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Original Article

Preliminary Investigation of CO_2 Sequestration by *Chlorella sorokiniana* TH01 in Single and Sequential Photobioreactors

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Abstract: Increasing accumulation of CO_2 in the atmosphere mainly caused by fossil fuels combustion of human activities have resulted in adverse global warming. Therefore, searching for treatment methods for effective utilization of CO_2 have received a great attention worldwide. Among various methods (e.g., adsorption, absorption, storage, membrane technologies, etc.) have been developed and applied, the sequestration of CO_2 using microalgae has recently emerged as an alternatively sustainable approach. In this work, a green microalgal strain *Chlorella sorokiniana* TH01 was used to investigate its capability in sequestration of CO_2 in laboratory scale. Results indicated that the *C. sorokiniana* TH01 grew well under a wide range of CO_2 concentration from 0.04% to 20% with maximum growth was achieved under CO_2 aeration of 15%. In a single photobioreactor (PBR) with 10 min empty bed residence time (EBRT), the *C. sorokiniana* TH01 only achieved CO_2 fixation efficiency of 6.33% under continuous aeration of 15% CO_2. Increasing number of PBRs to 15 and connected in a sequence enhanced mean CO_2 fixation efficiency up to 82.64%. Moreover, the CO_2 fixation efficiency was stable in the range of 78.67 to 91.34% in 10 following days of the cultivation. Removal efficiency of NO_3^--N and PO_4^{3-}-P reached 82.54 – 90.25% and 95.33 – 98.02%, respectively. Our trial data demonstrated that the *C. sorokiniana* TH01 strain is a promising microalgal for further research in simultaneous CO_2 mitigation via CO_2 sequestration from flue gas as well as nutrients recycling from wastewaters.

Keywords: Carbon dioxide, *C. sorokiniana* TH01, Photobioreactors, Sequestration, Nutrients removal.

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1. Introduction

Global warming caused by accumulation of billion tons of CO_2 in the atmosphere which is mainly attributed to the combustion of fossil fuels from industrial activities [1]. Hence, reducing the emissions of CO_2 is an urgently demand. Numerous technologies such as chemical adsorption, chemical absorption and storage have been applied for the purpose of treatment of CO_2 mostly discharging from industrial plants [1,2]. However, most of the developed technologies are costly and unsustainable. Biological method of capture CO_2 using microalgae have been considering as a promising technology [3]. Microalgae mostly grow via photosynthesis by consuming CO_2 and using solar energy at a rate of ten times greater than terrestrial plants with higher daily growth rate [4]. Capturing CO_2 by microalgae can be simultaneously integrated with wastewater treatment for nutrient removal while producing high-added value biomass which is promising feedstock for energy-related and bioproducts-related industries [3,5].

Various factors must be considered to successfully apply CO_2 sequestration using microalgae in industrial plants. The most important factor is the microalgal strain, which is need to be screened to find an excellent one based on main criteria such as highly adaptable to high concentration of CO_2, high growth, highly resistance to toxics (SO_x, NO_x, micro and nano dust), nutrient composition, light, pH, as well as reactor type [6]. Microalgae reported for biological carbon fixation include *Chlorella* sp. [7], *Scenedesmus* sp. [8], and *Dunaliella tertiolecta* [9]. Li et al. [10] developed a pilot-scale system for CO_2 fixation from actual flue gas using *Scenedesmus obliquus*, which revealed to tolerate high CO_2 concentration of 12% with optimal removal efficiency of 67%. *Scenedesmus obliquus* and *Chlorella pyrenoidosa* could grow at 50% CO_2 and obtain biomass concentration of 0.69 g/L, although the best growth was observed at 10% CO_2 with biomass concentration of > 1.22 g/L [11]. Ho et al. [12] studied CO_2 mitigation from gas stream containing 10% CO_2 using *Scenedesmus obliquus* CNW-N via two-stage cultivation strategy for algal biomass production. Carbon dioxide consumption rate was reported as 549.9 mg/L/d, while biomass and lipid productivity were estimated as 292.5 and 78.73 mg/L/d, respectively. In Vietnam, *Spirulina platensis* has been mainly used for CO_2 fixation coupling with high nutritive biomass production for functional foods from pretreated coal-fired flue gas (of tunnel brick factory) [13-15]. The harvested biomass had highly nutritive profile (62.58% protein, 8.72 % fatty acids) and met Vietnam national standard of functional food. The results indicated that CO_2 originated from industrial activities in Vietnam (e.g. coal-fired power plants, cement plants, natural gas processing plants, etc.) is a potential carbon source for production of high value algal biomass from cyanobacteria (e.g., *S. platensis*) and green microalgae (e.g., *Chlorella*, *Scenedesmus*). Although good results were achieved for *Spirulina* with respect to utilization of industrially discharged CO_2 for algal biomass production, many microalgae species from natural habitants of Vietnam have yet been explored for CO_2 sequestration and biomass production study.

In this work, a green algal strain *C. sorokiniana* TH01 isolated from wastewater of a coal-fired power plant in Quang Ninh province, Vietnam was used to explore its capability in growth and CO_2 sequestration via cultivation under a range of CO_2 concentration of 0.04 – 20% as carbon sources in a single photobioreactor. To improve CO_2 fixation efficiency, a sequence of fifteen photobioreactors connected in a series was also constructed to evaluate stable growth and efficiency of CO_2 fixation of the algal under the optimal CO_2 concentration. Furthermore, overall removal efficiency of nutrients such as NO_3^--N and PO_4^{3-}-P and algal biochemical compositions were also determined.

