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Nutrient removal of nursery and municipal wastewater using *Chlorella vulgaris* microalgae for lipid extraction

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ABSTRACT

Microalgae grown in wastewater media can not only be exploited for the nutrient removal from the wastewater, but also for the production of biofuels. In this paper, we investigated the growth of *Chlorella vulgaris* in iceberg lettuce nursery and municipal wastewater in a batch reactor. We analyzed the microalgal growth rate, nutrient removal rate and lipid production along with real-time monitoring of pH and dissolved oxygen (DO) dynamics during the culture period, which is rarely reported. NH_4-N was found to be the preferred form of nitrogen among different species of nitrogen for the growth of microalgae, and total specific nitrogen depletion rates of 33.0 and 39.6 mg TN/gSS/d were observed for nursery and municipal wastewater, respectively. The specific phosphate removal rates were 3.4 and 10.8 mg PO_4-P /gSS/d for nursery and municipal wastewater, respectively. The algal growth in nutrient-rich media resulted in increase in pH and DO concentration concurrently. The online measurements including pH and DO proved to be real-time indicators of algal growth not only during the different stages of culture period but also during dark and light hours in a day indicating definite variations in measured values depicting the photosynthesis dynamics.

Keywords: Microalgae; *Chlorella vulgaris*; Nutrient removal; Online-monitoring; Wastewater; Biofuel

1. Introduction

For a number of years, there has been a strong movement toward developing environmental-friendly technologies to minimize waste products in water bodies, greenhouse gas emissions, and the use of fossil fuels. In the wastewater industry, it is important to remove the nutrients from the effluents to reduce the possibility of nutrients triggering artificial algal blooms leading to eutrophication. Eutrophication can

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make the water unfit for any beneficial uses and can cause drastic reduction of oxygen in the water body, adversely affecting aquatic life. Traditionally, nutrient removal from wastewaters has been achieved by activated sludge. But technology has shown that microalgae can be exploited for simultaneous nutrient removal from wastewaters and subsequent lipid production for biofuel extraction. In addition, nutrient-rich wastewaters that are polished by algal treatment can be enriched with CO_2, providing the inorganic carbon source for algae to perform photosynthesis and hence recycling some of the greenhouse gases. Therefore, as carbon taxes are introduced, biofuel production through algae can potentially become very lucrative as the fuel is virtually carbon neutral, when used in conjunction with CO_2 removal [1–3]. When burnt, biofuels from algae can produce 70% less greenhouse gas emissions compared with fossil fuels [4] and minimize the release of nitrous oxides, sulfur, and other gaseous pollutants [1,2,5,6].

There are studies available on algae production using piggery [7–10], dairy [11,12], municipal [11,13–17] wastewater, and even wastewater from a steel making facility [18]. These studies have evaluated the quality of the effluents treated by the microalgae and some also estimated the lipid content of the harvested algae. From a nutrient removal perspective, most of these studies focus only on total nitrogen depletion, failing to give a complete breakdown of different nitrogen forms such as organic, ammonia, nitrite, and nitrate nitrogen during the algal growth. However, the wastewater from municipal wastewater plants and animal farms contains all forms of nitrogen including organic, ammonia, and nitrate nitrogen. When these wastewaters act as the feed for the microalgae, how these different species of nitrogen are consumed during the growth of microalgae needs further research.

Furthermore, the algal growth in nutrient-rich media often brings about pH dynamics and dissolved oxygen (DO) production concurrently. Axelsson [19] investigated changes in pH and DO as a measure of photosynthesis by marine macroalgae in a continuous flow reactor. However, real-time monitoring of pH and DO dynamics has not been extensively investigated in conjunction with algal growth.

Therefore, in this work, we investigated not only the removal of individual species of nutrients during an algal growth, but also monitored the real-time dynamics of pH, and DO during the monitoring period to give a complete picture of nutrient removal and growth dynamics in a batch bioreactor. The experiments were performed on two different wastewaters collected from a leafy vegetable nursery and a wastewater reclamation facility (municipal wastewater) using *Chlorella vulgaris* microalgae. This paper also discusses the experimental results on algal lipid yields in the selected wastewaters.

2. Materials and methods

2.1. Selected algal strain and growth conditions

For this research, *C. vulgaris* microalgae have been selected. *C. vulgaris* microalgae have a lipid content of 20–30% of their dry weight [20], extremely fast growing and relatively robust [5].

