Arnas Svaldenis

Cultivating Algae in a Photobioreactor

CO_2 fixation, synthetic wastewater nutrient removal and biomass production using the green algae species *Chlorella pyrenoidosa*

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Abstract

The most common way of producing energy worldwide is by utilizing fossil fuels, a finite resource that is diminishing rapidly. Many developing countries have various problems with their waste disposal practices and treating their wastewater. Persistent power outages in the power distribution network add to the problems. In most cases power is generated by burning coal, the dirtiest of fossil fuels, which emits flue gases into the atmosphere. By utilizing algae, a photosynthetic microorganism, it is possible to scrub heavy metals and CO_2 from the flue gases. Algae can also absorb various nutrients found in wastewater and convert them into useful by-products. We are experiencing a shift from humanity trying to dominate nature to humanity trying to preserve parts of nature and now trying to reach reconciliation with nature. Ever increasing pollution, population and demands for clean water and energy are issues of vital importance in today's global environment.

This thesis project investigates wastewater nutrient removal with carbon dioxide capture using microalgae. It also proposes a way to maintain a carbon neutral loop and utilization of waste products to produce biomass and biofuels. Algae provide an efficient way to prevent waste and pollution by utilizing resources efficiently, keeping majority of resources in a closed loop cycle. The aim of this project was to create a model bioreactor which showcases the concept. For this purpose a small scale photobioreactor was built under laboratory conditions.

A photobioreactor is a closed system which provides a controlled environment and enables high productivity of algae. Pure CO_2 and synthetic wastewater were used to model flue gas

and actual wastewater conditions. This way the bioreactor resembles the actual large scale process without posing a risk of process interference. When tested, the bioreactor exhibited promising results for synthetic wastewater nutrient removal. In subsequent studies investigations or a proof of concept phase it would be necessary to use real wastewater and industrial CO_2 sources. Ideally, this system would run fully automated on solar energy. This would be extremely useful to developing countries that rely heavily on energy from diesel and have no sufficient wastewater treatment facilities.

Keywords	Fossil fuel economy, Wastewater, Nutrient removal, Carbon capture, Biomass, Algae, Phytoremediation, Air pollution, Climate Change, Biofuels.

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Glossary and Abbreviations

PBR's	Photobioreactors
Microalgae	unicellular photosynthetic organism
CO_2	greenhouse gas
Photosynthesis	$6CO_2 + 6H_2O$ (+ light energy) → $C_6H_{12}O_6 + 6O_2$
Biomass	organic matter used as a fuel
CH_4	methane, a greenhouse gas
Raceway ponds	open-air method of cultivating algae
HARP	high rate algal ponds/photobioreactors
PAR	a spectrum of light that falls under 400-700nm range
SYKE	Finnish environmental institute
T2	algae growth promoting liquid medium
M8	algae growth promoting liquid medium
Protozoa	a unicellular eukaryotic organism
BOD_5	5 day dissolved oxygen concentration difference
DO	dissolved oxygen
COD	chemical oxygen demand
Carbon capture	ability to sequent carbon dioxide
TN_b	total nitrogen
WWTP	wastewater treatment plant
WehoPuts	small scale wastewater treatment unit
CHP	Combined heat and power unit
FT liquids	Fischer Tropsch liquids
Synthetic wastewater	water that resembles nutrient composition found in wastewater

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1 Introduction

Algae is a eukaryotic photosynthetic organism which has various environmental engineering applications including sewage treatment, eutrophication prevention and fertilizer recovery, CO_2 scrubbing and can also be used as a fertilizer, a food source for people and animals and a source of biofuel. [1] Microalgae are phototrophic microorganisms. Wastewater can be used to supply nitrogen and phosphorus, the main nutrients needed for the cultivation of algae. [2] The use of residual algal biomass is then refined to other usable products such as biofuels. The biomass accumulated can be fermented to produce methane or ethanol. The drawbacks of the current state of microalgae biotechnology are the high investment costs and the high demand on auxiliary energy for biomass production and for lipid processing to biodiesel, leading to high costs for biomass and biodiesel; a step that can be overtaken by using alternative sources of energy such as tidal, solar or wind power. [3]

The objective of the project was to determine the feasibility of using Baltic Sea algae (*Chlorella pyrenoidosa*) to mitigate carbon dioxide emissions effectively and efficiently, to achieve high nutrient removal efficiency from synthetic wastewater and to produce substantial amount of biomass.

2 Theoretical background

Algae are emerging as one of the most promising long-term, sustainable sources of biomass and oils for fuel, food, feed, and other co-products. Waste to energy, wastewater nutrient removal and flue gas scrubbing are just a few of the many benefits that algae can provide. [3]

2.1 Wastewater treatment with algae

Wastewater treatment plants have been using bioremediation techniques involving algae for years. [5] Algae are an excellent way to remove nitrogen and phosphorus from wastewaters. Algae can also remove heavy metals such as cadmium zinc, nickel, and lead. [6] This process is considered environmentally sound, recycles nutrients efficiently, does not lead to secondary pollution, and produces biomass and oils that can be harvested to make useful products such as biodiesel. By combining algae and wastewater treatment, low effluent nitrogen and phosphorous concentrations can be achieved. The process then recycles nutrients from wastewater treatment sludge to

produce biomass and oils that can be harvested to make useful products such as biodiesel.

2.2 Culturing techniques

There are two ways for algae cultivation. One is using the open water resources, and the other is setting the photobioreactor in the lakes or ponds. Sea cannot be used for the cultivation of the algae since the algae requirements are impossible to be fulfilled in the sea. Providing enough light and carbon dioxide and maintaining an even temperature is impossible in the sea or other huge areas. Algae cultivation can be performed in the open water resources like lakes or ponds. Large ponds have the largest production capacities in comparison to other systems in the same cost range. [4], [7], [8], [9], [11]

2.2.1 Open ponds

On a large industrial scale algae are cultivated in open raceway ponds. This has lower capital costs than cultivations in photobioreactors (PBR's). However, being in open air, raceway ponds are more susceptible to contamination for example by other algal species. Precipitation and temperature fluctuation also interfere with the number of species that can be cultivated successfully. Raceway ponds are also inefficient due to evaporation. [4]

2.2.2 Closed photobioreactors

Another common way used to cultivate algae on a mass scale is using photobioreactors of some sort, either tubular, flat or bubble column. PBR's have numerous advantages over raceway pond cultivation. They offer space saving, better gas control and transfer and are better protected from outside contamination for example other algae species that are not favorable. It can be used in a closed area like pond, but the cultivator must provide all nutrients including carbon dioxide, light and maintain temperature in balance. It requires a precise control of all elements to prevent any culture collapse. The advantage of the photobioreactor is that the algae grown in this environment are of highest quality. This way the algae consume more carbon dioxide and produce more oxygen. They have high nutrient content and can be cultivated for various purposes such as healthy food, fuel from biomass or manufacturing medicines and can be also used as fertilizer. [1] Tubular PBRs have been successfully used for indoor and outdoor microalgae cultures. In photobioreactors, harmful chemicals or bacteria which create problems for algae can

be removed, and a suitable environment can be created for the algae growth. However, there are disadvantages in using a PBR, primarily due to high capital cost. [4], [7], [10]

2.3 CO_2 and wastewater sources

CO_2 capture is one of the most critical challenges today for businesses and governments worldwide. Thousands of CO_2 emitting power plants and industries face the costly challenge to reduce CO_2 emissions or to pay penalties. There are many different sequestration techniques such as deep saline injection into depleted oil fields under the sea bed, or the use of energy intensive amine scrubbers. These methods face significant challenges; they are not environmentally sound and are relatively expensive. The ability to capture CO_2 makes algae the more environmentally sustainable way of mitigating carbon dioxide emissions. Industries that emit large amounts of CO_2 during their operations can use algae for CO_2 capture and generate useful by-products at the same time. The uncontrolled disposal of manure from companies that produce large quantities of animal waste causes surface water eutrophication and groundwater pollution due to their high concentration of organic matter and nutrients. Many piggeries use waste digesters to produce methane, which in turn is used as a heating fuel. Using large quantities of methane gives out CO_2, which can be used to grow algae. Liquid effluents released from the anaerobic digesters can also be treated by means of microalgae-based processes such as high rate algal ponds (HRAP) or other types of photobioreactors, thus providing two benefits: cost effective sequestration of CO_2, and manufacture of fuel feedstock. The combination of the three roles of microalgae – CO_2 fixation, wastewater treatment and biofuel production has a tremendous potential for biofuel and biomass production systems. [5], [16], [13], [14]

