# How effective is microalgae treatment of nursery wastewater for nutrient removal?

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*Abstract*— **Nutrient removal from wastewaters has been traditionally achieved by activated sludge. But, in recent years, microalgae have been exploited for nutrient removal from wastewaters with the aim of producing fatty acid for use as bio-fuel. There are studies available on algae production using piggery, dairy and municipal wastewater, however, the rates of nutrient removal and algal growth have not been fully investigated. Furthermore, the growth rates of algae in different wastewaters for potential lipid production have not been fully established. Hence, in this paper, it was aimed to investigate the nutrient removal and algal growth using wastewater collected from a leafy vegetable nursery. Experiments were conducted using a batch reactor, having a capacity of 3.5 L. The reactor was filled with the nursery wastewater and inoculated by *Chlorella vulgaris* microalgae. Both compressed air and $CO_2$ were supplied to provide the inorganic carbon necessary for the algal growth. The required illumination for growth was provided by florescence light. Algal growth was monitored by measuring the volatile solid concentration and cell density using spectrophotometer. Algae were harvested soon after the peak growth for future fatty acid measurement. In this paper, the experimental results on nutrient removal, algae growth in nursery wastewater along with online measurements including pH and dissolved oxygen dynamics during the culturing period are discussed.**

*Keywords- Microalgae; Nutrient removal; Fatty acid; Bio-fuel*

## I. INTRODUCTION

In recent years, there has been a strong movement towards developing environmental-friendly renewable sources of energy, sparked by the need of sustainability. A key focus in the development of new technology has been the identification of "green" solutions which minimize greenhouse gas emissions. Currently 80% of the main energy source comes from fossil fuels [1]. New alternative energy sources are sought to reduce the need for fossil energy resources. Another important move towards sustainability has been to emit cleaner waste products across all industries. In the wastewater industry, the focus is on nutrient removal. One option to produce a sustainable fuel source and to achieve nutrient removal in waste streams at the same time is growing microalgae for bio-fuel production. The removal of nutrients in effluents is important to further reduce the potential for an algal bloom that can make the water unfit for any beneficial use and that can also cause drastic reduction of oxygen in the water

body. Nutrient removal from wastewaters has been traditionally achieved by activated sludge. But, in recent years, microalgae have been exploited for simultaneous nutrient removal from wastewaters and fatty-acid production for bio-fuel. The effluent that has been polished by algae treatment can be recycled for different beneficial uses. If carbon taxes are introduced, biodiesel production through algae can potentially become lucrative as this process is virtually carbon neutral when used in conjunction with $CO_2$ removal [2].

There are studies available on algae production using piggery, dairy and municipal wastewater, however, the rates of nutrient removal and algal growth have not been fully investigated. Furthermore, the growth rates of algae in different wastewaters for potential lipid production have not been fully established. Hence, this paper reviews some of the research currently conducted in the field of nutrient removal through microalgae and algal dry weight production for the bio-fuel industry. In addition, it is aimed to investigate the rates of nutrient removal and algal growth using wastewater collected from a nursery. The experimental results on nutrient removal and algae growth in nursery wastewater as well as algae mass for a future fatty acid production are discussed. In addition, the pH and dissolved oxygen (DO) data collected from real-time monitoring of processes are also analyzed with respect to growth. The research gives insight into the feasibilities of using small scale effluent cleaning via algal growth in decentralized businesses. Such businesses can potentially have their own on-site algae farm for polishing effluent and producing algae for bio-fuel.

## II. BACKGROUND

### A. Microalgae as a Fuel Source

Microalgae can be grown in wastewaters, coal seam gas waters and heavy metal contaminated waters, which means that no primary farmland is required [3]. Hence, algae do not compete with food crops. Algae have higher lipid content and grow much faster compared to the traditionally grown oil crops such as sunflower and oil palm [4]. Because of that, microalgae were identified as the only possible alternative fuel sources to one day replace all of the United States' fossil diesel demand [2] [4]. When burnt, bio-fuels from algae produce 70% less greenhouse gas emissions than fossil fuels [5] and minimize the release of nitrous oxides, sulfur and other gaseous pollutants [2][6] [7] [8].

## B. Microalgae Research to Date

Microalgae were first grown in the 1950's, when they were initially identified as a potential food source for humans and animals [3] [7]. During the fuel crisis in the 1970's, researchers began to evaluate the potential for using algae in the production of bio-diesel [2] [7]. Now microalgae have been rediscovered as a fuel source, wastewater polisher and $CO_2$ sink. The potential as a carbon dioxide sink may be interesting in the context of a carbon trading system [7] [9]. Research has shown that microalgae require less water than terrestrial plants [2]. This is because the cells are grown in suspension; hence they have more efficient access to water [6] [26]. For the same reason they perform well in taking up $CO_2$ and other nutrients.

## C. Chlorella vulgaris as a Typical Algal Strain Used

*Chlorella vulgaris* belongs to the green freshwater microalgae strain [6] [7]. *Chlorella vulgaris* microalgae have a lipid content of 20 – 30% of their dry weight [10]. This may be less than other algae strains; however, *Chlorella vulgaris* is extremely fast growing and relatively robust [6]. Unfortunately *Chlorella vulgaris* is often smaller than 10 μm in diameter, which can cause difficulties when harvesting the algae [11].

