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# Coal seam gas water as a medium to grow *Dunalliella tertiolecta* microalgae for lipid extraction

Vasantha Aravinthan[a]*, Daniel Harrington[b]

[a]*Australian Centre for Sustainable Catchments, University of Southern Queensland (USQ), Toowoomba, Queensland 4350, Australia*
*Email: aravintv@usq.edu.au*
[b]*Faculty of Engineering and Surveying, University of Southern Queensland (USQ), Toowoomba, Queensland 4350, Australia*

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ABSTRACT

The coal seam gas (CSG) industry in Queensland, Australia is expected to produce about 300 GL of CSG water annually as a by-product of its methane extraction processes. Surat basin aquifers typically produce sodium bicarbonate/chloride type water, having high concentration of salts making it unfit for direct beneficial use. The objective of this bench-scale investigation is to assess the potential of using bicarbonate-rich CSG water as a medium for growing *Dunalliella tertiolecta* for biofuel production. The rates of microalgal growth and carbon sequestration were found to be 49.7 mg SS/L/d and 29 mg C/L/d, respectively, with an average total lipid content of 22% in CSG medium enriched with nutrients and amended for a salinity concentration of 10 gNaCl/L and 200 mg carbon/L in nonaerated batch reactor. In summary, the brine resulting from reverse osmosis treated CSG water could be trialed as an ideal medium to grow the microalgae *D. tertiolecta*.

*Keywords:* Microalgae; Nitrogen removal; Carbon sequestration; Biofuel

## 1. Introduction

The coal seam gas (CSG) industry in Queensland offers substantial economic benefits to Australia. However, they also inevitably bring about certain environmental impacts and challenges that need to be addressed to maintain a viable and sustainable economy in the region. Current projections indicate that the Australian CSG industry could extract in the order of 7,500 gigaliters (GL) of co-produced water from groundwater systems over the next 25 years, equivalent to approximately 300 GL per year. In comparison, the current total extraction from the Great Artesian Basin is approximately 540 GL per year as a by-product of its methane extraction processes [1]. Typically, CSG water has been disposed in large evaporation ponds as a waste product. In October 2008, the Queensland government released "CSG management policy" outlining the strategies for CSG water management. Central to the policy was the discontinuation

*Corresponding author.

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of evaporation ponds as the primary means of CSG water disposal, and remediation of existing ponds advised to occur within three years. Department of Environment and Resource Management, as administering authority, currently implements the policy. The purpose of which is twofold, to ensure that CSG activities do not contaminate the environment and to encourage the beneficial use of CSG water. The policy states that preferred management options for CSG water utilization are waste reuse to create useable or saleable products such as soda-ash, injection of brine into a natural underground structure that is geologically isolated and treatment methods for various applications [2]. It further states that an alternate use approval can be granted where CSG water can be converted from waste to a resource that can be used for the beneficial use of CSG water.

CSG water contains variable levels of salinity; the concentrations depend on the geological conditions. Surat basin aquifers in Queensland typically produce sodium bicarbonate/chloride type water (cited in [3]), having high concentration of salts making it unfit for direct beneficial use. Due to its high bicarbonate concentration, CSG water has the potential to be used as a medium for growing salinity–tolerant microalgae for the dual purpose of biofuel production and carbon sequestration.

With the expected increases in world energy usage in the coming years, a number of new green renewable energy sources must be developed to meet future demands, to reduce greenhouse gas emissions and to limit the potential of future fossil fuel source dependency [4]. Microalgae derived biofuels have recently emerged as a potential attractive fuel source, which can overcome many of the environmental and economical limitations of traditional crop-based biofuel methods [5–7]. This fuel production method requires significantly less land area, does not require arable land and can utilize saltwater, brackish water or wastewater for production. Fundamentally, this process replaces higher order plant crops with microalgae as the biomass for fuel production. Microalgae have an extractable oil content of between 10 and 80%, with oil contents of 20–50% being the most common [7].

Microalgal production requires sunlight for energy, nutrient rich water for algal growth and $CO_2$ for cell synthesis. There are studies available on algae production using nutrient-rich piggery [5], dairy [6] and municipal [6], wastewater. However, there are no experimental investigations conducted on the utilization of CSG water as a medium for microalgae biofuel production. CSG water quality characteristics indicate that it is rich in bicarbonate which can be converted by algae for biodiesel but lacks essential nutrients that are needed for the algal growth. Pratt et al. [8] explored a conceptual model for using CSG water to produce algae derived biofuels. They suggested the addition of agricultural waste produced in regional Queensland to CSG water to achieve the correct nutrient requirements for the desired algal growth. With no published research pertaining to algal growth for biofuel production and consequent carbon sequestration from CSG water media, the feasibility of this process is not yet known without any experimental investigations. The first challenge would be in identifying the appropriate microalgal strain that could be successfully grown in CSG water medium. In this study, the microalgae *Dunalliella tertiolecta* was chosen due to its high oil content and fast-growing ability in saltwater. Hence, the objective of this bench-scale investigation was to assess the potential of using CSG water as a medium to grow *D. tertiolecta* for the purpose of producing biofuel in a batch reactor. Furthermore, the growth rates, carbon sequestration and nutrient removal capacity of microalgal strain *D. tertiolecta* were also evaluated and presented.

## 2. Materials and methods

### 2.1. Selected algal strain and growth conditions

The microalgae *D. tertiolecta* was selected for this research as it is fast-growing, relatively robust and able to grow in saltwater, wastewater or brackish water [9]. The microalgae *D. tertiolecta* is a unicellular green algae 9–11 μm in size, with a reported oil yield of 36–42%. As such, *D. tertiolecta* was considered as an ideal candidate for application in an untested medium such as salty CSG water. A sample (20 ml) of microalgae strain *D. tertiolecta* was ordered from the Commonwealth Scientific and Industrial Research Organisation (CSIRO's) Australian National Algae Culture Collection, located in Hobart, Tasmania. The strain was first precultured in test-tubes and then in 250- ml flasks in an f/2 medium (1 ml/L) [10]. Culturing was initially conducted in two different salinities, with NaCl levels of 3.5 g/L (average reported CSG water equivalent), and 35 g/L (Seawater equivalent).

