acid input was required to maintain pH levels within the optimal growth range. Consequently algae growth conditions that facilitate a steady and prolonged pH increase time may prove to be advantageous. Overall growth results from section 5.2 suggest only limited benefit exists in implementing aeration.

5.6 DISSOLVED OXYGEN VARIATION

Dissolved oxygen (DO) readings were recorded in real-time, and measurements were logged on a per minute basis. DO variability was influenced predominantly by light source intensity and aeration. An abundance of light facilitated photosynthesis, and hence oxygen production (Moheimani 2005).

5.6.1 EXPERIMENT 1: DO VARIATION

Figure 5.15 shows the DO concentrations for Experiment 1, a light and dark phase line has been included. As expected higher DO values were recorded during the light periods. Results show DO readings peaking at (max 10.55 mg/L) day 2, after which levels declined. The instantaneous spikes in DO readings (approximately 1.5mg/L increase), observed during the light period on the first four days of the experiment, resulted from the experimenter removing algae that had clumped to the probe.

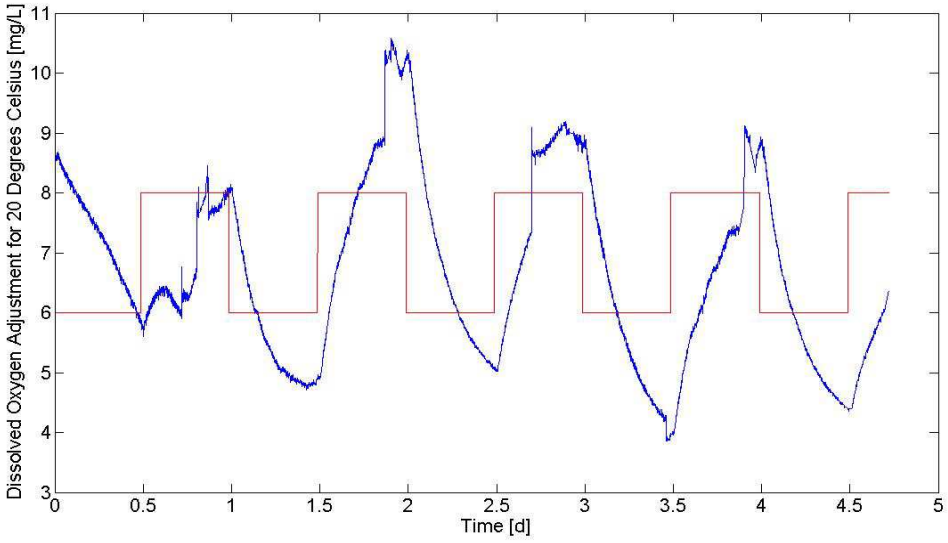


FIGURE 5.15 Experiment 1 – Bioreactor DO readings for the 6 day trial.

Figure 5.16 compares average maximum DO readings during the light period with average maximum DO readings in the dark period, for Experiment 1. The relative difference between these two sets of values provides an estimate of the net oxygen produced during each of the light periods. The saturation value of DO at 20°C is 9.17 mg/L, this is also indicated on the graph, as is the measured optical density readings for this trial.

The highest recorded DO production reading was 5.6 mg /L, on day 2. Dissolved oxygen production levels increased until the end of day 2 (linear), before declining. This increase in DO production is likely the result of a growing volume of active biomass during the lag, exponential and linear phases.

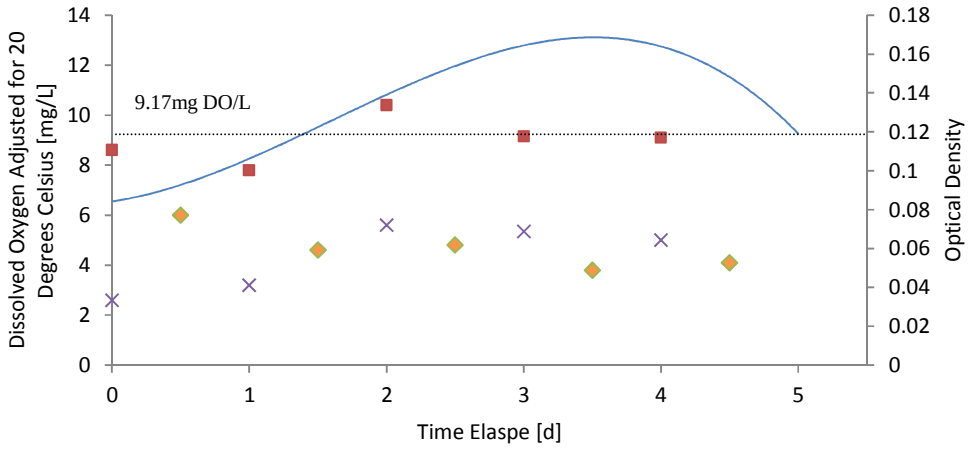


FIGURE 5.16 Experiment 1 –Average maximum DO readings during light period (■), average minimum DO readings during dark period (◆), Net oxygen produced during the day time (X) and Optical Density results (—).

Figure 5.17 shows the estimate oxygen production quantities in the light periods, for each day of Experiment 1. Quantities were derived using equation 3.8. Results show a peak oxygen production of 1.74 mg, produced on day 3.

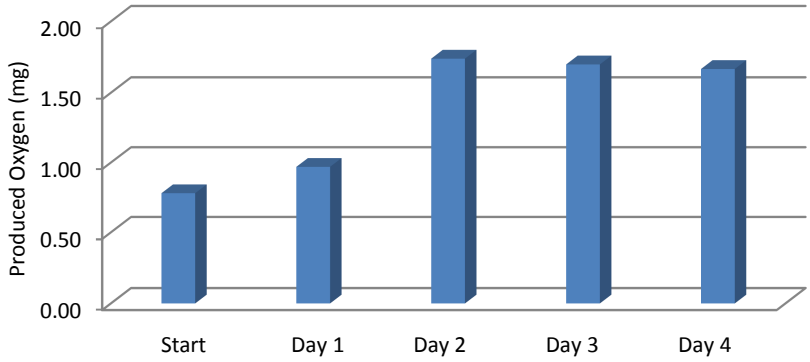


FIGURE 5.17 Experiment 1 – Daily oxygen quantities produced during the light period

5.6.2 EXPERIMENT 2: DO VARIATION

Figure 5.18 shows the DO dynamics for Experiment 2, a line indicating light and dark periods has been included. The results show an increase in DO readings until day 3 (max 8.35 mg/L), followed by a rapid decline in DO variability as the trial reaches the death phase. The instantaneous spikes in DO readings (approximately 1 to 1.5mg/L), observed during the first five days of the experiment, resulted from the removal of algae that had clumped to the DO probe.

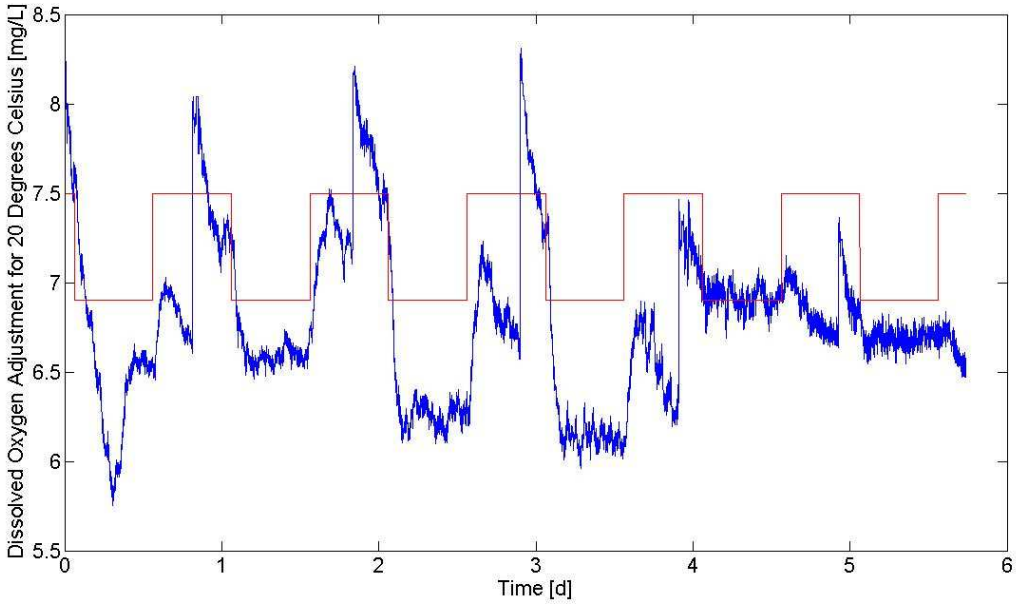


FIGURE 5.18 Experiment 2 – Bioreactor DO readings for the 6 day trial.

Figure 5.19 shows the average maximum light and dark period readings for Experiment 2, and the derived relative DO production during the light periods. A maximum DO production reading of 2.4 mg/L was recorded on day 3 (linear/stable). Results show a slight increase in DO production until day 3, after which reading reduce.

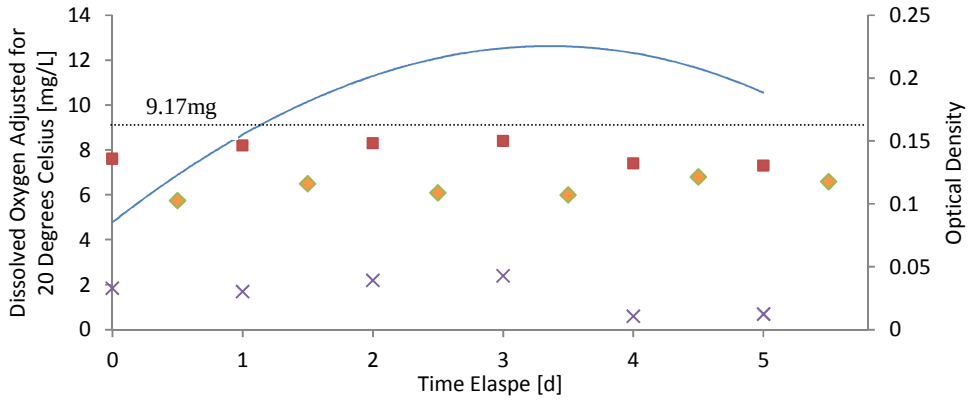


FIGURE 5.19 Experiment 2 –Average maximum DO readings during light period (■), average minimum DO readings during dark period (◆), Net oxygen produced during the day time (X) and Optical Density results (—).

Figure 5.20 shows the estimate oxygen production quantities in the light periods, for each day of Experiment 2. Quantities were derived using equation 3.8. Results show a peak production of 0.75 mg of oxygen produced on day 4.

