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Utilisation of Tris(hydroxymethyl)aminomethane as a gas carrier in microalgal cultivation to enhance CO_2 utilisation and biomass production

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Abstract

CO_2 supplement is usually a limiting factor in microalgal culture systems, especially when flue gases are used as the carbon source. In this study, Tris(hydroxymethyl)aminomethane (THAM) was applied as a gas carrier to increase CO_2 storage capacity in the culture medium and enhance microalgal biomass production. Abiotic experiments showed that the amount of CO_2 absorbed by the medium within the same absorption time increased with increasing THAM concentration, and the gas absorptivity maintained a higher level (> 60%) under the neutral pH conditions. Cell growth in shake flasks illustrated that the biomass productivities of *Scenedesmus dimorphus* were significantly improved (31 to 66%) when the medium contained 2-8 $mmol \cdot L^{-1}$ THAM. Meanwhile, THAM was not biodegraded during the cultivation. The biomass productivities achieved with 6 $mmol \cdot L^{-1}$ THAM were much higher (32 to 33%) than that with routine culture when it was applied into an open raceway reactor system (2 m^2), and the CO_2 utilisation efficiency also increased by 6 to 12%. The results show that the addition of THAM as a gas carrier for enhancing CO_2 input could be an easy-to-use and cost-effective approach for mass cultivation of microalgae.

Keywords: *Scenedesmus dimorphus*; Flue gas; CO_2 utilisation efficiency; Gas carrier; Biomass production

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1 Introduction

Microalgae-based biofuels have received increasing attention over the last few decades because of their higher productivity potential compared with traditional terrestrial feedstocks [1, 2]. During the history of microalgal mass cultivation, a variety of photobioreactors has been developed, among which the open raceway pond is the most widespread, and one of the few industrialised configurations to date, due to its simple structure, low construction and operating cost, and ease of scaling-up of its features [3-6]. However, it also encounters some drawbacks, one of which is an insufficiency of carbon supplement [7-9].

Chemical analysis has estimated that the microalgal biomass consists of almost 50% carbon [7]. Therefore, it is necessary to supply sufficient CO_2 into the culture medium to satisfy the carbon requirement of cell rapid growth; however, the open raceway pond cannot effectively absorb, and store CO_2 in a microalgal growth medium due to its shallow depth and poor gas/liquid mass transfer, which results in a low CO_2 utilisation efficiency and a low biomass productivity [2, 4]. Cong et al. investigated different alternatives for supplying CO_2 to the culture broth to improve CO_2 utilisation efficiency, such as including a carbonation sump in the raceway channel or incorporating a submerged horizontal carbonator [10, 11]. Pilot-scale experiments demonstrated that these systems could obtain a favourable CO_2 utilisation efficiency (> 80%) in the outdoor cultivation of *Spirulina platensis* when using pure CO_2 [12]. Putt et al. designed and investigated a carbon absorption column combined with open ponds and achieved 83% CO_2 gas absorptivity [13]. However, the capacity for CO_2 storage in the growth medium, which influences the number of CO_2 injection points that are required for a large plant, or, alternatively, the maximum microalgal productivity of the pond with one CO_2 station, was not improved in these studies, due to the water chemistry [8, 9, 14].

Although the gas mass transfer efficiency can be improved by increasing the area of the gas-liquid interface and prolonging the residence time of the bubbles, the

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amount of CO_2 absorbed in the medium sees no appreciable enhancement due to the poor solubility of CO_2 in water, especially when flue gases are used as carbon sources. Upon dissolution in water, three carbon chemical species are formed: CO_2 (actually a small amount of H_2CO_3 + $CO_{2\,(aq)}$), HCO_3^-, and CO_3^{2-}. The relative proportions of these species depend on the pH, salinity, and temperature [15, 16]. In previous studies, sodium hydroxide and sodium carbonate were added into the culture medium to enhance CO_2 absorption [15, 17]. However, the associated increment of the ionic strength in the culture medium may lead to some longer-term problems such as water recycle [18]. On the other hand, part of the CO_2 supplied is lost to the atmosphere along the pond channels because of the higher inorganic carbon concentration, especially under neutral pH conditions [17, 19, 20]. An alternative way of increasing the CO_2 storage capacity of raceway ponds, while maintaining higher utilisation efficiency, is to add an absorbent as gas carrier, thereby introducing another chemical reaction to produce a fourth carbon species [21]. The added absorbent should not be consumed during cultivation.