Most algae require substrate in C/N/P ratio of 50:8:1 [21]. The requirement for carbon component is very high, and therefore, CO_2 enrichment is essential to achieve optimal growth conditions. It is important to note that unlike many bacterial strains, microalgae are autotrophic, even though some species of microalgae can make use of organic carbon to certain extent for their growth. For that reason, algae and bacteria can, to some extent, live in symbiosis, where bacteria can utilize algal end- and by-products along with DO released by the algae and algae can utilize CO_2 produced by the bacteria. Nevertheless, they both compete for nutrients such as nitrogen and phosphorous.

C. vulgaris requires high light intensity, warm temperatures of 20–26˚C [6], a pH of near 7.5 [11,17] and some trace elements such as iron [7]. In addition, it was found that ammonia is often the preferred nitrogen form by *C. vulgaris* [16,18,22,23]. 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 at a minimum if algae need to be selectively grown. Substantial bacterial growth may also lead to elevated turbidity and hence hinders algal growth. *C. vulgaris* was obtained from CSIRO's Australian National Algae Culture Collection, located in Hobart, Tasmania, and initially grown in MBL media according to the purchase instructions [24] with the ratio of 1:10 (inoculum: MBL) in test tubes. The culture was then transferred in Erlenmeyer flasks of 250 mL in desired wastewater in triplicates, during when algal growth was monitored. Then, the culture was transferred in 3.5 L bioreactors for monitoring daily nutrient consumption profiles, pH, and DO dynamics along with algal growth.

2.2. Wastewater characteristics

The wastewater from leafy vegetable nursery water of about 50 L was collected from a nursery located in Cambooya, Queensland, at the point of downstream drain from run-off irrigation. The

domestic wastewater of about 50 L was collected from Wettala wastewater reclamation facility located in Toowoomba, Queensland at the point of effluent discharge to stream. These waters were filtered with 0.45-μm filter paper and stored at 4°C until further use. Raw wastewater characteristics were tested for organic strength and nutrients in order to gauge their concentrations for adequate growth conditions needed for *C. vulgaris*.

Organic strength of the wastewater was measured both by 5 day BOD according to standard methods [25] and total nitrogen using total organic carbon/total nitrogen analyzer (TOC-VCPH/CPN) by Shimadzu Corporation. Nutrients present in the wastewater such as NO_3–N, NO_2–N and PO_4-P in the liquid phase were measured using Ion Chromatography system (IC, Dionex ICS 2000) with an anion (AS-18) column during the analytical process.

2.3. Bioreactor design

A microalgae based titrimetric bioreactor was installed in the Environmental (water) laboratory, Faculty of Engineering and Surveying, University of Southern Queensland that enabled the real-time data collection corresponds to growth of *C. vulgaris* microalgae (Fig. 1).

The batch study was conducted using a single reactor having a capacity of 3.5-L. 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. Carbon dioxide was fed continuously at a rate of about 10 mL/min from the bottom of the reactor. When the microalgae were adjusted to the new conditions and growth was established (after 4 days), additional CO_2 feeding occurred every two hours for 15 s during the light-period at a flow of 56 mL/min, which was optimized based on the desired pH range. Some authors found that it was important that carbon dioxide was continually available during daylight hours to ensure optimal growth [26]. There were two florescent light sources (2,000 lux each) placed on two sides of the reactor to provide the required light intensity. Light was supplied from 5 am to 9 pm, operated on a 16:8 h light/dark cycle. 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. 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 transmitted the signals to the computer equipped with a Labview software package (National Instruments). In addition, the reactor was assembled with a DO electrode (YSI). The Labview software was used for monitoring the DO as well as temperature serial output from DO meter (TPS 90-D), and pH data (TSP Mini Chem) with high frequency. The Labview package also controlled 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 was recorded in a Microsoft excel sheet format. The users can set the parameters

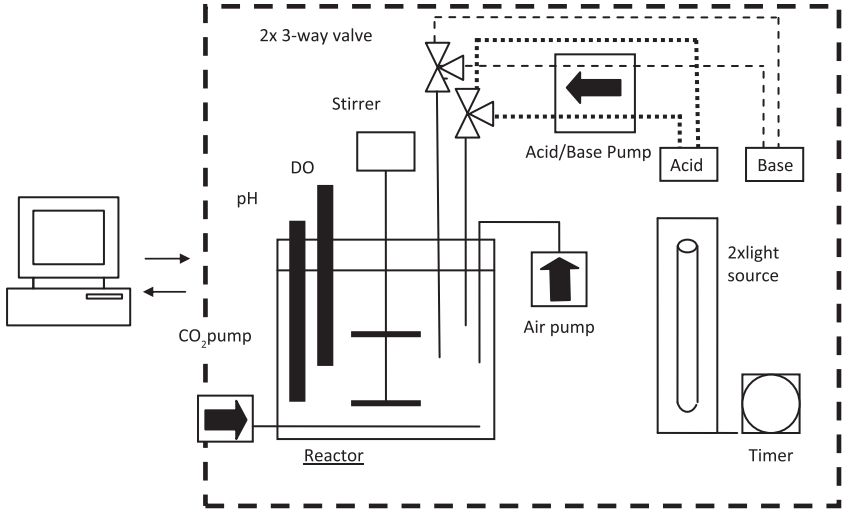

Fig. 1. Schematic diagram of bio-reactor.