Most algae require substrate in N:P:C ratio of 8:1:50 [12]. Other authors used the Redfield C:N:P ratio of 106:16:1 [13]. The carbon component is very high and hence needs to be enriched by $CO_2$ to achieve optimal growth conditions. It is important to note that unlike many bacterial strains, algae are autotrophic. That means algae cannot make use of organic carbon. For that reason algae and bacteria can to some extent live in symbiosis where bacteria can utilize algal end- and by-products and algae can utilize $CO_2$ produced by the bacteria. Nevertheless they both compete for nutrients such as nitrogen and phosphorous. *Chlorella vulgaris* also requires high light intensity, warm temperatures of 20 to 26°C [8], a pH of near 7.5 [3] [14] and some trace elements such as iron [3]. Since these conditions are also optimal for bacteria that achieve nutrient removal, it is important to keep the organic content of the wastewater measured in terms of 5-day BOD or COD at a minimum if algae need be selectively grown. It was found that *Chlorella vulgaris* performed best in 250 mg/L COD piggery wastewater [15]. Substantial bacterial growth may also lead to elevated turbidity and hence hinders algal growth.

## D. Review of Nutrient Removal Research

While some research of microalgae in wastewater has been conducted in recent years, the nutrient removal of ammonia-N, nitrate-N, nitrite-N, organic nitrogen and phosphate-P and dry weight of the harvested algae has rarely been reported. Table 1 shows nutrient removal reported in the literature. Researchers grew *B. braunii* in a continuous batch system with secondary treated sludge as the growing medium [16]. They fed the algae for 11 days and kept growing them without additional feed for an additional 19 days. During their test *B. braunii* reduced nitrate from 7.67 mg/L to none within 6 days. Phosphorus was reduced from 0.02 mg/L to none within 1 day. Nitrite was also found to be consumed, but ammonium remained untouched. Algal growth was also tested in municipal wastewater and diluted dairy wastewater in a semi continuous reactor [14]. These researchers utilized naturally occurring algae. They found that the ammonium-N removal and phosphate removal for the municipal wastewater was 84 to >99 % and 93 to >99 % respectively. The differences in removal efficiency were due to different hydraulic retention times along with $CO_2$ and air feeding. They also achieved a 96 % ammonium removal and a >99 % orthophosphates removal for the diluted dairy wastewater. Other researchers also grew *Chlorella vulgaris* in 3 liter municipal wastewater bioreactors [17]. These researchers found that the algae removed 60.1 ± 13.7 % of ammonia-N. The nitrate-N removal was about 5 %. Some other researchers tested three different microalgae strains, including *Chlorella vulgaris* [3]. Their animal wastewaters included aerobic effluent and anaerobic effluent. For the aerobic and anaerobic effluent, total nitrogen was removed by about 38 % and total phosphorus was removed by about 24 %.

While nutrient removal is often reported in terms of ammonium-N and phosphate-P removal, little information was given for nitrate-N, nitrite-N and organic nitrogen. It is important to monitor the fate of all nitrogen forms in order to gain a complete picture of nutrient removal occurring in a bioreactor.

## III. METHODOLOGY

### A. Wastewater Characteristics

The wastewater from a leafy vegetable nursery was collected, filtered with 0.45 μm filter paper and stored at 4°C. Raw wastewater characteristics were tested for organic strength and nutrients in order to gauge their concentrations for adequate growth conditions needed for *Chlorella vulgaris*.

Organic strength of the wastewater was measured both by 5-day BOD according to Standard Methods [18] and total nitrogen using Total Organic Carbon/ Total Nitrogen analyzer (TOC-VCPH/CPN).

TABLE I.    PREVIOUSLY RESEARCHED ALGAE GROWTH AND NUTRIENT REMOVAL IN DIFFERENT WASTEWATERS

| Algae strain | Growth medium | Ammonia removal | Nitrate removal | Phosphate removal | Growth period | Reference |
|---|---|---|---|---|---|---|
| *B. braunii* | secondary treated sludge | 0% | 100% | 100% | 30 days | [16] |
| range of algae strains | municipal wastewater | 84 - >99% | - | 93 - >99% | 15-18 days | [14] |
| range of algae strains | diluted dairy wastewater | 96% | - | > 99% | 15-18 days | [14] |
| *C. vulgaris* | urban wastewater | ≈60.1% | ≈5% | - | 50 hrs | [17] |
| *C. vulgaris* | diluted aerobic effluent | ≈38% TN | | ≈24% | 10 days | [3] |
| *C. vulgaris* | diluted anaerobic effluent | ≈38% TN | | ≈24% | 10 days | [3] |

Nutrients present in the wastewater such as nitrate nitrogen, nitrite nitrogen and phosphate phosphorous in the liquid phase were measured using Ion Chromatography system (IC, Dionex ICS 2000) using an anion (AS-18) column during the analytical process.

