O 39. INCREASING CARBON DIOXIDE AVAILABILITY IN MICROALGAE CULTIVATION SYSTEMS BY USING TRIETHANOLAMINE

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ABSTRACT: In this study, we investigated the use of triethanolamine (TEA), a well-known carbon dioxide (CO_2) capturing chemical, to increase CO_2 availability in microalgae growth media. Microalgae culture used in the study was a mixed culture, in which the dominant species were determined by microscopic analysis as *Chlorococcales* order of the *Chlorophyceae* class (i.e. *Scenedesmus sp*., *Chloroccum sp.*). The non-toxic triethanolamine concentration was determined by measuring the specific growth rates of microalgae cultures grown in BG11 medium spiked with 1, 2, 5, 10, 20 and 40 mM TEA. It was observed that the growth rate started to drop at TEA concentrations higher than 20 mM. the biomass growth rate as well as nitrate and phosphate consumption rates of microalgae at 20 mM TEA concentration were observed to be similar to those grown without any TEA addition. Based on GC-FID analysis, a decrease in TEA concentration was detected after 6th day of the growth period, but a separate study indicated that this decrease could not be associated with abiotic oxidation, biosorption or sorption by microalgae. Using pre-CO_2 loaded BG11 mediums with 20 mM TEA concentration resulted in higher biomass production rates compared to those without TEA addition, when the cultures were not aerated during cultivation. When the cultures were subjected to aeration, the biomass production rates of the cultures with and without TEA addition became close to each other. It was found that most of CO_2 loaded in the medium released within a few days of the growth period. Further studies are being conducted to reveal CO_2 release mechanisms from CO_2 loaded TEA solution to microalgae medium.

Keywords*: Carbon dioxide capture, microalgae, alkanolamine, triethanolamine, biomass production*

1. INTRODUCTION

Carbon dioxide (CO_2) emissions from large stationary sources, such as fossil fuel-fired power plants, cement kilns, refineries, and manufacturing facilities, contribute significantly to the increase of CO_2 level in the atmosphere. The current approach to reduce CO_2 emissions from these large point sources is to use amine-based post-combustion CO_2 capture systems followed by sequestration of the captured CO_2 into underground geological structures such as saline aquifers after transporting at high pressure via pipelines (Plaza and Pevida, 2019).

Amine-based CO_2 capture systems include an absorption unit in which flue gas CO_2 is absorbed into aqueous amine solutions by forming an intermediate compound, and a stripping unit in which the aqueous amine solutions are regenerated by stripping CO_2 from the intermediate structure using heat or pressure-swing method. The CO_2 released from the stripping unit is then compressed to 100 to 150 bar for industrial use or geologic sequestration (Rochelle, 2009). Although amine-based CO_2 capture processes could capture more than 90% of CO_2 from flue gas streams, there are still some remaining challenges regarding the high energy penalty of the process and the limited use of high-purity CO_2 captured in other industrial processes (Rochelle, 2009; Bara, 2012). Currently, installation of an amine-based CO_2 capture system to an existing power plant is estimated to increase the electricity usage in the plant by 75–80 % (Idem et al., 2006). In addition, the high-purity CO_2 produced from flue gas has also limited usage due to relatively low CO_2 demand (about 230 million tonnes per year) in various industrial applications, such as urea manufacturing, oil recovery, beverage production, metal fabrication, cooling and fire suppression (IEA, 2019). Furthermore, despite of the extremely large storage capacities of existing saline aquifers, geological sequestration has still several uncertainties and risks associated with long-term CO_2 storage due to possible CO_2 leakages and long-term maintenance and monitoring requirements (Anderson, 2016).

An alternative to the current approach is to assimilate CO_2 emissions from flue gases biologically by using photosynthetic microorganisms, such as microalgae. It is already known that microalgae is highly capable of utilizing flue gas CO_2 as an inorganic carbon source to produce new biomass. Capturing and assimilation of CO_2 by microalgae offer some advantages compared to the current approach, including low energy and chemical requirements, and production of commercially viable

biomass. Yet, this technology has also some drawbacks to be overcome before its full implementation. For example, effective delivery of gaseous CO_2 into the algal ponds is still a challenging task. Since, typical algal ponds are designed as shallow in order to maximize light penetration, when CO_2-rich flue gas is bubbled into such algal ponds, most of the CO_2 delivered escapes to the atmosphere, and only between 10 and 30% of the supplied amount could be utilized by microalgae (Li et al., 2013; Apel and Weuster-Botz, 2015). In addition, from practical perspective, it is difficult to find specific locations where sufficient and low-cost sources of both CO_2 and nutrient solution (wastewater, etc.) are located in close proximity to each other. Therefore, flue gas CO_2 must be compressed and transported from the CO_2 point source to the algal pond, which would increase operational costs drastically. It must also be taken into account that low or no usage of captured CO_2 during the night or winter periods when algae have no or low photosynthetic activity would lower the overall utilization of CO_2 in the process (Benemann and Oswald, 1994).