2.4 Growth phases

There are five phases in growing algae in batch cultures: (1) lag, (2) exponential, (3) phase of declining growth rate, (4) stationary phase and (5) death. An algae culture is most productive when it is maintained in constant exponential growth.
Figure 1. shows the five different phases of growth in logarithmic vertical axis plotted against time. [17]

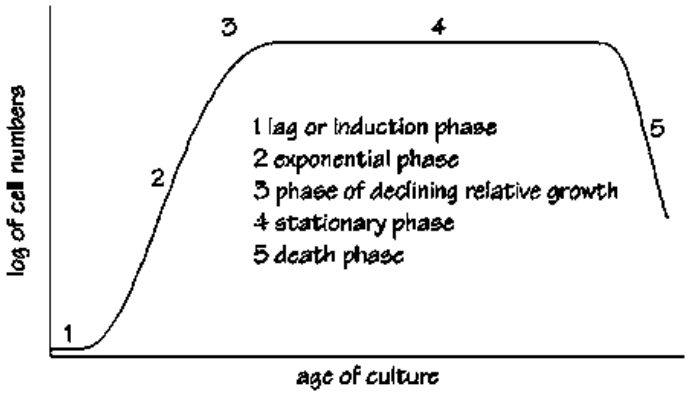

Figure 1. Algae growth phases [17]

2.5 Factors influencing growth

The most essential requirements for algae are nutrients, carbon-dioxide, minerals and light. Different algae species have different requirements. The common reaction that takes place for the growth of algae is as follows:

Water + carbon dioxide + light energy = glucose + oxygen + water

This reaction is called autotrophic growth. Algae can also grow in the darkness in the presence of glucose in water; this is called heterotrophic growth. One important factor for maintaining algae growth is temperature. The most favorable temperature for algae is between 20 - 25°C. Regarding light, algae can only use 1/10 of the light received. Therefore, more exposure to light leads to faster the growth of algae. It is also important to maintain the depth of the water to prevent photoinhibition. [18]

Algal growth is affected by nitrogen, phosphorous and potassium. Algae growth also depends on complex interactions among physical factors such as pH, light intensity, temperature and other biotic factors. A CO_2-enhanced aerated culture achieves high nutrient removal and an accelerated growth rate. Waste streams can be highly concentrated such as sewage, manure and industrial waste or more diluted such as wastewater effluent or eutrophic surface water. Algae require a photosynthetically active radiation (PAR) that is the part of the spectrum from 400nm to 700nm. [16], [12]

The growth rate will increase with increases in temperature up to its optimum, and once it reaches the optimum, the growth rate will decrease substantially with further increases in temperature. Finding and maintaining a suitable temperature for an algal species is significant to achieve a high growth rate. As algae grow, they draw all available CO_2 out of water, causing its pH to increase. When carbon dioxide dissolves in water, carbonic acid is formed, which has a pH of less than 7, thus pH tends to fall

when carbon dioxide is high. This is what happens during the night while the opposite occurs during daylight hours.

3 Prototyping

The primary reason for the prototyping a bioreactor is to showcase the possibilities of using algae to mitigate CO_2 emissions whilst simultaneously providing wastewater nutrient removal. The reactor itself had to be easy to transport within the campus area without the loss of function. The photobioreactor itself is used to promote biological growth by controlling environmental parameters including light. The tubes are made of acrylic and are designed to have light and dark cycles to enhance the growth rate. When drawing initial sketches the aim was to create a design that would be portable and easy to assemble and maintain, as well as look presentable to showcase the concept. Figure 2 presents a schematic of the reactor. [19], [21]

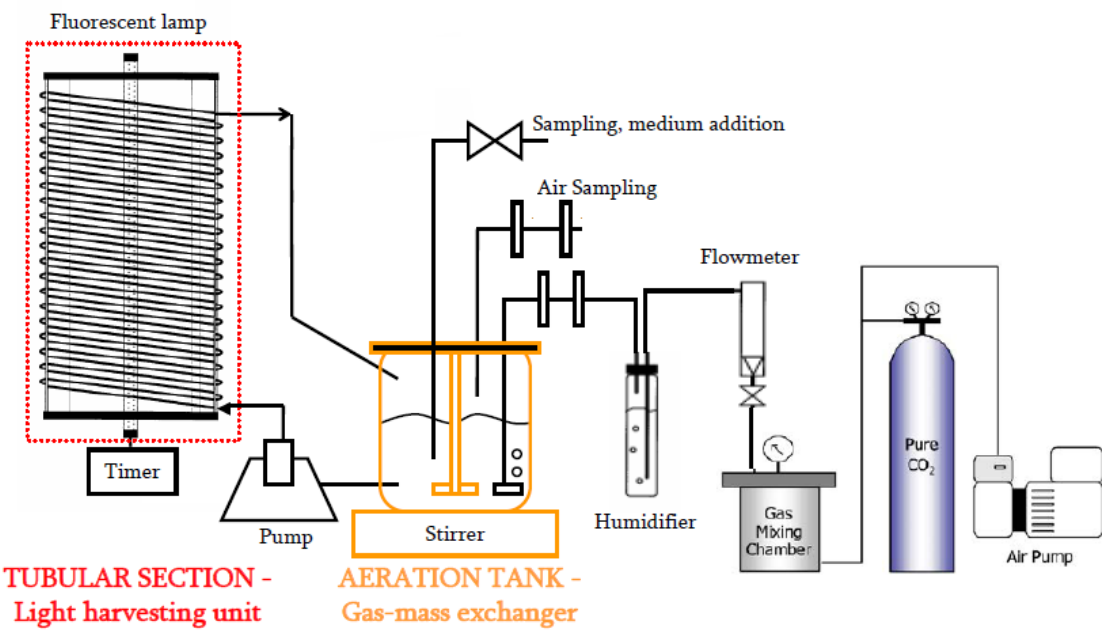

Figure 2. Bioreactor design [19]

Inside the cylinder tube two photosynthetic lamps were installed. A total of 15 m of 8mm tube was wrapped around it (Figure 5, Figure 3). In this structure algae were mixed and provided with light energy. The main cultivation tank was an aquarium the dimentions of which were 60cm x 30cm x 30cm. The top of the aquarium had to be modified with PVC pipes to house the gas sensors for inlet and outlet gas (Figure 4).

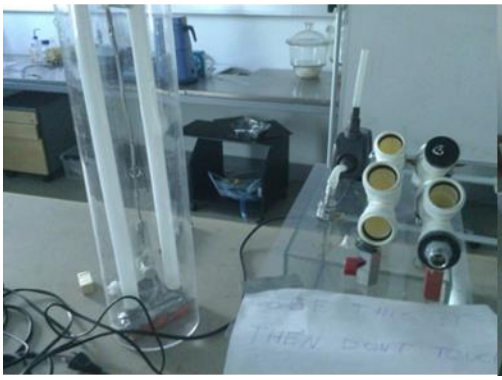

Figure 3.

Figure 4.

Figure 5.

Some holes were made in the lid for a heating element and a pH electrode. Mixing of algae was originally thought to be an issue; thus, for that reason there were three ways of mixing applied; a water circulation pump that provided liquid flow at 1350 l/h through the cylinder tube, an aquaball at 6 l/min and CO_2 injection through a PVC pipe diffuser with drilled 1mm holes. All components were working together to provide as even mixing of algae as possible. Figure 6 shows the components of the bioreactor, excluding the light harvesting unit. It also shows the gas sensors at the inlet and outlet PVC pipes.

Figure 6. Components of bioreactor

The completed bioreactor can be seen in Figure 7.

Figure 7. Finished bioreactor model

Before conducting any experiments, all equipment with surfaces that would come in contact with algae were washed using 1M HCl to mitigate the risk of contamination. The test run was successful using ion exchanged-water and no leaks occurred. Sensors worked fine and no leaks were detected. Leak test spray was used to check for possible gas leaks. The same ion exchanged water was used to cultivate the main algae culture with added nutrient concentrations.

4 Algae species and growth media

In this project, a species of *Chlorella pyrenoidosa* green algae native to the Baltic Sea, was obtained from the SYKE Finnish environmental institute, Helsinki. It was supplied in a bottle containing 165ml of algae solution with T2 media (Figure 8). Studies have shown that these organisms can grow on a wide variety of complex and synthetic media [20]. Since this strain of algae is extracted from the Baltic Sea, it requires a saline environment although some studies suggest it can grow in freshwaters as well. The salt used in algae cultivation was Tropic Marin – Reef actif.