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 consistency.

2.4. Measurement of nutrient depletion and algal growth

The batch experiments were conducted lasting for 13–18 days in batch reactor of 3.5 L. 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 aeration 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 10–15 days after inoculation of *C. vulgaris* into the reactor.

The algal growth was measured in terms of suspended solids and by quantifying the cell density using spectrophotometry (Jenway 6705 UV/Visible Spectrophotometer) at the wavelength of 505 nm. Suspended solids were measured using Standard Methods [25]. At the same time, pH, and DO profiles were also automatically logged into the system that gives an indication of growth in real-time.

2.5. 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 8,000 rpm for 10 min with a 3 min cool-down. The algae were washed once with distilled water and then underwent centrifugation again at 4,000 rpm for 10 min. The algae pellets were then freeze dried and stored in a desiccator at room temperature until they underwent lipid measurement.

2.6. Lipid extraction

The freeze-dried algae underwent the Folch method [27] to determine the total algal lipid content. The freeze-dried algal cells were first homogenized with chloroform and methanol (2:1 ratio) to a final volume of 20 times the algal weight in grams. A 20 min agitation of the mix was undertaken at 25°C and 150 rpm, after dispersion. The mix was then filtered through fluted filter paper to recover only the liquid phase. The test tube was rinsed with an additional 1.5 mL methanol to recover more liquid phase if necessary. Then, one fifth of the total volume was added as water to the flask to wash the solvent. The mix was then placed into a vortex for 10 s to allow full mixing. To separate the liquid and the chloroform phase, the mix was then centrifuged at 2,000 rpm for 5 min. The upper phase/nonchloroform phase was then siphoned off. The chloroform phase, which contains the lipids, was poured into preweighted flasks and dried under a nitrogen stream. The flasks containing the lipids were then weighted again. The lipid content was measured as a percentage of lipid weight to algal dry weight.

2.7. Volumetric and specific nutrient depletion rate

The volumetric nutrient depletion rate was calculated based on the slope of the nutrient consumption over time [8]. For the calculation of the specific nutrient depletion rate, the slope of the nutrient depletion over a time period was divided by the slope of the suspended solids produced over the culture period. Unless otherwise specified, the rates were calculated based on at least 6 points in the linear range of curve as shown in figures by solid line. Corresponding correlation coefficient was given as R^2, which is a statistical measure of how well the regression line approximates the real data points. These statistical evaluations were done using Microsoft Excel software.

3. Results and discussion

3.1. Raw wastewater characteristics

The leafy vegetable nursery wastewater was collected from an iceberg lettuce producing nursery during the fertilizer application. The municipal wastewater for this research was prepared by collecting and mixing the wastewater from the influent and effluent obtained from the plant. The measured TN/P ratio in the municipal wastewater after mixing was found to be 20:1 with the PO_4-P concentration of 0.9 mg/L. Since PO_4-P concentration was not sufficient for substantial algal growth, we synthetically added KH_2 PO_4 of final intended concentration of 10 mg/L. Since we intended to maintain a ratio of TN:P as 10:1, and there was only about 60 mg/L of organic + ammonia nitrogen available as total nitrogen, we added an additional 60 mg/L of NO_3–N by means of $NaNO_3$. The artificial addition of $NaNO_3$ enabled us to observe

the consumption of different forms of nitrogen during the algal growth. The prepared wastewater characteristics are given in Table 1.

3.2. Algal growth characteristics

The growth characteristics of the algae in nursery wastewater in terms of optical density and suspended solids are shown in Fig. 2. The relationship between suspended solid concentration and optical density can be described as suspended solids = 0.2691 optical density + 0.0659 (correlation coefficient $R^2 = 93.6\%$) in case of the nursery wastewater. Once the relationship is established, the concentration of suspended solids indicating the algal growth can be predicted by using the much quicker method of measuring optical density.

3.3. DO change with time

The concentration of DO for the 18 days culturing period in nursery wastewater is shown in Fig. 3. During the light period, the algae photosynthesis exceeded the respiration causing the release of oxygen into the liquid phase. On the contrary, during the dark period, there is a net consumption of oxygen as a consequence of respiration [28]. As shown in Fig. 3, the oxygen production increased until day 8 and reached a plateau until day 10 and then reduced gradually until day 18.