A viable solution to the drawbacks associated with the use of gaseous CO_2 in microalgae CO_2 assimilation process might be to supply CO_2 into microalgae media in the form of dissolved bicarbonate/carbonate by absorbing flue gas CO_2 in aqueous solutions using conventional absorption processes. This alternative could reduce the CO_2 loss encountered when gaseous CO_2 is used directly; furthermore, could decrease CO_2 transportation and storage costs substantially. Both microalgae and cyanobacteria are capable of uptake of bicarbonate ions through their membranes using a special enzyme called carbonic anhydrase. Earlier studies showed that various microalgae and cyanobacteria species could reach significant biomass production rates when bicarbonate salts (mostly sodium bicarbonate) are used as a sole carbon source instead of gaseous CO_2 supply (White et al., 2012; Chi et al., 2014; Kishi and Toda, 2017). Furthermore, different types of aqueous amine solutions were used in several studies to deliver bicarbonate ions to microalgae without affecting biomass growth rates up to certain amine concentrations (Choi et al., 2012; Kim et al., 2013; Sun et al., 2015; Sun et al., 2016). In these studies, mostly aqueous alkanolamine solutions were used to capture the CO_2 gas delivered to the growth medium by diffusers. Alkanolamines, which have long been used to remove CO_2 from flue gases in industrial processes, are chemical compounds that contain both hydroxyl and amino functional groups on an alkane backbone. They are classified into primary, secondary and tertiary alkanolamines based on the number of the attached amine group. CO_2 capture by amines can be described by two different mechanisms: (1) for primary and secondary alkanolamines, the mechanism involves a carbamate formation through reactions with CO_2 (Caplow, 1968), (2) for tertiary alkanolamines, the amine group acts as a base catalyst, increases the reactivity of water towards CO_2 and consequently forms bicarbonate ions by reacting with CO_2 (Donaldson and Nguyen, 1980). In earlier studies involving the use of alkanolamines for microalgae growth, the aqueous amine solutions were mixed with growth media in different proportions while supplying CO_2 to the media in gaseous form (Choi et al., 2012; Sun et al., 2015; Kim et al., 2013). In our study, we differed from these earlier studies by focusing on absorbing CO_2 in aqueous amine solutions in a separate gas absorber unit, and then feeding the CO_2-loaded amine solutions with growth medium. Furthermore, in our study we monitored the change in the amine concentration in the microalgae cultivation medium to determine whether the amine compounds are subjected to biotic or abiotic degradation during the cultivation step The fate of the amine compounds has not been the subject of the earlier studies.

In addition, the earlier studies on using aqueous amine solutions to deliver CO_2 to microalgae cultivation systems seemed to have focused on how to increase CO_2 utilization efficiency of conventional CO_2 delivery systems, rather than how to integrate current amine-based CO_2 capturing systems with microalgae cultivation systems. The present study, on the other hand, aims to investigate the effect of using CO_2 loaded amine solutions on microalgae cultivation process in comparison with conventional gaseous CO_2 delivery systems in order to see feasibility of integration of these two very different processes (CO_2 absorption and CO_2 assimilation by microalgae).

2. MATERIALS AND METHODS

2.1. Aqueous amine solution

In this study, triethanolamine (TEA), a tertiary alkanolamine, was used to prepare the aqueous amine solution for capturing CO_2. A tertiary alkanolamine was selected to capture CO_2 because tertiary alkanolamines are expected to result in less pH changes after addition to the growth medium compared to primary and secondary alkanolamines which contain higher number of hydroxyl groups. Furthermore,

previous studies showed that tertiary alkanolamines have less toxic effect on microalgae compared to primary and secondary alkanolamines, which produce toxic carbamate intermediates during reactions with CO_2 (Kim et al., 2013; Rayer et al., 2014). The aqueous TEA solutions were prepared by using analytical grade triethanolamine purchased from Sigma-Aldrich (USA).

2.2. Culture media and microorganisms

A mixed microalgae culture was used in this study. The culture was obtained by inoculating the water samples collected from nearby water bodies to a standard BG11 medium (Rippka et al., 1979). After an acclimation period, the dominant species in the culture was determined as *Chlorococcales* order of the *Chlorophyceae* class (i.e. *Scenedesmus* sp., *Chloroccum* sp.). The same culture was successfully used in our previous studies (Keris-Sen et al., 2014; Keris-Sen et al., 2019). The mixed culture was cultivated in the laboratory, in a 5-L glass tank, under a light intensity of 150 µmol photon m^{-2} s^{-1} in photosynthetically active radiation (PAR) spectrum, at 12 h light - 12 h dark illumination cycle and at 25 °C ± 2. The air diffusers located at the bottom of the tank both provided sufficient CO_2 to the culture and enhanced nutrient and light availability throughout the tank.