In principle, an amine, with its $–NH_2$ functional group, always shows a reversible reaction for the absorption and desorption of CO_2, which can enhance CO_2 absorption rate and capacity [22-24]. Our previous study showed that monoethanolamine (MEA) could notably increase CO_2 utilisation efficiency and microalgal biomass productivity, although high dosages (> 150 $mg \cdot L^{-1}$) caused cell injury [21]. Therefore, there is a need to explore new gas carriers with high carrying capacity and low bio-toxicity. Tris(hydroxymethyl)aminomethane (THAM), an organic amine, has typically been used as a scavenger of CO_2 from the atmosphere. The reaction between THAM and CO_2 produces a charged carbamate product, similar to that of MEA and CO_2 [25]. Meanwhile, it is a highly biocompatible substance and is usually used in the fish transportation [26, 27]. Also, it is often used as a buffer in microorganism collection or bench-scale cultivation of some algal genera without CO_2 supply in order to prevent the precipitation of dissolved nutrients [7, 28]. As yet, however, the addition of THAM to microalgal culture media for improvement of CO_2 input and biomass production has

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not been studied.

In this study, THAM was added to the growth medium to act as a gas carrier for effective use of CO_2 in different culture systems. Initially, abiotic absorption experiments were conducted to explore CO_2 absorption efficiency under different THAM concentrations in the medium; then the effects of THAM addition on biomass production of *Scenedesmus dimorphus* was investigated under indoor and outdoor conditions. Additionally, the effects of this addition on the lipid content and fatty acid profiles of the cells were also determined.

2 Materials and methods

2.1 Abiotic absorption experiments

CO_2 absorption experiments were carried out in a bench-scale carbonator (20 cm length, 25 cm depth, and 10 cm width), which was made of Perspex and constituted a trap container, and a gas distributor (Fig. 1a). A CO_2:N_2 gas mixture (10:90, v/v) was injected continuously at a volumetric flow rate of 0.8 $L \cdot min^{-1}$, through four gas distributors (40~60 μm pores) which positioned in proportional spacing on the absorber's bottom. On the upper side, it was sealed but left two openings, for the exhaust gas flow and the pH electrode, respectively (Fig. 1b). A thermostatic jacket was coupled to a water bath to maintain the water temperature at 25 ±1 °C. The carbonator was operated at a constant depth of 20 cm with a working volume of 4 L for each experiment. After being mixed in the mixing vessel, the gas was fed into the tap container and the effluent gas was continuously analyzed by a quadruple mass spectrometer (GAM200, IPI) to determine the CO_2 volume fraction. Before each test, the mass spectrometer was purged with high-purity nitrogen for 6 h. During this absorption process, the pH of the liquid was also monitored, using appropriate electrodes (Mettler-Toledo, CH). All electrical signals were recorded in situ by a data acquisition system (Fig. 1b).

THAM was purchased from Aladdin (Shanghai, China). To investigate its influence on the efficiency of CO_2 absorption, different concentrations of THAM

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were added to the blank culture medium.

Fig. 1 Sketch of (a) the gas-carbonator and (b) a schematic diagram of the abiotic experiments

2.2 Microalgae strain and culture systems

Scenedesmus dimorphus, classified as Chlorophyta, Chlorophyceae, was obtained from a culture collection of Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences. This microalga was grown in a modified BG-11 medium [29], which contained ($mg \cdot L^{-1}$): $NaNO_3$, 1500; $MgSO_4 \cdot 7H_2O$, 75; $CaCl_2 \cdot 2H_2O$, 36; citric acid, 6.0; Na_2EDTA, 1.0; ferric ammonium citrate, 6.0; Na_2CO_3, 20.0; $KH_2PO_4 \cdot H_2O$, 40.0; $ZnSO_4 \cdot 7H_2O$, 0.222; $CuSO_4 \cdot 5H_2O$, 0.079; $MnCl_2 \cdot 4H_2O$, 1.81; $Na_2MoO_4 \cdot 2H_2O$, 0.39; $Co(NO_3)_2 \cdot 6H_2O$, 0.0494; and H_3BO_3, 2.86.