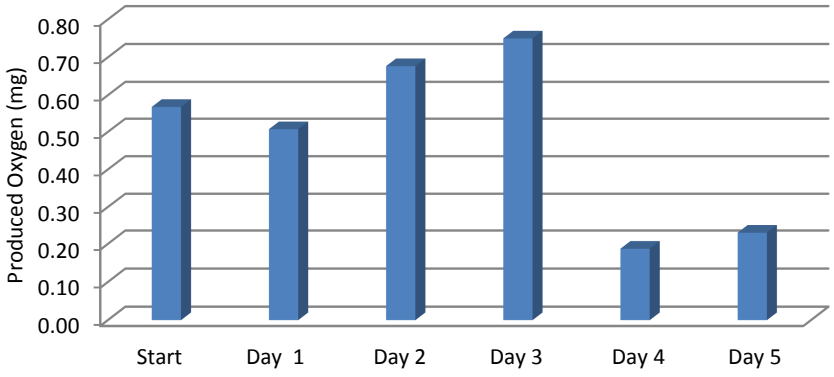


FIGURE 5.20 Experiment 2 – Daily oxygen quantities produced during the light period

5.6.3 DISSOLVED OXYGEN MEASUREMENT SUMMARY

Peak DO readings and estimated DO production levels were higher in Experiment 1, suggesting that aeration restricted the DO variability within the bioreactor for the growth of *Dunaliella Tertiolecta*. A review of the literature found no explanation for this occurrence. The difference may result from oxygen stripping in the aeration trials, similar to the carbon stripping observed in section 5.3.

5.7 ALGAE DRY MASS AND LIPID CONTENT

Algae were not harvested in either trial, due to poor growth performance.

5.8 ENCOUNTED PROCEDURAL ISSUES

5.8.1 ALGAL SETTLEMENT AND CLUMPING

Algal clumping onto the DO probe affected DO readings for both experiments. Attempts were made to lessen algae clumping by positioning the probe within areas of high turbulence, created by the stirrer, however this proved to be unsuccessful. The only method for removing clumps was to manually tap the probe. The resulting spike in DO levels after removing algal clumps (1 to 1.5mg/L) is documented in Figures 5.15 & 5.18. Algal clumping on the pH probe was not significant, and no effect on recorded results was observed.

5.8.2 POOR GROWTH RESULTS FOR DUNALIELLA TERTIOLECTA IN CSG WATER

The growth performance of *Dunaliella tertiolecta* in CSG media, in each of the trials was poor. Subsequent assessment studying the effect of salinity on growth found significant improvements in growth for both trials under modified salinity levels (refer Chapter 6).

5.9 CONCLUSIONS

Research has provided insight into the growth characteristics of microalgae *Dunaliella tertiolecta* in a CSG medium. The algae grew, although poorly, in both Experiments 1 and 2.

Optical density results suggest a slight overall benefit in aeration to facilitate the growth. The assessment of pH variability found that aeration significantly increased the pH gradient, and this was likely a result of the combination of algal growth and the removal of H^+ through carbon stripping. The subsequent steep rises in pH resulted in significant acid addition to moderate pH levels. Furthermore, aeration was found to cause losses of carbon from the media through stripping. Results suggest a potential for algal farming in CSG water without aeration.

CHAPTER 6 GROWTH CHARICTERISTICS AND LIPID PRODUCTION OF MICROALGAE DUNALLIELLA TERTIOLECTA GROWN IN SALINITY MODIFIED CSG WATER MEDIA AND THE EFFECTS OF AERATION.

This chapter presents the results and discussions on the optimal salinity, growth behaviour, carbon sequestration and nutrient removal of *Dunaliella Tertiolecta* in CSG water, using batch experiments. The discussions further extend to interpret the observations on real-time monitoring using the dissolved oxygen and pH probes during the growth period. This chapter ends with the assessment of lipid production from the harvested trials.

6.1 OPTIMAL SALINITY IDENTIFICATION

Initial assessment was made to identify the approximate optimal salinity level to facilitate growth of *Dunaliella tertiolecta* in a CSG medium. Four batch trials were conducted with salinity levels (NaCl) of 3.5g/L (CSG water), 10 g/L, 20 g/L and 35 g/L (seawater equivalent). At the commencement of the trials 250 ml of CSG water with modified salinity levels, were inoculated with 10 ml of *Dunaleilla tertiolecta* culture (0.28 mg of biomass), 5 ml/L of F2 concentrate and 1344mg/L of Sodium Bicarbonate ($NaHCO_3$).

Trials were carried out simultaneously in 250ml flasks, over a 7 day period. Photo-energy was provided by means of a light source, implemented on a 12 hour on (6am to 6pm) /off cycle. Optical density reading results (Figure 6.1) show optimal growth was achieved in the 10g NaCl/L trial. The trial with unmodified salinity levels (3.5g Nacl/L) performed comparatively poorly. Therefore it was concluded from these preliminary investigations that *Dunaliella Tertiolecta* would optimally grow in a salinity of 10 g/L, and subsequent trials were conducted using the modified CSG water with 10 g NaCl/L.

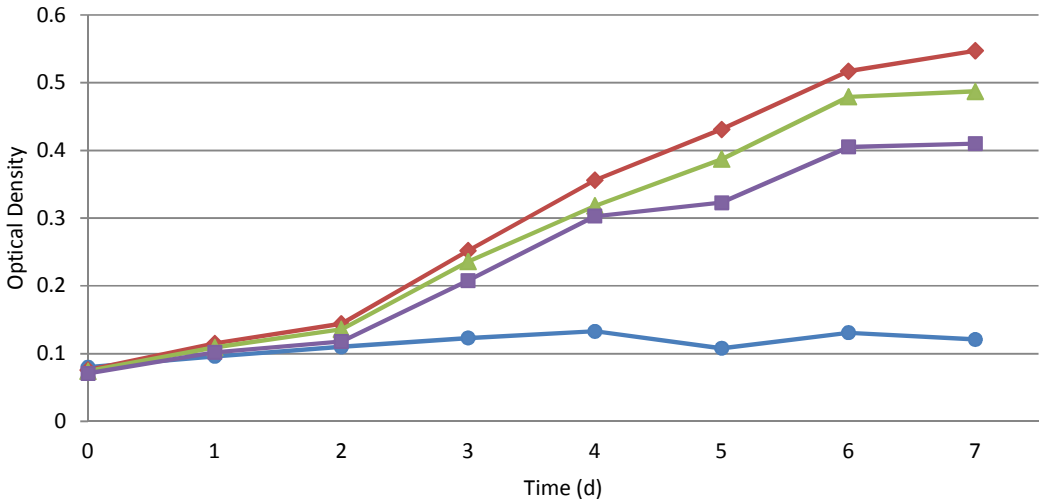


FIGURE 6.1 Algal growth in CSG water media measured with Optical Density at 505 nm with salinity levels of 3.5g NaCl/L (●), 10g NaCl/L (◆), 20g NaCl/L (▲) and 35g NaCl/L (■).

6.2 BATCH EXPERIMENTS

Batch experiments were repeated using microalgae *Dunaliella Tertiolecta* in a CSG water media, with the modified salinity of 10g NaCl/L). Trials were differentiated by the inclusion of aeration input in Experiment 4. The purpose of this distinction was twofold, to determine the effects of aeration on algal growth, and to test for a carbon stripping effect through aeration.

6.2.1 EXPERIMENT 3 (SALINITY MODIFIED CSG WATER MEDIA – NON-AERATION)

Experiment 3 was conducted to assess the growth capacity of microalgae *Dunaliella Tertiolecta* in a salinity modified (10g NaCl/L) CSG water medium, without aeration input. The experiment was intended as a lab scale assessment, to evaluate the algae's capacity to be grown in an open pond setting. Monitoring of growth and nutrient removal was conducted for the entirety of the trial. Photo-energy required for the algal growth was provided by means of a light source, implemented on a 12 hour on (6am to 6pm)/off cycle.

At the commencement of the trial, 3 litres of filtered CSG water was inoculated with 250 ml of *Dunaliella tertiolecta* culture (0.193 g of biomass), 7.5 g/L of NaCl and 5 ml/L of F2 concentrate. Sodium Bicarbonate ($NaHCO_3$) was added to the media (1344mg/L) to increase carbon levels to average CSG water conditions. The resulting C:N:P ratio was 192:85:8.5. Refer to Section 3.3 for further details.

6.2.2 EXPERIMENT 4 (SALINITY MODIFIED CSG WATER MEDIA – AERATION)

Experiments 4 replicated Experiment 3, except for the addition compressed air, which was fed into the bioreactor at a constant rate of 250ml/min. It was predicted that growth, and subsequent lipid production and nutrient removal would increase with a constant aeration feed. Furthermore, it was hypothesised that aeration would significantly reduce bicarbonate levels within the media through stripping. Refer to Section 1.7.4 for details of aeration properties.

At the commencement of the trial, 3 litres of filtered CSG water was inoculated with 250 ml of *Dunaliella tertiolecta* culture (0.198 g of biomass), 7.5 g/L of NaCl and 5 ml/L of F2 concentrate. Sodium Bicarbonate ($NaHCO_3$) was added to the media (1344mg/L) to increase bicarbonate levels. The C:N:P ratio for the batch experiment was 192:85:8.5. Refer to Section 3.3 for further details.

6.3 ALGAL GROWTH

Algal growth was quantified using daily Spectrometer measurements. Photos were taken to document the phase transition of algal growth.

6.3.1 EXPERIMENT 3: GROWTH CHARICTERISTICS

Figure 6.2 shows the recorded growth trends of *Dunaliella tertiolecta* in Experiment 3, represented through optical density measurements. The added trend line is based on a 3rd order polynomial. Results suggest a lag phase occurring within the first day of the experiment. The exponential growth period appears to take place between days 1 and 2, with a linear growth phase from days 2 to 5. Results suggest a possible transition to the stationary phase at the end of day 5. The trial was stopped for harvesting before the death phase was reached.

Approximately 100ml of media in the bioreactor was lost per day as a result of evaporation and sampling. Once the total volume of the media reduced to 3L, distilled water was used to replace any further losses within the bioreactor.

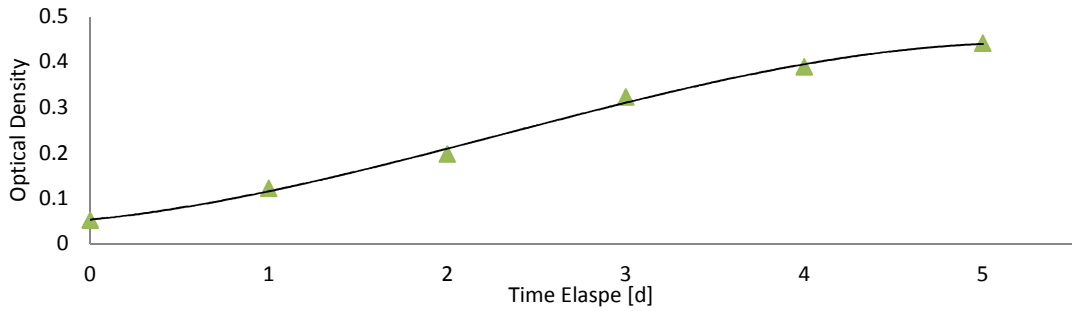


FIGURE 6.2 Experiment 3 – Algal growth in salinity modified CSG water media without aeration addition measured with Optical Density (▲) at 505 nm

Figure 6.3 shows the estimated algae growth rate in terms of suspended solids for Experiment 3 (0.935 g SS/L/d). The initial suspended solids reading was obtained at the commencement of the trial, through the process of measuring biomass inoculation quantities. All other values were derived from optical density readings, based on an assumed equal correlation between measurement methods. Refer to section 6.5, for application of the algal growth rate to determine nutrient removal rates.