Most algae require substrate in a C:N:P ratio of 50:8:1 [11]. Carbon is a key parameter for intensive culturing of unicellular algae [12]. $CO_2$ levels of between 2–6% (far greater requirement than the amount available in air which is 0.03%) were required to produce optimal growth rates for *D. tertiolecta*, with sharp reductions in growth outside of this optimal range [13]. Most species of alga are capable of importing either $CO_2$ or $HCO_3^-$ through the cell membrane for photosynthesis [14]. However, the utilization of $CO_2$ or $HCO_3^-$ as the preferred source for photosynthesis has been

found to be species dependent [15]. The microaglae *D. tertiolecta* has been shown to have good capacity to extract carbon from $CO_2$ and $HCO_3^-$, although uptake for $HCO_3^-$ is pH dependent. *D. tertiolecta* was unable to uptake carbon in the form of bicarbonates at a pH greater than 8.3 [16]. Since the requirement for carbon component is very high, $CO_2$ enrichment or bicarbonate must be provided to achieve high algal growth rates for most of the wastewaters. However, in this research, instead of providing artificial $CO_2$ feed, the available $HCO_3^-$ in the CSG water was used as a carbon source with a boost provided with $NaHCO_3$.

The key nutrients required for algal growth are nitrogen, phosphorus, and iron. While nitrogen is an essential component of structural and functional proteins within an algal cell and generally accounts for 7–10% of cell dry weight, phosphorus facilitates cellular metabolic processes by forming a number of structural and functional components needed for the development of microalgae. Iron has an important role in cellular biochemical composition, specifically for its role in photosynthesis, respiration, nitrogen fixation and DNA synthesis [17]. Chen et al. [9] found that *D. tertiolecta* was able to use either ammonium or nitrate as a nitrogen source. However, high levels of ammonium inhibited the growth, while in contrast high levels of nitrate increased cell density. Deprivation of nitrate or iron resulted in a sudden increase in lipid content. They also found that limiting of phosphate had little effect on growth, due to intra cellular phosphate storage. In our experiments, f/2 media (Algaboost) ordered from AusAqua Pty Limited was used to provide the key nutrients and other vital vitamins to facilitate the algal growth in CSG water as suggested by CSIRO scientists. The measured composition of f/2 medium indicated the presence of 1.7 P g/l and 17 g/L $NO_3^-N$ indicating N:P ratio to be 10:1. The media f/2 mainly contained nitrate nitrogen as nitrogen source, as indicated by measurements. In reality, the water can be enriched with agricultural waste full of nutrients to supply the nutrients needed for the algal growth.

The optimal salinity level for growth is different for every algal strand. The optimal growth of *D. tertiolecta* was found to be at a NaCl concentration of 0.5 M [18]. The maximum NaCl tolerance range for *D. tertiolecta* was found to be 0.05–3 M [19].

*D. tertiolecta* requires light intensity between the ranges of 100–200 μE/($m^2$s) [13] and optimum warm temperatures of 23°C [9] and a pH of near 7.5 [6].

### 2.2. CSG water characteristics

The CSG water was initially acquired by the National Centre for Engineering in Agriculture located at the University of Southern Queensland (USQ) Toowoomba campus, from a CSQ exploration company operating in Roma, Queensland. The collected CSG water was filtered using 0.45-μm filter paper, with the aid of a vacuum pump system. The water was then stored between 20 and 25°C in the Environmental Engineering laboratory, before being utilized in the bioreactor experiments. The obtained CSG water was tested for total carbon (TC) and total nitrogen (TN), using a Total Organic Carbon/Total Nitrogen Analyzer (TOC-VCPH/CPN). Anion compositions for selected ions such as $Cl^-$, $NO_2^-$, $NO_3^-$ and $PO_4^{3-}$ were measured using Ion Chromatography system (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, USQ that enables the real time data collection corresponding to the growth of *D. tertiolecta* microalgae as shown in Fig. 1.

Batch experiments were conducted using a single reactor having a capacity of 4 L. Compressed air was supplied continuously at a rate of 250 ml/min for aeration (when supplied) and the reactor contents were continuously mixed using an overhead stirrer. There were three florescent light sources (2000 lux each) placed on three sides of the reactor at a distance of 5 cm away to provide the required light intensity. Light was supplied for 12 h/day from 6 am to 6 pm. A titrimetric unit, consisting of an 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 rates. 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 dissolved oxygen electrode (YSI). The Labview software was used for monitoring dissolved oxygen as well as temperature serial output from dissolved oxygen meter (TPS 90-D) and pH data (TSP Mini Chem) with high frequency. The Labview package also controlled both 3-way solenoid valves in the titrimetric respirometer for acid and base pulsing respectively to keep the pH in the reactor constant. The 0–1 V 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,