T2 media	Concentration g/165ml
Biotin	8.25E-14
B12	8.25E-14
Thiamine	1.65E-11
$CoCl_2$ $6H_2O$	4.125E-15
$CuSO_4$ $5H_2O$	3.63E-14
$ZnSO_4$ $7H_2O$	1.65E-14
$MnCl_2$ $4H_2O$	2.97E-13
$NaMoO_4$ $2H_2O$	3.1185E-14
H_2SeO_3	2.145E-15
K_2CrO_4	3.201E-15
Na_3VO_4	3.036E-15
$NiSO_4$ $4H_2O$	4.455E-15
Na_2EDTA	7.194E-11
$FeCl_3$ $6H_2O$	5.1975E-11

Figure 8. Chlorella pyrenoidosa culture supplied by SYKE. Initial algae culture on the right and ingredients of the T2 media with their concentrations on the left.

Algae cultivation was started in a smaller 2L bottle containing M8 Medium initially (Figure 9). The M8 media was prepared using ion-exchanged water to ensure the nutrient composition is accurate.

M8 media	g / 2L
KNO3	1.5
KH2PO4	0.37
NaHPO4	0.13
CaCl2 2H2O	0.0065
FeSO4 7H2O	0.065
MgSO4 7H2O	0.2
Tropic Marin – Reef actif salt	12

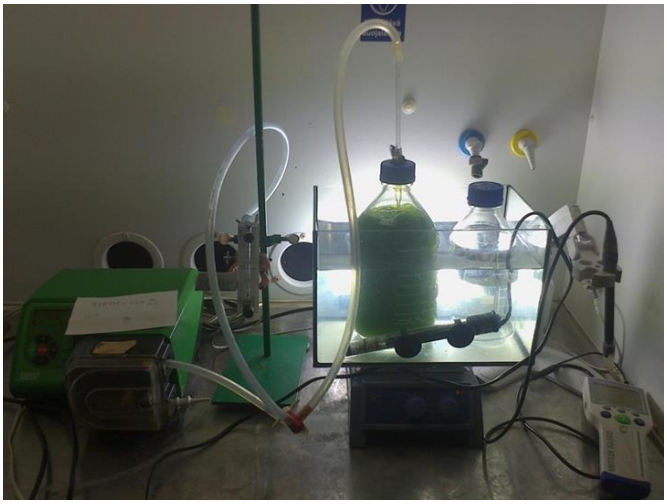

Figure 9. M8 growth media constituents on the left [22], and initial experimental setup on the right.

A water bath was used to keep the temperature constant, and aeration was provided using an air pump at 0.2 L/min. The water bath was placed on a magnetic stirrer to provide even mixing for algae. The algae were kept under 16 hour light/ 8 hour night cycle with a 6500K illumination at 125 watts. The algae were kept in 2 liter bottle until the cell concentrations measured were too dense to calculate cells accurately without dilution (Figure 10). After six days they were inoculated to the 25L main tank containing synthetic wastewater (Figure 11). A pH of 7 was kept throughout, with the help of incremental CO_2 addition.

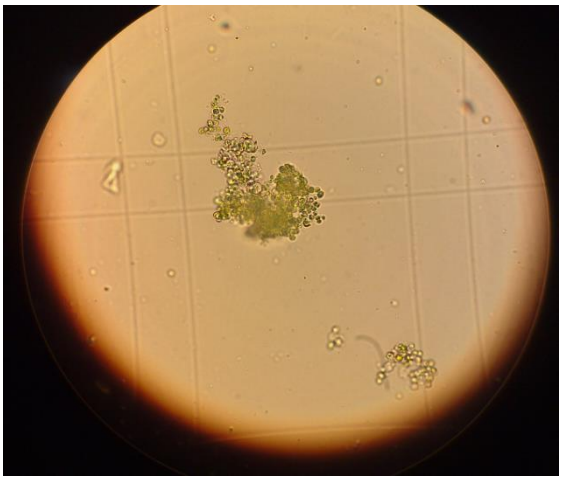

Figure 10. Algae culture ready for main tank cultivation

Synthetic Waste water	g / 25L
Peptone	4
Meat extract	2.75
$CO(NH_2)_2$	0.75
NaCl	0.175
$CaCl_2\ 2H_2O$	0.1
$MgSO_4\ 7H_2O$	0.05
K_2HPO_4	0.7
Surface active agent	0.275
Tropic Marin – Reef actif salt	150

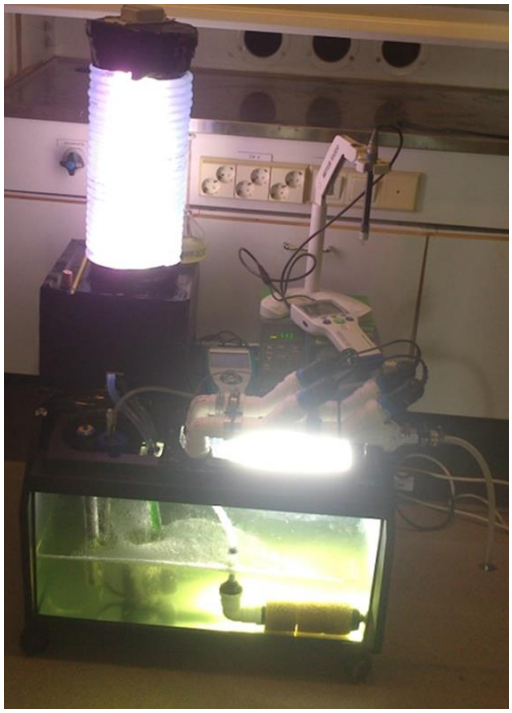

Figure 11. Synthetic wastewater composition on the left and algae growing in cultivation tank with synthetic wastewater on the right.

5 Experimental procedures

This chapter gives a detailed description of what was researched in this thesis project. It describes the procedures how the algae cell concentrations were measured. It also provides growth rate measurements with temperature variation.

5.1 Cell count

Monitoring algae growth rate is important as this indicates on how well the microorganisms are adapted to the environmental conditions provided. There are many factors that prohibit algal growth such as light limitation and poor mixing, which cause photoinhibition. In this thesis project the only limiting factor inhibiting algal growth was nutrient concentration. By monitoring the algal growth it could be seen when the algae growth remained stationary or when it started to decline. When the algae growth rate started to decline the process was stopped, and nutrient concentrations were measured, biomass analysis was made.

By measuring cell concentrations using a Brüker cell count tray and a microscope it was possible to graph the growth rate. Before taking each sample it was made sure the algae were as evenly mixed as possible by agitating the tank. Samples were taken from the main tank with a graduated pipette onto a cell count tray of 0.08mm^3. Using x40 magnification the cells were counted in a manner presented in Figure 12.

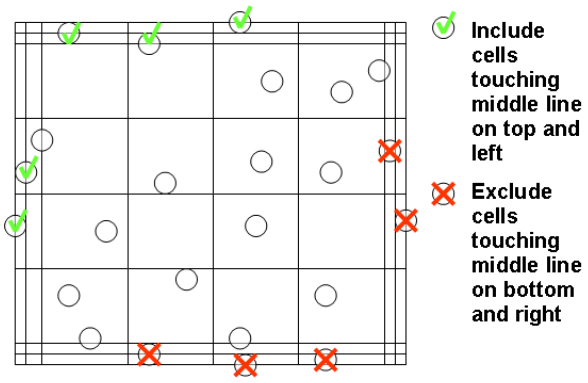

Figure 12. Cell counting procedure

The cell concentration per 1 ml were estimated by using the following arithmentic.

$$\#\,\boldsymbol{cells} \;=\; \mathbf{0.08}\boldsymbol{mm^3}$$

$$\boldsymbol{x} \;=\; \mathbf{1000}\,\boldsymbol{mm^3}$$

5.2 Growth rate

Growth rate is one important way of expressing the relative ecological success of a species or strain in adapting to its natural environment or the experimental environment imposed upon it. [15] Cell concentrations were measured every working day for 36 days. The maximum growth rate achieved was K' = 0.51 after 20 days of growth. The growth rate was calculated using equation given below:

$$K' = Ln \frac{(\frac{N2}{N1})}{(T2 - T1)}$$

Where N1 is biomass at time1 (T1) and N2 is biomass at time2 (T2) [15]

Results are shown in Figure 13 and Figure 14.

