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Increasing CO_2 concentration impact upon nutrient absorption and removal efficiency of supra intensive shrimp pond wastewater by marine microalgae *Tetraselmis chui*

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ABSTRACT
The objective of this study was to investigate the effect of increasing CO_2 concentration on the growth and the capability of *Tetraselmis chui.* in removal of nitrate, ammonium and phosphate from shrimp pond wastewater (SPWW). The factorial experimental design was used with the treatment of SPWW percentage in culture medium, namely: 100% SPWW, 75% SPWW + 25% Sea Water (SW) and 75% SW + 25% SPWW coupled with three CO_2 concentration treatments: 390 ppm, 550 ppm and 1000 ppm using CO_2 system. Growth of *T. chui.* for lengh of cultivation period tended to be higher at treatments of 390 ppm CO_2 and 100% SPWW, however there was a declining growth over period of cultivation for both treatments. The growth rate *of T. chui* was higher for all percentage of SPWW treatments in culture medium at 390 ppm CO_2 concentration compared to other percentage of SPWW treatments and CO_2 concentration treatments. There was a decreasing of growth rate with increasing CO_2 concentration at 100% SPWW and 75% SPWW + 25% SW in culture medium. Nitrogen removal efficiency and removal rate by *T. chui.* were strongly affected by CO_2 concentration. However, there was no significant effect of increasing CO_2 concentration to removal efficiency and rate of PO_4 by *T. chui.*

KEYWORDS
microalgae Tetraselmis chui and CO_2 concentration; nutrient removal; shrimp pond wastewater; supra intensive aquaculture technique

Introduction

Nowadays, there is a serious environmental problem faced by most countries around the globe in the form of increasing concentration of greenhouse gases, CO_2 in particular. Anthropogenic activities contribute more than 7% (v/v) of global CO_2 emissions from burning coal at power plants (Ramanathan 1988), and De Morais *et al.* (2007) found that around 10–15% (v/v) of exhaust gases from power plants is carbon dioxide. Photosynthetic microorganisms can convert CO_2 from sources into biomass (De Morais and Costa 2007a) and microorganisms are most effective in the absorption of CO_2 from the atmosphere. Comparing microalgae and higher plants, there are several advantages of microalgae in terms of a high level of photosynthesis efficiency, higher biomass production and faster growth (Tang *et al.* 2011). CO_2 fixation by algae through photosynthesis is estimated to be a flexible technology with energy storage and more environmentally friendly (Dote *et al.* 1994; Minowa *et al.* 1995; Miao and Wu 2006).

Some previous research results show several species of microalgae can reduce CO_2 concentrations, such as *Chlorocuccum littorale* (Skjånes *et al.* 2007), *Chlorella kessleri, Scenedesmus obliquus* (Ota *et al.* 2009), *Chlorella vulgaris* (de Morais and Costa 2007a) *Dunaliella tertiolecta, Botryococcus braunii, Spirulina platensis* (De Morais *et al.* 2007), *Chlorella* sp. (Sydney *et al.* 2010), *Nannochloropsis oculate* (Chiu *et al.* 2008). Chiu *et al.* (2008, 2009) reported the growth of *Chlorella* sp. and *N. oculata* was inhibited when CO_2 concentrations are above 5%. De Morais and Costa (2007a, 2007b) and De Morais *et al.* (2007) also found that four microalgae *C. kessleri, S. obliquus, Spirulina* sp. and *C. vulgaris* at their best growth when CO_2 concentrations are below 6%, and Sydney *et al.* (2010) calculated carbon dioxide assimilation for four species of microalgae, *D. tertiolecta, B. braunii, S. platensis* and *C. vulgaris* at 5% CO_2.

Intensive aquaculture at the coastal area has a potential for increasing seafood biomass production, such as shrimp and fishes. However, this activity has led to an environmental problem due to increasing organic waste. Gondwe *et al* (2012) and Vezzulli *et al.* (2008) indicated that aquaculture is the major contributor to the increasing levels of organic waste and toxic compounds. Without proper treatment, aquaculture waste would potentially cause harmful algal bloom (Hegaret 2007). Wastewater effluent from aquaculture industry contains nitrogenous compounds (ammonia, nitrite and nitrate), phosphorus and dissolved organic carbon which may lead to environmental deterioration at high concentration (Ali *et al.* 2005). Ammonia (NH_3) is the product of fish respiration and decomposition of excess organic matter (Lananan *et al.* 2014). Lananan *et al* (2014) found nitrogenous compounds present in excess amount are responsible

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for generating eutrophication which disrupt the aquatic ecosystem balance and could leads to massive mortality of aquatic fauna.