## B. Bioreactor Design

A microalgae based titrimetric bioreactor was installed in the Environmental (water) laboratory, Faculty of Engineering and Surveying, University of Southern Queensland that enables the real time data collection corresponds to growth of *Chlorella vulgaris* microalgae (Fig. 1). The batch study was conducted using a single reactor having a capacity of 3.5 liters. Compressed air was supplied continuously at a rate of 0.25 L/min for proper aeration and the wastewater was continuously mixed using an overhead stirrer. $CO_2$ was fed continuously at a rate of about 10 ml/min from the bottom of the reactor. When the microalgae was adjusted to the new conditions and growth was established (after 4 days), $CO_2$ feeding occurred every two hours for 15 seconds during the light-period at a flow of 56 ml/min. Some authors found that it was important that carbon dioxide was continually available during daylight hours to ensure optimal growth [4]. There were two florescent light sources (2000 lux each) about 10 cm from the reactor to provide the required light intensity. Light was supplied for a duration of 16 hours starting from 5am to 9pm.
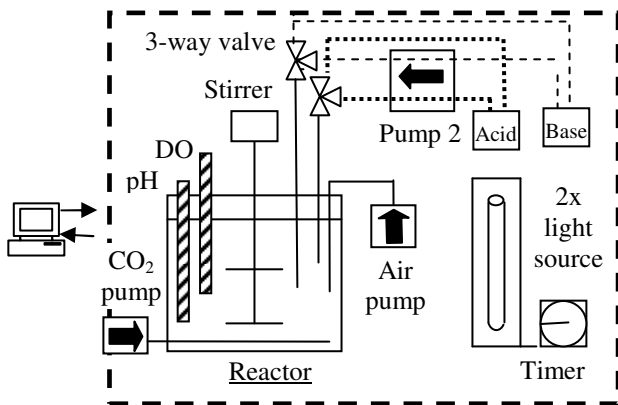


Figure 1.   Schematic diagram of bio-reactor

A titrimetric unit, consisting of Ionode pH electrode connected with the pH transmitter (TSP Mini Chem), two 3-way solenoid valves, an acid tank and a base tank, were installed in order to monitor and control the pH of the system during the experimental run. The acid and base were continuously pumped around by a peristaltic pump to keep a constant liquid pressure in the dosage system and to maintain constant dose rate. The data acquisition unit transmited the signals to the computer equipped with the Labview software package (National Instruments). In addition, the reactor was assembled with a dissolved oxygen electrode (YSI). The Labview software was used for monitoring the dissolved oxygen as well as temperature serial output from dissolved oxygen meter (TPS 90-D) and pH data with high frequency. Labview package also controled both of the 3-way solenoid valves that were assembled in the titrimetric respirometer for acid and base pulsing respectively to keep the pH in the reactor constant. The 0-1 volt signals from the transmitter were logged by a PC equipped with the Labview software package and a combined A/D I/O card (National Instruments, PCI-6013). All

data acquired from the experiment were recorded in a Microsoft Excel sheet format. The users can set the parameters on the front panel with the tolerance set- point limit. During the batch experiments, both pH and DO profiles were monitored every minute and pH was commonly controlled at a set point of 7.5 ± 0.3 by automatic addition of base (0.1 N NaOH) or acid (0.05 N $H_2SO_4$) solutions with two 3-way solenoid valves. Temperature was controlled in the laboratory using the air conditioning system at 25°C. However, the reactor temperature fluctuated between 21 and 24°C. Therefore, a temperature correction was performed on the experimental data to a base of 20°C to maintain the consistency.

## C. Inocculation by Chlorella vulgaris

*Chlorella vulgaris* was initially grown in 250 ml Erlenmeyer flasks with 150 ml MBL and 15 ml *Chlorella vulgaris* in MBL from an older culture (usually 3 weeks old culture). The entire content in the Erlenmeyer flask was used to inoculate the bio-reactor.

## D. Measurement of Nutrient Depletion and Algal Growth

We conducted the batch experiments lasting for 15 to 18 days. During this time, liquid samples amounting to 20 ml were collected from the reactor every day and filtered for measurements of nutrients and organic strength. Prior to sampling, the microalgae were suspended by air bubbling and overhead mixing to ensure getting a representative sampling for measurement purposes. The batch experiments were terminated when the death phase was entered which was found to start after 12 to 15 days after inoculation of *Chlorella vulgaris* into the reactor. In this paper, we are presenting the data we collected from the second of two such batch experiments.

The algal growth was measured in terms of suspended solids and by quantifying the cell density using spectrophotometry. Suspended solids were measured using Standard Methods [1]. At the same time, pH and DO profiles were also automatically logged into the system that gives an indication of growth in real-time.

For the spectrophotometry (Jenway 6705 UV/Vis. Spectrophotometer), the wavelength was set at 505 nm. The baseline was determined with the filtrate from the 0.45 μm suspended solids filtration.

Nutrient depletion was measured as described under section III A.

## E. Microalgae Harvest

Algae were harvested after the death phase had occurred. Centrifugation was used to separate the algae from the wastewater. The centrifugation occurred at 8000 rpm for 10 minutes with a 3 minute cool-down. The algae were washed once with distilled water and then underwent centrifugation again at 4000 rpm for 10 minutes. The algae pellets were then freeze dried and stored in a desiccator at room temperature for lipid measurement later on.

## F. Specific Growth Rate Calculation

The specific growth rate was calculated for nutrient depletion and oxygen release. For the first case, the slope of the nutrient change over a time period was divided by the slope of the suspended solids over a time period as shown in (1). For the oxygen production during the day time, the area under the dissolved oxygen graph over 24 hours was multiplied by the

volume of wastewater in the bio-reactor and divided by the light period time as shown in (2). The DO values during the dark period were considered to form the baseline, where the increase above the baseline was considered the DO production by the algae which resembled the area under the DO graph.

$$specific\ growth\ rate = \frac{slope\left(\frac{nutrient}{time}\right)}{slope\left(\frac{suspended\ solids}{time}\right)} \quad (1)$$

## IV. RESULTS AND DISCUSSIONS

### A. Raw Wastewater Characteristics

The wastewater used had a 5-day BOD of 113 mg/L. The nutrients in the wastewater had an average concentration of 116 mg TN/L, 0.508 mg $NO_2$-N/L, 41.1 mg $NO_3$-N/L and 23 mg $PO_4$-P/L, which resulted in a TN:P ratio of 5:1.