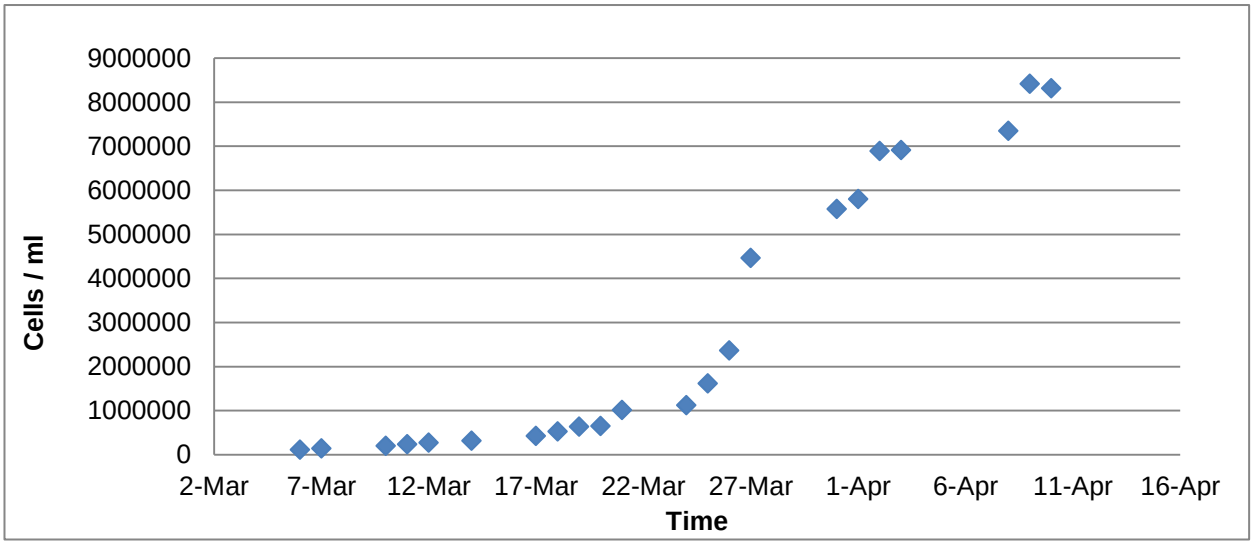

Figure 13. Chlorella pyrenoidosa growth rate

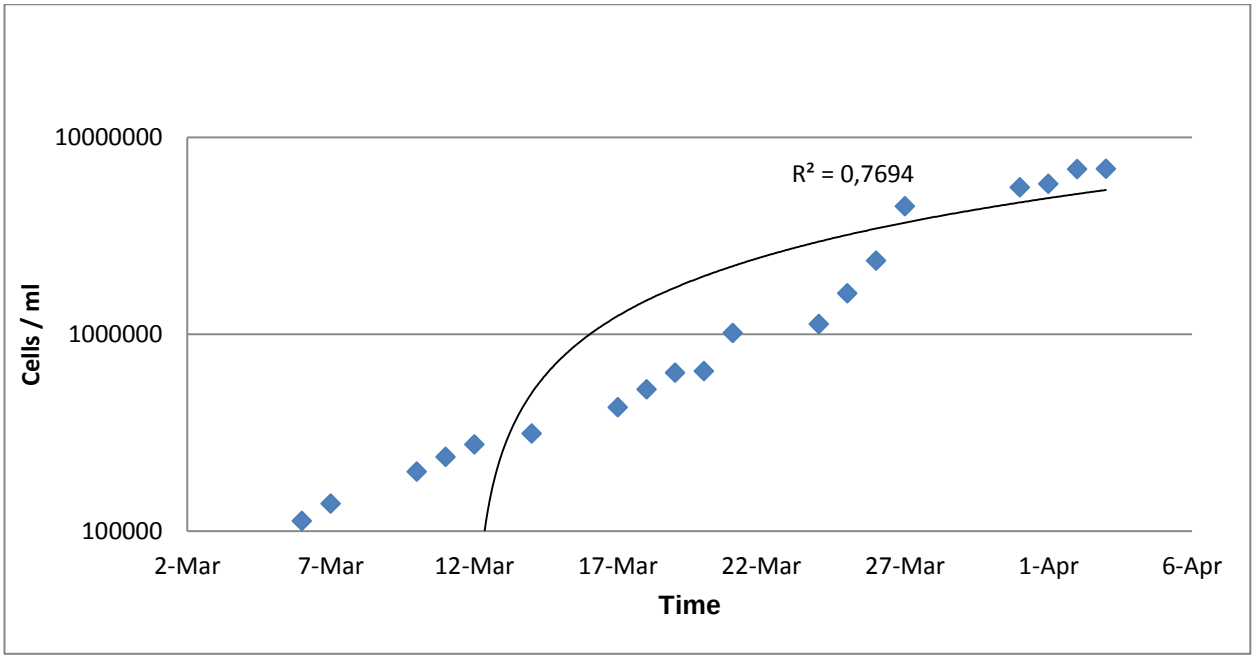

Figure 14. Chlorella pyrenoidosa growth rate on logarithmic scale

A maximum division of 0.73 per day and a doubling time of 32 hours were also calculated once the specific growth rate was known using the equations below:

$$Divistions\ per\ day; Div.day^{-1} = \frac{K'}{Ln(2)}$$

$$Doubling\ time = \frac{1}{Div.day^{-1}}$$

Temperature is an important element for growing algae. It strongly influences cellular chemical composition, the uptake of nutrients, carbon dioxide fixation, and the growth rates [22]. Bioreactor used in this project had a heating element that was able to adjust the temperature in the main tank. Temperature variation and the corresponding growth rates were recorded until a satisfactory growth rate was reached. Figure 15 shows growth rates against temperature variation.

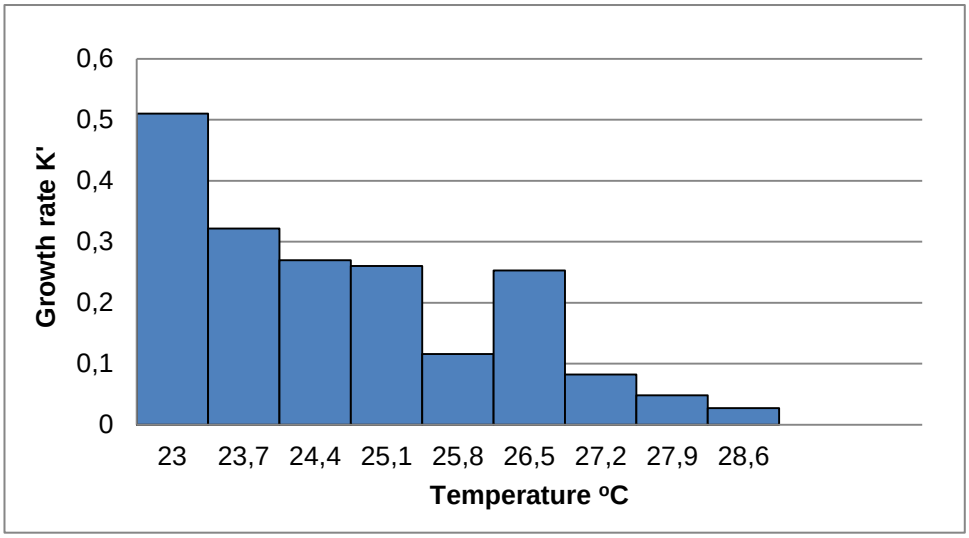

Figure 15. Algae growth against temperature variation

In 1958 research conducted by Samejima and Myers [23] reported a maximum growth rate of 0.65 at 25°C in the laboratory of Algal Physiology at the University of Texas in 1958. The experimental setup used in this project had the maximum growth rate of 0.51 at 23°C.

Algae cultivation in the main tank started on 6 March 2014 and ended on 10 April in 2014. On the 28th day of growth protozoa was discovered in a sample. It is quite common to find protozoa within algae growth environments. A sample of protozoa under x40 magnification can be seen in Figure 16.

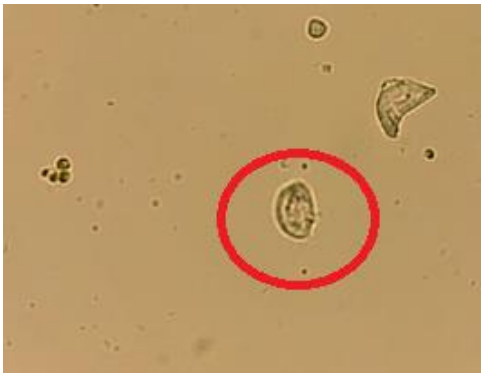

Figure 16. Protozoa in a sample

The unwanted species may have been introduced into the main algae tank via an improperly washed pipette or the air.

5.3 Biomass

This section describes the procedure used to determine the algae biomass dry weight. The laboratory work was conducted with the help of first-year students Sarlena Häninnen, Tongtong Gao and Shailesh Pandey. Glass fibres filters with a 1.6 µm pore size were placed in the oven overnight at 105^{O}C and then weighed on analytical scales (Figure 17). Weight of each individual glass fibre filter was written down, and then algal liquid was filtered using a Büchner flask. The filtered papers were then placed in the order they were weighed; then they were weighed after drying in a furnace at 105^{o}C (Figure 18). The difference obtained was the dry weight.