Besides having the ability to absorb CO_2, microalgae also has the ability to remove nitrogen and phosphorus from wastewater efficiently, so that microalgae can also be used as organisms for the bioremediation of tertiary treatments of liquid waste. Biological treatment using algae has several advantages, such as high efficiency, minimum cost, easy and simple operation and only require small concentration (Sabeti *et al.* 2019). Aslan and Kapdan (Chiu *et al.* 2009) found that *C. vulgaris* very effective in removing nutrient concentrations as NH_4-N0 < 22 mg L^{-1} and PO_4-P0 < 7.7 mg L^{-1}. The use of a wide range of microalgae such as *Chlorella, Scenedesmus, Phormidium, Botryococcus, Chlamydomonas* and *Spirulina* for treating domestic wastewater has been reported that those microalgae showed an effective nutrient removal (Olguin 2003; Chinnasamy *et al.* 2010; Kong *et al.* 2010; Wang *et al.* 2010). Microalgae require high amounts of N and P for proteins (40–60% of dry weight) so they could potentially be a nutrient removal from organic waste water (Olguin 2003; Chinnasamy *et al.* 2010; Kong *et al.* 2010; Wang *et al.* 2010).

Several studies have been conducted on the use single species of microalgae (Silva-Benavides and Torzillo 2012) and cyanobacteria (Tam and Wong 1996; Voltolina *et al.* 2005; de-Bashan *et al.* 2008) for waste water applications, and focused more on the function of microalgae as CO_2 absorbents and nutrients removal separately. However, research on combining the function of microalgae as CO_2 absorbent and absorbent of organic waste are still scarce. The aim of this study was to investigate the effect of increasing CO_2 concentration on the growth and the capability of *Tetraselmis chui* in nitrate, ammonium and phosphate removal from shrimp pond wastewater. While the effect of increasing CO_2 concentration on algal growth and nutrient removal, the cultured media enrichment with CO_2 system was examined, and pH was controlled daily. Different percentages of shrimp pond waste waters were used as culture media to examine nutrient removal efficiency.

Material and methods

Microorganisms and culture medium

The microalgae *T. chui* were obtained from Culture Collection of Algae, Research Center for Coastal Aquaculture and Fisheries Extension, Maros, South Sulawesi, Indonesia. Stock solution of Conway for microalgae stock culture media was made with material composition (per liter): 15 mg $NaNO_3$, 12 mg $MgCl_2 \cdot 6H_2O$, 18 mg $CaCl_2 \cdot 2H_2O$, 15 mg $MgSO_4 \cdot 7H_2O$, 1.6 mg KH_2PO_4, 0.08 mg FeCl3 $\cdot 6H_2O$, 18 mg $CaCl_2 \cdot 2H_2O$, 15 mg $MgSO_4 \cdot 7H_2O$, 1.6 mg KH_2PO_4, 0.08 mg $FeCl_3 \cdot 6H_2O$, 0.1 mg $Na_2EDTA \cdot 2H_2O$, 0.185 mg H_3BO_3, 0.415 mg $MnCl_2 \cdot 4H_2O$, 3 µg $ZnCl_2$, 1.5 µg $CaCl_2 \cdot 6H_2O$, 0.01 µg $CuCl_2 \cdot 2H_2O$, 7 µg $Na_2MoO_4 \cdot 2H_2O$ and 50 mg NaCO. Microalgae stock was incubated at 2000-mL flasks at room temperature, under continuous fluorescence lighting. Stirring was done by an aerator. Initial concentration of *T. chui* inoculated into aquarium was 9976 ind/ml.

Characteristic of wastewater

Wastewaters were collected from supra intensive shrimp pond of Research Center for Brackishwater Aquaculture and Fisheries Extension, Maros, South Sulawesi. Waste water from the pond were then filtered with 200 µm mesh-size to remove large particles and indigenous bacterium. Ammonia nitrogen (NH_3–N), nitrate nitrogen (NO_3–N) and total phosphorus (TP) were determined for all samples using Spectrophotometer.

Experimental setup and cultivation condition

Experiments were performed in 4-L glass aquarium with working volumes of 3.6 L. CO_2 system were used to manipulate CO_2 concentration in the culture media. CO_2 concentration treatment consisted of three treatments, namely 390 ppm as a control treatment (concentration of CO_2 in the water), 550 ppm pCO_2 as a prediction CO_2 concentration at 2050 based and 1000 ppm pCO_2 as a prediction CO_2 concentration at 2100 (based on IPCC CO_2 prediction). The CO_2 system is a tool that was assembled from a number of components, including supporting equipment such as a CO_2 supply, an O_2 compressor and a mass flow controller (MFC). This system functioned as a regulator of carbon dioxide (CO_2) concentration in the water. Carbon dioxide from CO_2 gas cylinders and oxygen from the O_2 compressors both enter the mass flow controller. The MFC regulates the flow rate and has CO_2 meters with digital displays so that the rate of carbon dioxide concentration flowing into the aquaria can be measured and regulated. In this research, the mass flow controller was set for two concentrations: (i) 550 ppm pCO_2 (CO_2 gas flow rate range $7.95 - 8$ mL min^{-1} and O_2 range 2:49 to 2:55 L min^{-1}) and (ii) 1000 ppm pCO_2 (CO_2 gas flow rate range $9.95 - 10$ mL min^{-1} and O_2 range 1:10 L min^{-1}). Waste water of supra intensive shrimp pond were used as culture medium. Filtered seawater (SW) was used to dilute SPWW accordingly to achieve percentages of SPWW used in this work. Experimental design used is completely randomized design with three replicates for each treatment. There were two factors of treatment, namely: CO_2 concentrations (390 ppm, 550 ppm and 1000 ppm) and supra intensive pond waste water (SPWW) concentrations were 100% SPWW, 75% SPWW+ 25% SW and 25% SPWW + 75% SW). The experiment was conducted for 21 days. The schematic of experiment setup was presented in Figure 1.