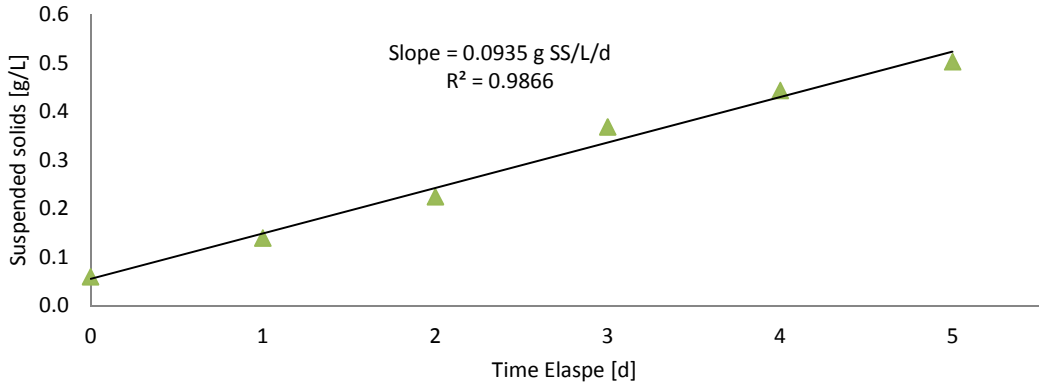


FIGURE 6.3 Experiment 3 – Algal growth in salinity modified CSG water media without aeration addition measured with derived Suspended Solids readings (▲).

6.3.2 EXPERIMENT 4: GROWTH CHARICTERISTICS

Figure 6.4 shows the recorded growth characteristics of *Dunaliella Tertiolecta* with compressed air input. The trend line was again derived using a 3rd order polynomial. The data suggests a possible lag phase early on day 1, an exponential growth phase between days 1 to 3, and a linear growth trend from day 3 to the end of the trial. The trial was stopped for harvesting before the stationary and death phase were reached.

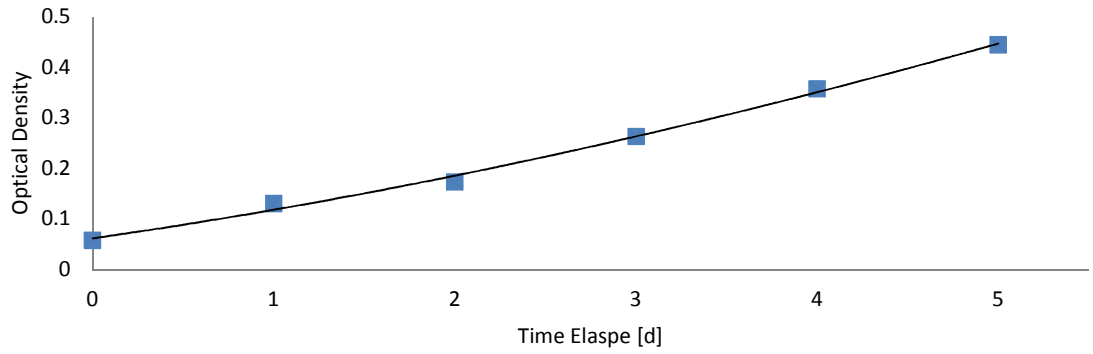


FIGURE 6.4 Experiment 4 - Algal growth in salinity modified CSG water media with aeration measured with Optical Density (■) at 505 nm.

Figure 6.5 shows the estimated algae growth rate in terms of suspended solids for Experiment 4 (0.081 g SS/L/d). Refer to section 5.4, for application of the algal growth rate to determine nutrient removal rates.

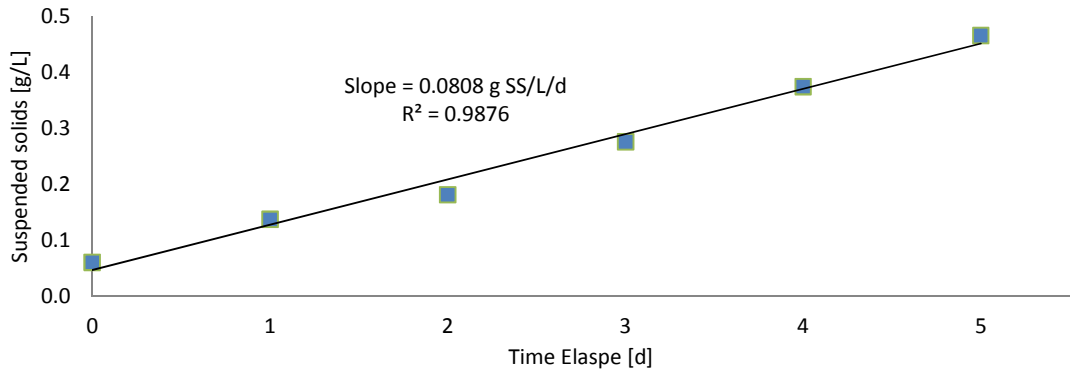


FIGURE 6.5 Experiment 4 – Algal growth in salinity modified CSG water media with aeration addition measured with derived Suspended Solids readings (■)

6.3.3 LOW BICARBONATE CONTROL TRIAL

A 5 day control trial with reduced bicarbonate levels was conducted to validate the benefit of growing microalgae *Dunaliella tertiolecta* in a carbon rich solution. Refer to Section 6.3.4 for growth results. The control trial replicated the parameters of Experiment 3, with the exception of the reduced bicarbonate concentration of the media.

In preparation for the trial, 4 L of CSG media was aerated in the bioreactor tank for 3 days to reduce the bicarbonate concentration of the solution. At the commencement of the trial the carbon concentration was 33.6 mg C/L. No aeration was used during the trial period. Refer to Section 6.4 for details of carbon variability during the trial.

At the commencement of the trial, 3 litres of filtered CSG water was inoculated with 250 ml of *Dunaliella Tertiolecta* culture (0.245 g of biomass), 7.5 g/L of NaCl and 5 ml/L of F2 concentrate. The C:N:P ratio for the batch experiment was 33.6:85:8.5.

6.3.4 ALGAL GROWTH SUMMARY

Comparison was made of the growth characteristics between Experiment 3, Experiment 4 and the low bicarbonate control trial (Figure 6.6). Results show both Experiment 3 and 4 performing significantly better than the control trial, indicating a distinct benefit in growing *Dunaliella tertiolecta* in a carbon rich solution. Furthermore, growth measurements for Experiments 3 and 4 were comparatively similar, suggesting no identifiable benefit in implementing aeration.

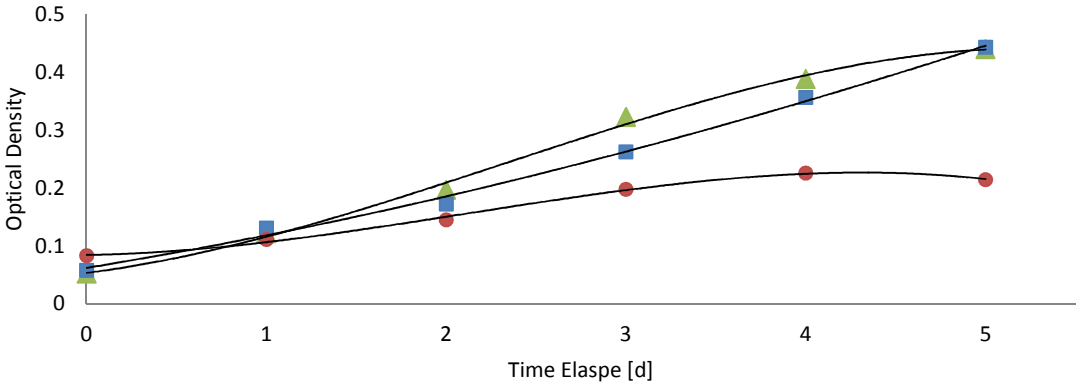


FIGURE 6.6 Experiment 3, 4 & Low Bicarbonate Control Trial – Comparison of *Dunaliellla tertiocleta* growth in a salinity modified CSG water media measured with Optical Density readings (Experiment 3 = ▲, Experiment 4 = ■ and Low Bicarbonate Control Trial = ●) at 505 nm

Figure 6.7 compares growth trends for Experiments 1, 2 in the salinity level 3.5g NaCl/L (conducted in previous trials), with growth trends for Experiments 3 and 4, with salinity level 10 g NaCl/L. Results clearly demonstrate a benefit in increasing the salinity level to 10g NaCl/L.

The capacity for increasing the salinity levels in an outdoor pond setting could be achieved through the implementation of reverse osmosis (RO) processes. Brine output from RO treatment could be inputted into CSG water pond to increase salinity levels to optimise algal growth conditions.

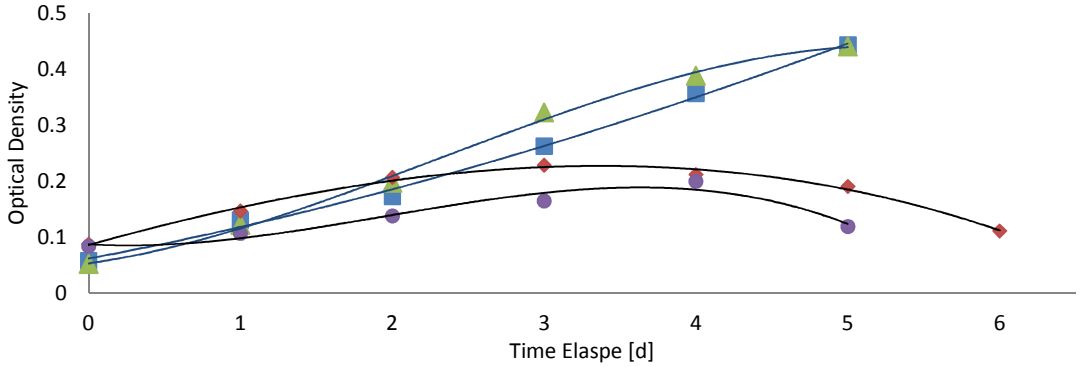


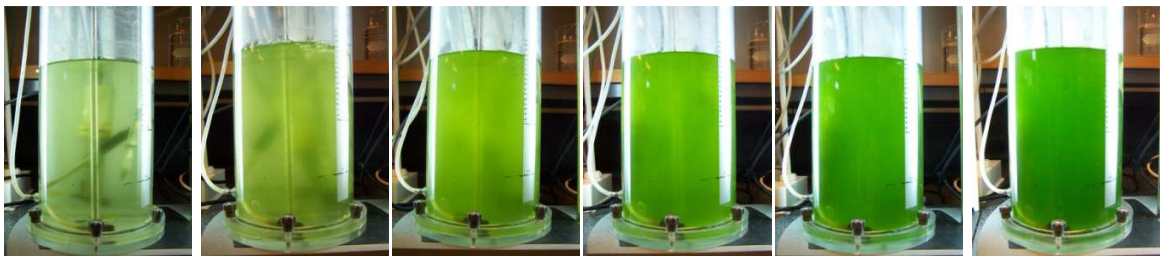
FIGURE 6.7 Experiment 1, 2, 3 and 4 – Comparison of *Dunaliella tertiolecta* growth in CSG water with salinity levels 3.5g NaCl/L (Experiment 1 = ● & Experiment 2 =◆) and 10g NaCl/L (Experiment 3 = ▲ and Experiment 4 = ■) measured with Optical Density readings at 505 nm

A comparison of the photographic documentation of *Dunaliella Tertiolecta* growth in the bioreactor for Experiments 3, Experiment 4 and the low bicarbonate control trial is shown in Figure 6.8. Photos illustrate high growth in both Experiments 3 & 4, and moderate growth in the control trial (low bicarbonate).