2. Methods

2.1. Microalgal strain and media

The microalgal strain used in this study was identified and named as *Chlorella sorokiniana* TH01 (*C. sorokiniana* TH01) which was

obtained from microalga collection of Department of Applied Analysis, Institute of Chemistry, Vietnam Academy of Science and Technology, Vietnam. The strain was isolated and purified from wastewater of a Cam Pha's coal-fired power plant, Quang Ninh province, Vietnam. The strain was maintained on solid agar BG-11 medium which consists of (g/L) $NaNO_3$, 1.5; K_2HPO_4, 0.04; $MgSO_4 \cdot 7H_2O$, 0.075; $CaCl_2 \cdot 2H_2O$, 0.036; Citric acid, 0.006; Ferric ammonium citrate, 0.006; EDTA (Ethylenediaminetetraacetic acid), 0.001; Na_2CO_3, 0.02; mix A5 solution, 1 mL/L; agar, 10. Mix A5 consists of H_3BO_3, 2.86 g/L; $MnCl_2 \cdot 4H_2O$, 1.81 g/L; $ZnSO_4 \cdot 7H_2O$, 0.222 g/L; $Na_2MoO_4 \cdot 2H_2O$, 0.39 g/L; $CuSO_4 \cdot 5H_2O$, 0.079 g/L; $Co(NO_3)_2 \cdot 6H_2O$, 0.0494 g/L) [16] under continuous light intensity of 60 µmol/m^2·s at 25°C.

The seed *C. sorokiniana* TH01 culture was made by transferring solid algal on agar plate into 100 mL flask containing 50 mL sterilized BG-11 medium and culturing in one week to obtained cell concentration of 4.8×10^4 cells/mL, followed by further growth in 250 mL flaks containing 150 mL BG-11 medium under shaking rate of 150 rpm and light illumination of 110 µmol/m^2·s at 25°C for another week to reach cell concentration of 5.7×10^5 cells/mL. The obtained seed culture of *C. sorokiniana* TH01 was used for following CO_2 sequestration experiments.

2.2. Growth experiments of C. sorokiniana TH01 in single PBR

All experiments were performed under irradiation of LED system (light intensity of 110 µmol/m^2·s) at 27-28°C (Fig. 1). Duran glass bottles (D × H = 182 mm × 330 mm, 5 L**)** containing 4 L BG-11 were used as photobioreactors (PBRs) which were inoculated with 150 mL of the seed culture of *C. sorokiniana* TH01. The bioreactors were connected with industrial CO_2 tank (99,99%, Indochina Gas JSC, Hanoi, Vietnam) and air pump via a long stainless steel pipe (450 mm × ϕ3 mm) to the bottom for gas bubbling in. Carbon dioxide and air flowrates were controlled by flow meters to yield different concentration of CO_2 of 0.04%, 5%, 10%, 15% and 20% aerating the PBRs. Detail of industrial CO_2 and air flowrate were designed in Table 1. Exactly 400 mL/min mixtures of CO_2 and air of different CO_2 concentrations controlled by a flow meter (DFG-6T, 0.1-0.8 L/min scale, Darhor Technology Co., Limited, Hangzhou, Zhejiang, China) were continuously aerated into the inlet of the PBR and flow out into an infrared online CO_2 analyzer (SERVOMEX4100, Servomex, UK) to monitor CO_2 concentration for measurement of CO_2 fixation efficiency (Fig. 1).

Table 1. Different concentration of CO_2 made from industrial CO_2 flow and air flow employed as carbon sources for cultivation of *C. sorokiniana* TH01 in single PBR

CO_2 concentration (%)	Industrial CO_2 flowrate[a] (L/min)	Air flowrate[b] (L/min)	CO_2+Air mixture flowrate[c] (L/min)
0.04	0	0.4[d]	0.4
5	0.5	9.5	0.4
10	0.5	4.5	0.4
15	0.5	3.0	0.4
20	1.0	4.0	0.4

[a]*Industrial CO_2 (99.99%) flowrate controlled with a flowmeter (DFG-6T, 0.1 – 0.8 L/min scale, Darhor Technology Co., Limited, Hangzhou, Zhejiang, China)*
[b]*Air flowrate controlled with a flowmeter (DFG-6T, 2 – 20 L/min scale, Darhor Technology Co., Limited, Hangzhou, Zhejiang, China)*
[c]*CO_2+Air flowrate controlled with a flowmeter (DFG-6T, 0.1 – 0.8 L/min scale, Darhor Technology Co., Limited, Hangzhou, Zhejiang, China)*
[d]*Air flowrate controlled with a flowmeter (DFG-6T, 0.1 – 0.8 L/min scale, Darhor Technology Co.,Limited, Hangzhou, Zhejiang, China)*