Growth monitoring and kinetic growth parameters

Cell density of *T. chui* was monitored by calculating microalgae cell numbers every 3 days using a Sedgwick Rafter Counting Cell. The growth rate was calculated using the formula (1):

$$\mu = (\ln N_t - \ln N_o)/t \tag{1}$$

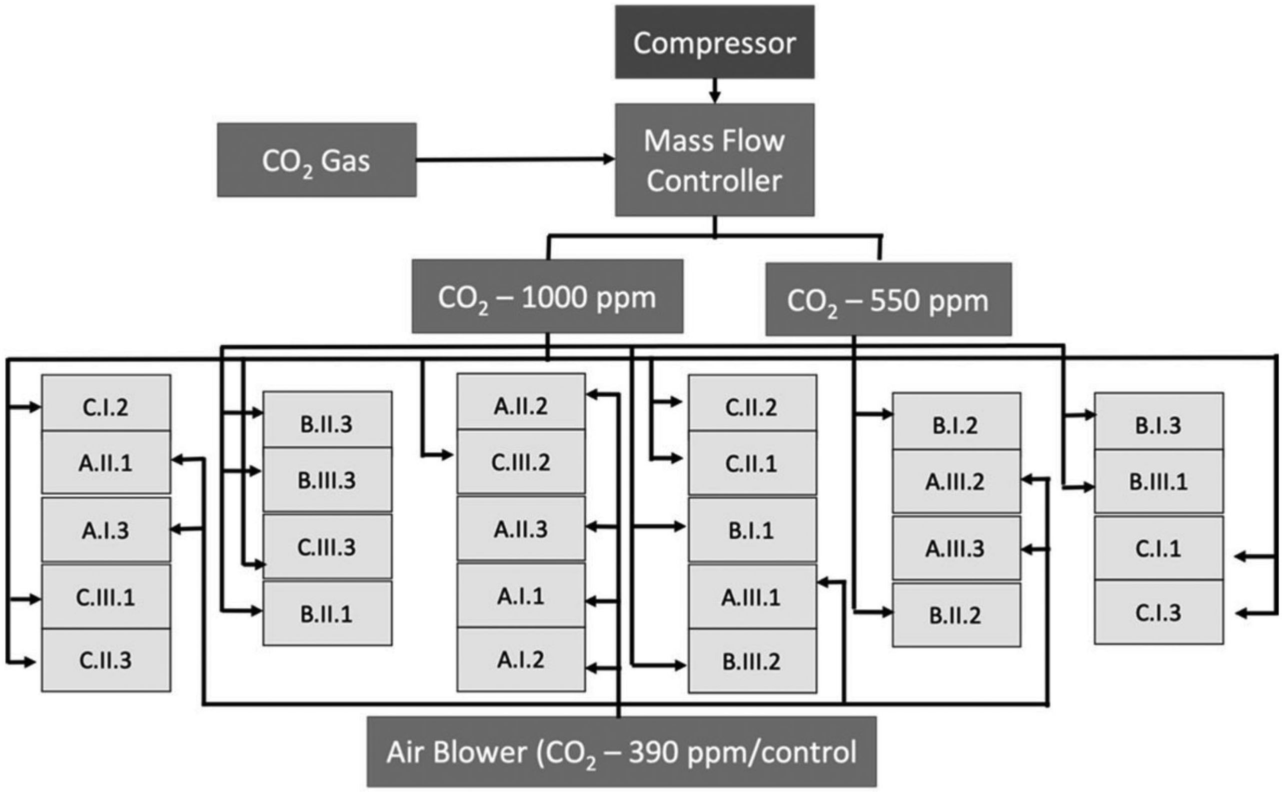

Figure 1. CO_2 system and schematic experimental design. Note: A: CO_2 concentration (390 ppm), B: CO_2 concentration (550 ppm), C: CO_2 concentration (1000 ppm); I : 100% shrimp pond wastewater (SPWW), II : 75% SPWW + 25% Seawater (SW), III: 25% SPWW + 75% SW; 1, 2 and 3 : replicate number.

where:

μ = growth rate (cell.day^{-1})
N_t = number of cells at time t (t)
N_o = number of cells at time 0 (to)
t = culture duration (hour.day^{-1})

Nutrient removal

Nutrients removal was determined by quantification of nitrate, ammonia and phosphate in the culture medium within the cultivation time. For nutrient analysis, 100-mL samples from each culture were weekly collected. Ammonium content was quantified by using Nessler's reagent with using colorimetric method, 0.3 ml of Nessler's reagent was added to 1 ml of wastewater and the resulting orange-red color was measured in the UV/Vis spectrophotometer at 420 nm. The standard curve was prepared from NH_4Cl to calculated ammonium content (Pouliot *et al.* 1989; Chevalier *et al.* 2000; Mallick 2002; Olguin 2003). Nitrate concentration was determined through UV spectroscopy at 220 nm using a T80 UV/VIS Spectrophotometer (PG Instruments, UK), according to Brucine Method. On the other hand, inorganic phosphate quantification was performed by measuring absorbance at 650 nm of an ammonium phosphomolybdate complex formed by reaction of inorganic phosphate with ammonium molybdate in spectrophotometer. Nutrient concentrations within the cultivation time were then used to determine nutrients removal efficiencies (R, in %).