2.3. Determination of non-toxic TEA concentration

The non-toxic triethanolamine concentration was determined by measuring the specific growth rates of microalgae cultures grown in BG11 medium spiked with 1, 2, 5, 10, 20, and 40 mM TEA. The experiments were conducted in duplicates, in 500 mL glass reactors, under a light intensity of 150 µmol photon m^{-2} s^{-1} in PAR spectrum, at 12 h light - 12 h dark illumination cycle and at 25 °C ± 2. The growth of microalgae in the reactors was monitored on daily basis by measuring the optic density of the samples at a wavelength of 682 nm with a UV-VIS spectrophotometer (Thermo Scientific). The spectrophotometric analysis was calibrated with respect to total dissolved solids (TSS) measurements of the mixed culture according to the Standard Methods 2540-D (APHA, 2005).

The effects of the non-toxic TEA concentration on nutrient consumption of microalgae cultures were also determined by monitoring nitrate (NO_3^-) and phosphate (PO_4^{3-}) concentrations in the growth medium. The nitrate and phosphate concentrations were measured using an ion chromatography system (Shimadzu Prominence) equipped a conductivity detector and an anion-exchange column. The samples of 5 mL were taken from each reactor daily and the samples were filtered thorough 0.45 µm syringe filter before injected into the column.

2.4. Determination of TEA degradation

Since TEA is a nitrogen containing organic compound, it is expected to be susceptible to biodegradation by microalgae during the cultivation period. Furthermore, TEA could also be stripped and/or degraded by abiotic environmental conditions like the oxidative environment created in the reactor by aeration. Thus, TEA concentration during microalgae growth was monitored daily by taking 2 ml samples from each reactor. Two control reactor sets were run simultaneously in order to distinguish the degree of degradation caused by biotic and abiotic factors: (1) two identical reactors filled with BG11 medium spiked with same TEA concentration and aerated at the same rate as the reactors containing microalgae culture, (2) two identical reactors filled with BG11 medium spiked with the same TEA concentration as the reactors containing microalgae culture.

TEA concentrations in the samples taken from the reactors were filtered thorough 0.45 µm syringe filter and analysed by gas chromatography (GC) by using an Agilent 6890 N series gas chromatograph equipped with a flame ionization detector (FID), a DB-5HT capillary column (30 m x 0.53 mm I.D. x 0.1 µm), a hydrogen generator (Shimadzu), and an auto-injector. The temperature in the column was set initially to 150 °C for 1 min, then it was ramped to 200 °C at 65 °C min^{-1} and then it was ramped to 330 °C at 40 °C min^{-1}, and finally it was hold for 3 min. The total run time was 9 min. The detector temperature was 335 °C, and the air and hydrogen flows to the detector were set as 450 mL min^{-1} and 45 mL min^{-1}, respectively. In each analysis, 1 µL of sample was injected into the inlet module at 260 °C and the injected sample was spilt at a ratio of 1:10 before entering the column. The carrier gas in the column was helium at 1.7 mL min^{-1} flow and at 80 kPa constant pressure, while the make-up gas was nitrogen.

2.5. Comparison of CO_2 delivery approaches

The investigation of the effect of using CO_2 loaded TEA solutions on microalgae cultivation process in comparison with conventional gaseous CO_2 delivery systems was performed by setting up four types of reactor sets with changing parameters as depicted in Table 1.

Table 1. Experimental parameters for each reactor setup

Parameters	Reactor Set No			
	R1	**R2**	**R3**	**R4**
50 mg L^{-1} initial biomass concentration	√	√	√	√
BG11 medium (including no sodium carbonate)	√	√	√	√
375 mL medium volume	√	√	√	√
150 µmol m^{-2} s^{-1} PAR illumination	√	√	√	√
Addition of CO_2-loaded TEA	√		√	
Aeration at 50 mL min^{-1} with air containing CO_2		√		
Aeration at 50 mL min^{-1} with air containing no CO_2	√			