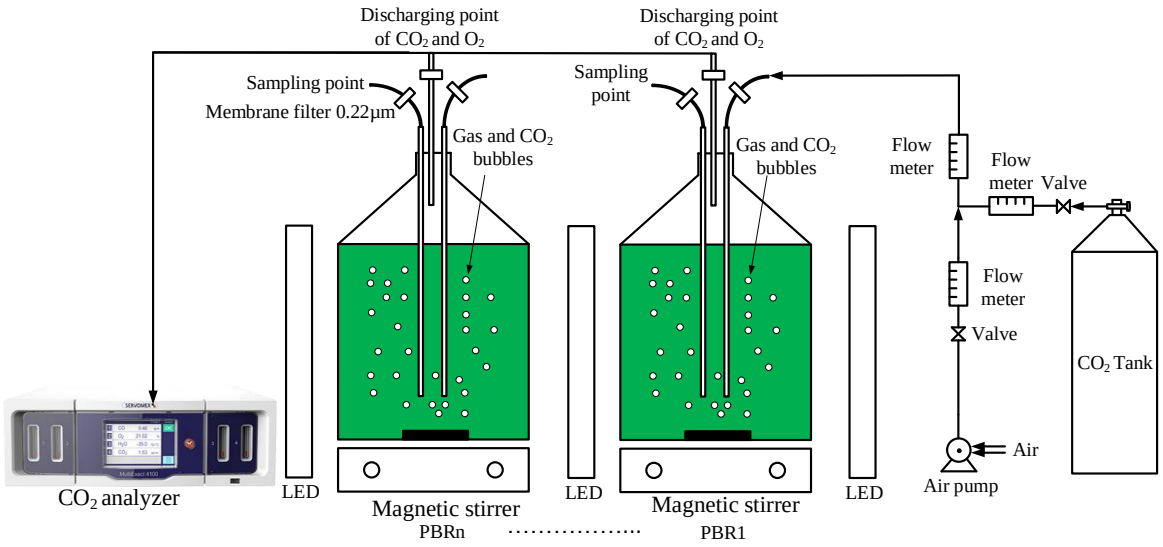

Fig. 1. Schematic diagram of CO_2 sequestration using *C. sorokiniana* TH01 in a single and sequence of photobioreactors (PBRs).

2.3. Growth experiments of C. sorokiniana TH01 in a sequence of PBRs

Based on experimental data achieved from section 1.3, the CO_2 concentration resulted in maximum growth of C. sorokiniana TH01 was applied for further investigation of *C. sorokiniana* TH01's growth and its stability in CO_2 sequestration in a sequence of 15 PBRs connected in a series under the same light and temperature conditions employed in section 1.3 (Fig. 1). The optimal mixture of CO_2 and air was continuously aerated the system at a rate of 400 mL/min while biomass growth, pH trend of algal culture and CO_2 fixation efficiency were regularly monitored in ten days.

2.4. Analysis

2.4.1. Algal growth monitoring and biomass productivity

The growth of *C. sorokiniana* TH01, including dry weight and chlorophyll *a* concentration were simultaneously determined. Dry weight was determined with filter paper (Whatman 0.45 µm, 47 mm, UK). Dry weight (DW, g/L) was calculated using equation (1).

$$DW = \frac{m_a - m_b}{V} \quad (1)$$

Where m_a and m_b are the weights of oven-dried filter at 105 ^oC for 24 h after and before filtration, respectively, and V is the volume of the microalgal suspension filtered.

The specific growth rate (µ, day^{-1}) was determined from the linear coefficient of the equation modelling (2), which was described in [17] of the exponential phase of the growth curve.

$$\mu = \frac{(\ln X_2 - \ln X_1)}{t_2 - t_1} \quad (2)$$

Where X_2 and X_1 are biomass concentrations (g/L) measured at time slot t_2 (day) and t_1 (day), respectively.

Pigments were determined using a slightly modified method which was described elsewhere in a recent study [18]. Briefly, Pigments were extracted by pure methanol at 60 ^oC for 30 min, and the amount of chlorophyll *a* (Chl-*a*, mg/L) was calculated using equation (3).

$$\text{Chl-}a = \frac{(15.65OD_{666} - 7.34OD_{653})V_{MeOH}}{V_{algal\ suspension}} \quad (3)$$

Where OD_{666} and OD_{653} are optical Densities at 666 nm and 653 nm, respectively; V_{MeOH} and $V_{algal\ suspension}$ are the

volumes of methanol and microalgal suspension used for extraction of pigments, respectively.

The biomass productivity was calculated using equation (4):

$$P=\frac{C}{t} \quad (4)$$

Where C is biomass concentration (g/L), t is cultivation time (day) and P is a real productivity (g/L·day).

The concentration of CO_2 was monitored at inlet and outlet of the PBRs by CO_2 analyzer (SERVOMEX4100, UK), which was then used to calculated CO_2 removal efficiency according to the following equation (5) that was described in [19].

$$E_{CO_2}=\left(1-\frac{CO_{2outlet}}{CO_{2inlet}}\right)\times 100\% \quad (5)$$

Where CO_{2inlet} and $CO_{2outlet}$ are the CO_2 concentration measured at inlet and outlet point of the PBRs.

2.4.2. Nutrients removal efficiency

For nutrient concentration measurement, 250 mL of each sample was filtered by VWR Sterilie 0.45 µm cellulose acetate membrane syringe filters (VWR, Radnor, PA) and diluted to concentrations within the reasonable detection range of anions, including nitrate and phosphate. Concentration of NO_3^--N and PO_4^{3-}-P were determined using standard methods for the examination of water and wastewater published by American Public Health Association, American Water Works Association, Water Environment Federation [20]. The removal efficiency of NO_3^--N and PO_4^{3-}-P was determined by equation (6).

$$H_i=(1-\frac{C_i}{C_{i0}})\times 100(\%) \quad (6)$$

Where, C_i and C_{i0} (mg/L) are concentration of NO_3^--N and PO_4^{3-}-P measured at cultivation time (t) and initial time (t_0), respectively.