Experiment 3 (No Aeration)

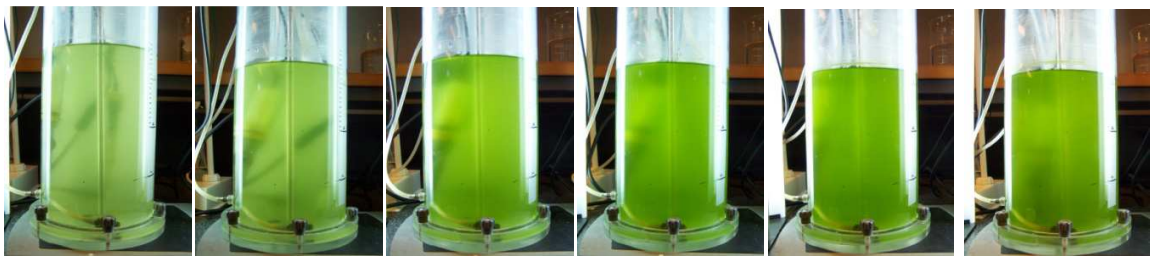
Day 1 Day 2 Day 3 Day 4 Day 5 Day 6

Experiment 4 (Aeration)



Day 1 Day 2 Day 3 Day 4 Day 5 Day 6

Control Trial (Low Bicarbonate)



Day 1 Day 2 Day 3 Day 4 Day 5 Day 6

FIGURE 6.8 Photographic comparison of algae growth in the Bioreactor for Experiments 3, Experiment 4 and the Low Carbon Control Trial over a 6 day trial period.

6.4 CARBON SEQUESTRATION

Carbon levels within the media were assessed through daily measurements of Total Carbon (TC), using the Nitrogen Analyser (TOC-VCPH/CPN). Results for Experiments 3, 4 and the low bicarbonate control trial are shown in Figures 6.9 a), b) and c) respectively. Readings have been adjusted to accommodate tank volume variability, during sampling.

6.4.1 EXPERIMENTS 3, 4 AND CONTROL TRIAL: CARBON LEVELS

Recorded carbon levels in Experiment 3 were steady for the first day of the trail. After day 1 carbon levels decreased at a uniform rate for the remainder of the trial. The total carbon reduction for this 4 day period was 120.9mg/L and the carbon removal rate was 29.29 mg C/L/d.

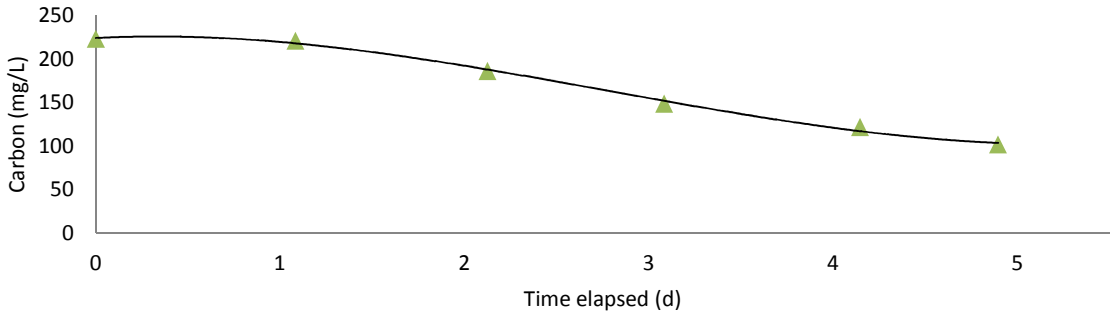


FIGURE 6.9 a) Total Carbon (TC) readings for Experiment 3

In Experiment 4, results show a steady decline in carbon levels over the first three days (148.3 mg/L reduction) coinciding with the identified lag, exponential and linear growth phases. The carbon removal rate for this period was 49.4 mg C/L/d. Only a minimal carbon reduction was recorded for the remainder of the trial (3.37 mg C/L) with a rate of reduction of 1.98 mg C/L/d.

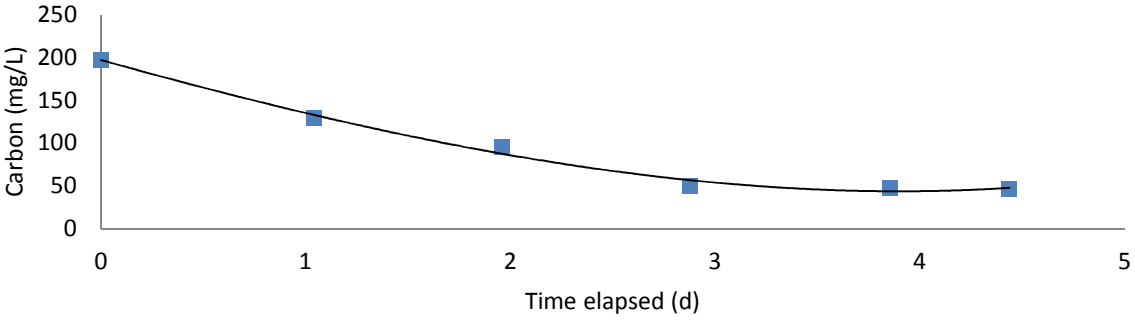


FIGURE 6.9 b) Total Carbon (TC) readings for Experiment 4

In the control trial a steady decline in Carbon levels was observed over the first three days (29.1 mg/L reduction) coinciding with the identified lag, exponential and linear growth phases. The carbon removal rate for this period was 9.1 mg C/L/d. No carbon reduction was recorded for the remainder of the trial.

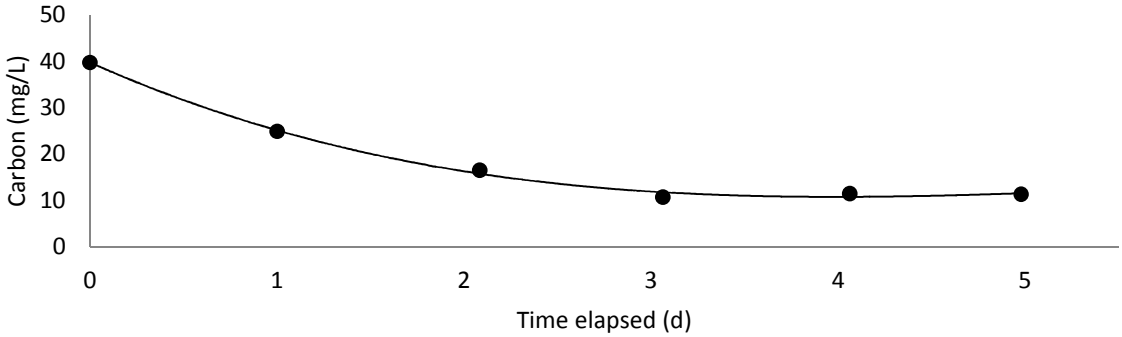


FIGURE 6.9 c) Total Carbon (TC) readings for the Low Bicarbonate Control Trial

6.4.2 CARBON SEQUESTION ASSESSMENT

Figure 6.10 shows the TC rates for Experiments 3, Experiment 4, the low bicarbonate control trial and the carbon loss control trail (Section 5.3.2). Nutrient addition, algal inoculation and salinity adjustment are the only differing factors between Experiment 3 and the carbon loss control trial, therefore the resulting difference in carbon levels between the trials (82.2 mg/L) is likely the result of carbon sequestration by the algae. Similarly, as there was no significant difference in the growth rate between Experiments 3 and 4, it is likely that the aeration process is the main contributor to the observed difference in carbon reduction between these trials. The final difference in carbon levels between Experiment 3 and Experiment 4 was 72.40 mg/L.

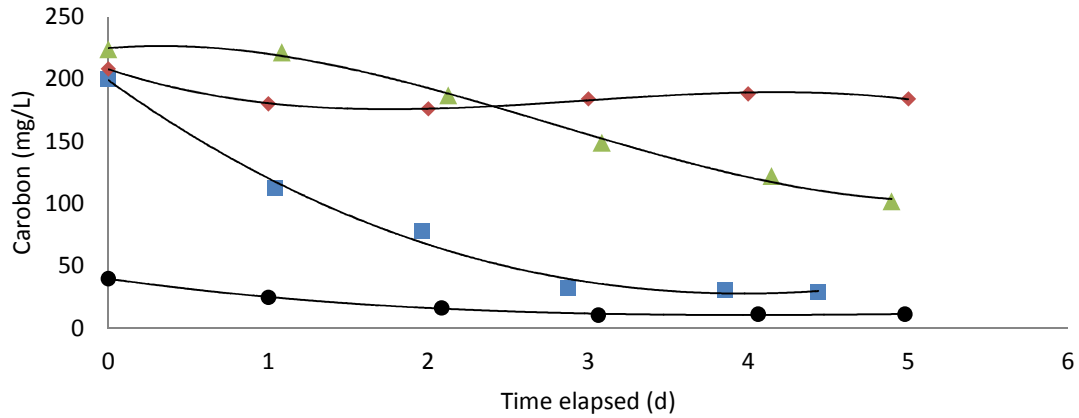


FIGURE 6.10 Experiment 3, Experiment 4, Carbon Loss Control Trial and Low Bicarbonate Control Trial – Carbon Depletion Rates (Experiment 3 = ▲, Experiment 4 = ■, Carbon Loss Control Trial = ● and Low Bicarbonate Control Trial = ◆).

6.5 NUTRIENT REMOVAL

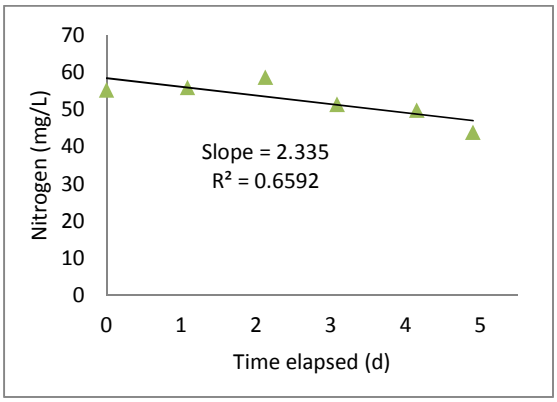
Nutrient depletion was assessed by measuring daily total nitrogen and phosphorous levels in the bioreactor tank. Figures 6.11 a) & b) show the recorded nitrogen levels for Experiment 3 and Experiment 4, respectively. Results have been adjusted to accommodate volume variability within the bioreactor tank, when sampling.