Nutrients removal efficiencies were determined according to Eq. (2) (Nayak *et al.* 2016):

$$\%R = \frac{S_i - S_f}{S_i}.100 \qquad (2)$$

where S_i and S_f correspond to nutrients concentration (in mg L^{-1}) in the beginning and at the end of cultivation time, respectively.

The rate of nutrient removal was calculated using the following equation (3) (Nayak *et al.* 2016):

$$\text{Removal rate } \left(\text{mg d}^{-1}\text{L}^{-1}\right) = (S_i - S_f)/Dt. \qquad (3)$$

where, S_i and S_f are the mean values of nutrient concentration at the beginning (t_o) and at the end (t_i) time respectively. Δt is the cultivation time in days.

Statistical analysis

For each parameter, the average and the standard error values were calculated. The statistical significance of the results was evaluated using two ways ANOVA to investigate whether the differences between treatments could be considered significant and Tukey's multiple comparison test was performed to examine the difference between two treatments. This analysis was performed using the statistical software GrapPad Prism 7.05. Statistical tests were carried out at a significance level of 0.05.

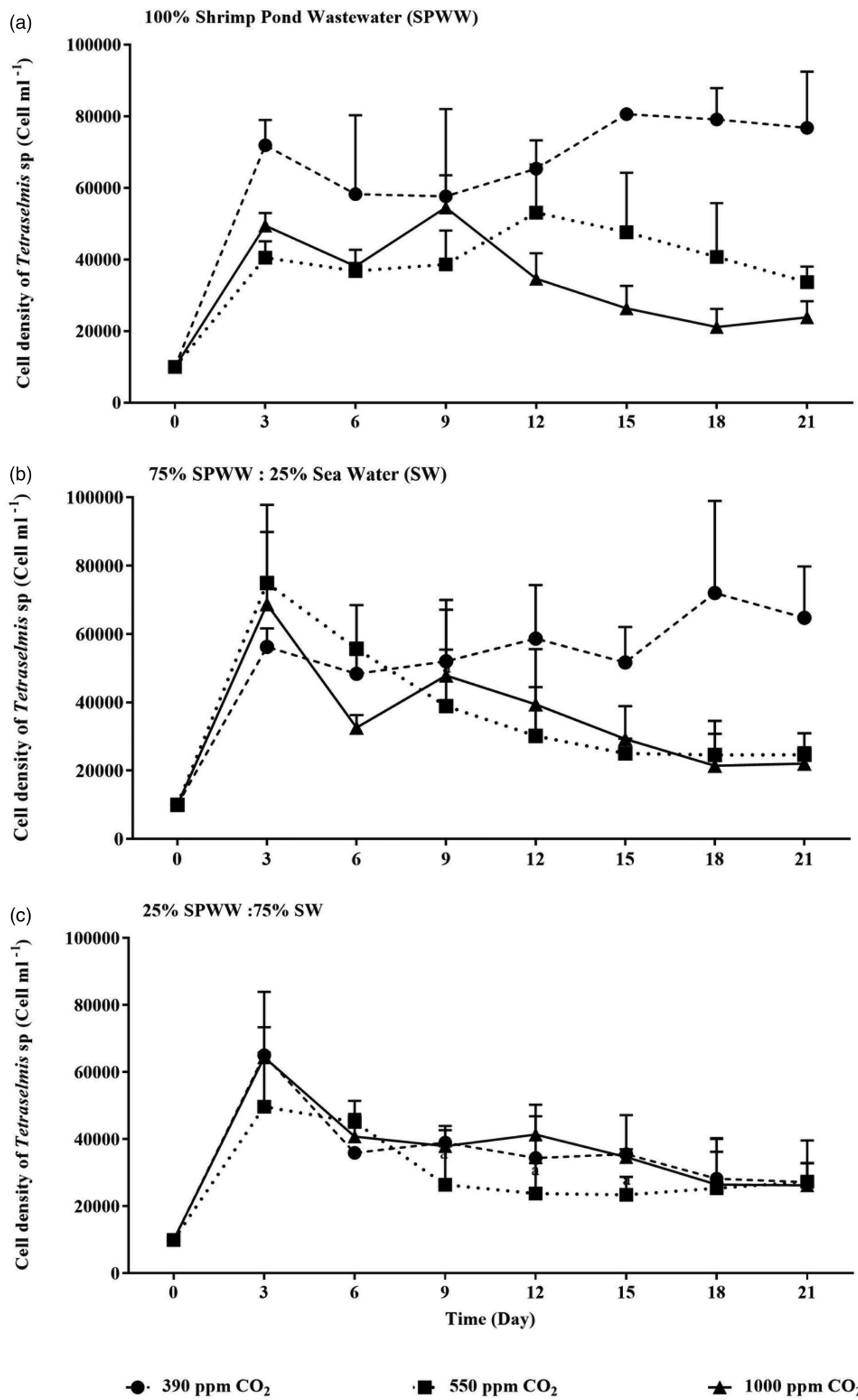

Figure 2. The growth curves obtained for *Tetraselmis chui*. (x ± SE, *n* = 3). (a) 100% SPWW, (b) 75% SPWW + 25% SW and (c) 25% SPWW + 75% SW.

Results and discussion

Microalgal density and growth rate

In this study, the selected microalgae, *T. chui* was cultivated in small aquarium using shrimp pond wastewater (SPWW) as a culture medium, supplemented with different concentration of CO_2; 390 ppm, 550 ppm and 100 ppm. The growth profile of *T. chui* in various CO_2 concentration with the incubation time of 21 days was assessed. *T. chui* density was examined every third day of culture for 21 days for determining growth rate and the pattern of *T. chui* and the study also examined the effect of increasing CO_2 concentrations and percentage of SPWW on the growth behavior of *T. chui*. (Figure 2). Figure 2 showed that the density of *T. chui* for all cultivation periods tended to higher at treatments of 390 ppm CO_2 and 100% SPWW, however there was a declining density over period of cultivation for both treatments (CO_2 concentrations and percentage of SPWW in culture medium). The increasing CO_2 concentration at 100% SPWW in the culture medium of *T. chui* caused decreasing cell density over period of culture. This finding showed that *T. chui* was less tolerance to high concentration of CO_2, unlike other species of marine green algae, such as *Chlorella* sp. which showed high tolerance to CO_2 level (*Nayak et al.* 2016). The growth profile of *T. chui* at most of SPWW percentage in culture medium and CO_2 concentration treatment showed a dramatic increased at the first three days of culture and gradually decreased in the following day. This finding also indicated that there was an increasing growth pattern in different of SPWW percentage. This finding was in line with previous study by (Tripathi *et al.