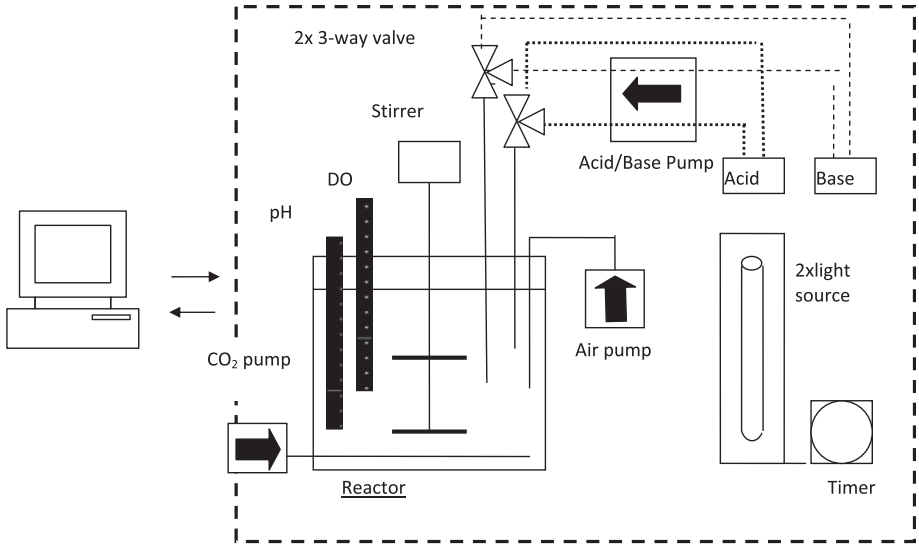


Fig. 1. Schematic diagram of bio-reactor.

PCI-6013). All data acquired from the experiment was 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 $7.7 \pm 0.6$ by automatic addition of base (0.1 N NaOH) or acid (0.05 N $H_2SO_4$) solutions. 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. Experimental runs

Experimental runs/setups that were conducted to assess the optimal growth conditions of *D. tertiolecta* in CSG medium are shown in Table 1. At the commencement of all the trials (except Control 2), $NaHCO_3$ was added to the filtered CSG water to increase the bicarbonate concentrations to the average CSG values and 5 ml/L of f/2 medium to enrich the water with nutrients and vitamins required for the algal growth. Control 2 was performed in order to compare the growth of microalgae in media having low bicarbonate concentrations.

As can be seen in Table 1, Experimental trials were conducted with and without aeration within the bioreactor. Aeration in a batch reactor can aid in mixing the algae with the nutrients present in the media and help expose them to the light energy provided external to the bioreactor. While aeration can provide a limited amount of carbon dioxide from the air (300 ppm) for the algal growth and remove the excess oxygen produced during the photosynthesis process, it can also strip off carbon dioxide if excess bicarbonate is present, resulting in reduction of the bicarbonate concentration in the liquid phase. Therefore, these batch experimental trials were performed in a laboratory to evaluate the capacity of growing algae with and without aeration in order to evaluate the feasibility of growing algae in open-pond setting in bicarbonate rich CSG medium without the need for aeration. The aeration was performed at a rate of 250 ml/min for experimental setups of 2 and 4.

For the experimental trials as described in Setups 1 and 2, the bioreactor was fed with enriched CSG water of 3 L, and then inoculated with *D. tertiolecta*, which was cultured in 250 ml of medium, prepared using the raw CSG water (1.3 g NaCl/L equivalent) and 1 ml/L f/2 medium. A control trial (named as Control 1 in Table 1) was also conducted to identify whether there is any carbon lost from the CSG media within the bio-reactor tank, through processes other than aeration stripping or algal consumption. The control trial replicated the parameters of Setup 1, except for the omission of nutrition addition and algal inoculation. The media was stirred at a constant rate during the trial period, and no aeration was used.

Setups 3 and 4 were conducted with similar conditions as Setups 1 and 2 respectively, except that the salinity concentrations of CSG media were increased to 10 g NaCl/L to assess the improvement in algal growth conditions. For the experimental trials as described in Setups 3 and 4, the bioreactor was fed with enriched CSG water of 3 L, and then inoculated

Table 1
Experimental Conditions

| Experimental setup | Total carbon mgC/L | Salinity g NaCl/L | Aeration | Added mass of microalgae* in 3 L of bioreactor (mg) |
|---|---|---|---|---|
| Setup 1 | 200 | 1.3 | No | 6.95 |
| Setup 2 | 200 | 1.3 | Yes | 7.12 |
| Control 1 | 200 | 1.3 | No | No |
| Setup 3 | 200 | 10 | No | 193 |
| Setup 4 | 200 | 10 | Yes | 198 |
| Control 2 | 33.6 | 10 | No | 245 |

Notes: *A batch volume of 3 L was inoculated with 250 ml of cultured *D. tertiolecta* for the experiments. The added mass is given in the table.

with *D. tertiolecta*, which was cultured in 250 ml of medium, prepared using the raw CSG water (10 g NaCl/L equivalent) and 1 ml/L f/2 medium. Note that the difference in mass of cultured *D. tertiolecta* added to the reactors attributed to the difference in growth in different media.

### 2.5. Measurement of algal growth, nutrient depletion, and carbon sequestration levels

Algal growth and liquid-phase concentrations of carbon, nitrogen, and phosphorus were monitored for the entire duration of experimental trials by withdrawing samples on a daily basis. Prior to sampling, algae were put into suspension by temporarily accelerating the rotational speed of the stirrer to ensure getting a representative sampling for measurement purposes. Before sampling, the volume of the media was recorded, concentration adjustment was subsequently made to all results at the data analysis stage.

Algal growth was initially quantified through both spectroscopy measurements and suspended solids. Suspended solids were measured using Standard Methods [20], and cell density by spectroscopy (Jenway 6705 UV/Vis. Spectrometer) at a wavelength of 505 nm. Initially, a relationship was established between suspended solids and optical density as follows: (suspended solids (g/L) = 0.6058 optical density). In some experiments, only spectroscopy method was adopted to quantify the algal growth. At the same time, pH and DO profiles were also automatically logged into the system providing an indication of growth in real-time.

The samples were filtered using 0.45-μm filter papers, and the liquid-phase TC, total organic carbon (TOC), inorganic carbon (IC), and TN were measured using TOC and nitrogen analyzer (TOC-VCPH/CPN). The nutrient levels of $NO_2^-$, $NO_3^-$, and $PO_4^{3-}$ were measured using Ion Chromatography system (IC, Dionex ICS 2000) with an anion (AS-18) column during the analytical process.