Fig. 4 shows the average maximum DO reading during the light period and the average minimum DO reading during the dark period in nursery wastewater. The wastewater was supersaturated (DO above 9.17 mg/L at 20°C) for 6 consecutive days during the

Table 1
Wastewater characteristics

Wastewater characteristics	Lettuce nursery wastewater	Municipal wastewater
BOD (mg/L)	113.0	60.7
Organic + ammonium N (mg/L)	72.2	56.6
Nitrite nitrogen (mg/L)	0.5	0.0
Nitrate nitrogen (mg/L)	38.8	60.2*
Total nitrogen (mg/L)	111.0	116.8
Phosphate as P (mg/L)	22.0	12.8*
TN:P ratio	5.0:1	9.1:1

*Municipal wastewater was synthetically modified using $NaNO_3$ as nitrogen source and KH_2PO_4 as phosphate source as described in the methodology.

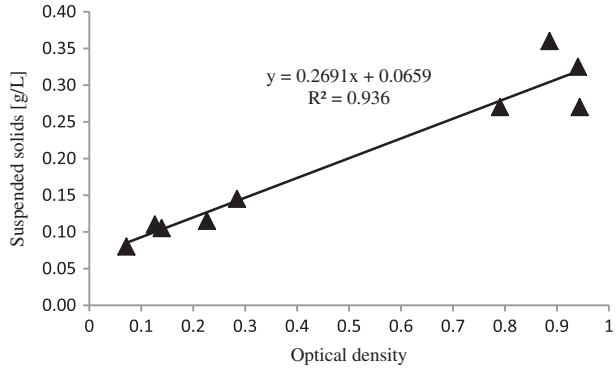

Fig. 2. Relationship between suspended solids measurements and optical density at 505 nm in lettuce nursery wastewater.

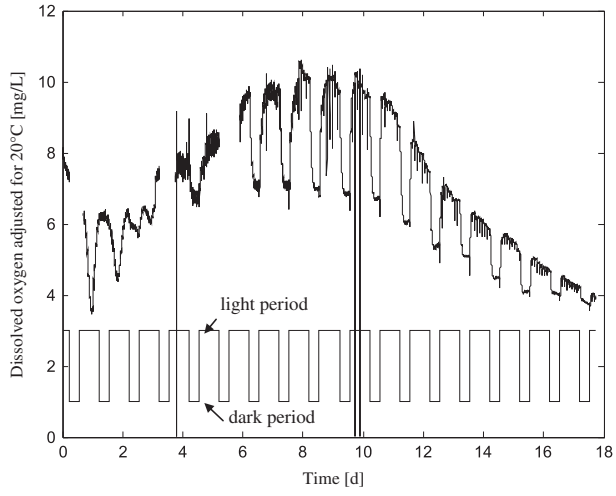

Fig. 3. Change in DO with time for lettuce nursery wastewater.

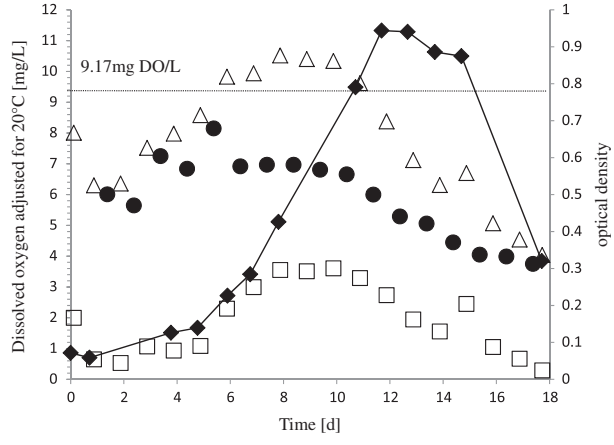

Fig. 4. Average maximum DO during the light period (Δ), the average minimum DO during the dark period (●), the net DO produced during the daytime (□) and the optical density readings (◆) in nursery wastewater.

light period. The maximum DO readings during the light period changed at a much higher rate over the entire growth period compared with the minimum DO readings at night. The daytime net oxygen graph shows that maximum oxygen production occurred during the linear growth phase.

The secondary *Y*-axis shows the optical density depicting the different algal growth phases. According to the optical density readings, the lag phase that occurred during the acclimatization period could be identified approximately until day 4. The reason may be due to nonviable cells or spores in the growth medium, changed culture conditions, or change in nutrient levels [29]. When the cells have adjusted to the new conditions, they enter an accelerated growth phase where they grow and divide. The exponential phase occurred between day 4 and 6, followed by the linear growth phase, which lasted until day 12. The stationary phase remained until approximately day 15, followed by the death phase.