6.5.1 NITROGEN REMOVAL

Results from Experiment 3, shows uniform reduction in nitrogen levels over the duration of the trial period. The nitrogen removal rate for the total trial was 2.335 mg TN/L/d. The algae growth rate in terms of suspended solids for Experiment 3 was 93.5 mg SS/L/d, giving a specific nutrient removal rate of 0.025 mg TN/SS.

The nitrogen removal rate for Experiment 4 was 2.808 mg TN/L/d. The algal growth rate for Experiment 4, in terms of suspended solid increase (derived from optical density readings) was 80.8 mg SS/L/d, giving a specific nutrient removal rate of 0.035mg TN/SS.

a)



b)

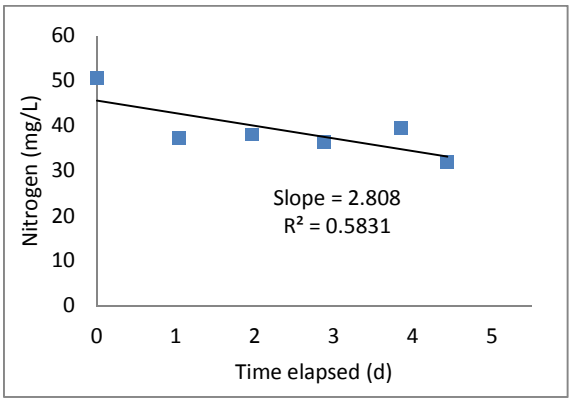


FIGURE 6.11 a) & b) Nitrogen readings for (a) Experiment 3 (▲) and (b) Experiment 4 (■)

6.5.2 PHOSPHOROUS REMOVAL

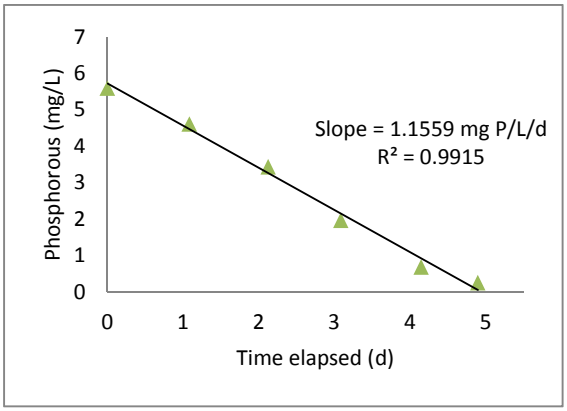
Figures 6.12 a) & b) show the recorded phosphorous levels for Experiment 3 and Experiment 4, respectively. The phosphorous depletion rate for Experiment 3 was 1.156 mg/L/d giving a specific growth rate of 0.012 P/SS. The ratio of phosphorous to nitrogen depletion was

1.156:2.335. The phosphorous depletion rate of Experiment 4 was 0.959mg/L/d, with a specific growth rate of 0.012 P/SS, giving a P:N depletion rate of 0.959:2.808.

The theoretical depletion rate of P:N is between 1:8 and 1:16 (Grobbellar 2004). Current results range from 1:2 to 1: 3, this is possibly due to poor correlation coefficient (r^2) in fitting the graphs especially for nitrogen depletion, indicating that the consumption of N and P did not occur linearly throughout the experiments.

The observed low Phosphorous levels at the end of the trial period may have attributed to the transition to a stationary phase, observed in experiment 3.

a)



b)

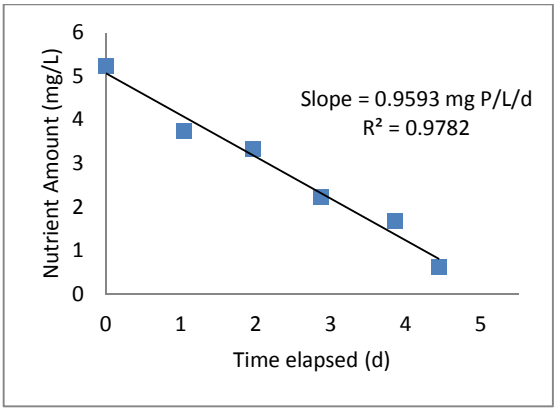


FIGURE 6.12 a) & b) Phosphate readings for (a) Experiment 1 (▲) and (b) Experiment 2 (■)

6.6 pH VARIATION

No CO_2 addition was provided in Experiments 3 or 4. Therefore pH variability within the bio-reactor, resulting from algal behaviour, was influenced predominantly by algal growth depending on the light source availability. The light source was set on a 12hr on/off cycle. The controlled pH range was set at 7.7, with a tolerance of ±0.6, allowing a maximum pH value of 8.3.

6.6.1 EXPERIMENT 3: pH VARIATION

The pH fluctuations for Experiment 3 are shown in Figure 6.13. Dark (troughs) and light (peaks) periods are shown by the added red line. In the light period, pH levels rose at an increasing rate as the trial progressed. In the dark period a gradual increase in pH levels was observed on all except the last day. The obtained dark period results, again suggested that the algae *Dunaliella*

Tertiolecta still had photosynthesis capacity under the limited light source conditions of the libratory.

The increasing number of acid inputs required to maintain a pH of < 8.3 was another indication of the growth performance. One day 1 only two inputs were recorded, on 4 day there were five acid inputs.

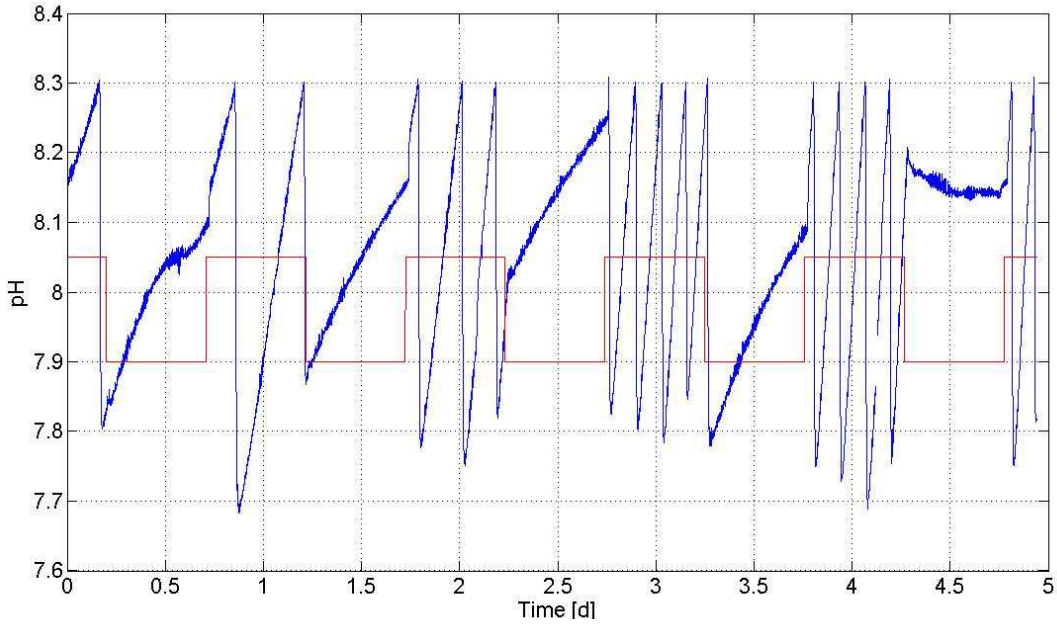


FIGURE 6.13 Experiment 3 – Bioreactor pH readings for the 5 day trial.

Figure 6.14 shows the pH readings for days 1, 2 & 4 of Experiment 3, over a 24 hour period. Based on observations from the growth behaviour shown in Figure 6.2, these days correspond respectively with the identified lag/exponential, exponential and linear growth phases. All 24 hour readings start at midnight, splitting the dark period into two 6 hour sections.

The pattern of pH variability was characteristically similar for all growth phases of the experiment, with shaper rises in pH levels in the light periods. For all readings no reduction in the pH gradient was observed when pH levels approached 8.3, suggesting that the upper limit of the pH range could potentially be increased.

In the light periods, the sharpest increase in pH levels was observed on day 4 (linear), approximately 0.194 pH unit/h. The shallowest light period gradient occurred on day 1 (lag/exponential), approximately 0.077 pH unit/h. In the dark period, the steepest pH gradient

occurred at the start of day 4 (linear), approximately 0.023 pH unit/h, a negative pH gradient was observed at the end of day 4, approximately -0.005 pH unit/h. The Experiment 3 growth pattern (Figure 6.2) suggests the alga is making a transition into the steady growth phase at this time period.

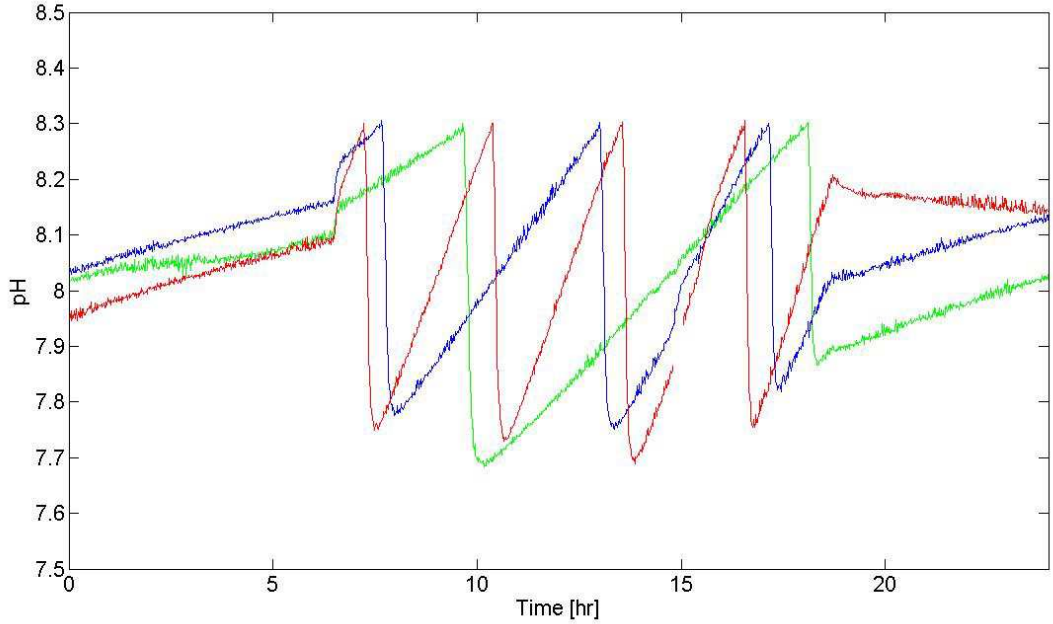


FIGURE 6.14 Experiment 3 – Bioreactor pH readings over a 24 hour period for Day 1 (—), Day 2 (—), and Day 4 (—).