Empty bed residence time (EBRT, min) of CO_2 passed through a single PBR was determined by equation (7).

$$EBRT=\frac{V}{Q} \quad (7)$$

Where, V is working volume of a single PBR (mL) and Q is flowrate of air + CO_2 mixture (mL/min).

Total EBRT (T-EMBRT) of CO_2 passed through PBRs system was determined by the following equation (8).

$$T\text{-}EBRT=\sum_{i=1}^{n}\frac{V_i}{Q_i} \quad (8)$$

Where V_i is working volume of PBR number i in the PBRs system (mL), Q_i is aeration rate of air + CO_2 mixture (mL/min).

2.4.3. Harvesting biomass

The *C. sorokiniana* TH01 biomass was harvested at the end of cultivation by centrifugation method at 4000 rpm for 5 min using a centrifuge (TDL-5A, Zenith Lab Inc., Brea Blvd.Brea, CA92821, USA). The dewatered biomass was dried at 25 ^oC for 24h using a cool dryer (MSL300MT, Mactech Co., Ltd, Vietnam) to obtain flake biomass. The flake form was further ground by a mini grinder (800A, LaLiFa Co., Ltd, Vietnam) to obtain fined algal powder (< 5 µm). The biomass powder was used for analysis of biochemical composition.

2.4.4. Biochemical composition and lipid characterization of C. sorokiniana TH01

The major biochemical compositions of *C. sorokiniana* TH01 biomass include carbohydrates, proteins and lipids. Moisture of *C. sorokiniana* TH01 was determined by drying the biomass at 105 ^oC overnight that was weighed against the original weight of biomass [21]. The amount of total carbohydrate of *C. sorokiniana* TH01 was measured by phenol-sulfuric acid assay [22]. Total protein was determined following procedure which was described in [23]. The total fatty acid methyl esters (FAME) derivation content of *C. sorokiniana* TH01 was derived using *in situ* transesterification method of the algal biomass with HCl/methanol (5% v/v) as homogeneous catalyst at 85^oC for 1 h and quantified using gas chromatography-flame ionization detector (GC-FID) as described in [21].

2.5. Statistical analysis

The experiments carried out in duplicate with two replicates measurements and the results were presented as mean ± S.D. of all four biological replicates ($n = 4$). Statistical analysis was done using one-way ANOVA followed by post hoc Tukey's test (Graph pad V7) and a *p*-value of <0.05 was taken as significant. The statistical analysis was conducted using SPSS 22.0 (IBM, USA).

3. Results and discussion

3.1. Effect of CO_2 concentration aeration on the algal growth in single PBR

Fig. 2A shows that BG-11 medium inoculated with *C. sorokiniana* TH01 was saturated with CO_2 after 2 – 3 days aeration. The initial pH of BG-11 medium was 7.74±0.17 which is preferable for the most of *C. sorokiniana* TH01 growth. The pH of the culture increased from 7.74 to about 8.6 under aeration of air with 0.04% CO_2. The increasing CO_2 concentration by mixing air with industrial CO_2 from 0.04% through 5%, 10%, 15% and 20% resulted in decreasing of pH of the algal culture. The decrease of pH was due to the increase HCO_3^- and H^+ production via reaction of $CO_2 + H_2O \rightarrow HCO_3^- + H^+$ when concentration of CO_2 increased. The higher concentration of CO_2 the faster and deeper decreased of pH of the algal culture. However, dissolution of CO_2 in the liquid media tends to reach equilibrium (depend on temperature and pressure) which is controlled by Henry's Law. Thus, under a specific CO_2 concentration, pH of the algal culture tended to reach a specifically stable value. In practice, the stable pH values of the algal culture measured under aeration of CO_2 concentration of 0.04%, 5%, 10%, 15% and 20% were 8.6, 7.0, 6.6, 6.5 and 5.8, respectively.

It is observed that *C. sorokiniana* TH01 adapted well under CO_2 concentration range of 0.04 – 20%. The increasing biomass concentration was recorded when CO_2 concentration increased from 0.04 to 15%. Particularly, maximum CO_2 concentration was achieved at 2.04±0.21 g/L when 15% CO_2 was applied. The increasing biomass production when CO_2 concentration aerated from 0.04 to 15% was attributed to addition of inorganic carbon source for enhancement of photosynthesis process of the *C. sorokiniana* TH01. However, further increase CO_2 concentration to 20% caused significant decrease of the pH of the algal culture (from 7.74 to 5.8) which inhibited the algal growth leading to decreasing of biomass concentration (Fig. 2B). Thus, it was summarized that optimal CO_2 concentration for the *C. sorokiniana* TH01 growth is 15%, which is a popular proportion of CO_2 in flue gas, whereas pH of the algal culture should be maintained between 6 and 9 for better algal growth.