As shown in Fig. 4, the measurements of DO during this period can also confirm the observation that the lag phase remained approximately until day 4 since no considerable DO was produced. The exponential growth phase occurred between day 4 and 6 according to the optical density measurement which can be validated using DO measurements as well. However, linear growth trend lasted until day 8 according to DO measurements as the plateau was achieved starting from approximately day 8, whereas the optical density measurements indicated this phase continued until day 12. While this discrepancy cannot be explained, DO measurements can still be indirectly used for predicting the different algal growth phases.

Fig. 5 shows online DO measurements as recorded on different days during the culturing period over 24 h, with $t = 0$ indicating 12 am. DO remained unchanged at a baseline value during the dark period until 5 am. When the light was turned on at 5 am, the DO gradually started to increase and eventually reached a maximum value. When the light was switched off at 9 pm, the DO started to gradually decrease to a similar baseline value. However, during day 2, there was a drop in DO profile during the daytime as opposed to the observations on day 8, 12, and 17. This corresponds to the lag period as identified from the algal growth measurements both by suspended solids and optical density, where algae still gets acclimatized to the new environment.

It can be seen from Fig. 5 that there was difference in oxygen production by algae during different growth phases. The sudden reduction in oxygen which occurred every two hours during the light period was due to oxygen stripping instigated by CO_2

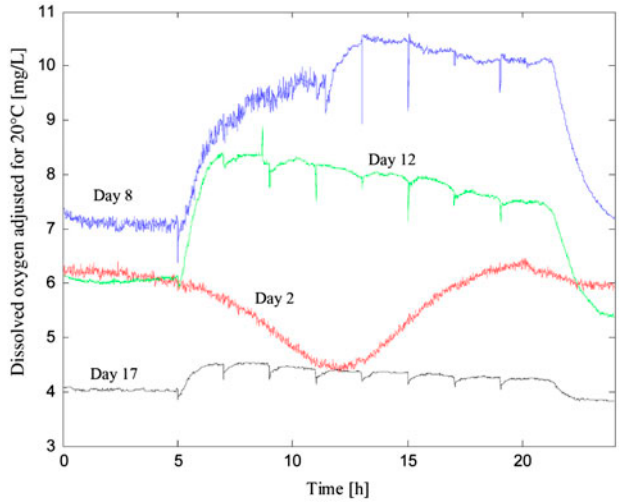

Fig. 5. DO dynamics in the bio-reactor in nursery wastewater on different days.

pumping. The baseline DO values were different depending on which growth phase the algae experienced. For example, the DO concentration remained 6.2, 7.3, 6.1, and 4.1 mg DO/L for days 2, 8, 12, and 17, respectively. It should be noted that the highest baseline recorded on day 8 corresponds to the vigorous growth of algae, whereas the lowest baseline corresponds to day 17 indicating the death phase. The DO increase above the baseline during the light period was considered to be the net DO production by the algae as it corresponds to the difference between the oxygen produced during photosynthesis and that consumed by respiration. The net DO produced during days 8, 12, and 17 corresponds to 334.9, 220.9, and 38.2 mg DO, respectively.

3.4. pH change with time

Carbon dioxide was continuously supplied at a rate of 10 mL/min. In addition, 56 mL/min CO_2 was supplied for 5–15 s (depending on the wastewater type and its buffering capacity) every two hours between 5 am and 9 pm. The pH dynamics in nursery wastewater as recorded in different days during the culturing period over 24 h is shown in Fig. 6, with $t = 0$ indicating 12 am. Observations indicate that there was minimal pH change during the night time when neither light nor CO_2 supply was provided from 9 pm to 5 am. When the light was turned on at 5 am, the solenoid valve that connects the CO_2 supply to the reactor was programmed to automatically activate so that the algae received the required carbon for growth.

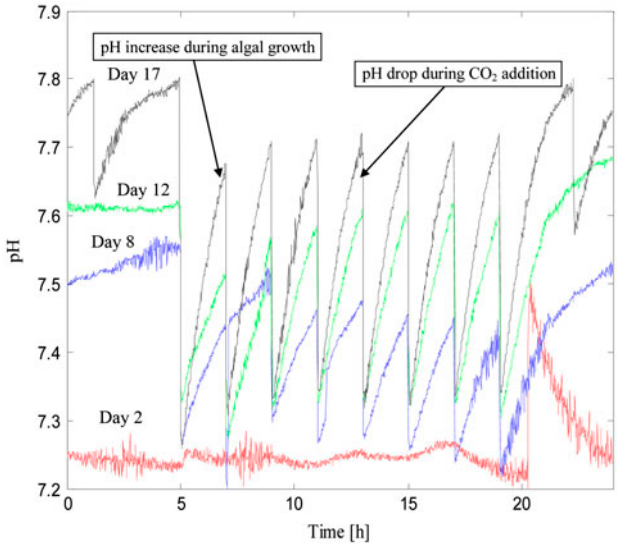

Fig. 6. pH dynamics in the bio-reactor in nursery wastewater on different days.