6.6.2 EXPERIMENT 4: pH VARIATION

The pH levels for Experiment 4 are presented in Figure 6.15. Sharp steady increases in pH were observed for the first 3 days of the experiment. Dark and light periods are indicated by the added red line. The pH increased during both periods until the end of day 3, with slightly steeper gradients observable during light periods.

Similar to Experiment 2, the steep pH gradients observed in both the day and night periods, may be attributable to a combined effect of algal growth and a reduction in H^+ within the solution resulting from carbon stripping through aeration. Refer to section 5.5.2 for further details.

With reference to figure 6.1, the pH variability for the first 3 days coincide with the lag/exponential, exponential and exponential/linear growth periods, as identified in Figure 6.4. As with Experiment 2, results for this period clearly demonstrated a reduction in the buffering capacity of the solution, suggesting a reduction in the bicarbonate concentration of the media. By day 4, the buffer capacity of the solution had reduced to a level such that, the 10ml acid addition caused the pH to drop below the lower range limit of 7.1, which led to the automated base addition. The acid/base input quantity was subsequently adjusted to 6ml, steady results were generally achieved for the remainder of the trial.

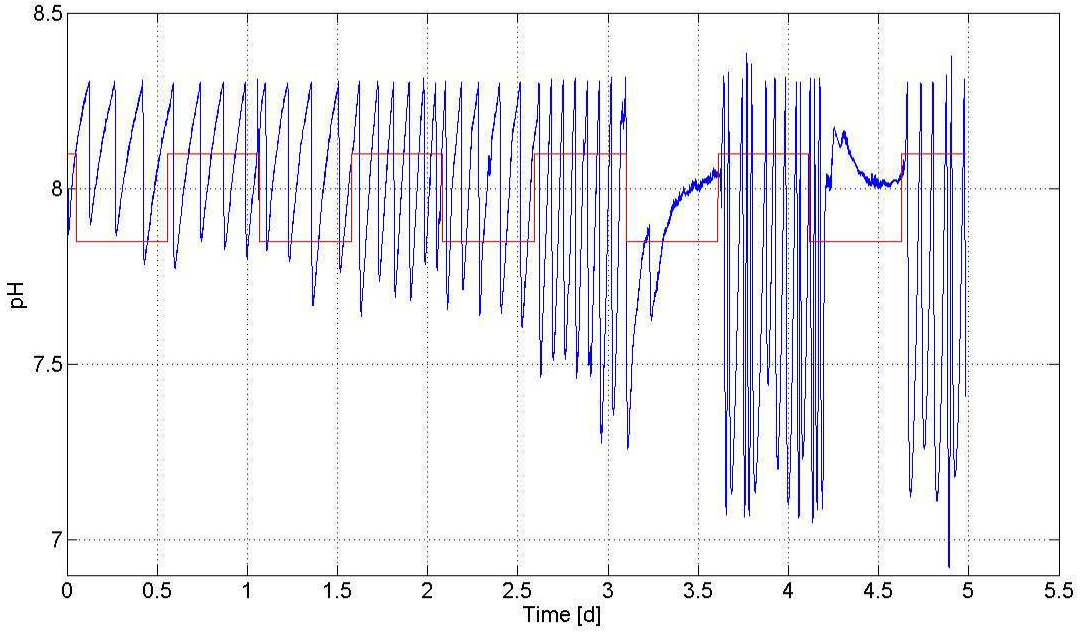


FIGURE 6.15 Experiment 4 – Bioreactor pH readings for the 5 day trial.

Figure 6.16 shows the pH results over a 24 hour period for days 1, 2, and 3, corresponding with the identified lag/exponential, exponential and exponential/linear growth phases respectively. All 24 hour readings start at midnight, splitting the dark period into two 6 hour sections. Results show relatively consistent pH patterns for all three days, with some erratic results at the end of day 3. A reduction in the gradient, above a pH reading of 8 was frequently observable on day 1, 2 and the start of day 3.

Significant increases in pH were observed in both dark and light periods. In the light periods the steepest average pH gradients were observed on day 3 (linear), approximately 0.651 pH unit/h.

The shallowest average gradients occurred on day 1 (lag/exponential), approximately 0.099 pH unit/h. In the dark period the steepest average pH gradient was recorded at the start of day 3 (linear), approximately 0.274 pH unit/h. The shallowest pH gradient was observed at the end of day 3 (linear), approximately 0.194 pH unit/h.

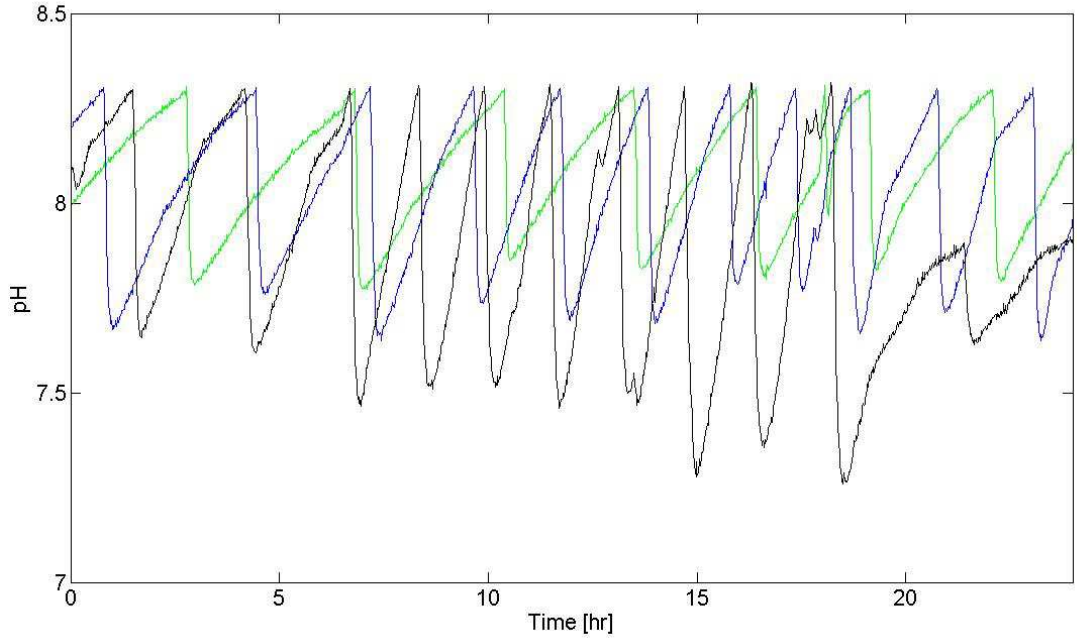


FIGURE 6.16 Experiment 4 – Bioreactor pH readings over 24 hour period for Day 1 (—), Day 2 (—) and Day 3 (—).

6.6.3 pH VARIATION SUMMARY

Similar to Section 5, the pH results of the 2 experiments show significantly sharper increases in pH levels in aeration trial. The cause of this steeper gradient is likely due to a combination of algal growth and the removal of H^+ from the solution through carbon stripping.

The sharper increases in pH, may again prove to be problematic as significant acid input was required to maintain pH levels within the optimal growth range. Consequently algae growth conditions that facilitate a steady and prolonged pH increase time (Non-aeration trials) may prove to be advantageous. Growth results from Section 6.3 suggest no overall benefit in implementing aeration.

6.7 DISSOLVED OXYGEN VARIATION

Dissolved oxygen (DO) readings were recorded in real-time, and measurements were logged on a per minute basis. DO variability was influenced predominantly by light source intensity and aeration. An abundance of light facilitated photosynthesis, and hence oxygen production (Ferdnandes 2010).

6.7.1 EXPERIMENT 3: DO VARIATION

Figure 6.17 shows the DO concentrations for Experiment 3, a light and dark phase line has been included. As expected higher DO values were recorded during the light periods. Results show DO readings peaking at 30.00 mg/L on days 3 and 4. Note that the upper limit reading capacity of the dissolved oxygen meter (TPS 90-D) is 30.00 mg/L, suggesting that higher DO levels may have occurred on these days due to extremely fast growing algae producing oxygen. Due to these very high observed readings, a calibration check of the DO meter was conducted on day 3. Calibration testing results suggested that the meter was functioning correctly.

The instantaneous spike in DO readings (approximately 2.5mg/L increase), observed during the light period on the first day of the experiment, resulted from the experimenter removing algae that had clumped to the probe.

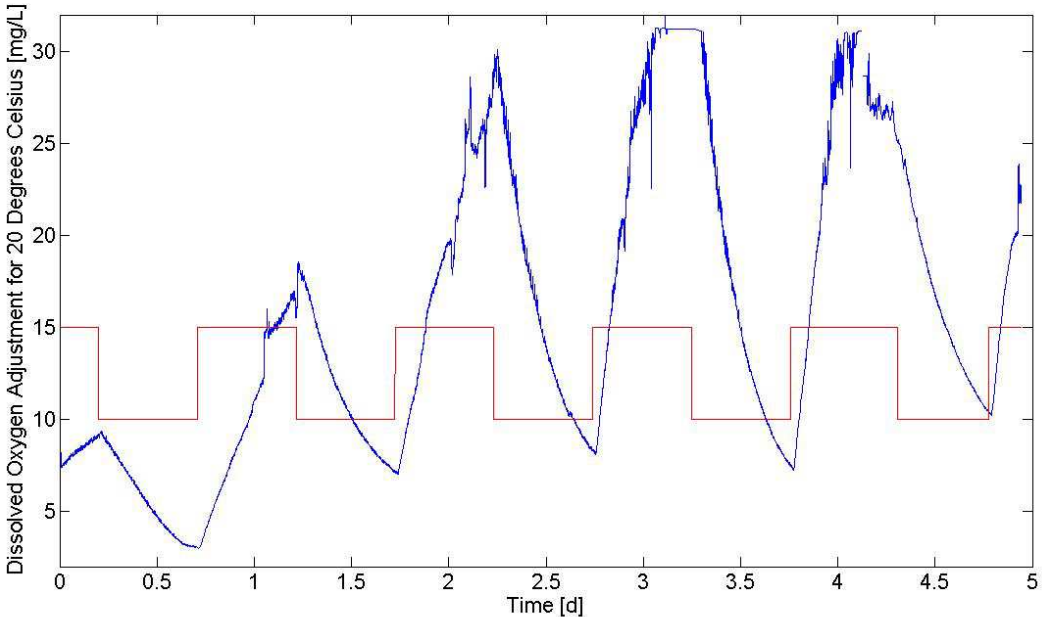


FIGURE 6.17 Experiment 4 – Bioreactor DO readings for the 5 day trial.

Figure 6.18 compares average maximum DO readings during the light period with average maximum DO readings in the dark period, for Experiment 3. The relative difference between these two sets of values provides an estimate of the net oxygen produced during each of the light periods. The saturation value of DO at 20°C is 9.17 mg/L, this is also indicated on the graph, as is the measured optical density readings for this trial.