All reactor sets were run in 500 mL glass reactors (including 375 mL of BG11 medium), under a light intensity of 150 µmol photon m^{-2} s^{-1} in PAR spectrum, at 12 h light - 12 h dark illumination cycle and at 25°C ± 2. All experiments were conducted in duplicates with an initial biomass concentration of 50 mg L^{-1}. Additionally, reactor sets R1 and R3 were spiked with CO_2-loaded TEA solutions. The CO_2-loaded TEA solution was prepared by passing analytical grade CO_2 gas (at 99.9% purity) for 2 hours through a gas washing bottle which includes 2 M TEA solution. Then, the CO_2-loaded concentrated TEA solution was diluted with BG11 medium before starting up the reactors. Different from than the reactor set R3, R1 was aerated at 50 mL min^{-1} flow rate by a diffuser, but to prevent CO_2 input to the system, the air was pumped to the reactor after passing through a gas washing bottle including 1 N NaOH solution. By doing this, it was aimed to compare the system with TEA to the conventional CO_2 bubbling system (reactor set R2) by adding the mixing effect coming from the aeration itself. The reactor set 4 was executed as a control experiment.

Along the cultivation period, microalgae growth and nutrient consumption in the reactors were monitored on daily basis with the spectrophotometric and the ion chromatographic methods explained in earlier. In addition to them, total organic carbon (TOC) and total inorganic carbon (TIC) contents in the reactors were measured by using a TOC analyser (Shimadzu) by taking 5 mL samples on daily basis. The change in the temperature and pH of the growth medium were also followed daily using a multi-parameter meter (Mettler Toledo).

3. RESEARCH FINDINGS

3.1. Determination of non-toxic TEA concentration

The specific growth rates of microalgae cultures grown in BG11 medium and spiked with 1, 2, 5, 10, 20, and 40 mM TEA are illustrated in Figure 1. As depicted in the figure, the specific growth rates started to drop significantly at TEA concentrations higher than 20 mM. Although the highest growth rate occurred around TEA concentration of 5 mM, still high growth rates were observed at the high TEA concentration of 20 mM. Since the objective was to maintain high TEA concentration to be able to increase the CO_2 capturing capacity of the system, subsequent experiments were performed at 20 mM TEA concentration.

The effect of 20 mM TEA content in the growth medium on biomass production and nutrient consumption of the microalgae was also determined by a separate set of experiments and the results are presented in Figure 2.

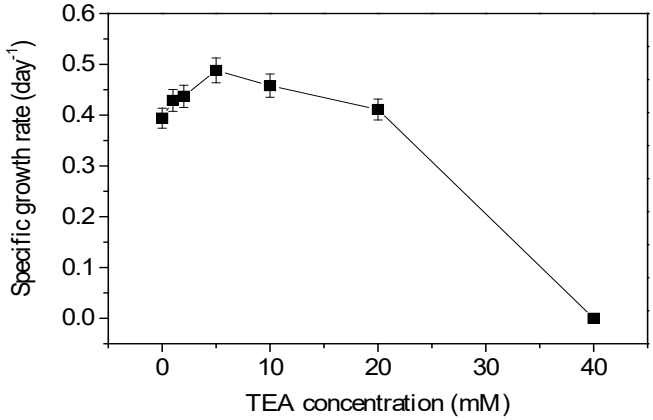

Figure 1. Specific growth rates of microalgae cultures for different initial TEA concentrations in the growth medium