### B. Growth Characteristics

At first, the algae are likely to experience some shock when transferred from the stock solution to the growth solution. During this acclimatization period, the algae will grow very little or not at all, hence it is referred to as the lag phase. The phase can last up to a few days. The shock may be due to non-viable cells or spores in the growth medium, changed culture conditions or change in nutrient levels [19]. When the cells have adjusted to the new conditions, they enter an accelerated growth phase where they grow and divide. If nutrition and light is provided in excess, the algae start growing exponentially [19]. As the algae cell concentration increases, the quantity of light energy absorbed reduces and the algae enters a linear growth rate.
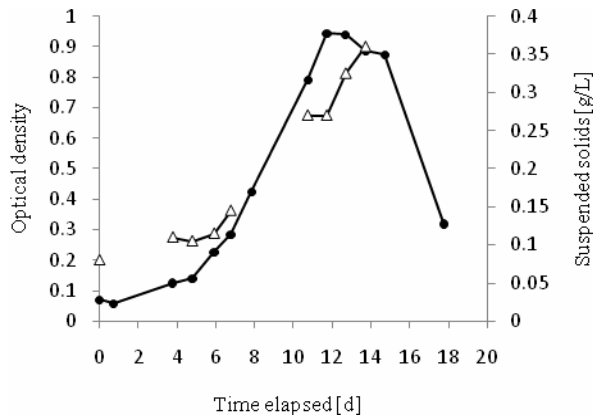


Figure 2. Algal growth was monitored by observing daily changes by measuring the absorbance at the optical density (●) at 505 nm with a spectrophotometer (Jenway 6705 UV/Vis.) and suspended solids (Δ)

The phase remains until nutrients or light energy become the limiting factor or if some inhibitors develop [19]. At that point, the death phase begins.

Fig. 2 shows the growth characteristics of the algae in terms of optical density and suspended solids. The lag phase could

be identified approximately until day 4. The exponential growth phase occurred between day 4 and 6; followed by a linear growth trend until day 12. The stationary phase remained until approximately day 15, followed by the death phase.

A relationship between suspended solids and optical density can be seen in Fig. 3. The relationship between the two can be expressed as; suspended solids = 0.2691 optical density + 0.0659 with the correlation coefficient of 93.6 %. Now, the concentration of suspended solids can be predicted by using the much quicker technique of measuring optical density.

$$mg\ Oxygen\ produced\ during\ daytime = \frac{area\ under\ DO\ graph}{light\ period}$$
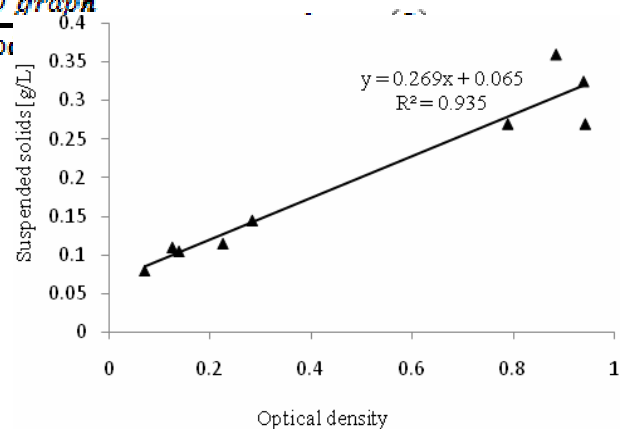


Figure 3. Relationship between suspended solids measurements and optical density at 505nm.

Fig. 4 shows filter papers from the suspended solids measurements. It shows the initial discoloration of the algae during the lag phase and the increase in algae mass during the linear phase. The maximum algae mass was achieved in the stationary phase. The death phase again shows discoloration on the filter paper.
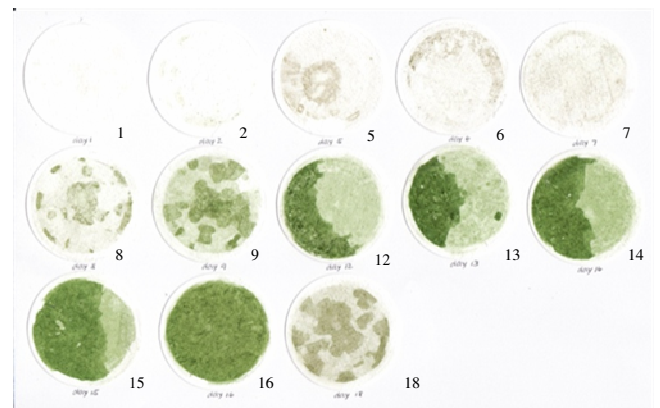


Figure 4. Filter paper collected over the growth period, where the numbers represent the day of filtration.

### C. Nutrient Removal

Fig. 5 shows the change in nitrogen concentration in the wastewater during the culturing period. Total nitrogen (TN) decreased by 2.64 mg/L/d. The algal growth in terms of suspended solid increment is 28.9 mg SS/L/d. Assuming all the suspended solids are from algal cells, the specific growth rate of algae can be deduced as 0.091 TN/SS. Organic-N and

NH$_4$-N decreased at a rate of 2.04 mg/L/d with a specific growth rate of 0.071 (Org-N + NH$_4$-N)/SS. Other researchers found NH$_4$-N to be depleted at a rate of 20.64 mg/L/d in wastewater of a steel making facility [20]. This can be attributed to the different wastewater characteristics.