* 2019) who found that growth pattern of *Scendesmus* sp. was increasing when they growth in different percentage of wastewater (25 − 100%). Statistical analysis showed that there was a significant difference of *T. chui* abundance between CO_2 concentration treatments and percentage of SPWW (100% SPWW and 75% SPWW + 25% SW) for every third days of observation. However, there was no significant difference of *T. chui* density between CO_2 concentration treatments at 25% SPWW + 75% SW for all days of observation. This finding showed that increasing CO_2 concentration and decreasing SPWW percentage in culture medium did not affect cell density of *T. chui* over period of culture. It assumed that *T. chui* could adapt well when there was low nutrient concentration event they were exposed by a high CO_2 concentration. There are two factors affecting the growth of microalgae when excess CO_2 is added to the mass culture, i.e., a) supply of carbon to the cell, and b) the pH (Raeesossadati *et al.* 2014), however species tolerant to a lower pH can grow at higher CO_2 concentrations (Moheimani 2013).

The growth rate of *T. chui* cultivated in SPWW under different CO_2 concentration were determined after 21 days cultivation and were used to describe the influence of different CO_2 concentration on this kinetic growth parameter (Figure 3). The result showed that the growth rate of *T. chui* was higher for all percentages of SPWW in culture medium at 390 ppm CO_2 compared to other percentages of SPWW treatment in the culture medium with other CO_2 concentration treatments. The highest growth rate was found at 100% SPWW in the culture medium at 390 ppm CO_2 concentration accounting for 0.096 cell day^{-1}. On the other hand, the lowest growth rate of *T. chui* was found at 75% SPWW + 25% SW treatment at 1000 ppm CO_2 concentration, accounting for 0.044 cell day^{-1}. The range of growth rate for different percentage of SPWW in the culture medium and CO_2 concentration treatments from 0.037 ± 0.0058 cell day^{-1} (1000 ppm CO_2 concentration at 75% SPWW + 25% SW in culture medium) and 0.096 ± 0.006 cell day^{-1} (390 ppm CO_2 concentration at 100% SPWW in culture medium). This finding showed a lower growth rate of *T. chui* compared to other marine green algae species *Spirulina* sp. (Keffer and Kleinheinz 2002). In general, there was a decreasing of growth rate with increasing of CO_2 concentration at 100% SPWW and 75% SPWW + 25% SW in culture medium. This finding was in line with previous study which found that the growth rate of *Scenedesmus* sp. was lower at 80% CO_2 than other percentage of CO_2 treatment (de Morais and Costa 2007b). The decreasing growth rate of *T. chui* was assumed due to decreasing pH with elevating concentration of CO_2 in culture media as a result of increasing acidification. Raeesossadati *et al.* (2014) found that microalgae could grow under 100% CO_2, however their growth was inhibited due to acidification. Microalgae consumed CO_2 in photosynthesis process resulted in increasing pH, this changes condition may affect growth rate of microalgae species (Pires *et al.* 2012)