### 2.6. Microalgae harvest

Algae were separated from the water using a two-step centrifugation process. Initially, algae underwent centrifugation at 8,000 rpm for 10 min, with a 3 min cool down period (Beckman Avanti CentrifugeJ-25 I). Excess water was then removed, and the algae were washed with distilled water, before undergoing a second stage of centrifugation at 4,000 rpm for 10 min (Eppendorf Centrifuge 5810 R). Once separation was complete, the algae pellets were frozen for 24 h and then freeze dried at −56 °C and 30 torr (VirTis 2KBTES-55). They were then stored at room temperature in a desiccator until they underwent lipid measurement. The timing and the growth phase at which the algae were harvested was specific to each experiment.

### 2.7. Lipid extraction

The freeze-dried algae underwent the Folch method [21] 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 2000 rpm for 5 min. The upper phase/non-chloroform phase was then siphoned off. The chloroform phase, which contains the lipids, was poured into pre-weighted 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.

## 3. Results and discussion

### 3.1. CSG water characteristics

The typical CSG water characteristics can vary greatly depending on the aquifer from which the water

is drawn. The raw water properties for several wells from Roma and Dalby, Queensland, indicated that all yielded sodium bicarbonate—chloride type waters typical of Surat basin aquifers in the near-surface recharge areas [3]. In addition, detailed listing of CSG water characteristics for various sample sites within the Surat basin confirms the presence of high concentrations of sodium, bicarbonate and chloride [22]. The CSG water obtained from a site near Roma was tested for the required water characteristics needed for this research, and found to have TC and TN of 23.5 mg/L, and 0.3 N mg/L respectively, after the pH adjustment to 7.5. The pH adjusted CSG water had only 23.5 mg TC/L. However, according to raw water characteristics [3], typical bicarbonate and carbonate alkalinity of CSG water from Roma region varied between 800–950 and 17–39 mg/L expressed as $CaCO_3$ with pH ranges from 8.24 to 8.55. To emulate the real life CSG water conditions having an average of 900 mg/L bicarbonate alkalinity expressed as $CaCO_3$ (equivalent to 216 mgC/L), the total IC in the water was increased to 200 mgC/L through the addition of $NaHCO_3$.

CSG water anion chemistry analysis indicated that it did not have any nutrients such as $NO_2^-$, $NO_3^-$ and $PO_4^{3-}$ respectively, however contained 805.5 mg/L of Chloride ($Cl^-$). Based on the measurements, the water was deduced to have 1.3 g NaCl/L equivalent of salinity corresponding to 805.5 mg/L of Chloride ($Cl^-$). In our experiments, therefore, the CSG water was enriched with required nutrients for algal growth with f/2 medium.

### 3.2. Preliminary trials of algal growth (Setups 1 and 2 and Control 1)

Fig. 2(a) shows the recorded growth trends of *D. tertiolecta* during Setups 1 and 2 using the optical density measurements. The trials lasted only for 6 days with a transition to the death phase commencing from Day 4. Comparing the growth characteristics between the two trials, Setup 2 with aeration input experienced faster initial growth and recorded the highest optical density readings however, difference in growth between the trials was not significant. Fig. 2(b) shows the TC concentration (since there was no organic carbon in CSG medium, TC measurements indicate the IC present in the medium) for the three trials.

The TC reduced significantly over the first two days in Setup 1 and stabilized at approximately 95 mg/L for the remaining duration suggesting no further carbon uptake by the algae. In Setup 2, results show a steep decline in carbon levels over the first two

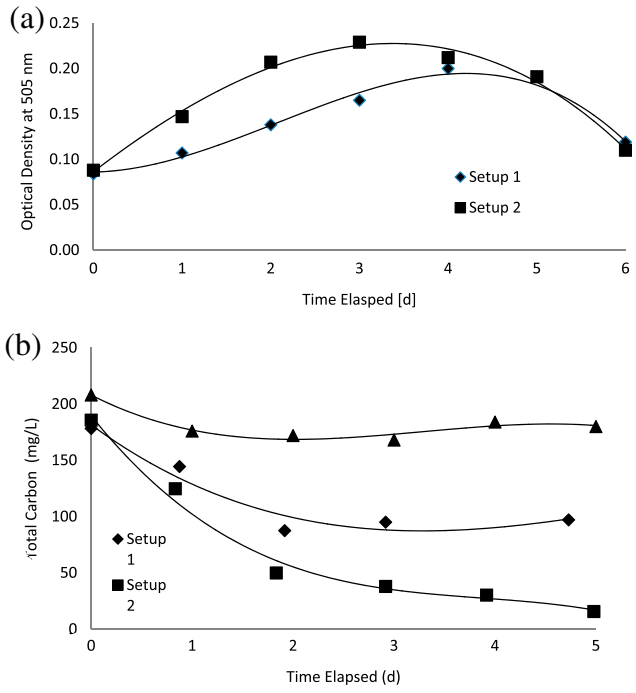


Fig. 2. Comparison of *D. tertiolecta* growth in CSG water (a) optical density readings at 505 nm (b) TC profiles during Setups 1 and 2.

days (135.8 mg/L reduction) coinciding with the identified exponential and linear growth phases. The rate of reduction then changes to a shallower grade for the remainder of the trial, a final reading of 15.7 mg/L was recorded. The carbon removal rate was 74.07 mg C/L/d for first two days of the trial, and 11.99 mg C/L/d for the remainder of the trial. The control trial identified the rate at which carbon was lost from the CSG media through processes other than aeration stripping or algal absorption was minimal (about 12 mg/L) over the duration of a 6-day trial. As nutrient addition and algal inoculation are the only differing factors between Setup 1 and the control trial, the resulting difference in carbon levels between the trials (90.9 mg/L) is likely the result of carbon sequestration by the algae. Similarly, as there was no significant difference in the growth rate between Setups 1 and 2, it is likely that the aeration process is the main contributor to the observed difference in carbon reduction between these trials. The final difference in carbon levels between Setup 1 and Setup 2 was 79.3 mg/L.