The highest recorded DO production reading was 23.1 mg /L, on day 3. Dissolved oxygen production levels increased until the end of day 3 (linear), before a suggested decline. This increase in DO production is likely the result of a growing volume of active biomass during the lag, exponential and linear phases.

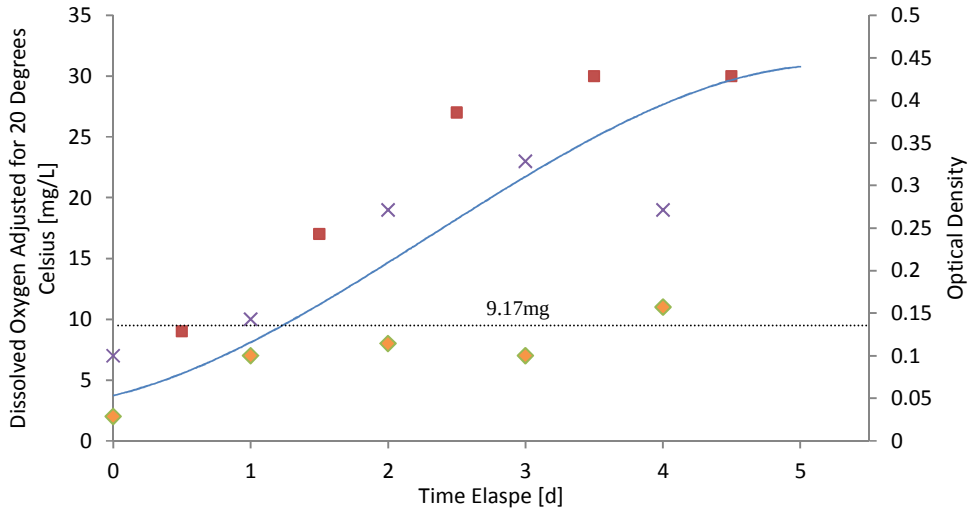


FIGURE 6.18 Experiment 3 –Average maximum DO readings during light period (■), average minimum DO readings during dark period (◆), Net oxygen produced during the day time (X) and Optical Density results (—).

Figure 6.19 shows the estimate oxygen production quantities in the light periods, for each day of Experiment 3. Quantities were derived using equation 3.8. Results show a peak oxygen production of 7.23 mg, produced on day 3.

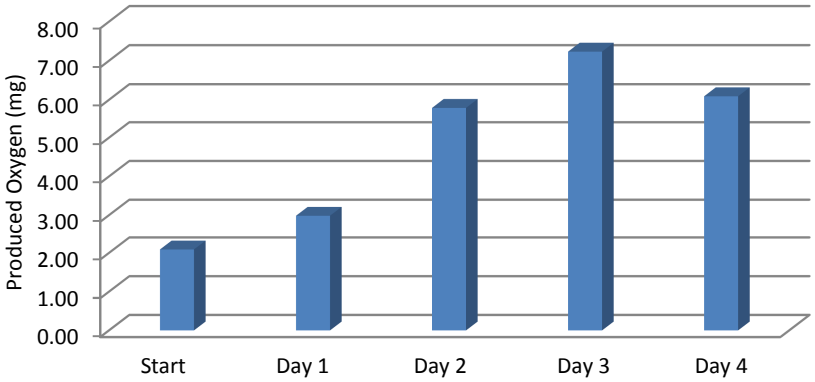


FIGURE 6.19 Experiment 3 – Daily oxygen quantities produced during the light periods

6.7.2 EXPERIMENT 4: DO VARIATION

Figure 6.20 shows the DO dynamics for Experiment 4, a line indicating light and dark periods has been included. The results show an increase in DO readings until day 2 (max 10.5 mg/L), followed by steady DO results for the remainder of the trial.

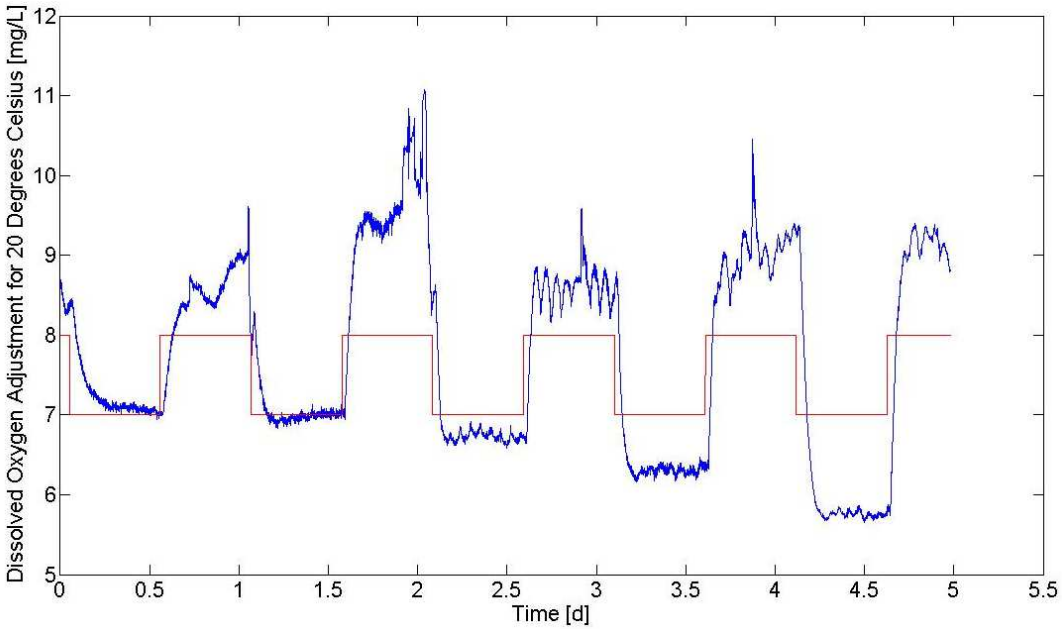


FIGURE 6.20 Experiment 4 – Bioreactor DO readings for the 5 day trial.

Figure 6.21 shows the average maximum light and dark period readings for Experiment 4, and the derived relative DO production during the light periods. A maximum DO production reading

of 3.7 mg/L was recorded on day 4(linear). Results show relatively stable DO production throughout the trial period.

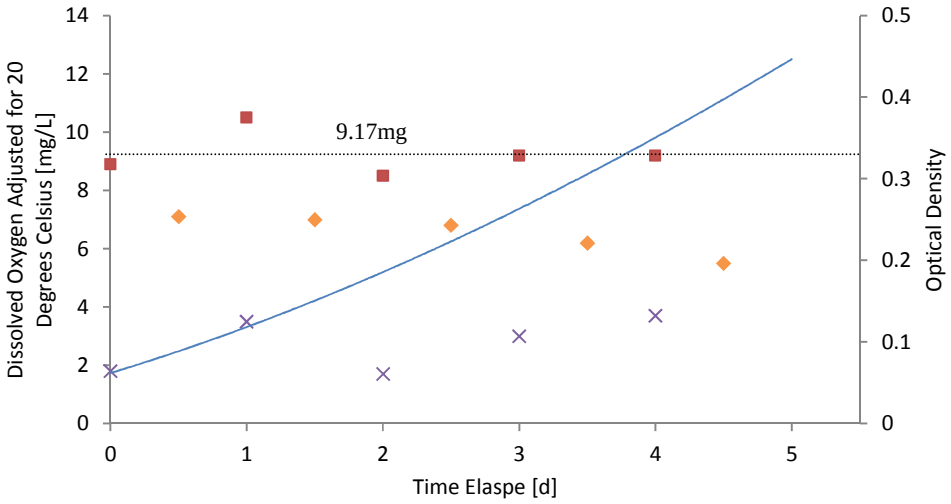


FIGURE 6.21 Experiment 4 –Average maximum DO readings during light period (■), average minimum DO readings during dark period (◆), Net oxygen produced during the day time (X) and Optical Density results (—).

Figure 6.21 shows the estimate oxygen production quantities in the light periods, for each day of Experiment 4. Quantities were derived using equation 3.8. Results show a peak production of 1.04 mg of oxygen produced on day 4.

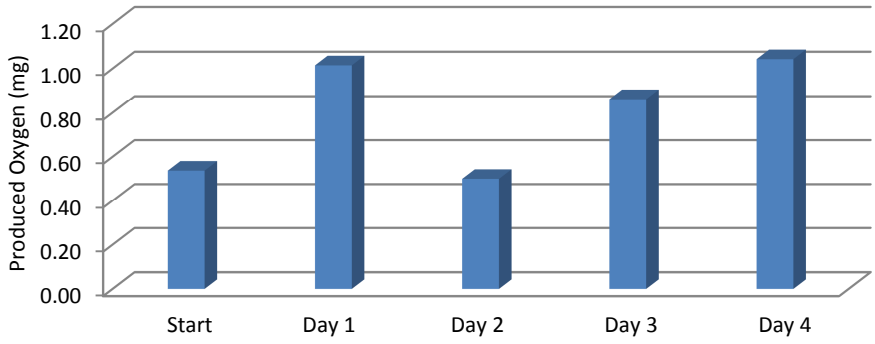


FIGURE 6.22 Experiment 4 – Daily oxygen quantities produced during the light periods

6.7.3 DISSOLVED OXYGEN MEASUREMENT SUMMARY

Peak DO readings and estimated DO production levels were significantly higher in Experiment 3, suggesting that aeration restricted the DO variability within the bioreactor for the growth of *Dunaliella tertiolecta*. Results were similar to the findings in Section 5. The disparity of the DO results could possibly be attributable to stripping of the oxygen through aeration, similar the observed carbon stripping effect.

6.8 ALGAE DRY MASS AND LIPID CONTENT

Algae were harvested after 5 days of growth. The algal dry mass and the total lipid content for Experiment 3, Experiment 4 and the low carbon control trail are shown in Table 6.1. Results indicate no definitive advantage between Experiment 3 and 4 processes. Furthermore results further demonstrate the advantage of using a bicarbonate rich solution for oil production.

TABLE 6.1 Algal dry mass and lipid content

Trial	**Algal Dry Mass (g)**	**Total Lipid Content (%)**
Experiment 3	0.39	24
Experiment 4	0.41	20
Low Carbon Control Trial	0.28	25

6.9 ENCOUNTED PROCEDURAL ISSUES

6.9.1 ALGAL SETTLEMENT AND CLUMPING

Minimal algal clumping onto the DO probe, affecting DO readings was observed in the early stages of Experiment 3. By positioning of the probe within the area of high turbulence created by the stirrer, further clumping was avoided.

6.10 CONCLUSIONS

Research has provided insight into the growth characteristics of microalgae *Dunaliella tertiolecta* in a salinity modified CSG medium. High growth rates were observed in both Experiments 3 and 4, and moderate growth was observed in the low bicarbonate control trial. Results suggest a clear benefit in adopting a bicarbonate rich solution for the growth of *Dunaliella tertiolecta.* The assessment of pH variability found that aeration significantly increased the pH gradient, and this was likely a result of the combination of algal growth and the removal of H^+ through carbon stripping. The subsequent steep rises in pH resulted in significant acid addition to moderate pH levels. Furthermore, aeration was again found to cause large losses of carbon from the media through stripping. Results suggest a potential for algal farming in CSG water without aeration.