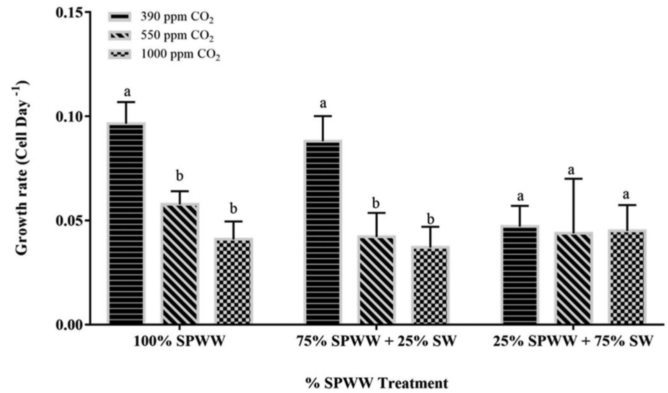

Figure 3. The growth rate of *Tetraselmis chui* at different CO_2 concentrations and treatment of SPWW percentage in culture medium ($x \pm SE$, $n = 3$). The same lowercase letter denotes a non- significant difference and different lowercase letter denotes a significant difference of growth rate between CO_2 concentration treatment ($p < 0.005$).

The effect of CO_2 concentration on nutrient removal and removal rate

The integration of wastewater with CO_2 sequestration is encouraging for higher growth rate of microalgae (Gonçalves *et al.* 2016). Moreover, the use of SPWW as a culture medium will minimize the requirement for nutrient. Figue 4 showed the removal efficiency of nitrate (NO_3), ammonium (NH_4) and phosphate (PO_4) by *T. chui* at different CO_2 concentration using different percentage of SPWW in culture medium. The concentration of CO_2 was varied (390 ppm, 550 ppm and 1000 ppm) and their effect on nutrient removal was assessed. The efficiency of bioremediation by *T. chui* was determined by measuring the overall nutrient removal percentage and nutrient removal rate as shown in Figure 4. Nutrient removal efficiency was higher for NH_4 than for NO_3 and PO_4 and the lowest nutrient removal was found for PO_4 at all CO_2 concentration and treatment of SPWW percentage. Nitrate removal efficiency was higher at treatment of SPWW percentage in culture medium than other SPWW treatments. NO_3 removal efficiency for this treatment was accounting for 99%, 98% and 89% for 390 ppm, 550 ppm and 1000 ppm CO_2 concentration treatment, respectively. This study found that NO_3 removal efficiency was slightly increasing with increasing CO_2 concentration for the treatment of SPWW percentage in culture medium, except the opposite trend was occurred for the treatment of 25% SPWW + 75% SW. This finding indicated that CO_2 concentration affected NO_3 removal efficiency by *T. chui*. It was increasing NO_3 removal efficiency with increasing CO_2 concentration. Our