As can be observed from Fig. 6, addition of CO_2 instantly reduced the pH in all of the observation days. However, the reduction in pH was not observed on day 2, since no CO_2 was supplied during the lag phase. In addition, all the other days, after 9 pm when both the light and CO_2 supply were switched off, the pH seemed to have been slowly increasing to reach its approximate baseline value. However, the increase in pH was not as steep as that occurred during the light period due to absence of CO_2 utilization during the dark period of photosynthesis. Since algae gain energy from light and without this energy source they consume nutrients at a much lower rate.

The pH on day 2 remained largely unchanged and reached the lower end of the allowable pH range, which happened just after 8 pm. Since the pH set point was maintained at 7.5 ± 0.3, the pH controller automatically dosed the base to increase the pH. After day 4, the automatic CO_2 addition was performed every two hours during the light period from 5 am to 9 pm. This can be seen from Fig. 6 as sudden pH drops every two hours starting from 5 am. In days 8, 12, and 17, pH consistently increased between CO_2 supplies indicting algal growth. The pH rises as the algae consume CO_2 and reduce its concentration [19,26,30]. This is because the photosynthetic CO_2 fixation causes OH^- to accumulate in the wastewater [22]. On days 8 and 12, the slope indicating the increase in pH was slightly shallower for the first two hours until when the second CO_2 addition occurs. This may be due to algae adjusting to the light period which started at 5 am after undergoing the dark cycle of photosynthesis during the night time. This confirms findings that the algae stop growing during dark periods and start growing exponentially as soon as the light period starts again [5].

The steepness of the slope, which indicates utilization of CO_2, and hence algal growth differs for different days in the culture period. For example, the pH increase was 0.0945 (pH unit/h) for day 8 compared with 0.1428 (pH unit/h) for day 12 indicating the difference in growth rates during these days. This explains a larger biomass was available for the consumption of CO_2 during day 12 in comparison with day 8. This is evident on all days except during the death phase. It is not clear why there was an increase in pH during the death phase. The pH dynamics occurring during a death phase cannot be interpreted as logical as DO dynamics due to several other complex biological reactions that may be occurring in the bioreactor along with changes in the buffer capacity.

3.5. Nutrient removal

In the investigation using lettuce nursery wastewater as a growth medium, the variation of nutrients during the growth period is shown in Fig. 7. In this figure, markers denote the raw data points, whereas the straight lines corresponding to the specific markers indicate the points that were taken for the calculation of slope corresponding to nutrient depletion or accumulation rate.

As shown in Fig. 7, total nitrogen in the lettuce nursery wastewater was depleted at a volumetric rate of 2.64 mg/L/d ($R^2 = 95.9\%$) which was calculated

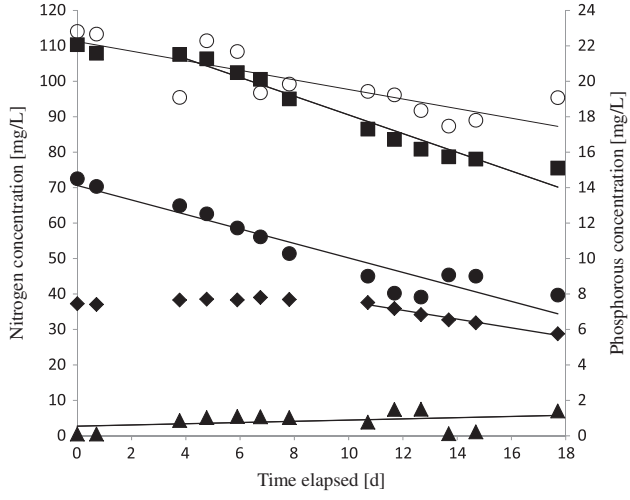

Fig. 7. Change in dissolved nitrogen concentration in lettuce nursery wastewater with time; Org-N + NH_4-N (●), TN (■), NO_2–N (▲), NO_3–N (◆) on primary *Y*-axis and PO_4-P (○) on secondary *Y*-axis.