While the algal growth was promising in CSG water, the preliminary Setups indicated that neither trial achieved high levels of growth, with both trials failing within 6 days. The poor performance of the trials was attributed to the less than optimal salinity levels

required for the growth and therefore, new Setups were designed to identify the optimal salinity levels required for the maximum growth rate of *D. tertiolecta* in CSG medium.

### 3.3. Optimal salinity assessment for successful growth of *D. tertiolecta* using CSG water (enriched by f/2 medium and bicarbonate)

In order to identify the approximate optimal salinity level to facilitate growth of *D. tertiolecta* in a CSG medium, four preliminary batch trials were conducted with salinity levels of 1.3 g NaCl/L (CSG water), 10 g NaCl/L, 20 g NaCl/L, and 35 g NaCl/L (seawater equivalent) in 250-ml Erlenmeyer flasks. At the commencement of the trials, 250 ml of CSG water (enriched with $NaHCO_3$ and f/2 medium as described in Section 3.2) with modified salinity levels, were inoculated with 10 ml of *D. tertiolecta* culture equivalent to 0.28 mg of biomass. Photo-energy was provided by means of a light source, implemented on a 12 h on (6 am–6 pm)/off cycle. Algal growth measurements as indicated by optical density readings results are shown in Fig. 3.

This clearly indicates that the optimal algal growth could be achieved when the CSG water is amended to a maximum salinity concentration of 10 g NaCl/L trial. The trial with unmodified salinity levels with CSG water alone (1.3 g NaCl/L) performed comparatively poorly. Therefore, it was concluded from these preliminary investigations that *D. tertiolecta* would optimally grow in a salinity of 10 g/L, and subsequent trials were conducted using the amended CSG water with 10 g NaCl/L in 3 L batch reactor.

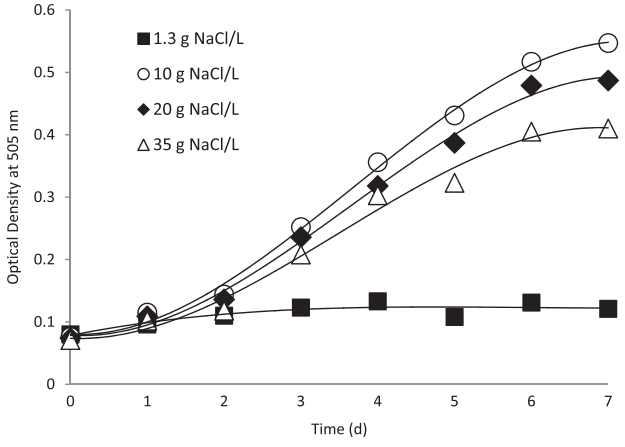


Fig. 3. Algal growth in amended CSG water with different salinity concentrations.

### 3.4. Experimental trials of algal growth using CSG water with modified salinity of 10 g/L

Two batch experiments were conducted to assess the growth capacity of microalgae *D. tertiolecta* in salinity modified (10 g NaCl/L) CSG water medium with initial carbon concentration of 200 mg TC/L with aeration (Setup 4) and without aeration input (Setup 3). A control experiment (Control 2) was conducted at an initial bicarbonate concentration of 40 mg/L to find out the effect of low carbon on algal growth. The results and discussions on algal growth characteristics, carbon sequestration and nutrient removal during the algal growth in CSG water in batch rectors are discussed in the following sections.

#### 3.4.1. Algal growth

Fig. 4(a) shows the recorded growth trends of *D. tertiolecta* in Setups 3, 4 and control trials represented through optical density measurements. All the trials were stopped around the 6th day in order to harvest the algae for lipid measurement as previous trials proved that stationary phase occurred starting around the day 5 followed by death phase.

Results suggest that algae grew in the new environment with minimal lag phase, showing almost linear growth rates until the 5th day. Results show both Setup 3 and 4 performing significantly better than the control trial, indicating a distinct benefit of growing *D. tertiolecta* in a bicarbonate rich solution. Furthermore, growth measurements for Setups 3 and 4 were measured to be 49.7 and 46.3 mg SS/L/d, respectively, suggesting no identifiable benefit exists in implementing aeration. Comparing the growth trends of Setups 1, 2 with the salinity level 1.3 g NaCl/L with those of Setups 3 and 4, with salinity level 10 g NaCl/L, the algal growth improved greatly in increased salinity level of 10 g NaCl/L.

#### 3.4.2. Carbon sequestration

Fig. 4(b) shows the TC concentrations for Setups 3, 4, Control 1 and 2. Recorded carbon levels in Setup 3 were steady for the first day of the trial, which decreased at a uniform rate for the remainder of the trial. The TC reduction for this 4 day period was 120.9 mg/L and the volumetric carbon removal rate was 29.29 mg TC/L/d. In Setup 4, results show a steady decline in carbon levels over the first three days (148.3 mg/L reduction) coinciding with the identified linear growth phase. The carbon removal rate for this period was 49.4 mg TC/L/d. Only minimal carbon reduction was recorded for the remainder of