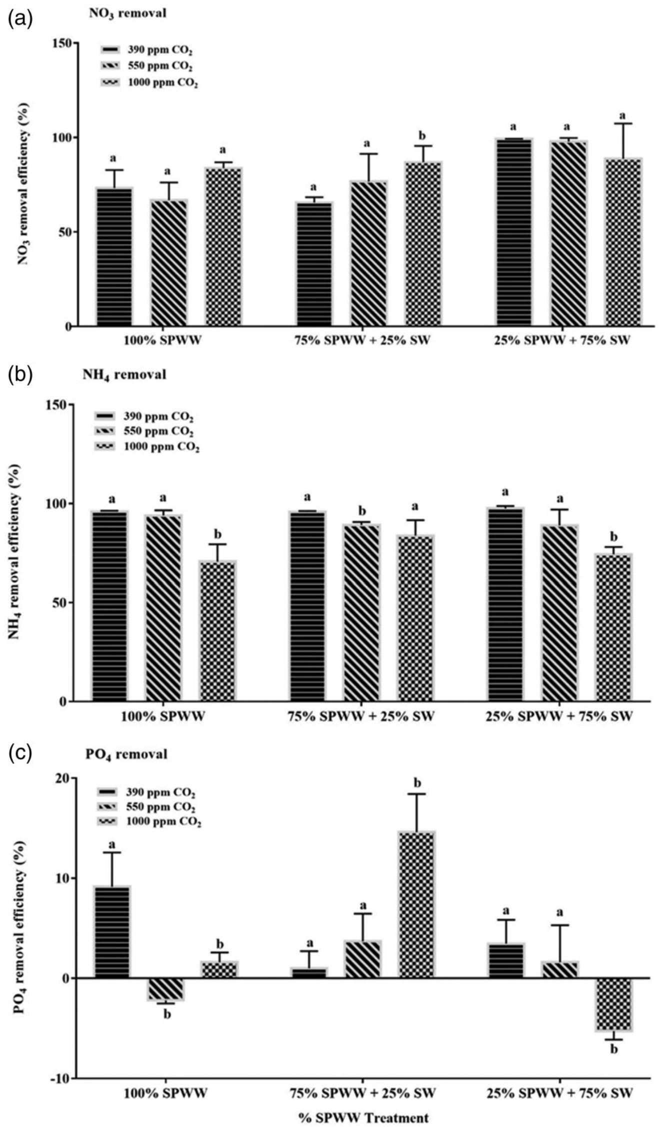

Figure 4. Nutrient removal efficiency at different CO_2 concentrations and treatment of SPWW percentage (x ± SE, $n = 3$). The same lowercase letter denotes a non- significant difference and different lowercase letter denotes a significant difference of nutrient removal efficiency between CO_2 concentration treatment ($p < 0.005$).

finding was in line with previous study by (Pires *et al.* 2012) and Nayak *et al.* (2016) who found that *Chlorella vulgaris* which was cultured with non-enriched-air stream had a lower nitrogen removal than those of cultured with CO_2 enriched-air and nutrient removal capacity improve with increasing CO_2 concentration. Our finding also indicated that the initial concentration of NO_3 will affect NO_3 removal efficiency. It showed at Figure 4a which was the treatment of 25% SPWW+ 75% SW in culture medium with average initial NO_3 concentration accounting for 0.092 mg L^{-1} resulting in 99.23% NO_3 removal efficiency. This finding was supported by Hariz *et al.* (2019) who found that there was increasing nutrient assimilation when CO_2 was adding into the microalgae culture media.

NH_4 removal efficiency tended to higher at 390 ppm CO_2 concentration for all treatment of SPWW in culture medium than those of other CO_2 concentration treatments. The highest NH_4 removal efficiency was found at the treatment of 25% SPWW + 75% SW at 390 ppm CO_2 concentration accounting for 97%. On the other hand, the lowest NH4 removal efficiency was 74% for the treatment of 75% SPWW + 25% SW in culture medium at 1000 ppm CO_2 concentration (Figure 4b). This finding indicated that NH_3 removal efficiency was decreasing with increasing CO_2 concentration. Our finding was also in agreement with previous study by Nayak *et al.* (2016) who found NH_4 removal efficiency by *Chlorella vulgaris* reached 98%. Moreover, Tam and Wong (1996) found that *Chlorella vulgaris* could remove NH_4 efficiently at 95% in batch culture.

PO_4 removal efficiency was the lowest than other nutrient removal efficiency for all the treatments of SPWW percentage in culture medium and CO_2 concentration treatments. The highest PO_4 removal efficiency was found at 1000 ppm CO_2 treatment for 75% SPWW + 25% SW in culture medium with mean value of 36.76 ± 11.99%. However, our PO_4 removal efficiency was lower compared to previous study by Gonçalves *et al.* (2016) who found that PO_4 removal efficiency at *Chlorella vulgaris* ranged between 49.0 ± 4.3% and 83.5 ± 0.3%. This finding indicated that there was a species-specific response on absorbing $PO_{4,}$ depending on environmental condition and media composition. Aslan and Kapdan (2006) reported that media composition and environmental condition (such as the initial nutrient concentration, the light intensity, the nitrogen/phosphorus ratio, the light/dark cycle or algae species) were factors affecting nitrogen and phosphorus removal efficiency by microalgae. Interestingly, we found a negative value of removal efficiency at 100% SPWW and 25% SPWW + 75% SW in culture medium at high concentration of CO_2 (550 ppm and 1000 ppm), with mean values −2.19% and −5.77%, respectively. This finding indicated that initial PO_4 concentration in the culture medium affected the efficiency of PO_4 removal by *T. chui.* Furthermore, Amini *et al.* (2019) found that up-take capacity and removal efficiency of NO_3^- and PO_4^- by *Dunaniella salina* increases with an elevated initial NO_3^{-} and PO_4^{3-} concentrations. It showed that with elevated ions concentration in solutions, the removal efficiency by algae was increased (Amini *et al.* 2019). In this study, there was no significance different of nutrient removal between CO_2 concentration and the treatment of SPWW percentage except for 75% SPWW + 25% SW treatment, there was a significant difference of nutrient removal between CO_2 concentration treatment.