using the slope from 4th to 18th day. There was negligible consumption of nitrogen until day 4. The combined organic nitrogen and NH_4-N was depleted at a volumetric rate of 2.04 mg/L/d ($R^2 = 90.1\%$) during the culture period. The graph also shows that NO_3–N remained unchanged until day 11, after which it decreased at a rate of 1.24 mg/L/d with the correlation coefficient of 97.6%. NO_2–N was found to increase from 0.52 to 5.1 mg/L until the 4 day, which corresponds to the decrease in NH_4-N during the lag phase, when there was no algal growth and minimal change in total nitrogen. Therefore, this could be due to NH_4-N being oxidized to NO_2–N during this period, rather than NH_4-N being used for algal growth. However, there was no evidence that NO_2–N was oxidized to NO_3–N since the profile of NO_3–N remained same. The concentration of NO_2–N increased subsequently and reached 7.51 mg/L by day 12. It was obvious from Fig. 7 that NH_4-N was the preferred nitrogen source for algal growth. This outcome supports the findings by a range of researchers who discovered that *C. vulgaris* consumes ammonia first before any other form of nitrogen in the wastewater [16,18,22,23].

PO_4-P depleted at a rate of 0.27 mg/L/d ($R^2 = 66\%$) as shown in Fig. 7 on the secondary *Y*-axis. Generally, total nitrogen (TN) decreased at the rate of 2.64 mg/L/d. Algae require only 1/8th–1/16th of P for every part of N, which explains the slow consumption rate of PO_4-P [11,22]. In this experiment, the TN and P depletion rates result in a N:P ratio of 9.8:1.

Fig. 8 shows the variation of nutrient concentrations in the municipal wastewater during the algal growth. In the municipal wastewater, the total nitrogen decreased by 1.59 mg/L/d ($R^2 = 78.5\%$). Organic-N and NH_4-N decreased at a rate of 1.46 mg/L/d ($R^2 = 51.5\%$). The correlation was not as expected due to great fluctuations for the first 4 days, which were also taken into account in the slope calculation. NO_3–N remained largely unchanged until day 5, after which it decreased at a rate of 1.89 mg/L/d ($R^2 = 90.4\%$). Nitrite was found to increase from 1.59 to 2.99 mg/L from day 3 to 12 (Fig. 8 second *Y*-axis) at a rate of 0.38 mg/L/d ($R^2 = 99.2\%$). The conditions provided are ideal for nitrification. However, bacterial nitrification could not be verified as no attempt was made to identify the bacterial strain.

PO_4-P depleted at a rate of 0.43 mg/L/d. The depletion of PO_4-P is shown in Fig. 8 on the secondary *Y*-axis. Interestingly TN was depleted at 1.59 mg/L/d corresponding to a P use of 0.43 mg/L/d resulting in a N:P ratio of 3.7:1, which shows a much higher use of P compared with what was found by Lundquist [21] and Grobbelaar [22]. While the volumetric nutrient removal rate indicates the nutrient uptake by algae, it makes the comparison difficult due to different growth rate of algae with different mass concentration as measured by suspended solids. Therefore, we made an attempt to calculate the specific nutrient removal rate in terms of mg nutrient/g suspended solids/day. Here, the nutrient depletion rate was divided by the initial algal concentration as measured by suspended solids to give the specific nutrient removal rate. According to Fig. 9, specific nutrient removal rates in terms of total nitrogen, and phosphorus were higher for municipal wastewater compared with lettuce nursery wastewater. This could be due to municipal wastewater having several other micronutrients, and enzymes needed for algal growth compared with lettuce nursery wastewater.

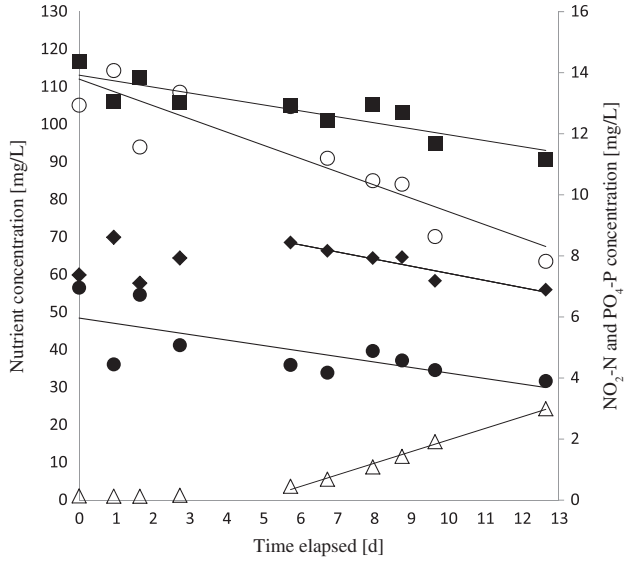

Fig. 8. Change in dissolved nitrogen concentration in municipal wastewater with time; Org-N + NH_4-N (●),TN (■), NO_3–N (◆) on primary *Y*-axis and NO_2–N (Δ) and PO_4-P (○) on secondary *Y*-axis.