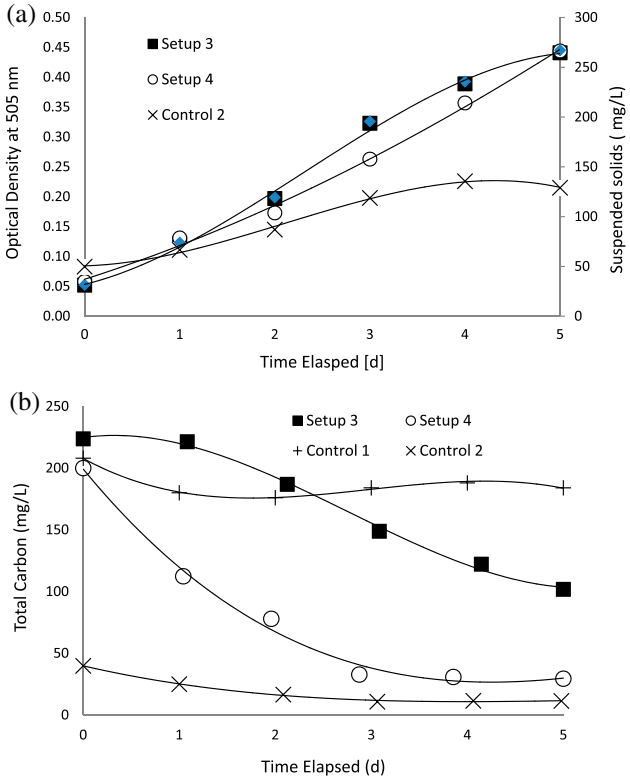


Fig. 4. Comparison of *D. tertiolecta* growth in ameded CSG water (a) optical density readings and (b) TC profiles during the Setups 3 and 4.

the trial (3.37 mg C/L) with a rate of reduction of 1.98 mg TC/L/d. In the control trial, a steady decline in carbon levels was observed over the first three days (29.1 mg/L reduction) coinciding with the identified growth phase. The carbon removal rate for this period was 9.1 mg TC/L/d. No carbon reduction was recorded for the remainder of the trial. Nutrient addition, algal inoculation, and salinity adjustment are the only differing factors between Setup 3 and Control 1; therefore, the resulting difference in carbon concentrations between the trials (82.2 mg/L) is likely the result of carbon sequestration by the algae. Similarly, as there was no significant difference in the growth rate between Setups 3 and 4, it is likely that the aeration process is the main contributor to the observed difference in carbon reduction (72.4 mg/L) between these trials.

### 3.4.3. Nutrient removal

TN and phosphorus removals were monitored during the algal growth period as shown in Fig. 5. Results show uniform nitrogen and phosphorus removal occurring during the growth period.

Volumetric nitrogen removal rates of 2.335 and 2.808 mg/L/d were observed for Setups 3 and 4 with correlation coefficient of 66 and 58% respectively. Recorded phosphorus concentrations show that depletion rate occurs at the rate of 1.156 and 0.959 mg/L/d for Setups 3 and 4 with high correlation coefficient of 97–99%. The theoretical depletion rate of P:N is between 1:8 and 1:16 [23]. Interestingly, our results show that P:N ratio is in between 1:2 and 1:3 indicating higher consumption of phosphorus in comparison with nitrogen.

### 3.4.4. pH variation with time

Gaseous $CO_2$ was not provided for both Setups 3 (without aeration) and 4 (with aeration). Therefore, pH variability within the bioreactor, resulting from algal behavior, was influenced predominantly by algal growth depending on the light source availability. The controlled pH range was set at 7.7, with a tolerance of ±0.6, allowing a maximum pH value of 8.3.

The pH dynamics occurring in the bioreactor during algal growth in amended CSG water for Setup 3 as recorded in different days during the culturing period over 24 h is shown in Fig. 6(a). Dark (troughs) and light (peaks) periods are represented by the added discontinued line.

Observations indicate that there was gradual, but minimal pH increase during the night time when light supply was not provided from 6 pm to 6 am on all except the last day. During the light period, pH levels increased at an increasing rate as the trial progressed. This confirms findings that the algae stop growing during dark periods and start growing exponentially

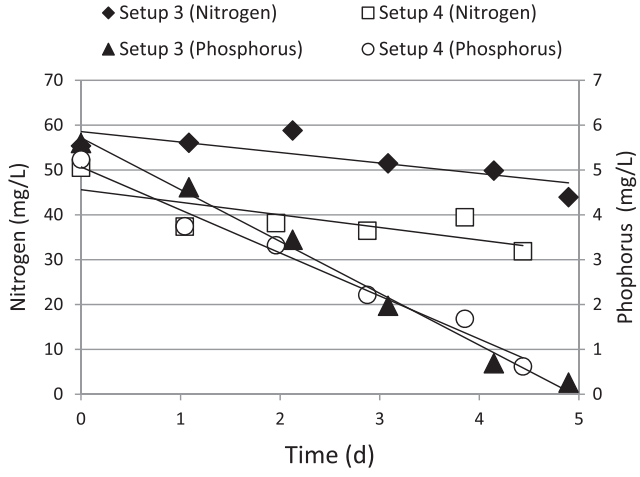


Fig. 5. TN and phosphorus concentration profiles during algal growth in Setups 3 and 4.

as soon as the light period starts again [24]. Since the pH set-point was maintained at 7.7 ± 0.6, the pH controller automatically dosed the acid to decrease the pH and control it within desirable range. The increasing number of acid inputs into the reactor to maintain the pH < 8.3 was another indication of the growth performance. While only one acid input was required on day 1, there were 5 inputs on day 3. The steepness of the slope, which indicates utilization of bicarbonate for algal growth, differs for different days in the culture period. In the light periods, the sharpest increase in pH levels was observed on day 4, approximately 0.194 pH unit/h. The shallowest light period gradient occurred on day 1 corresponding to lag phase, approximately 0.077 pH unit/h. The pH rises as the algae consume $CO_2$ and reduces its concentration [7,25]. This is because the photosynthetic $CO_2$ fixation causes $OH^-$ to accumulate in the wastewater [23]. This phenomenon has been confirmed in our experiments indicating near absence of photosynthesis during the night time with minimal pH change and high rate of photosynthesis during the day time with increasing rate as linear growth progresses.