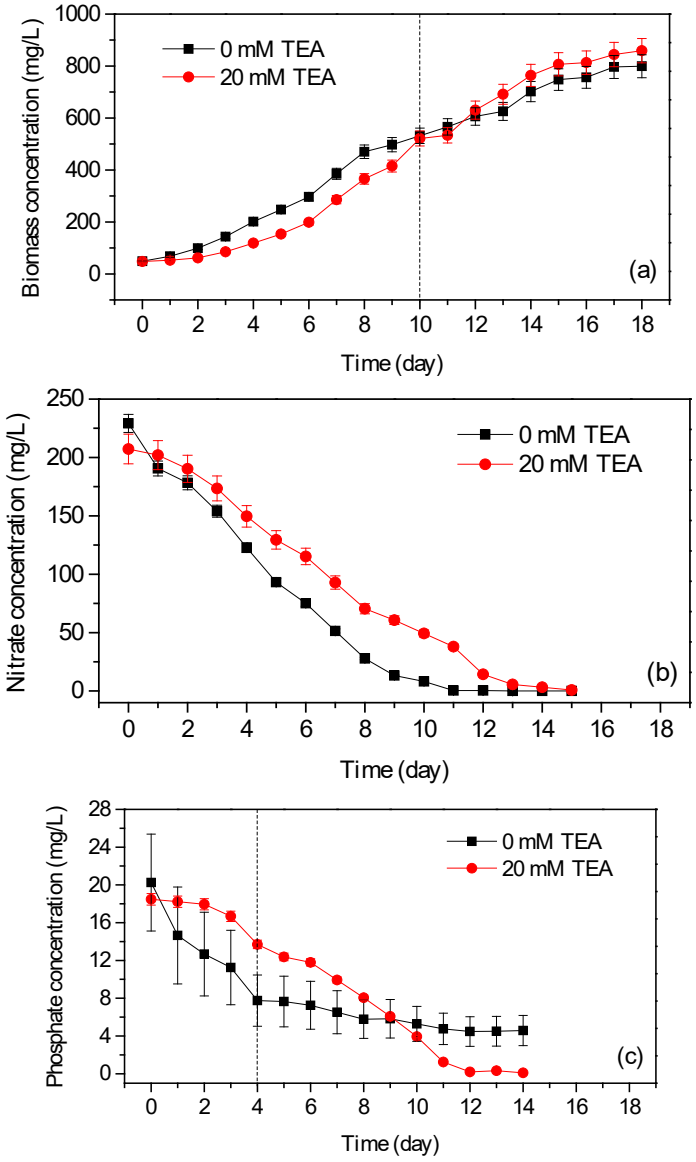

Figure 2. Comparison of the cultures grown in growth mediums including 0 and 20 mM TEA in terms of (a) biomass growth, (b) nitrate, and (3) phosphate consumptions

Figure 2a indicates that the biomass productions of those cultures with and without TEA addition have had quite similar biomass production rates. Although, the culture with 20 mM TEA showed a lower growth rate than those of the control culture in first 10 days, after an adaptation period, the TEA containing culture caught the biomass concentrations of the control culture. In overall culture period, both cultures reached similar daily biomass production rates (28.3 mg L^{-1} day^{-1} for the control culture, 26.0 mg L^{-1} day^{-1} for the culture with 20 mM TEA).
For nutrient consumption rates, similar patterns were observed between the two cultures. Although nitrate consumption rate of the control culture seemed to be higher, both cultures depleted their nitrate content at the same time (Figure 2b). Both cultures encountered a lag period for phosphate uptake (about 3 days) (Figure 2c). At the end of the experiment, the culture with 20 mM TEA consumed all phosphate content while the phosphate concentration in the control culture remained at about 4 mg L^{-1}

3.2. Determination of TEA degradation

TEA concentration in the growth medium was monitored during the growth experiments. The results are presented together with the results of nitrate concentration in the growth medium in Figure 3.

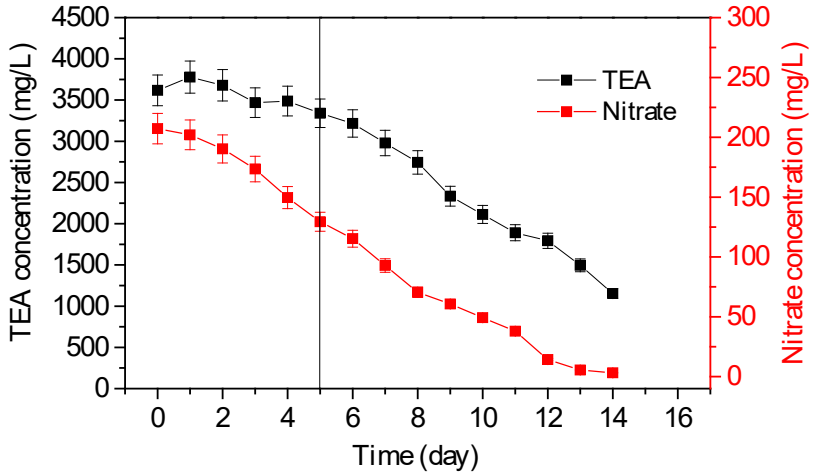

Figure 3. The change in TEA concentration in the growth medium in comparison with the nitrate consumption during microalgae cultivation

The TEA concentration remained stable for the initial 5 days and then started to drop. Since TEA has nitrogen groups in its molecular structure, it is plausible to expect that microalgae might change their nitrogen source from nitrate to TEA. However, the results show that the nitrate consumption rate didn't slow down after the 5th day, indicating that nitrate was still the preferred nitrogen source.

Two control experiments were carried out along with the growth experiments to reveal the reason for the drop in the TEA concentration. Percentile TEA degradations in the growth experiments and in these two control experiments were determined after 6 and 12 days of operation and compared with each other (Figure 4). The results reveal that TEA degradation in reactors with no biological activity was quite low. Even after 12 days of aeration, the TEA degradation reached about 10% only. On the other hand, the TEA degradation reached about 50% at the 12th day in reactors containing microalgae culture. Furthermore, in the first 6 days, the TEA degradation was only 12%, but the degradation accelerated during the second 6 day reaching 50%. These results could be explained by a possible heterotrophic biodegradation activity by microalgae and/or any other heterotrophic microorganism that might acclimate to the TEA and the growth medium.