Table 2 summaries that *C. sorokiniana* TH01 is ranked among the superior strains in adaption with high concentration of CO_2. The maximum CO_2 tolerance of *C. sorokiniana* TH01 is comparable to tolerant degrees of *Chlorella* PY-ZU1 (15% CO_2 after domestication period of 7 days) [19], but significantly higher than 10% CO_2 reported for *Scenedesmus obtusiusculus* [24] and 10% CO_2 for *Scenedesmus obliquus* CNW-N [25]. It is also noted that although the maximum biomass concentration of *C. sorokiniana* TH01 of 1.0-2.04 g/L is lower than 2.65 g/L, 2.7- 6.0 g/L and 3.51 g/L reported for *Chlorella* PY-ZU1, *Scenedesmus obtusiusculus* and *Scenedesmus obliquus* CNW-N, respectively, the specific growth rate of *C. sorokiniana* TH01 determined as 0.99 -1.4 day^{-1} was notable higher than 0.18 – 0.38 day^{-1} determined for *Scenedesmus obtusiusculus* and 1.19 day^{-1} reported for *Scenedesmus obliquus* CNW-N. However, the biomass productivity of *C. sorokiniana* TH01 of 0.23 – 0.49 g/L·day were comparable to 0.68 g/L·day, 0.25 – 0.52 g/L·day and 0.29 g/L·day reported for *Chlorella* PY-ZU1, *Scenedesmus obtusiusculus* and *Scenedesmus obliquus* CNW-N, respectively.

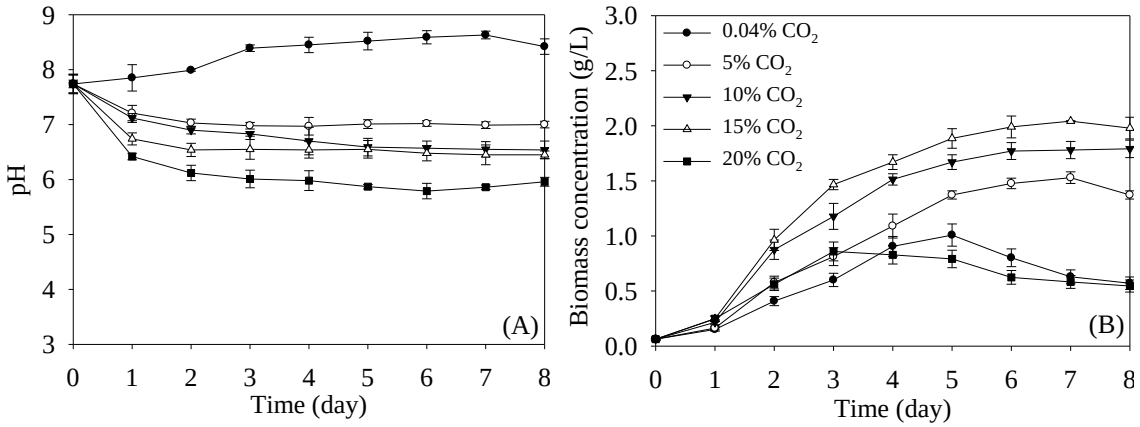

Fig. 2. Trend of pH of algal culture (A) and biomass concentration of *C. sorokiniana* TH01 (B) measured under aeration of different CO_2 concentration.

Table 2. Growth of microalgae under different CO_2 concentration aerated

Algal strain	CO_2 concentration (%)	Maximum specific growth rate (μ, day^{-1})	Maximum biomass concentration (g/L)	Maximum biomass productivity (g/L·day)	References
C. sorokiniana TH01	0.04	0.99±0.07	1.0±0.14	0.23±0.02	This study
C. sorokiniana TH01	5	1.26±0.11	1.53±0.17	0.29±0.04	This study
C. sorokiniana TH01	10	1.40±0.09	1.79±0.15	0.44±0.02	This study
C. sorokiniana TH01	15	1.36±0.12	2.04±0.21	0.49±0.03	This study
C. sorokiniana TH01	20	0.81±0.05	0.85±0.08	0.29±0.05	This study
Chlorella PY-ZU1	15	-	2.65	0.68	[19]
C. pyrenoidosa	6	-	1.59	-	[19]
Scenedesmus obtusiusculus[a]	5	0.37	6.0	0.5	[24]
Scenedesmus obtusiusculus[a]	10	0.38	5.7	0.52	[24]
Scenedesmus obtusiusculus[b]	5	0.23	3.12	0.32	[24]
Scenedesmus obtusiusculus[b]	10	0.34	2.7	0.26	[24]
Scenedesmus obtusiusculus[c]	5	0.18	3.37	0.25	[24]
Scenedesmus obtusiusculus[c]	10	0.18	3.1	0.26	[24]
Scenedesmus obliquus CNW-N	10	1.19	3.51	0.29	[25]

[a]*Light intensity irradiated at 134 μmol/m²·s*
[b]*Light intensity irradiated at 94.4 μmol/m²·s*
[c]*Light intensity irradiated at 54.7 μmol/m²·s*