Nutrient removal rate was calculated for determining the capability of *T. chui.* in absorbing nutrient for their growth. Results of the present study showed that there was a different pattern of nutrient removal rate between the treatment of SPWW percentage in culture medium and CO_2 concentration (Figure 5). The maximum removal rate in this study was 0.09 mg L^{-1} of N. This finding indicated that increasing CO_2 concentration could escalate the uptake of NO_4 by *T. chui.* On the other hand, for NH_4 removal rate increasing CO_2 concentration could decline ammonia uptake. Statistically, there was a significance different of nutrient removal rate for NO_3 and NH_4 between the treatment of SPWW percentage and CO_2 concentration treatments ($p < 0.05$). This finding indicated that CO_2 concentration was strongly affecting an uptake of nitrogen by *T. chui.* This result was contradicting with previous study by Gonçalves et al (2016) who found that the uptake rate of nitrogen by *Chlorella vulgaris* was not strongly depended on CO_2

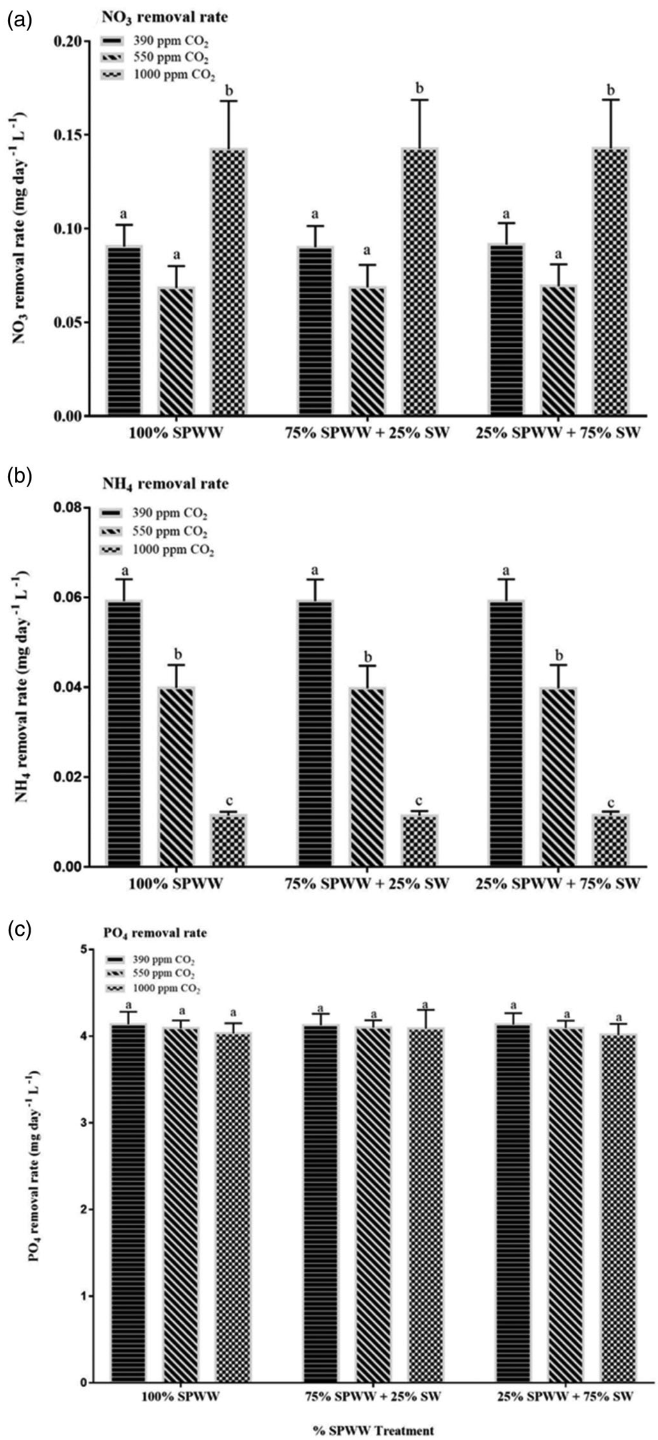

Figure 5. Nutrient removal rate at different CO_2 concentrations and the treatment of SPWW percentage ($x \pm SE$, $n = 3$). The same lowercase letter denotes a non- significant difference and different lowercase letter denotes a significant difference of nutrient removal efficiency between CO_2 concentration treatment ($p < 0.005$).

concentration. Contradictive result was presumably due to *T. chui*. was less tolerant to increasing CO_2 concentration, compared to *Chlorella vulgaris* which was more tolerant to increasing CO_2 concentration (Raeesossadati *et al.* 2014).

Nutrient removal rate for PO_4 tend to be similar in all treatments of SPWW percentage in culture medium and CO_2 concentrations. However, PO_4 removal rate was not significantly different between the SPWW percentage and CO_2 concentration treatments, indicating there was no significant effect of increasing CO_2 concentration to removal rate of PO_4. This finding was in line with previous study at *Chlorella vulgaris*, *Phormidium subcapitata* and *Spirulina salina* by (Raeesossadati *et al.* 2014) who found that phosphorus uptake rate was not strongly influenced by CO_2 concentration.

Conclusion

The results of this study demonstrated that *Tetraselmis chui*. was less tolerant to increased CO_2 concentration, mainly through decreasing growth rate in elevated CO_2 concentration. Although, *T. chui* could well adapt in a high CO_2 concentration when low nutrient concentration present. NO_3 removal rate by *T. chui* was increased with escalating CO_2 concentration, whilst NH_4 removal rate decreased with increasing CO_2 concentration. In conclusion, CO_2 concentration was significantly affecting nitrogen removal efficiency and rate by *T. chui*, but not significantly affecting removal efficiency and rate of PO_4 by *T. chui*.

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