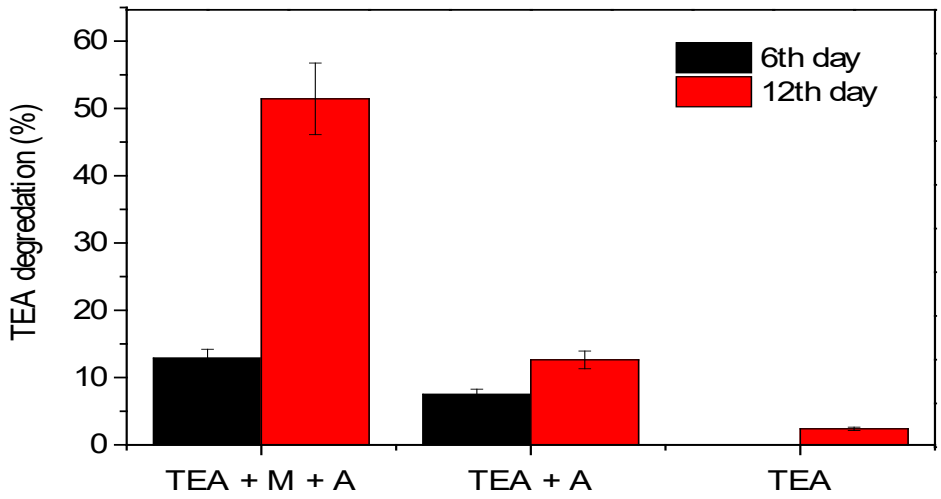

Figure 4. Comparison of TEA degradation during microalgae cultivation (M) and aeration (A) compared to blank TEA solution after 6 and 12 days of operation

3.3. Comparison of CO_2 delivery approaches

Another set of experiments were performed to distinguish the effect of TEA usage in the growth medium to the microalgae growth compared to those that could be achieved using the conventional CO_2 delivery approach. The results of the microalgae growth and nutrient consumption for four experimental sets, as presented in Figure 5a, indicate that both conventional aeration and TEA addition increased growth rates compared to the control culture in Reactor 4. When the CO_2-loaded TEA system is aerated (as in R1), the growth rate dropped due to disturbance by aeration (compared to R3). The rapid increase in biomass concentrations after the 6th day in R1 may be contributed to possible heterotrophic activity in the medium, as was seen in previous experiments. The specific growth rates corresponding to the CO_2-loaded TEA system in the reactor 3 and the gaseous CO_2 delivering system in the reactor 2 are very close to each other (Table 2). When the nutrient utilizations are compared, it is obvious that compared to control reactor (R4), the other three reactor sets have similar nutrient uptake rates (Figure 5a and 5b). Only difference can be seen on phosphate consumptions. While almost all the phosphate content in the TEA-added systems (R1 and R3) was consumed within 8 days, the final phosphate concentration in the aerated system only reached half of its initial phosphate concentration.

Table 2. Specific growth rate and corresponding lag period for each reactor

Reactor No	Specific growth rate (1/day)	Error (±)	Lag period (day)
R1	0.27	0.06	1
R2	0.33	0.05	1
R3	0.36	0.06	1
R4	0.26	0.04	5

During these experiments, total organic carbon (TOC) and total inorganic carbon (TIC) concentrations in the reactors were monitored to elucidate CO_2 utilization mechanisms. These results are presented in Figure 6 along with the pH measurements of the reactors. The very high TOC concentrations measured in TEA-added systems (R1 and R3) can be attributed to the organic carbon coming from the 20 mM TEA addition. These high TOC concentrations remain stable for 6 days, however, the TOC concentration in R1 starts to drop after the 6th day. As brought forward earlier, this drop in TOC levels may be the result of accelerated heterotrophic activity and degradation of organic carbon in the system, as depicted by the increase in TIC concentration in Figure 6b.