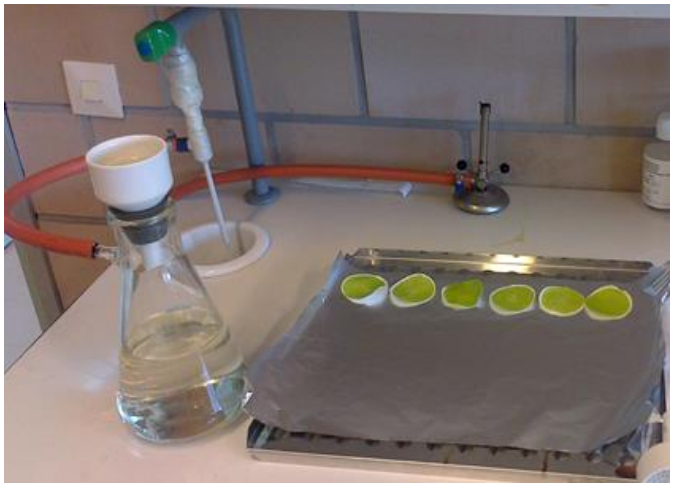

Figure 17. Biomass experimental setup

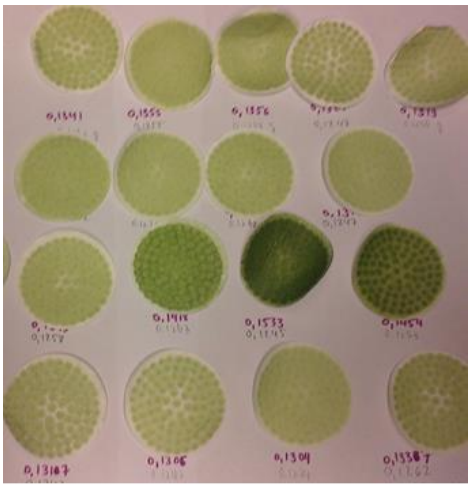

Figure 18. Filtered and dried algae cake

5.4 Nutrients and BOD_5

Biochemical Oxygen Demand or BOD is the quantity of dissolved oxygen consumed by microorganisms during the microbial and chemical oxidation of the constituents contained in a wastewater sample during an incubation period at a given temperature. The biochemical oxygen demand represents the oxygen utilized during the oxidation of both carbon and nitrogenous compounds. [24]

Dissolved oxygen was measured using a Hach LDO dissolved oxygen (DO) probe. BOD_5 was obtained by measuring the initial DO concentration and subtracting the one obtained after 5 days in a dark bottle at 20°C.
BOD_5 was calculated using the following equation:

$$DO_{initial}\left(\frac{mg}{l}\right) - DO_{in\ 5\ days}\left(\frac{mg}{l}\right) = BOD_5\left(\frac{mg}{l}\right)$$

Total nitrogen, phosphorous and COD were measured using a Hach DR 3900 spectrophotometer. To avoid over-measuring a range of 3 dilutions was prepared. Dilutions were prepared by taking 50mL, 20mL and 10mL of synthetic wastewater and algae treated water in separate 100mL volumetric flasks and diluted to the mark. Chemical Oxygen Demand (COD) is a measure of the amount of organic matter oxidized by a strong chemical oxidant. COD is used to measure organic matter in commercial, industrial, and municipal wastes that contain compounds toxic to biological life where the BOD_5 test would not work. The COD levels in a wastewater sample are almost always greater than BOD_5 levels because more compounds can be chemically oxidized than can be biologically oxidized. In most cases, once the COD/BOD_5 relationship is known for a particular facility, the COD concentration of a sample can be used to approximate the BOD_5 concentration. The COD test can generally be done within 2.5 hours, whereas a BOD_5 test takes five days. A COD test is performed when a quick determination of oxygen demand is needed. [24]

5.5 pH monitoring

Cell concentration samples were measured for ph as well. The pH was measured using a Mettler Toledo SG78 – SevenGo Duo pro™ portable pH meter. The graph in Figure 20 shows the pH fluctuation over time. To maintain a suitable pH, CO_2 was injected into the tank if pH went higher than neutral. It was concluded that such pH fluctuations had no significant role in algal growth rate.

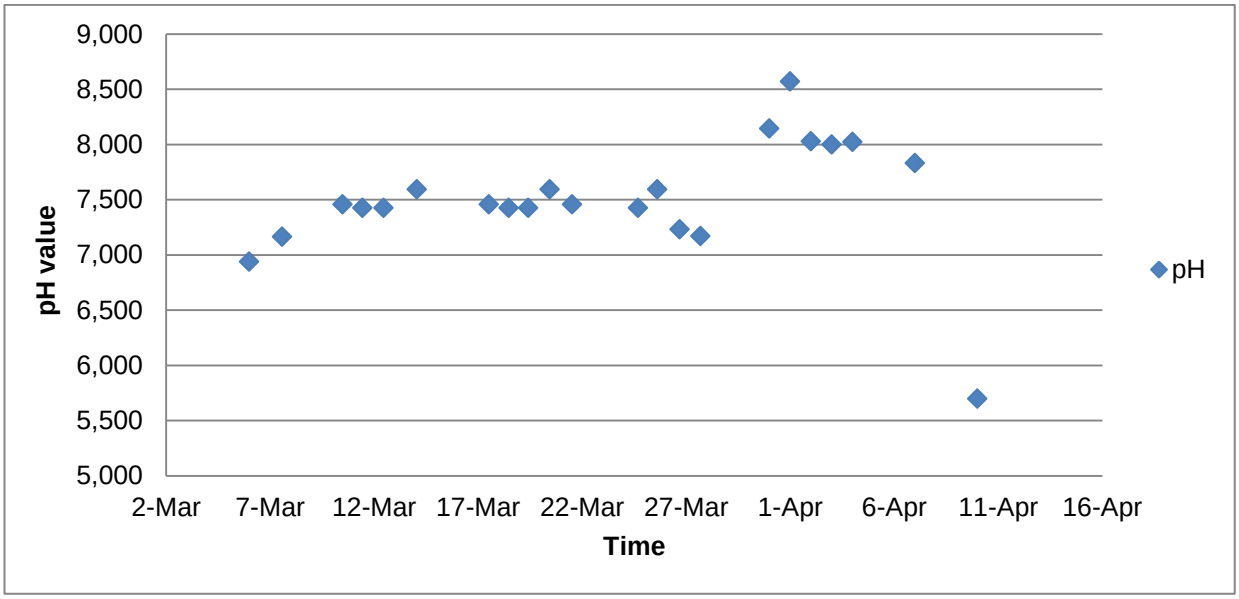

Figure 19. pH fluctuation in bioreactor

6 Analysis of Results

This chapter presents the results obtained for wastewater nutrient removal as well as for carbon capture efficiency. It also provides the results of biomass analysis and compares them with results from other sources. The gas inlet and outlet concentrations are also given.

6.1 Inlet and outlet gas

The bioreactor used a mixture of air and CO_2-enriched air to promote algal growth. Gas concentrations for inflow and outflow were measured using Vernier O_2 and CO_2 gas sensors. Unfortunately, these sensors failed to work properly after a few days in use. However, a fraction of data was obtained.

Air flow was measured to be constant at 0.2 $\frac{L}{min}$ or 288$\frac{L}{day}$.

With this information it was possible to calculate the total mass of CO_2 injected into the system over a period of time. Atmospheric CO_2 levels provided the total CO_2 injected in $\frac{g}{L}$. [25]

Current atmospheric CO_2 was 399.47 i.e. 399.47 $\frac{mg}{L}$ or 0.39947 $\frac{g}{L}$

Since air flow was constant, the total mass of CO_2 injected per day was calculated as follows:

$$0.39947\frac{g}{L} * 288\frac{L}{day} = 115.04\ \frac{gCO_2}{day}$$

After 15 days in the main tank pure CO_2 was combined with air injection using a three-way valve. This decreased the pH buildup in the main tank. Carbon dioxide was mixed with air at a $0.9\frac{L}{min}$ and injected into the tank until the pH reached around 7 again. This took approximately 3 minutes each time. The total CO_2 mass injected increased by $0.7190\frac{g}{day}$. On the basis of this the total theoretical CO_2 introduced to the tank was 2.5382 kg of pure CO_2.

With this set up, the sensors were not sensing the difference between input and output, but just the total input and output as Figure 20 illustrates.