CHAPTER 7 CONCLUSIONS AND FUTURE WORK

7.1 CONCLUSIONS

The production of biofuel from microalgae can reduce greenhouse gas emissions, help address future fuel shortages and create new industry in rural areas (CSIRO 2011). Over the next decade, up to 300 GL of CSG water will be produced as a by-product of methane extraction processes the in Surat and Bowen Basins. Typically this water is considered as a waste product and is disposed of in large evaporation ponds. However, by utilizing a combination of high carbon CSG water with nutrient rich agricultural waste to grow microalgae, there is the potential to create significant quantities of biofuel (Pratt 2011).

In order to evaluate the capacity of utilising existing open pond infrastructure to grow algae in a CSG water medium for the purpose of biofuel production, carbon sequestration and nutrient extraction (nitrogen and phosphorous), lab scale batch experiments were conducted using microalgae *Dunaliella tertiolecta* in this media, at the University of Southern Queensland. The study has shown that *Dunaliella tertiolecta* can be grown in the CSG water, and there is a capacity for lipid production, carbon sequestration and nutrient removal.

Initial trials using an unmodified CSG water sample with an F/2 nutrient supplement found poor growth for non-aeration and aeration scenarios, with initial rates of 0.0292 g SS/L/d and 0.0303 g SS/L/d, respectively. High growth was achieved once salinity levels were increased to 10 g NaCl/L, with non-aeration growth rates of 0.0935 g SS/L/d and aeration growth rates of 0.0808 g SS/L/d. Overall performance results indicated no clear benefit in adopting aeration for algal growth facilitation. A control trial with a lowered bicarbonate concentration and salinity level of 10 g NaCl/L, found only moderate grow, suggesting a distinct benefit in utilizing a bicarbonate rich solution for algal production.

Carbon sequestration levels were assessed for the non-aeration trials (Experiment 1 and Experiment 3). Results indicated substantial carbon absorption by the alga in both trials, with quantities of up to 90.9mg C/L for Experiment 1 and 82.2mg C/L for Experiment 3. Carbon stripping quantities for the aeration trials were found to be as high as 81.52 mg/L and 72.4 mg/L,

for Experiment 2 and Experiment 4 respectively. Overall results indicated a clear benefit in adopting a non-aeration setting for high levels of carbon sequestration and algal growth.

Nutrient removal rates were highest in the salinity modified trails, with nitrogen and phosphorous removal rates of 2.335 mg N/L/d and 1.156 mg P/L/d for Experiment 3, and 2.808 mg N/L/d and 0.959 mg P/L/d for Experiment 4. Nutrient depletion P:N ratios of 1:2 to 1:3 were observed.

The algal dry mass and lipid content for Experiment 3, Experiment 4 and the low carbon control trial were 0.39 g at 24%, 0.41 g at 20% and 0.28 g at 25%, respectively.

Overall results indicate that high levels of algal growth, carbon sequestration and nutrient removal are achievable in a salinity modified CSG water medium. Generally speaking this research has proven to be very successful, warranting further study of algal growth, lipid production, carbon sequestration and nutrient removal from a CSG water source.

7.2 SUGGESTIONS FOR FUTURE WORK

7.2.1 CLOSED LOOP AERATION SYSTEM

Building on from the assessment of the effect of aeration on carbon stripping, investigation could be made of the potential advantage of adopting a closed loop aeration system to facilitate algal growth. By creating a closed system, in which the air is recycled through the CSG water, it is hypothesised that carbon concentrations within the air and water would soon equalise. Therefore aeration could be used to stimulate growth without causing excessive carbon stripping. Furthermore, if carbon levels in the air supply did increase, there is the additional possibility that this air could aid to moderate pH levels within a solution.

A closed bio-reactor tank structure with a base inlet and top outlet, exists in the USQ (FoES) Environmental Engineering laboratory. A vacuum pumping system with inlet and outlet tubes, capable of running for extended period (> 1 week) would be required.

7.2.2 OPTIMAL BICARBONATE LEVEL IDENTIFICATION

It has been shown that algal growth is highly sensitive to the concentration level of CO_2 input, with poor growth in both low and high concentration settings (Tang el al. 2010). Developing on from this finding, assessment could be made to identify the optimal bicarbonate concentration for algal growth. This may not be practical for a batch experiment setup. Alternately bicarbonate concentrations could be monitored and adjusted daily, for set period trials, with varying bicarbonate levels tested.

7.2.3 ALTERNATE AGLAL STRAND ASSESSMENT

Due to budget and time constraints, only one algal strand was tested in the CSG water. Furthermore, because the CSG water source was an untested media, the prominent reason for the selection of microalgae *Dunaliella tertiolecta* was its hardy nature rather than its lipid production capacity. As this research has shown healthy algal growth was achievable in the CSG media, future studies may consider selecting an algal strand with great lipid production capacity.

7.2.4 IMPROVED pH CONTROL

The pH moderation system controlled by Labview, moderated pH levels by adding consecutive acid/base inputs until the level of the media returned to within a specified range. The period between consecutive inputs is equal to the time data logging period (1 minute for all trials). This was an issue, as a lag period existed between acid/base addition and the resulting final pH level of the media. This lag period could extend for up to 10 minutes. Consequently over-adjustment of pH levels by the automated system was an ongoing issue.

A preferable system would be to have the set minimum time period between consecutive acid/base inputs independent of the set time period between data logging. And to have the capacity to manually change both settings, as required.

7.2.5 IMPROVED REACTOR DESIGN

Suggested modifications to the bio-reactor include, adopting a tank structure that allows for better light penetration into the media. This would likely require a wide thin structure, increasing the surface area to volume ratio. Secondly the existing small rotor used for mixing

could be replaced with a larger rotor system. This may alleviate problems with clumping to the probes, and the rotation speed could be reduced, potentially improving algal survival rates.

7.2.6 FUTURE EXPERIEMENTS

Due to time constraints, and with the capacity to only run one batch experiment at a time, all experiments were conducted only once. It was therefore difficult to draw definitive conclusions from the obtained results. Future works that intend to build on from this research, should first attempt to replicate the relevant trials.

7.3 SUMMARY

This chapter concluded the results of the dissertation and made suggestions for future research. It was found that optimal growth of *Dunalella tertiolecta* in CSG water was achieved with a modified salinity level of 10 mg NaCl/L. Furthermore results demonstrated a significantly benefit in using a bicarbonate rich solution for algal growth. Aeration was shown to cause carbon stripping and did not appear to increase the overall growth. High levels of carbon sequestration and nutrient removal rates were documented. A number of future research suggestions were made including creating a closed aeration loop system, identifying the optimal bicarbonate level for algal growth, testing alternate algal strands, modifying the pH control system and improving the bio-reactor design shape and stirring method to facilitate algal growth.

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Appendix A

University of Southern Queensland

FACULTY OF ENGINEERING AND SURVEYING

ENG4111/4112 Research Project

PROJECT SPECIFICATION

FOR: DANIEL HARRINGTON

TOPIC: NUTRIENT REMOVAL FROM WASTEWATER BY MICROALGAE FOR USE IN BIO-FUEL

SUPERVISOR: Vasantha Aravinthan

PROJECT AIM: The aim for this project is to determine the whether bio-diesel feedstock Coal Seam Gas water could be use

Effect of varying salinity levels,

Programme: Issue A, 22nd March 2011

1. Research background information relating to: coal seam gas water, the process of deriving bio-fuels from microalgae, and the properties of relevant microalgae strands.
2. Research and select a microalgae strand best suited to produce bio-fuel derived from a CSG water medium.
3. Undertake laboratory analysis of

As time permits:

Appendix B

Manual Trail Logging Sample

Trial H - Dunaliella Teriolecta in CSG water con Sodium bicarbonate & Bubbles

time	Day	Date	Tank Volume (L)	TOC/TN Label	Ion	Spectrometer	Comments
4:30	1	14/9/2011	3.35	H1,H2	H1,H2	0.058	Dillution x 4
5:30	2	15/9/11	3.45	H3,H4	H3,H4	0.131	Dillution x 4
3:00	3	16/9/2011	3.4	H5,H6	H5,H6	0.173	Dillution x 4
1:30	4	17/9/2011	3.48	H7,H8	H7,H8	0.263	Dillution x 4
12:00	5	18/9/2011	3.55	H9,H10	H9,H10	0.357	Dillution x 4
2:30	6	19/09/2011	3.6	H11,H12	H11,H12	0.444	Dillution x 4

Trial Stopped

Nutrient Adjustment Method - Sample

Nitrogen

Date	*Time*	*Duration*	*Time elaps. (hrs, min)*	*Time elaps. (d, hrs)*	*Av. Read. (mg/L)*	*Reactor Vol. (L)*	*Av. Read. (mg)*	*Unconsum. (mg/L)*	*Consumed (mg/L)*	*Nutrients (mg/L)*
8/30/2011	18:00	0	0:00	0:00:00	61	3.32	203.007	61.147	0.000	61.147
8/31/2011	15:00	21:00	21:00	0:21:00	50	3.30	163.536	61.517	11.961	49.186
9/1/2011	16:00	25:00	46:00	1:22:00	58	3.22	187.632	63.046	4.775	56.372
9/2/2011	16:00	24:00	70:00	2:22:00	49	3.15	155.245	64.447	15.163	45.984
9/4/2011	11:30	43:30	113:30	4:17:30	62	3	187.324	67.669	5.228	55.919

Appendix B

MATLAB SCRIPT SAMPLE

```
%Trial H - pH Comparison of stages over 24hr

%lag
%DO_lag = xlsread('Total_Trial_H.xlsx',1,'A3:A1555');
%pH_lag = xlsread('Total_Trial_H.xlsx',1,'B498:B1938');
time = xlsread('Total_Trial_H.xlsx',1,'N2:N1466');

%exponential
%DO_expo = xlsread('Total_Trial_H.xlsx',1,'A1555:A2980');
pH_expo = xlsread('Total_Trial_H.xlsx',1,'B440:B1904');

%linear
%DO_linear = xlsread('Total_Trial_H.xlsx',1,'A2980:A13222');
pH_linear = xlsread('Total_Trial_H.xlsx',1,'B1904:B3368');


%stationary
%DO_stationary = xlsread('Total_Trial_H.xlsx',1,'A13222:A33333');
pH_stationary = xlsread('Total_Trial_H.xlsx',1,'B3368:B4832');


%death
%DO_death = xlsread('Total_Trial_H.xlsx',1,'A33333:A33406');
pH_death = xlsread('Total_Trial_H.xlsx',1,'B4832:B6296');


figure;
plot(time,pH_expo,'g')
grid off
hold on
plot(time,pH_linear,'b')
plot(time,pH_stationary,'k')
%plot(time,pH_death,'r')

set(gca,'FontSize',16)
xlabel('Time [hr]','FontSize',16)
ylabel('pH','FontSize',16)
axis([0 24 7 8.5])
```