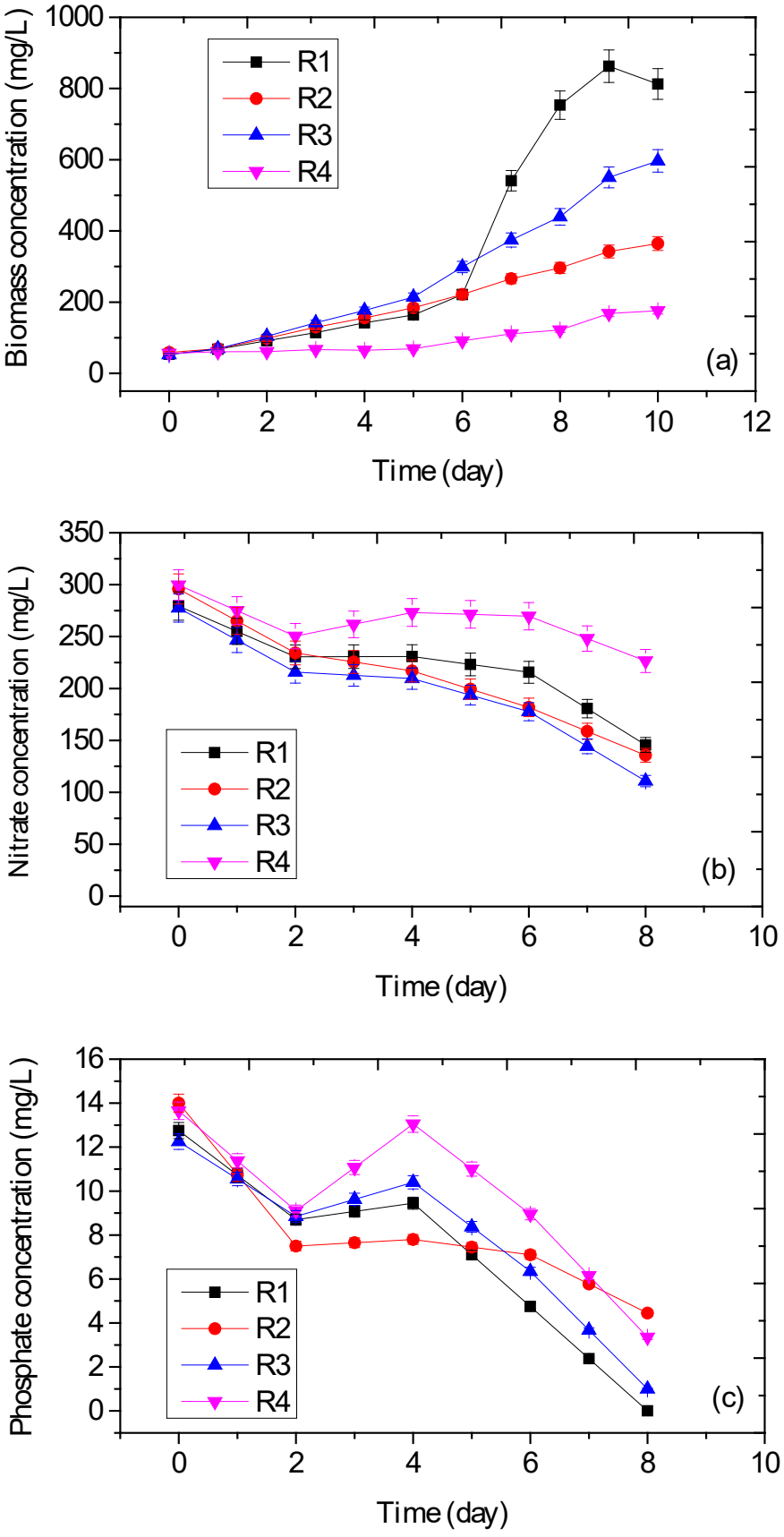

Figure 5. Comparison of the cultures grown in different reactors in terms of (a) biomass growth, (b) nitrate, and (3) phosphate consumptions