3.2. Nutrients removal efficiency

Nitrate and phosphate are two essential nutrients of microalgae to synthesize protein, DNA and ATP in microalgal cells. The rate of uptake of these two nutrients depends on cultivation conditions (light, temperature, CO_2 concentration). In this study, the initial concentration of NO_3^--N and PO_4^{3-}-P in BG-11 medium were determined as 247 mg/L and 7.13 mg/L, respectively. Under different CO_2 concentration, uptake rates of these nutrients are illustrated in Fig. 3. Data shown in Fig. 3A indicates that at CO_2 concentration aeration of 10% and 15% NO_3^--N concentration was sharply dropped from 247 mg/L to 87.68 mg/L and 83.17 mg/L in the 1st day, followed by gradually decreased to 27.68 mg/L and 24.08 mg/L at 8th day of the cultivation. Although the reduction curves of NO_3^--N concentration by the *C. sorokiniana* TH01 grown under CO_2 concentration of 0.04%, 5% and 20% were similar, the reduction magnitudes were different. The final NO_3^--N concentrations measured at 8th day were 43.13 mg/L, 35.61 mg/L and 32.86 mg/L for the CO_2 concentration supplied of 0.04%, 5% and 20%, respectively. The overall removal efficiency of NO_3^--N by *C. sorokiniana* TH01 determined at 8th day of the cultivation under CO_2 concentration of 0.04%, 5%, 10%, 15% and 20% were 82.54%, 85.59%, 88.80%, 90.25% and 86.70%, respectively (Fig. 3B).

The variation of PO_4^{3-}-P concentration is shown in Fig. 3C. Data reveals that PO_4^{3-}-P concentration was dramatically dropped from initial concentration of 7.13 mg/L to 3.5 – 3.9 mg/L during the 1st day of all cultivations with CO_2 aeration at 0.04 – 20%. The deepest reduction of PO_4^{3-}-P was observed when *C. sorokiniana* TH01 was grown under 15% CO_2, which is consistent with biomass growth as described in Fig. 2B. Concentrations of PO_4^{3-}-P measured at 8th day of the cultivation under CO_2 concentration of 0.04%, 5%, 10%, 15% and 20% were 0.33 mg/L, 0.32 mg/L, 0.17 mg/L, 0.14 mg/L and 0.36 mg/L, corresponding to PO_4^{3-}-P removal efficiency of 95.33%, 95.38%, 97.57%, 98% and 95%, respectively (Fig. 3D). The high NO_3^--N and PO_4^{3-}-P removal efficiency representing that the *C. sorokiniana* TH01 is a promising strain to grow in nitrogen- and phosphorous-rich wastewaters for simultaneous pollutants removal and biomass production.

3.3. Biochemical composition of algal biomass

The major biomolecules accumulated in microalgae are carbohydrates, proteins, and/or lipids. It was commonly reported in the literature that the variation in algal biochemical composition is responding results of the changing of cultivation conditions such as pH, temperature [26], light [26], salinity, metal contents and nutrients availability [27, 28]. Majority of studies investigating growth of microalgae in synthetic media demonstrated that nitrogen and/or phosphorous starvation improves accumulation of lipids and/or carbohydrates [28].

In this study, since phosphorous and nitrogen were exhausted from 6th day of cultivation (Fig. 3D), respectively, the *C. sorokiniana* TH01 experienced both phosphorous and nitrogen starvation stages and that lipids and carbohydrates content increased.

Data shown in Table 3 reveals that lipids and carbohydrates were accumulated at the largest proportion of 39.26% and 45.21%, respectively, whereas proteins content only reached 12.51% of dry cell weight when *C. sorokiniana* TH01 grown under 15% CO_2. The content of lipids, carbohydrates and proteins synthesized by *C. sorokiniana* TH01 grown under 0.04% CO_2, 5% CO_2, 10% CO_2 and 20% CO_2 were determined as 28.63%, 38.01% and 30.5%; 28.84%, 38.28% and 27.97%; 35.32%, 39.41% and 22.29% and 31.36%, 39.52%, and 25.17%, respectively. The chlorophyll *a* content is only measured at below 1% of the dry cell weight. Our achieved data is well comparable to the lipids (29.92%), carbohydrates (34.80%) and proteins (26.88%) contents measured for *Acutodesmus dimorphus* grown in BG-11 medium during 2 – 3 days of nitrogen starvation [29]. The data obtained for *C. sorokiniana* TH01 is also well agreed with lipids, carbohydrates and proteins determined as 50.12%, < 30%, and 10%, respectively, for

Scenedesmus acuminatus grown in the same BG-11 medium during 9 days of nitrogen starvation [28]. Accumulation of high lipids and carbohydrates contents verifying that the *C. sorokiniana* TH01 is ranked among superior strains for bioenergy production in the literature [30,31]. While lipids are used for biodiesel and bio-jet fuels synthesis, the carbohydrates are promising derived feedstocks for production of gas biofuel (bio-hydrogen) and liquid biofuels (bioethanol and biobutanol) via sequential chemical/biochemical methods [30,31]. Additionally, proteins of the *C. sorokiniana* TH01 was constituted up to 12.51-30.50% dry biomass (Table 3), revealing a highly promising application in animal feed and food production with potential health benefits [32,33].