Nitrate nitrogen remained unchanged until day 11, after which it decreased at a rate of 1.24 mg/L/d at a specific growth rate of 0.043 NO$_3$-N/SS. It should be noted that the conditions provided are ideal for both autotrophic nitrifying bacteria as well as algal growth. Nitrite was found to increase from 0.52 to 5.1 mg/L till the 4 day, which corresponds to decrease of ammonia during the lag phase when there was no algae growth. This could be due to nitrification where ammonia could be oxidized to nitrite. However, there was no evidence that nitrite nitrogen was oxidized to nitrate nitrogen since the profile of nitrate nitrogen remained same. After 12th day, the nitrite nitrogen increased to 7.51 mg/L again, indicating when the algae reached its death phase, the nitrifying bacteria could have started oxidizing ammonia. However, this cannot be verified unless we get repetitive results in from similar batch experiments.
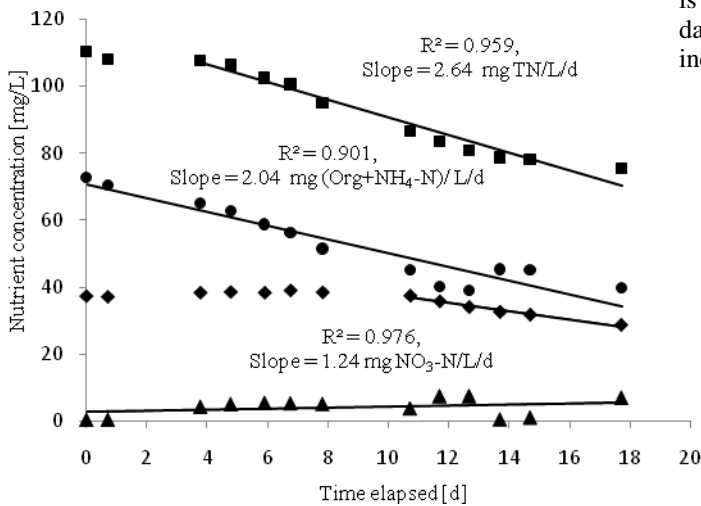


Figure 5. The graph shows the change in nitrogen concentration in form of Org-N + NH$_4$-N (●),TN (■), NO$_2$-N (▲), NO$_3$-N (◆).

Some researchers found that *Chlorella vulgaris* consumes ammonia first before any other form of nitrogen in the wastewater [13] [17] [20] [21]. The same behavior can be observed in this study as shown in Fig. 5. In our experiment too, ammonia nitrogen seems to have been the preferred nitrogen source until 11th day, after which some of nitrate nitrogen appeared to have been taken up.

Phosphate-P depleted at a rate of 0.27 mg/L/d with a specific growth rate of 0.0093 PO$_4$-P/SS. Algae require only 1/8th - 1/16th of P for every part of N, hence the slow consumption rate of P by the algae [12] [13]. In our experimental results, TN was used at 2.64 mg/L/d corresponding to a P use of 0.27 mg/L/d resulting in the ratio of 1:9.8. The depletion of PO$_4$-P is shown in Fig. 6.
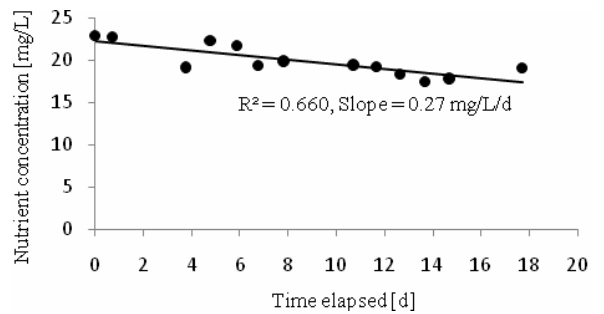


Figure 6. PO$_4$-P change with time.

### D. pH Change with Time

In addition to the continuous 10 ml/min CO$_2$ supply, CO$_2$ was provided every two hours for 16 hours from 5 am to 9 pm at a volume of 56 ml/min flow rate for 15 seconds. The pH rises as the algae consumes CO$_2$ and reduces its concentration [4][22], because the photosynthetic CO$_2$ fixation causes OH$^-$ to accumulate in the wastewater [13], while pH decreases as CO$_2$ is supplied. While Fig. 7 shows pH dynamics during the 18 days culturing period, in which the pH seems to have steadily increased after 4th day.
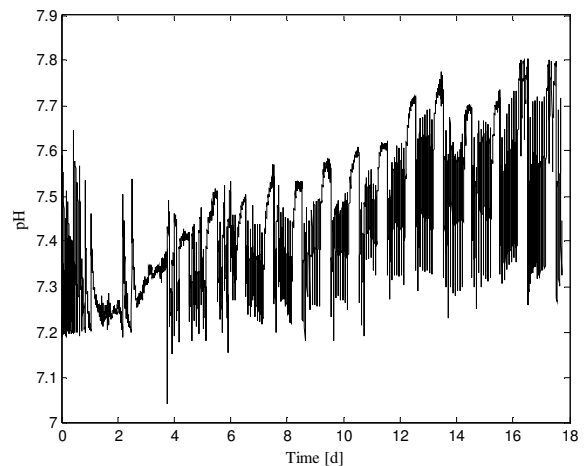


Figure 7. pH was monitored every minute for 18 days.