Inocula were prepared by according to Sun et al. [21]. The indoor cultivations were carried out in 500-mL flasks with 300 mL of culture medium, with shaking at 120 rpm on an orbital shaker at 25 ± 1 °C. Eight 30-W fluorescent lamps were mounted 30 cm above the rows of photobioreactors to provide a light/dark cycle of 12/12 h with 150 $\mu E \cdot m^{-2} \cdot s^{-1}$ illumination intensity. CO_2 was provided from a commercial cylinder and mixed with ambient air to give a simulated flue gas with 10% (v/v) CO_2. This gas was supplied to the flasks through a sintered glass plate once every 4 h during daylight, and was continuously bubbled for 5 min each time at a rate of 0.3 $L \cdot min^{-1}$. Different concentrations of THAM (0, 2, 4, 6, and 8 $mmol \cdot L^{-1}$) were added into the growth medium to investigate its effects on microalgal growth.

The outdoor cultivations were conducted under the natural temperature and light conditions prevailing in the Haidian district of Beijing, China (latitude 39° 59′ N, longitude 116° 19′ E) using two 2-m^2 raceway ponds (see Supplementary Material-Fig. SM1). A four-blade paddle wheel, powered by a three-phase AC synchronous motor (Zhe Jiang Xinling Motor Co., Ltd), was used to drive the fluid at a flow velocity of 20 $cm \cdot s^{-1}$ [30]. The total volume of culture broth was 200 L, corresponding to a depth of 10 cm. Simulated flue gas was supplied using an air sparger at the bottom of the

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ponds. To optimise CO_2 supply and avoid excessive waste, the supply system was controlled by a pH sensor (pH-stat) in the ponds. The controller unit continuously recorded the pH values measured by sensors submersed in the ponds, and as soon as the pH exceeded an upper limit (pH 8.0), a solenoid value opened to release CO_2 to the gas mixture which entered the reactor in a 10% concentration in air until the pH reached the lower value (7.0). The amounts of CO_2 addition were recorded. In this case, 6 mmol·L^{-1} of TRIS was added to the culture medium to evaluate its effects on biomass productivity and CO_2 utilisation efficiency.

2.3 Chemical analysis

Biomass concentration was determined by measuring the optical density at 680 nm (OD_{680}) and correlating it with a predetermined cell dry weight (CDW) standard curve: CDW = 0.4938 × OD_{680} (R^2 = 0. 998). Any optical density greater than 1.0 was first diluted to give an absorbance in the range 0.1-1.0, and then the CDW was calculated by multiplying the value given by the equation by the dilution factor.

At the end of each batch culture, algal cells were collected from the culture suspension by centrifugation, and then freeze-dried (LGJ10-C, Four-Ring Science Co., Beijing, China). Total lipids were extracted according to the method of Bligh and Dyer with a slight modification [21]. Fatty acid methyl esters were prepared by acidic transesterification of the total lipid extracts as described by Liang et al. [31]. The samples were then re-dissolved in hexane and analysed by a gas chromatograph (Agilent, 7890A, USA) equipped with an FID detector and an Agilent DB-17 column (30 m × 0.25 mm), according to the method described by Wang et al. [32].

The lyophilized biomass obtained from each batch culture was used to determine the elemental concentration of carbon using an Elemental Analyzer CHNS/ vario EI III (Elementar Analysensysteme, Germany).