3.6. Algal lipid content

The total lipid content as measured from the dry mass of the algae grown in nursery wastewater, and municipal wastewater were 9.7 and 4.5%, respectively. Normally, the algae would be harvested at the stationary phase, where algae mass and lipid content is at its peak. However, in our experiments, the algae were not harvested until the death phase was advanced. Generally, it can be said that nursery wastewater performed best in terms of lipid production followed by municipal wastewater. This lipid content was compared with those obtained from literature. Putt [20], Converti et al. [31] and Rodolfi et al. [32] have con-

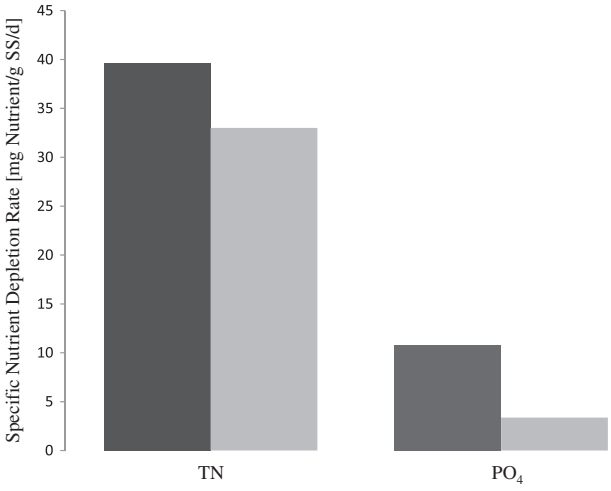

Fig. 9. Specific nutrient removal for municipal ■ and nursery ■ wastewater.

cluded that *Chlorella* have produced 30, 14.7, and 18.4% of total lipids, respectively.

3.7. Limiting factors of algal growth

In our experiments, the algal death occurred even though the macronutrients such as N, P, and C were not fully depleted as shown in Figs. 7 and 8. Therefore, it is unlikely that N, P, or C were the limiting factors of algae growth and ultimately the cause of 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 [33]. As there were no micronutrients and vitamins applied with the fertilizer for the nursery seedlings, it is unlikely that all essential micronutrients were available for the algal growth.

The quality and quantity of light, temperature, oxygen concentration, CO_2, pH, and micronutrients can be possible influencing factors of algal growth [33]. In addition, microorganisms, competition with other algae or shear through vigorous mixing, may contribute to the growth performance of the algae [33].

After light, temperature has the most significant effect of algae growth [6]. While most algae strains can tolerate up to 15˚C below their optimum temperature, the growth can be severely inhibited at a temperature rise of 2–4˚C above the optimum. Some researchers suggested a constant temperature between 20 and 26˚C, which was achieved for a majority of the time for this experiment [6]. Therefore, it is unlikely that premature algal death would have been caused by increased temperature.

Supersaturated oxygen in the bioreactor can cause decrease in photorespirance and photooxidative death of the algae. Elevated oxygen levels have significant effects on algal growth [33,34]. At 20˚C, the saturation value of DO is 9.17 mg/L, while the maximum DO reached on day 8 was 9.94 mg/L (Fig. 4) in nursery wastewater. This could have contributed to loss of some algal cells.

A certain degree of turbulence in the water can result in shearing and death of the algae [6,33]. In this experiment, turbulence occurs due to mechanical mixing, and repetitive aeration during sampling and could therefore have contributed to loss of algal cells. In addition, the light availability in the batch reactor would have been limited when algal mass increased in growth, which also would have resulted in the early death phase even though nutrients were still present.

4. Conclusion

This research has given insight into the algal growth rate in nursery wastewater and municipal wastewater. During these batch experiments, the online measurements including pH and DO proved to be real-time indicators of algal growth. These measurements correlate well with the different phases of algal growth during the culture period, and indicating definite variations in measured values during dark and light hours. The specific nutrient depletion rates were 33.0 mg TN/gSS/d and 3.4 mg PO_4-P/gSS/d for nursery wastewater and 39.6 mg TN/gSS/d and 10.8 mg PO_4-P/gSS/d for municipal wastewater. The total lipid content as measured from the dry mass of the algae grown in nursery wastewater, and municipal wastewater were 9.7 and 4.5%, respectively.

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