Fig. 8 shows the pH changes that occurred during different growth phases for 24 hours. There was no CO$_2$ supplied during the lag phase. Hence the pH during that phase remained largely unchanged and reached the lower end of the allowable pH range just after 8 pm, where it received a sodium hydroxide supply to increase the pH. The unchanged pH may have also been due to the consumption of ammonia by nitrifying bacteria, which release H$^+$ and hence decrease pH [9] and the offset of pH due to CO$_2$ consumption by algae from the continuous CO$_2$ supply and the atmosphere. In addition, it is possible to observe that no pH change occurs during the night time when the light was off from 21 hours to 5 hours in the morning. This is evident in all phases except in death phase. It is not clear why there was an increase in pH during the death phase, which is contrary to the expected behaviour.

Both the stationary and linear phase demonstrates the algae's growth between CO$_2$ supplies. The steepness of the slope indicates utilisation of CO$_2$ and hence algal growth. During the linear growth phase, the slope was 0.0945 (pH unit/h) where during the stationary phase the slope was 0.1428

(pH unit/h). This indicates that a larger biomass was available for the consumption of $CO_2$ during the stationary phase. For both phases, the slope was slightly shallower for the first two hours until when the second $CO_2$ addition occurs. This may be due to algae awakening to the light period which started at 5 am after having a rest period during the night time. The slope also decreased after the last $CO_2$ pumping after the light period has ceased. Algae gains energy from light and without this energy source they consume nutrients at a much lower level. This confirms findings that the algae stop growing during dark periods and start growing exponentially as soon as the light period starts again [6].
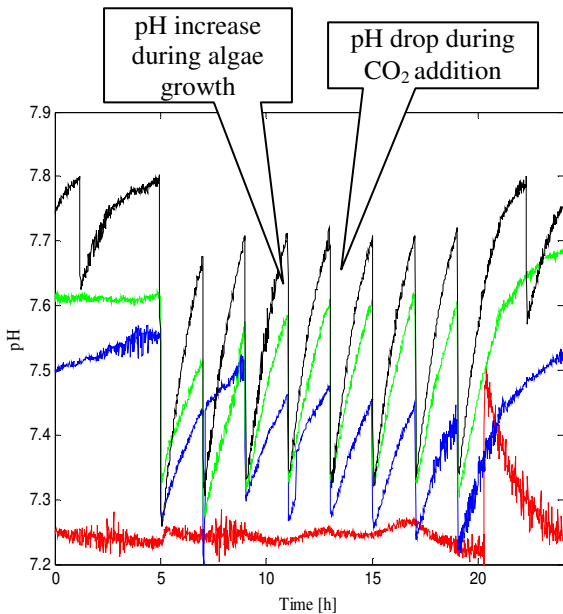


Figure 8. The graph shows the pH increase due to $CO_2$ consumption by the algae during the light period and the decrease of pH due to $CO_2$ addition for different growth phases: lag phase (——), linear phase (——), stationary phase (——) and death phase (——).

### E. Dissolved Oxygen Change with Time

Fig. 9 shows the concentration of dissolved oxygen for the 18 days culturing period. The bottom graph in Fig. 9 gives an indication for light and dark periods. While, during the light period, the algae photosynthesis exceeded the respiration causing the release of oxygen into the liquid phase, during the dark period there was a net consumption of oxygen as a consequence of respiration [23]. The oxygen production increased linearly until day 10 and then reduced exponentially until day 18.

Fig. 10 shows the average maximum dissolved oxygen reading during the light period and the average minimum dissolved oxygen reading during the dark period. The wastewater was supersaturated for 6 consecutive days during the light period. At 20°C, the saturation value of dissolved oxygen is taken as 9.17 mg/L. Supersaturation occurred during the linear growth phase as indicated by the optical density readings in the same graph, where DO produced exceeded this value. The maximum DO readings during the light period increased and decreased at a much higher rate over the entire growth period compared to the minimum DO readings at night. Fig. 10 also shows the net oxygen produced during the daytime. Again, the maximum oxygen produced occurred during the linear growth phase.
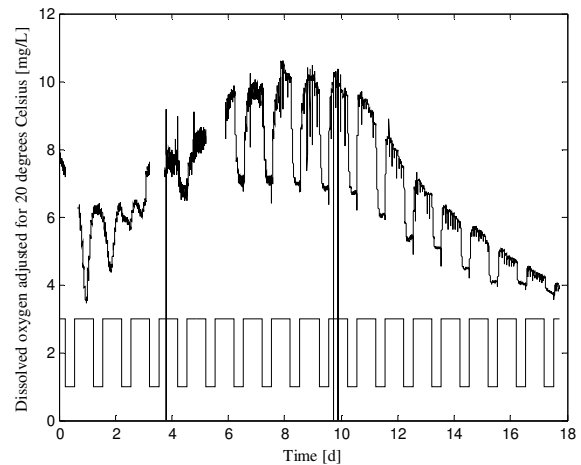


Figure 9. Dissolved oxygen was adjusted to 20 degrees Celsius and monitored every minute for 18 days. The bottom graph indicates light (peaks) and dark (valleys) periods.