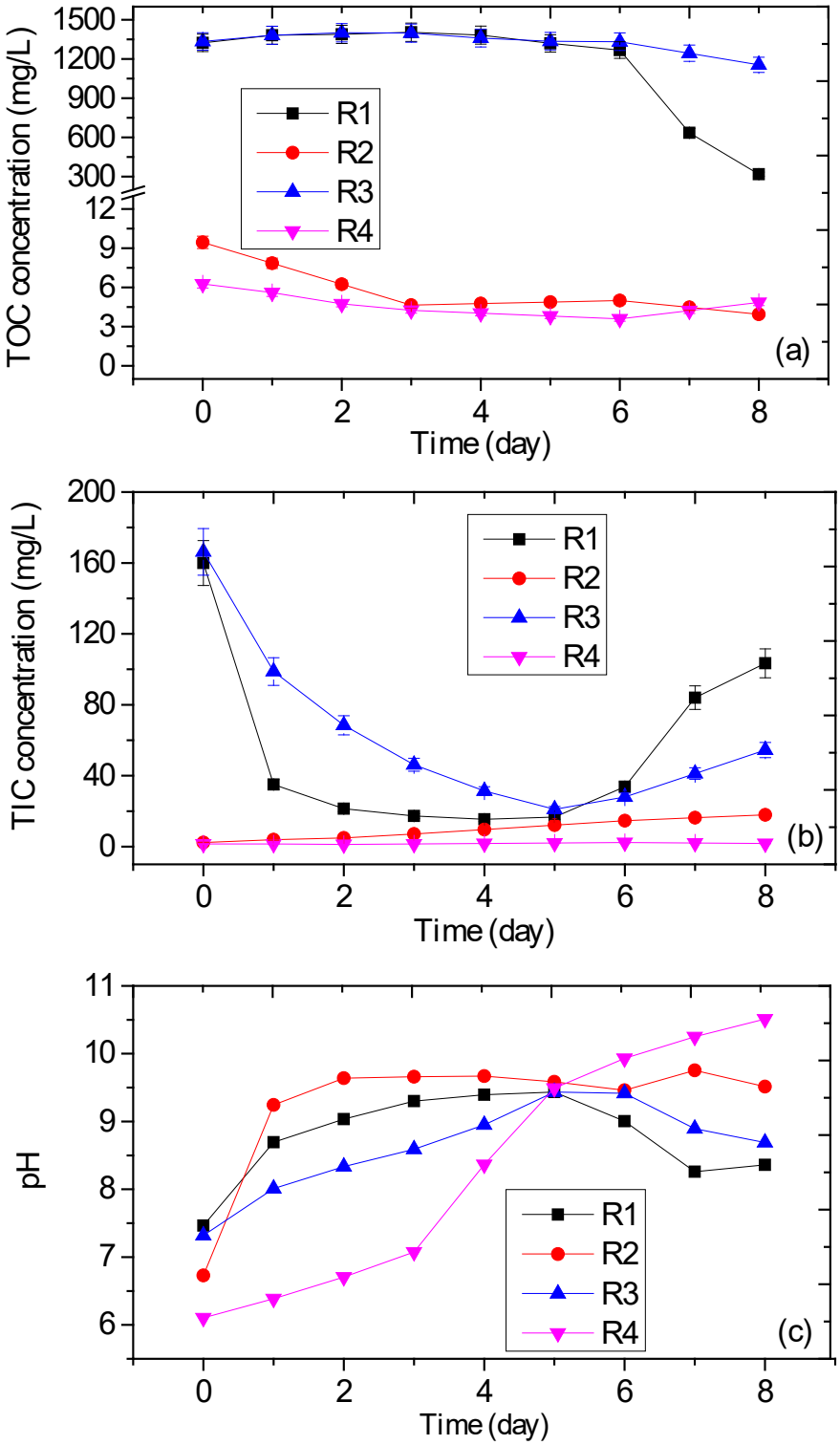

Figure 6. Comparison of the cultures grown in different reactors in terms of (a) TOC concentration, (b) TIC concentration, and (c) pH of the growth medium

Figure 6b demonstrates that the CO_2 captured by the TEA solution is released rapidly in aerated reactor sets (R1) while CO_2 release is delayed in non-aerated reactor sets (R3). This observation indicates that an acid-base equilibrium is formed between TEA and CO_2 when these are brought into contact at high CO_2 concentrations in the air (flue gas). However when the CO_2 loaded aqueous solution is added to a systems open to the atmospheric conditions (which means in equilibrium with a low CO_2 partial pressure) the CO_2 captured by the TEA could escape to the atmosphere in order to reach the new

equilibrium. Since TEA is a weak base, when it is in equilibrium with CO_2, they both behave as a buffer system and reduce the pH increase during microalgae growth (Figure 6c). However, this loose interaction between TEA and CO_2 can cause rapid decarbonisation of the system. Further studies should be conducted to understand the parameters affecting the CO_2 releasing mechanism when CO_2-loaded TEA solutions are mixed with growth media.

4. DISCUSSION AND CONCLUSION

This study investigates the use of TEA, a well-known CO_2 capturing chemical, to increase CO_2 availability in microalgae growth media. It was found that TEA addition to a certain concentration could enhance inorganic carbon availability in microalgae bioreactors and could result in a similar biomass productivity compared to those obtained by conventional gaseous CO_2 delivery systems. It was observed that due to high carbon content of TEA, a heterotrophic activity might be seen during the microalgae growth. Therefore, open growth systems can be an unsuitable option for operating systems using TEA as CO_2 transporter. It was also found that most of CO_2 loaded in the medium released within a few days of the growth period. Thus, CO_2-loaded TEA could a serve as a CO_2 transporter to microalgae cultivation system without affecting growth conditions of microalgae species. However, this release causes rapid decarbonisation of the system as it does in CO_2 bubbling systems. Thus, further studies are being conducted to reveal CO_2 releasing mechanisms from CO_2 loaded TEA solutions to microalgae media, and to increase CO_2 utilization efficiency of the proposed approach.

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