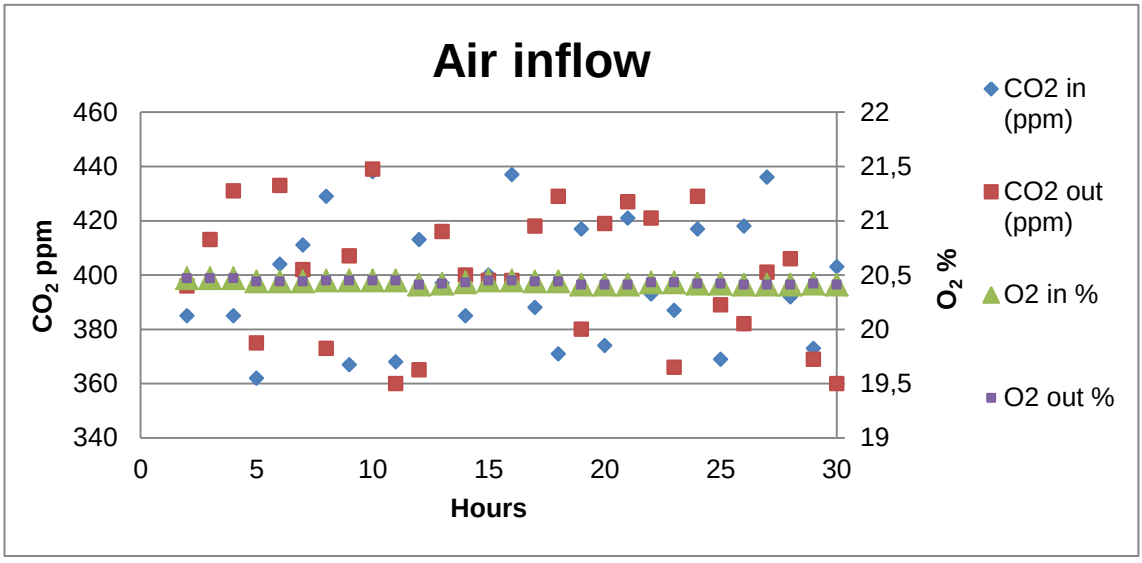

Figure 20. Injected air gas concentrations

Under ideal conditions using an airtight reactor and analytical gas sensors that are capable of measuring gas concentrations with sufficient resolutions, the difference obtained would show exactly how much O_2 algae produces during photosynthesis and how much CO_2 it absorbs. When using a splitter valve and combining air and pure CO_2, a noticeable difference between gas concentrations can be seen. In the secondary axis

of Figure 23 pH changes are graphed when injecting x11 elevated CO_2 concentration. The graph in Figure 21 shows the interval of 3:03 seconds.

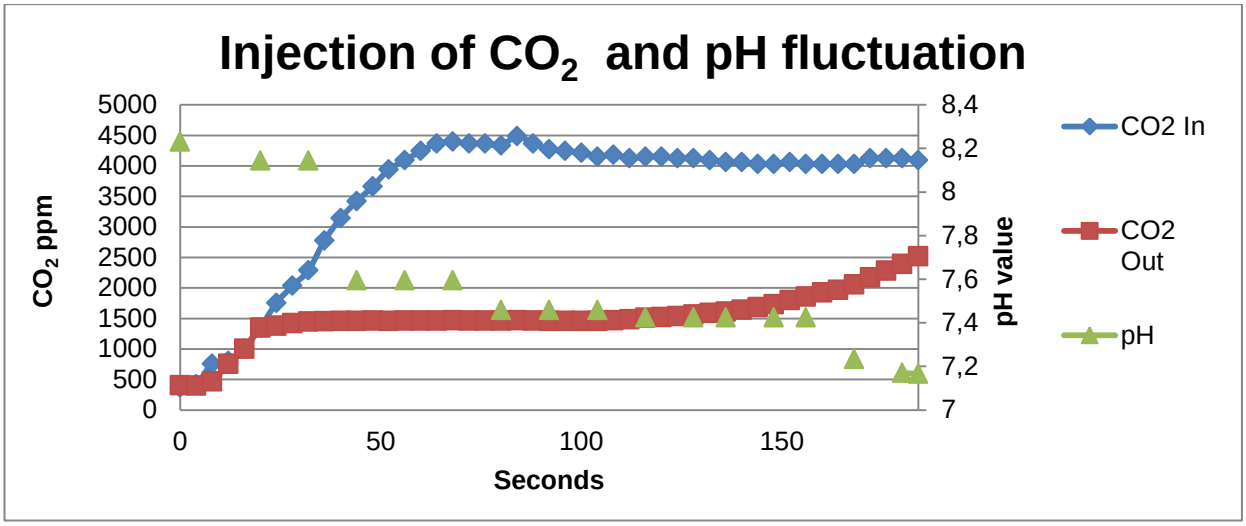

Figure 21. Graph depicts how CO_2 injection influences the value of pH.

6.2 Biomass analysis

The carbon content in microalgae under non-limiting nutrient conditions is about 50% by weight. Total biomass obtained was 7.603 g. Assuming a 50% carbon content, the total biomass obtained was divided by half. Actual carbon content may vary$\pm$ 5%.

$C_{captured} = \frac{Dry\ weight\ of\ biomass}{2} \rightarrow \frac{7.603\ g}{2} = 3.8015\ g$

$$\eta_{carbon\ capture\ efficiency} = \frac{C_{captured}}{Total\ gCO_2\ injected} \times 100 = \frac{3.8015\ g}{2538\ g} \times 100 = 0.14978\%$$

Knowing the total carbon captured, the theoretical rate at which oxygen is produced can be calculated. The process of photosynthesis shows that for every mole of carbon dioxide consumed, a mole of oxygen is produced:

$$6CO_2 + 6H_2O + light\ energy \rightarrow C_6H_{12}O_6 + 6O_2$$

Thus the O_2 production can be calculated by:

$$Total\ O_2 produced = \ Molar\ mass\ CO_2\ \div C_{Captured} = 44.10 \frac{g}{mol} \div 3.8015\ g$$

$$= 146.9\ mol\ O_2 \rightarrow 4.59\ g\ O_2$$

Using the equation below algal biomass productivity can be calculated.

$$Biomass\ productivity = \frac{Total\ biomass\ obtained}{Total\ volume\ of\ algae\ tank} = \frac{7.603\ g}{25\ L} = 0.30412\ \frac{g}{L}$$

7 Wastewater analysis

My bioreactor utilized synthetic wastewater as a growth medium for algae. Results obtained show the difference between initial synthetic wastewater nutrient composition and algae treated wastewater composition. Initial synthetic wastewater and algae treated wastewater results are given in Figure 22.

Nutrient concentrations mg-L^{-1}

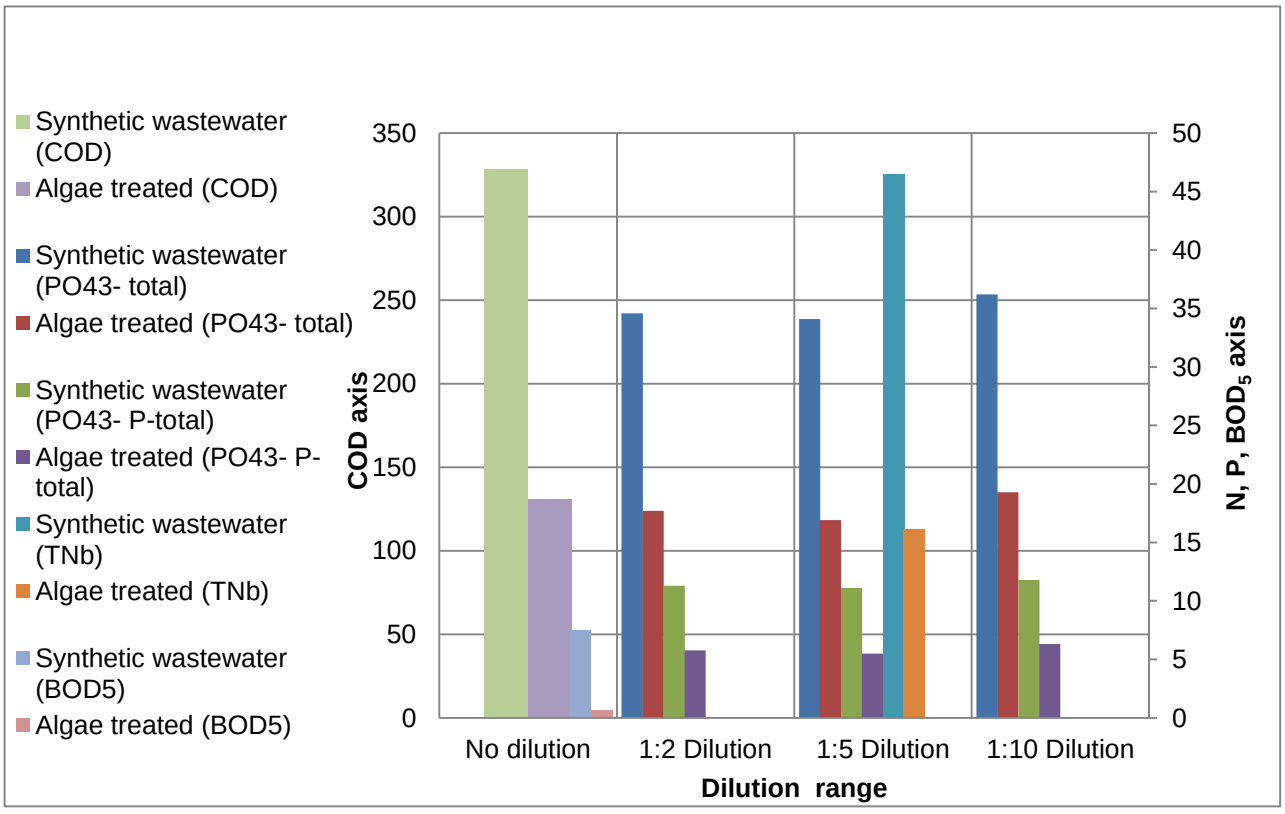

Figure 22. Nutrient concentrations in $\frac{mg}{l}$ in two separate vertical axis.