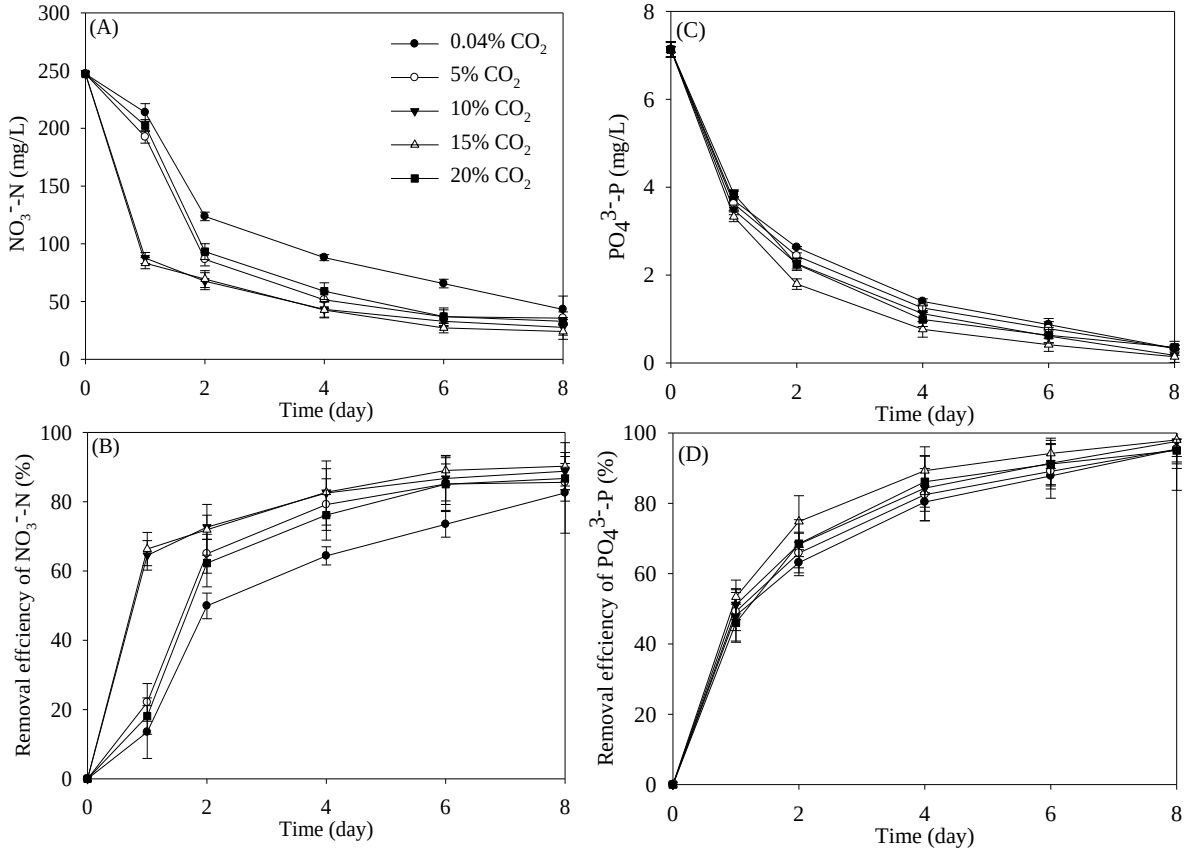

Fig. 3. Variation trend and removal efficiency of NO_3^--N and PO_4^{3-}-P by *C. sorokiniana* TH01 under different CO_2 concentration.

Table 3. Biochemical composition of *C. sorokiniana* TH01 biomass growth under different CO_2 concentrations (n=4)

CO_2 concentration feeding	0.04%	5%	10%	15%	20%
Chlorophyll a (%)	1.02±0.21	0.98±0.17	0.91±0.12	0.86±0.07	0.95±0.15
Lipids (%)[a]	28.63±2.45	28.84±2.84	35.32±2.78	39.26±3.21	31.36±2.82
Carbohydrates (%)	38.01±3.84	38.28±4.59	39.41±5.94	45.21±4.24	39.52±4.07
Proteins (%)	30.5±3.54	27.97±3.49	22.29±3.38	12.51±4.85	25.17±2.64

[a]*Lipids was determined as fatty acid methyl esters (FAME).*

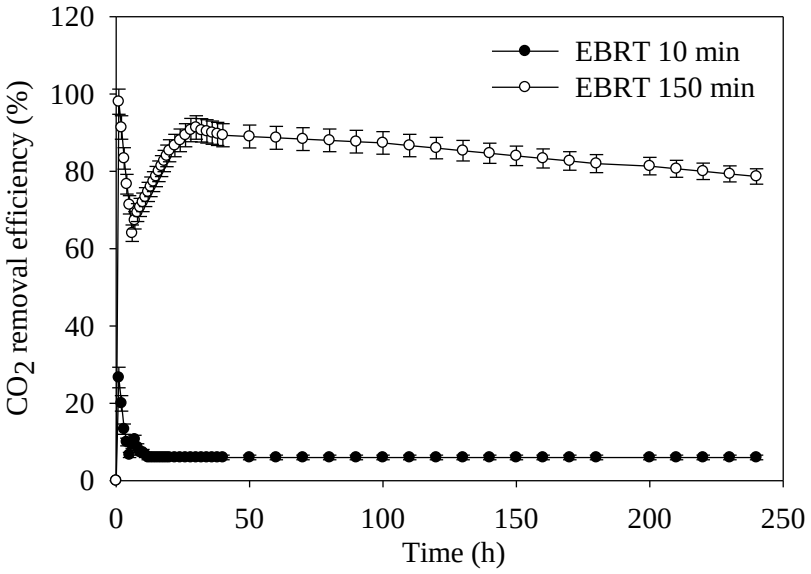

Fig. 4. CO_2 removal efficiency by *C. sorokiniana* TH01 grown under aeration of 15% CO_2 in single PBR and a sequence of 15 PBRs.