Fig. 11 shows the concentration of dissolved oxygen for different growth phases over 24 hours. The sudden reduction oxygen that occured every two hours during the light period was due to oxygen stripping instigated by $CO_2$ pumping. It can be seen that there was a much higher oxygen release during the linear growth phase, followed by the stationary phase and death phase, corresponding to the net oxygen release rates of 334.9 mg DO, 220.9 mg DO, and 38.2 mg DO respectively. During the dark period, the DO concentration remained between 6 and 7.5 mg DO/L for the linear and stationary growth phase and approximately 4 mg DO/L during the death phase. These values were considered to be the baseline, where the increase above the baseline was considered to be the net DO production by the algae (photosynthesis – respiration).
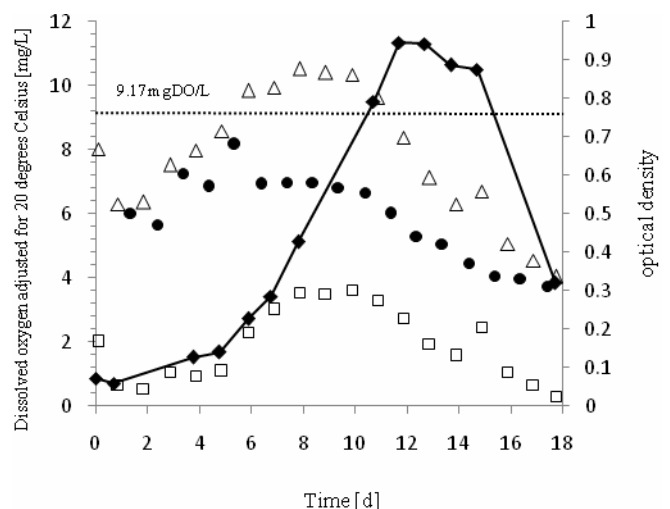


Figure 10. The graph shows the average maximum dissolved oxygen reading during the light period (Δ), the average minimum dissolved oxygen reading during the dark period (●), the net oxygen produced during the daytime (□) and the optical density readings (◆)
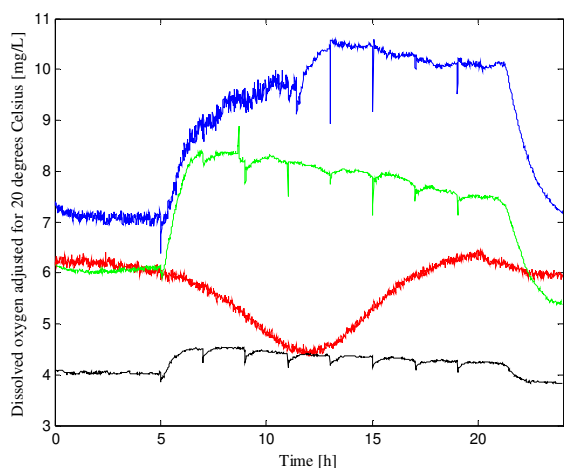
Figure 11. The graph shows the change in dissolved oxygen concentration in the bio-reactor for different growth phases: lag phase (—), linear phase (—), stationary phase (—) and death phase (—).

## F. Algal Dry Mass

The dry mass of the algae was measured to be 0.672 grams. It is important to note that the algae were not harvested until the death phase was advanced. Normally the algae would be harvested at the stationary phase where algae mass and lipid content is at its peak.

## G. Limiting Factors of Algal Growth

As the macronutrients where not fully depleted, it is unlikely that N, P or C were the limiting factors of algae growth and ultimately the cause of algae death. The quality and quantity of light, temperature, oxygen concentration, $CO_2$, pH and micronutrients can be possible influencing factors of algal growth [24]. In addition, microorganisms, competition with other algae or shear through vigorous mixing, may contribute to the growth performance of the algae [24]. After light, temperature has the most significant effect of algae growth [8]. While most algae strains can tolerate up to 15 degrees Celsius below their optimum temperature, the growth can be severely inhibited at a temperature rise of 2 to 4 degrees Celsius above the optimum. Some researchers suggested a constant temperature between 20 to 26°C, which was achieved for a majority of the time for this experiment [8]. Therefore it is unlikely for increased temperature to be a contributing factor. Supersaturated oxygen in the bioreactor can cause decrease of photorespirance and photooxydative death of the algae. At 20°C, the saturation value of dissolved oxygen is 9.17 mg/L, while the maximum DO reached on day 8 was 9.94 mg/L (Fig. 10). Elevated oxygen levels have significant effects on algal growth [24] [25]. This could be a cause of the early algal death. Another possibility could be the depletion of micronutrients and vitamins. Previous research has shown that micronutrients play an important part in the growth of algae [24]. As there were no micronutrients and vitamins applied with the fertiliser for the nursery seedlings, it is unlikely that all essential micronutrients were available for the algae. Unlike dairy, piggery and municipal wastewater where all the micronutrients will be available for the algae to grow, in the case of nursery wastewater, there is a lack of trace nutrients thus causing the death of algae in spite of the presence of nutrients. A certain degree of turbulence in the water can result in shearing and death of the algae [8] [24]. In this experiment, turbulence occurs due to mechanical mixing and repetitive aeration during sampling and could therefore have contributed

to the algal death. From microscope images it was found that at least one other algae strain (*Euglena*) and some bacteria were present in the wastewater at day 18. It is possible that these organisms competed with *Chlorella vulgaris*.

## V. CONCLUSIONS

This research has given insight into the algal growth rate in the nursery wastewater along with nutrient depletion rate of 2.64 mg TN/L/d and 0.27 mg $PO_4$-P/L/d, with the specific growth rate of 0.091 TN/SS. The online measurements including pH and dissolved oxygen can be a real-time indicator of different algal growth patterns. In this experiment, these measurements confirmed the algal growth pattern during the whole period, as well as the growth in 24 hours during light and dark hours. Further work involves measuring the lipid content of the produced algal cells and comparing those obtained from municipal and dairy wastewater. In addition, the algae growth models will be developed to interpret and calibrate the experimental observations in terms of nutrient depletion and real-time data collection.

REFERENCES

[1] Wiggers, VR, Meier, HF, Wisniewski Jr A, Chivanga Barros, AA, Wolf Maciel, MR 2009, Biofuels from continuous fast pyrolysis of soybean oil: A pilot plant study, *Bioresource Technology*, vol. 100, no. 24, pp. 6570-6577.