Table 1 gives the results of water analysis, (a) Synthetic wastewater and (b) Algae treated wastewater.

(a) Table 1. Synthetic wastewater nutrient concentrations

Dilution	Analyte						
	PO_4^{3-} total	PO_4^{3-} - P-total	TN_b	COD	DO $_{Initial}$	DO $_{Final}$	BOD_5
No dilution	-	-	-	328	9.23	1.8	7.43
1:2 Dilution	34.6	11.3	-	-	-	-	-
1:5 Dilution	34.1	11.1	46.5	-	-	-	-
1:10 Dilution	36.2	11.8	-	-	-	-	-

(b) Table 1. Algae treated wastewater nutrient concentrations

Dilution	Analyte						
	PO_4^{3-} total	PO_4^{3-} - P-total	TN_b	COD	DO $_{Initial}$	DO $_{Final}$	BOD_5
No dilution	-	-	-	131	7.64	6.98	0.66
1:2 Dilution	17.7	5.78	-	-	-	-	-
1:5 Dilution	16.9	5.5	16.1	-	-	-	-
1:10 Dilution	19.3	6.31	-	-	-	-	-

As seen from tables above and in Figure 22 it can be seen that sample dilution gave a marginal 3.75% error between results.

8 Discussion and Conclusions

The aim of this project was to determine the carbon dioxide capturing and simultaneous synthetic wastewater nutrient removal efficiency. Using a custom build bioreactor, it was possible to analyse algae growth rate and biomass production. Tests included basic cell counting and pH measuring techniques. Obtaining initial concentration of synthetic wastewater, made it possible to compare it to algae-treated results, thus obtaining the efficiency or carbon capture and nutrient reduction. Results showed that algae species *Chlorella pyrenoidosa* are indeed capable of absorbing N and P nutrients rather efficiently. The bioreactor design was successfully used to cultivate algae under laboratory conditions, using both air and CO_2. However, this model is just a small-scale model and more data is needed to develop it as a large scale pilot.

8.1 Wastewater

The results show that algae can grow in synthetic wastewater. These algae species also provided a satisfactory result absorbing nitrogen nutrients, although the minimum phosphorous removal efficiency was not achieved. This may be due to a initial phosphorous concentration, as algae favor it in excess. Initial and final nutrient concentrations were then used to calculate the removal efficiency. Table 2 gives some comparison data from two different sources: Viikki WWTP and a small scale WW treatment unit in KWH Freeze OY both located in the Helsinki region.

Table 2. Comparison of removal efficiencies.

	Nutrients and removal efficiencies			
	N	P	BOD_5	COD
Minimal removal %	>40%	>85%	>90%	-
Viikki WWTP (2010.10.16)	93%	98%	98%	95%
WehoPuts 70 KWH Freeze OY (2011)	86%	96%	96%	-
Algae bioreactor	65.38%	48.85%	91.19%	60.06%

The bioreactor met nitrogen and BOD removal requirements. According to Finlex [26] , the following minimum purification results must be achieved: P > 80%; N > 40%; BOD_5 > 90% under the law (196/2011). A study conducted by (Wong et al.) [27] suggests that *Chlorella pyrenoidosa* can achieve over an 90% nitrogen and a 62% phosphorous removal efficiency in concentrated municipal wastewater in 15 days. Another study by (Zheng et al.) [28] found that under optimal conditions, *Chlorella pyrenoidosa* could eliminate 75.2% of the active phosphorus, 100% ammonia nitrogen, 84.1% of the nitrite nitrogen and 52.8% nitrate nitrogen in the wastewater within 11 days.

Water quality results obtained in this thesis really show that *Chlorella pyrenoidosa* works well in removing nitrogen and reducing the BOD content of the synthetic wastewater. However, it was not as effective in achieving the minimum required phosphorous removal by 36%. It may be that the initial phosphorous concentration was not high enough, resulting in lower removal percentage.

8.2 Biomass

According to (Yang et al.) [29] *Chlorella pyrenoidosa* under 3000 lux illumination and 27°C could achieve 3.55 g/l biomass productivity after 30 days of cultivation. Depending on operational conditions, the theoretical efficiency of CO_2 use can range from 20 % to 90 %. In practice, the efficiency of CO_2 fixation in open raceways may be less than 10 %; for thin layer cultivation, the efficiency of CO_2 fixation is roughly 35%. In closed tubular photobioreactors (PBRs) CO_2 fixation efficiencies of approximately 75% have been reported: To reach such efficiencies with the bioreactor used in this thesis would require either expanding the bioreactor tank volume or to reduce inflow gas rates.

Since algae have environmental as well as commercial benefits, more researches are to be conducted in the field of algal cultivation. The modern technologies must be developed so that the algae can be produced in large water resources and more benefits can be achieved. Different ways to control the cost and to increase the productivity must be discovered. One of the ways to encourage researchers and investors in this field could be highlighting the commercial benefits that can be gained from algae such as biofuel, fertilizer, oil extraction and industrial use.

9 Future prospects

9.1 Integrating anaerobic digestion into the algal wastewater treatment process

From our biowaste and wastewater it is possible to obtain CH_4 and CO_2 gases. CH_4 can then be captured using selective membranes, pressurized and then burned in for example a CHP burner or a gas stove. CHP produces not only hot water but also a fair amount of electricity. Combustion of CH_4 produces more CO_2, which can then be combined with the CO_2 from the anaerobic process and transported to algae gas exchange tank to promote algal growth. Algae like nutrients found in wastewater; combined with additional CO_2 in exhaust gases and sunlight, algae produce O_2, (scrubs out emissions), biomass which can be used to make CH_4, FT liquids, ethanol and oils which then are processed to make alkanes (biodiesel). The positive outcomes of growing algae are essentially limitless. There exists septic tank systems for treating wastewater locally; unfortunately, those are not environmentally friendly solutions since they use various chemicals. They also do not provide any additional benefits. Using a

renewable source of energy (such as solar, wind, tidal) all of the processes can be automated to an unprecedented extent to achieve maximal sustainable efficiency. Thus, a fully carbon-neutral, sustainable economy that works in par with nature can be achieved. The scale of the bioreactor directly affects the removal efficiency. Extensive algae cultivation requires a suitable geographic location with high potential of using a renewable source of generating power. Figure 23 shows a conceptual sketch of a setup where anaerobic digestion is integrated into algae wastewater treatment process.