Fig. 6(b) shows the pH variation that occurred in Setup 4 with aeration. The trials with aeration indicate that the pH increases during the night time as well until day 3 and 4. However, increase in pH during the daytime has been higher as can be seen by the steeper pH increases as linear growth of algae progresses. In the light periods, the steepest average pH gradients were observed on day 3, approximately 0.651 pH unit/h. The shallowest average gradients occurred on day 1, approximately 0.099 pH unit/h. In the dark period, the steepest average pH gradient was recorded at the start of day 3, approximately 0.274 pH unit/h, with the shallowest pH gradient at the end of day 3, approximately 0.194 pH unit/h.

While the pH increase during the day time can therefore be attributed to both algal growth and aeration effects, that during the night time is solely due to the aeration. Aeration can strip off the high bicarbonate concentrations present in the liquid phase and remove it as $CO_2$ to the air, resulting in the accumulation of $OH^-$ resulting in pH increase. During Days 3 and 5, aeration did not increase the pH during the night time as in the first 3 days due to limited buffering capacity of the solution. By day 4, the buffering capacity of the medium has reduced to such an extent that any automatic addition of acid to maintain the pH within the desired levels has reduced the pH to less than the lower limit of tolerance level. This resulted in automatic base addition to raise the pH level. Fig. 4 reinforces this finding that TC reduction has been higher in Setup 4 in comparison with Setup 3 indicating possible carbon loss due to stripping effect caused by aeration. While there is no discernible difference in algal growth in Setups 4 and 5, there was an increase in volumetric TC depletion rate along with sharp pH dynamics in trials with aeration input. Aeration, therefore, is not considered being beneficial for algal carbon sequestration as it depletes the IC present in the media.

#### 3.4.5. DO variation with time

The concentration of dissolved oxygen for the culturing period during Setup 3 is shown in Fig. 7. Dissolved oxygen produced in the liquid phase is a reflection of algal photosynthesis (that elevates the DO), aeration (that can supply oxygen to the liquid phase and strip off excess oxygen that may be present) and respiration (that reduces the DO. During the light period, the algae's photosynthesis exceeds the respiration causing the release of oxygen into the liquid phase when growth occurs. On the contrary, during the dark period, there is a net consumption of oxygen as a consequence of respiration [26].

As shown in Fig. 7(a), the oxygen production during the light period increased until day 4 to the maximum of 30 mg/L (above 100% air saturation). These unusual sensor readings compelled us to check whether the sensor was functioning properly. Repeated calibrations ensured that the probe was functioning correctly. This phenomenon was verified with the literature, which suggested that DO readings of greater than 100% saturation can occur in environmental water because of the production of pure oxygen by photosynthetically active organisms and/or because of nonideal equilibration of DO between the water and the air above it [27]. Since there was no aeration supplied in Setup 3, the produced oxygen remained in the liquid phase causing supersaturation/over saturation even though there was mixing with overhead stirrer. Supersaturated oxygen in the bioreactor can cause decrease in photorespirance and photooxidative death of the algae. Elevated oxygen levels can have significant effects on algal growth [28,17]. However, during the short experiments, this effect was not observed. The online DO measurements confirm the observation of algal growth in different days as shown in Fig. 4, indicating that DO measurements can still be indirectly used for predicting the different algal growth phases.

DO dynamics during Setup 4 with aeration is shown in Fig. 7(b). There is clear distinction between the DO concentration during light and dark period with maximum and minimum DO concentration, it is evident that DO did not exceed above 11 mg/L. Even though the rate of algal production was almost the

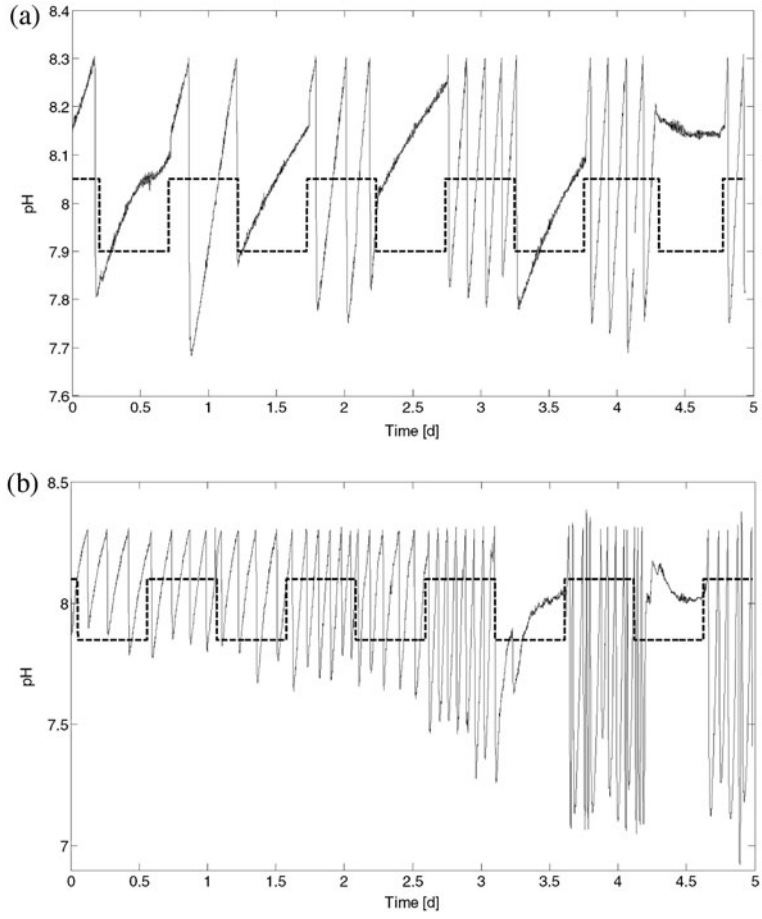


Fig. 6. pH dynamics during algal growth in (a) Setup 3 and (b) Setup 4 as observed using real-time measurements.

same for both Setups 3 and 4, the plateau of DO concentration observed in Setup 4 indicates that the excess oxygen produced during the light period as a consequence of photosynthesis and respiration has been stripped off due to the aeration provided at the rate of 250 ml/min.