[2] Li, Y, Horsman, M, Wu, N, Lan, CQ and Dubois-Calero, N 2008, Articles: biocatalysts and bioreactor design, biofuels from microalgae, *Biotechnology Progress*, vol. 24, no. 4, pp. 815-820.

[3] Park, KY, Lim, BR, Lee, K 2009, Growth of microalgae in diluted process water of the animal wastewater treatment plant, *Water Science and Technology*, vol 59, no. 11, pp. 2111-2116.

[4] Chisti, Y 2007, Biodiesel from microalgae, *Biotechnology Advances*, vol. 25,no. 3 pp. 294-306.

[5] SARDI 2009, *Algal production group, SARDI Aquatic Sciences*, South Australian Research and Development Institute, retrieved December 20, 2009, from http://www.sardi.sa.gov.au/aquaculture/aquaculture/microalgal_producti on_systems

[6] Widjaja, A, Chien, CC, Ju YH 2009, Study of increasing lipid production from fresh water microalgae Chlorella vulgaris, *Journal of the Taiwan Institute of Chemical Engineering*, vol. 40, no. 1, pp. 13-20.

[7] Sheehan, J, Dunahay, T, Benemann, J, Roessler, P, 1998, *A look back at the U.S. Department of Energy's aquatic species program – biodiesel from algae*, U.S. Department of Energy's Office of Fuels Development, retrieved 6 January, 2010, from http://www.nrel.gov/docs/legosti/fy98/24190.pdf

[8] Mata, TM, Martins, AA, Caetano, NS 2010, Microalgae for biodiesel production and other applications: a review, *Renewable and Sustainable Energy Reviews*, vol. 14, pp. 217-232.

[9] Pienkos, PT 2007, *The potentials for biofuels from algae*, Algae Biomass Summit, San Francisco, retrieved 15 February, 2010, from http://www.nrel.gov/docs/fy08osti/42414.pdf

[10] Putt, R 2007, *Algae as a biodiesel feedstock: a feasibility assessment draft*, MSU Department of Chemical Engineering, Auburn, Alabama, retrieved 15 February, 2010, from http://bioenergy.msu.edu/feedstocks/algae_feasibility_alabama.pdf

[11] Weber, H 1997, *Woerterbuch der Mikrobiologie*, Gustav Fischer, Ulm, Germany.

[12] Lundquist, TJ, 2006, *Production of algae in conjunction with wastewater treatment*, California Polytechnic State University, retrieved 5 January, 2010 from http://www.docstoc.com/docs/2988054/Production-of-Algae-in-Conjunction-with-Wastewater-Treatment-Tryg-J

[13] Grobbelaar, JU 2004, Algal nutrition, mineral nutrition, *handbook of microalgal culture: biotechnology and applied phycology*, Blackwell Publishing, Calton, Australia.

[14] Woertz, I, Feffer, A, Lundquist, T, Nelson, Y 2009, Algae grown on dairy and municipal wastewater for simultaneous nutrient removal and

lipid production for biofuel feedstock, *Journal of Environmental Engineering*, vol. 135, no. 11., pp. 1115-1122.

[15] Travieso, L, Benitez, F, Sanchez, E, Borja, R, Martin, A, Colmenarejo, MF 2006, Batch mixed culture of Chlorella vulgaris using settled and diluted piggery waste, *Ecological engineering*, vol. 28, no. 2, pp. 158-165.

[16] Tsukahara, K, Sawayama, S 2005, Liquid fuel production using microalgae, *Journal of Japan Petroleum Institute*, vol. 48, no. 5, pp. 251-259.

[17] Ruiz-Marin, A, Mendoza-Espinosa, LG, Stephenson, T 2009, Growth and nutrient removal in free and immobilized green algae in batch and semi-continuous cultures treating real wastewater, *Bioresource Technology*, vol 101, no. 1, pp. 58-64.

[18] APHA 1995, *Standard methods for the examination of water and wastewater, 19th Edition.*, American Public Health Association, Washington, DC.

[19] Lee, YN, Shen, H 2004, Basic culturing techniques, *handbook of microalgal culture: biotechnology and applied phycology*, Carlton, Australia.

[20] Yun, YS, Lee, SB, Park, JM, Lee, CI, Yang, JW 1997, Carbon dioxide fixation by algae cultivation using wastewater nutrients, *Journal of Chemical Technology and Biotechnology*, vol. 69, pp. 451-455.

[21] Graham, LE, Wilcox, LW 2000, *Algae*, Prentice Hall, New Jersey, USA.

[22] Fogg, GE, Collyer, DM 1953, *The accumulation of lipides by algae*, *algal culture from laboratory to pilot plant*, p. 177, Carnegie Institution of Washington Publication 600, Washington, D.C.

[23] Masojidek, J, Koblizek, M, Torzillo, G 2004, Photosynthesis in microalgae, *handbook of microalgal culture: biotechnology and applied phycology*, Blackwell Publishing, Calton, Australia.

[24] Moheimani, NR 2005, *The culture of coccolithophorid algae for carbon dioxide bioremeddiation*, thesis, Murdoch University.

[25] Richmond, A 2004, Biological principles of mass cultivation, *handbook of microalgal culture: biotechnology and applied phycology*, Blackwell Publishing, Calton, Australia.

[26] Bott, K, Nayar, S 2008, Biodiesel from microalgae, *Issues (South Melbourne)*, vol. 85, pp. 39-41.