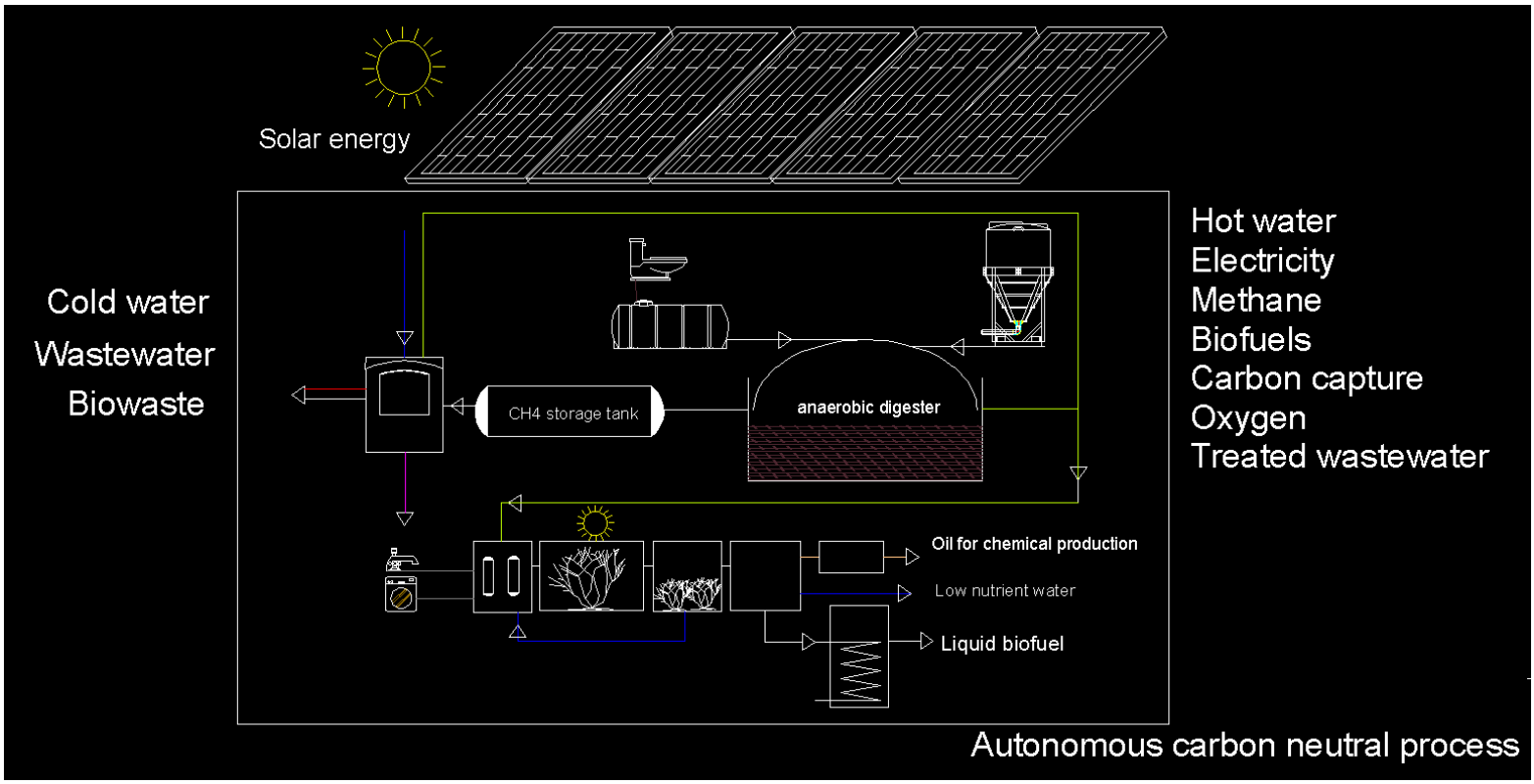

Figure 23. Integrating anaerobic digestion into algal wastewater treatment process.

There are numerous benefits such system could provide:

- Capturing, compressing and utilizing CH_4 gas from anaerobic reaction
- Utilizing the captured CH_4 in a CHP for electricity and how water
- Using microalgae reactor to capture CO_2 from both process and CHP
- Generating renewable energy

To achieve maximal positive impact of this incredible microorganism, such bioreactor systems should become a common way for households, neighborhoods, districts or even entire cities to clean their environment and produce their own fuel. I find this particularly applicable in densely inhabited areas e.g. Shanghai, Beijing and Guangzhou. Systems like this not only help clean the already heavily polluted air but also treat a fraction of the wastewater generated. The most cost effective way of cultivating algae is to establish a community that recycles their biowaste and wastewater primarily through the help of algae bioreactors. For example, nutrients from washing powder, laundry toilets are fed into a closed digester. A fraction of the wastewater is screened and fed in increments to the main algae reactor. Using the sun's energy, algae could be cultivated without the need of extra costs. Using the proposed design, developing countries have a good chance to start clean instead of going through a coal and fossil fuel phase.

9.2 Challenges and Risks for Algae Cultivation

Although the production of *Chlorella pyrenoidosa* looked promising and involved creative technology, it has not to date been cultivated on the scale as predicted. After a decade of experimentation, studies showed that, following exposure to sunlight, *Chlorella* captured just 2.5 percent more sunlight than other conventional crops. Therefore places where there is enough sunlight are favorable places for algae. A sophisticated process, and additional cost, is required to harvest the crop and, for Chlorella to be productive, its cell walls would have to be pulverized. The experiments which were carried out by scientists in laboratories proved that it is much more difficult to cultivate the algae than it seems to be. Tests conducted in this thesis work were time consuming. In any further research it is recommended to use more accurate, analytical gas sensors and measuring techniques such as light fluorescence for determining the growth rate and biomass production.

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Appendix 1. CO_2 risk assessment

1.1 Health risks

The gas cylinder contains compressed and liquefied carbon dioxide which is not classified as dangerous or toxic substance. It is a colorless gas or a colorless cryogenic liquid and at low concentrations the gas is also odorless. With higher concentrations odor will appear sharp and acidic and the gas will act as an asphyxiant and an irritant.

Contact with the gas can cause respiratory tract, eye and skin irritation. In an extreme case if the gas or liquid rapidly expands, due to a ruptured cylinder or a regulator failure, contact with exposed skin may lead to frost bites or a rapid suffocation.

Inhaling high concentrated carbon dioxide has various side effects like dizziness, vomiting, headaches and unconsciousness. Even though CO_2 is not flammable, exposing the cylinder to heat or fire may lead to an explosion or a rupture, due to the pressure the gas is under.

1.2 Handling

1.2.1 CO_2 cylinder should be safely used by folllowing the strutures that required for all gas cylinders:

- Never drag or physically carry cylinders
- Never pick up by the cap
- Never paint a cylinder.
- Never leave cylinders in areas where they will be subject to damage from falling objects, corrosion or public tampering.
- Never subject cylinders to artificially created low temperatures without approval from the supplier.

Reference: http://www.ehs.iastate.edu/publications/manuals/gascylinder.pdf

1.2.2 Accroding to the unique properties of CO_2 itself, some specific handle safety directions should be followed:

- Make sure the working area for CO_2 is properly ventilate
- Do not let CO_2 build up to unacceptable levels (above 5,000 ppm) in the work area.
- CO_2 cylinders must be away from water or aqueous fluids (beer, lemonade, etc.).
- Be sure of liquids(from other parts of our system) will not enter to the CO_2 cylinders
- Certain amount is mandatorily required when filling a CO_2 cylinder to strive against the pressure
- Make sure connect the hoses of CO_2 cylinder after a job to avoid liquid CO_2 flash-set to dry ice slugs.
- Backfeed into the container is not allowed.

1.3 Protection

Working with CO_2 gas requires proper protective clothing. CO_2 is more dangerous in liquefied form, which can causes frostbites by contacting with the skin. To avoid the damages, wearing laboratory coat and gloves is required during the experiments. If it contacts with eyes it may causes blindness, so safety goggles has to be worn as well. Since CO_2 founded in the air, in low concentration it does not causes problems by inhalation. The CO_2 tank contains higher concentration of liquefied gas, so the whole process has to be done in a fume cupboard to avoid the damages what the inhalation can cause. A high concentration can replace oxygen which leads to permanent damages in organs or even death.

	Catastrophic	Critical	Moderate	Minor	Negligible
Frequent	25	20	15	10	5
Probable	20	16	12	8	4
Occasional	15	12	9	6	3
Remote	10	8	6	4	2
Improbable	5	4	3	2	1

Taulukko 1. Ranking

Activity	Risk	Severity	Frequency	Total
Falling over that the top assembly to be knocked off	cylinder gets ruptured.	1	3	3
regulator failure.	explosion with possible death(high concentration only) or inury when the cylinder valve is opened	4	2	8
connecting failure	leakages	1	4	4
exposure to fire	cause the cylinder to rupture or explode	4	1	4
connect with water	frostbite	3	2	6

Taulukko 2. Above is the table of risk analyses

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Appendix 2. Bioreactor part list

Price	Unit
13.00 €	Lights 2x18W
11.00 €	2x SuperGlue
3.50 €	Duct tape
4.99 €	Extension cable
11.50 €	Vase
4.29 €	Fuse 1,6A
23.50 €	Lamp
23.50 €	5x Quick coupling nipples
2.50 €	Rubber plug
11.55 €	3x Drain's bushing corner
19.80 €	4x Drain's bushing branch
9.95 €	Drain's
6.60 €	Valve plug x3
4.40 €	2x Claw coupling rubber gasket
7.50 €	Mini ball valve
5.50 €	Quick coupling nipples
11.50 €	Quick coupling casing
5.00 €	2x Sauger gasket
179.58 €	**Grand total**