Table 4. Biomass productivity and CO_2 fixation efficiency of *C. sorokiniana* TH01 in single and 15 sequential bioreactors under 15% CO_2 (n=4)

EBRT (min)	Biomass concentration (g/L)	Maximum biomass growth rate (g/L·day)	Maximum/mean CO_2 fixation rate (g/day)	Mean CO_2 fixation efficiency (%)
10	2.89±0.12	0.29±0.03	15.82±1.09 (9.26)	6.33±0.38
150	2.53±0.27	0.25±0.02	135.41±1.64 (123.17)	82.64±5.75

EBRT: Empty bed residence time
Numbers in brackets are mean values

3.4. CO_2 fixation efficiency in single and sequential photobioreactors

C. sorokiniana TH01 was cultured in BG-11 medium and continuously aerated with 400 mL/min of 15% CO_2 to determine its biomass productivity and CO_2 removal capability in a single and a sequential of 15 photobioreactors. The empty bed residence time (EBRT) of single bioreactor and 15 sequential bioreactors are 10 and 150 min, respectively. Similar mixing of the culture caused by gas bubbles resulted in the same biomass productivities for each bioreactor in the multi-stage sequential bioreactors system.

Maximum biomass concentrations were 2.89 g/L and 2.53 g/L on 10^{th} day, respectively. The maximum growth rate of *C. sorokiniana* TH01 in single and 15 sequential bioreactors were 0.29 and 0.25 g/L·day, respectively (Table 4). The CO_2 concentration in single PBR and 15 sequential PBRs were measured at 11-14.1% and 1.3-5.4%, respectively, supporting excellent growth of the microalgal. The obtained data indicates that the most appropriate CO_2 concentration range for *C. sorokiniana* TH01 is about 1.3-14.1% demonstrating a wide adaptability of the microalgal in industrial CO_2 sequestration. The amount of CO_2 fixation exhibited a linearly proportional with cultivation time. The peak CO_2 fixation rate was increased from 15.82 g/day with EBRT of 10 min to 135.41 g/day with EBRT of 150 min (Table 4).

CO_2 fixation efficiency by *C. sorokiniana* TH01 cultured with EBRT of 10 min sharply increased to 26.67% as CO_2 quickly adsorbed and saturated in the culturing medium and gradually decreased to 6.67% within first 5 days, and then increased to 10.75% and stabilized at 6.0% to 7.75% within the following 10 days. The mean CO_2 fixation efficiency was calculated as 6.33%. When cultured *C. sorokiniana* TH01 in a sequence of 15 PBRs with EBRT of 150 min, the similar trend of CO_2 fixation curve was observed as *C. sorokiniana* TH01 grown in the single PBR, achieving the maximum CO_2 fixation efficiency of 91.34% within 26 h and then stabilizing at 78.67 to 91.34% in the following 10 days (Fig. 4). Although CO_2 removal efficiency achieved by *C. sorokiniana* TH01 in single PBR is comparable to that of *Chlorella* PY-ZU1 (7.6%) grown in soil extract medium under the same CO_2 concentration (15%), the maximum CO_2 fixation rate of *C. sorokiniana* TH01 of 15.82 g/day was significantly higher than 0.95 g/day determined for *Chlorella* PY-ZU1 [34] and 1.41-2.91 g/day reported for *Scenedesmus acuminatus* [24]. In sequential PBRs system, *C. sorokiniana* TH01 obtained CO_2 fixation rate and removal efficiency of 135.41 g/day and 82.64% were both considerable higher than 10.51 g/day and 70.48%, respectively, reported for *Chlorella* PY-ZU1 [34]. This is reasonable because our PBR system are much larger using larger volume of culture medium (13 times larger) than the reactors used by Chen et al. [34]. Moreover, our PBRs system required EBRT of 150 min for CO_2 passed through the system which is higher than the system used by Chen et al. [34] with EBRT of 140 min, therefore *C. sorokiniana* TH01 had a longer time to utilize HCO_3^- produced from CO_2.

4. Conclusion

C. sorokiniana TH01 was grown well in BG-11 medium under aeration of 0.04 – 20% CO_2 with optimal growth determined at 15% CO_2. Biomass production was peaked at 2.89 g/L and 2.53 g/L under CO_2 concentration of 15% within 8 days of cultivation in single PBR and a sequence of 15 PBRs, respectively. Biomass productivity measured in sequential PBRs system was 0.25 g/L·day, which was similar to that of single PBR (0.29 g/L·day). Increasing of EBRT from 10 min to 150 min considerably enhanced mean CO_2 fixation efficiency from 6.33% to 82.64%. When cultivated in a sequence of 15 PBRs, *C. sorokiniana* TH01 was stably grown under 15% CO_2 with CO_2 fixation efficiency of 78.67 to 91.34% in the following 10 days, demonstrating that the *C. sorokiniana* TH01 is a promising algal strain for application in industrial CO_2 sequestration. Algal biomass accumulated high content of lipids (28.63- 39.26%), carbohydrates (38.01-45.21%) and proteins (12.51 -30.50%) which are high-valued biomaterials for bioenergy and food/feed production.

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