### 3.4.6. Algal lipid content

Algae were harvested after 5 days of growth for lipid measurements just before reaching the stationary phase where algal mass and lipid content were at its peak. The dry mass of algae from Setups 3 and 4 were 0.39 and 0.41 g, respectively. The total lipid content as measured from the dry mass of the algae grown in Setups 3 and 4 were 24 and 20%, respectively. This lipid content was within the range reported from literature. Previous researchers have concluded that *Chlorella* have produced 30 and 18.4% of total lipids, respectively, in the experiments with different wastewaters [29,30].

### 3.4.7. Evaluation of CSG as a medium to grow the microalgae D. tertiolecta

Experiments indicated that *D. tertiolecta* would grow in a CSG medium amended to a final salinity concentration of 10 g NaCl/L and carbon concentration of 200 mg TC/L, and enriched with required nutrients as shown in Setups 3 and 4. Currently, CSG product water is increasingly treated by reverse osmosis (RO) desalination processes for other beneficial uses. However, RO produces large volume of salt and brine as a by-product, which needs further management. The brine output from RO treatment could be used as an ideal medium to optimize the growth of algae for the extraction of biodiesel purposes.

Experimental trials to grow *D. tertiolecta* in CSG medium with and without aeration indicates that there was no discernible difference in algal growth during the 5 days of batch experiments (Fig. 4(a)). However, aeration resulted in reduction in carbon concentration, indicating that bicarbonate concentration in the liquid phase was converted to carbon diox-

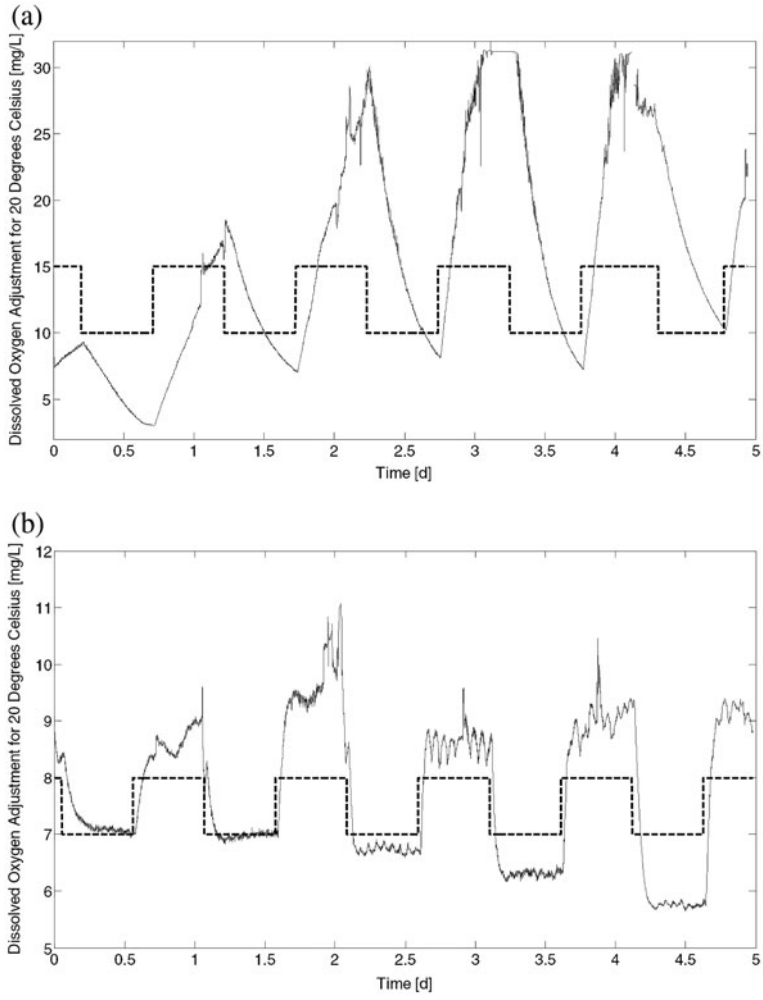


Fig. 7. DO dynamics during algal growth in (a) Setup 3 and (b) Setup 4 as observed using real-time measurements.

ide resulting in overall loss of carbon (Fig. 4(b)). This was confirmed by the pH observations as well (Fig. 6). Hence, aeration needs to be limited to increase the availability of carbon present in the CSG medium for use by algae for carbon sequestration. Aeration also removed excess oxygen present in the liquid phase as shown in Fig. 7. While there is no evidence in Setups 3 and 4 that supersaturation of DO affected the overall algal growth, aeration at a milder form helps ward off the excess oxygen present in the medium as a result of algal photosynthesis. This suggests that *D. tertiolecta* can be trialed to grow in CSG medium in open-pond setting without carbon dioxide and aeration inputs. This needs to be confirmed by pilot-scale testing.

## 4. Conclusions

CSG water amended to a salinity concentration of 10 g NaCl/L and 200 mg TC/L enriched with nutrients supplied by f/2 media was used as a medium to grow the microalgae *D. tertiolecta*. The rates of algal growth and carbon sequestration rates in a non-aerated reactor were found to be 49.7 mg SS/L/d and 29 mg C/L/d respectively with an average of 22% total lipid content. The bench-scale investigations suggest that the CSG brine resulting from the RO treatment could be trialed as an ideal medium to facilitate the growth of *D. tertiolecta* for biofuel production.

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