To evaluate the CO_2 storage capacity in the culture medium, 2 mL samples were taken periodically during the culture process to determine the total inorganic carbon

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(TIC) content, using a LiquiTOC II analyser (Elementar, Germany). The THAM content in the supernatant was analysed by a pre-column derivatisation high-performance liquid chromatography (HPLC) method [33-35]. Briefly, 2 mL culture samples were collected every two days and filtered through a 0.22 μm membrane. The soluble fraction (0.5 mL) was then mixed with 0.5 mL $NaHCO_3$ (0.5 $mol \cdot L^{-1}$) solution and reacted with 0.1 mL 2,4-dinitrofluorobenzene (DNFB, 5 $g \cdot L^{-1}$ acetone) at 75 ℃ for 60 min. After derivatisation, samples were diluted with K_2HPO_4 solution (50 $mmol \cdot L^{-1}$) to 10 mL. They were then analysed by HPLC with a UV detector according to the conditions described in Liu et al. [33].

Three replicates were performed for all analyses and the experimental data are shown as mean values. All chemicals used were of analytical grade.

2.4 Parameter calculation

In abiotic absorption experiments, the CO_2 gas absorptivity (η_{CO2}) was calculated according to the following equation:

$$\eta_{CO_2} = \frac{C_{CO_2,in} - C_{CO_2,out}}{C_{CO_2,in}(1 - C_{CO_2,out})} \quad (1)$$

where $C_{CO2,in}$ (%) is the CO_2 volume fraction of inlet gas, $C_{CO2,out}$ (%) is the CO_2 volume fraction of effluent gas.

The initial biomass concentration of inoculum and the biomass concentration at time t under indoor and outdoor cultivations were designated as X_0 and X_t ($g \cdot L^{-1}$), respectively. The increment of biomass concentration ΔX ($g \cdot L^{-1}$) over culture time Δt was calculated as $\Delta X = X_t - X_0$. Thus, the biomass volumetric productivity (P_v, $g \cdot L^{-1} \cdot d^{-1}$) of indoor cultivations was calculated as:

$$P_v = \Delta X / \Delta t \quad (2)$$

The biomass areal productivity (P_A, $g \cdot m^{-2} \cdot d^{-1}$) of the outdoor ponds was calculated as:

$$P_A = P_v \times V / A \quad (3)$$

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where V (L) is the working volume in outdoor cultivations and A (m^2) is the lighted area of the ponds.

The CO_2 utilisation efficiency (E_{CO2}, %) was calculated according to the following equation:

$$E_{CO2}=\Delta X \times V \times f_C / M_C \quad (4)$$

where f_C (%) is carbon fraction in the algal biomass determined by elemental analyzer, and M_C (g) is the mass of carbon calculated according to the consumption of CO_2 over the culture time, Δt.

3 Results and discussion

3.1 CO_2 mass transfer within the carbonator

The effect of different THAM concentrations on the CO_2 absorption capacity in the bench-scale carbonator was determined experimentally using an area integral calculation method [36]. Due to the additional chemical reaction, both the gas solubility and absorption efficiency were higher in the presence of THAM (Fig. 2).

Fig. 2 Effect of time and concentration of THAM on (a) CO_2 gas absorptivity, (b) total inorganic carbon absorbed and (c) pH of the liquid phase

When the blank medium was used as the liquid phase, the CO_2 gas absorptivity decreased significantly and was lower than 10% after 15 min (Fig. 2a). Meanwhile, the TIC absorbed by the liquid phase was lower than 5.0 $mmol \cdot L^{-1}$, which was the solubility limit of CO_2 in the BG-11 medium (Fig. 2b). In spite of the low CO_2 absorption, the pH of the liquid phase decreased from 8.0 to 5.4 (Fig. 2c). This was due to the low alkalinity of the blank medium, which was exhausted immediately. When THAM solutions were used, the CO_2 gas absorptivity remained above 60% until the added absorbent was exhausted (Fig. 2a). The TIC absorbed by the liquid phase increased with the increase of THAM concentration (Fig. 2b). In particular, the TIC value reached 17.4 $mmol \cdot L^{-1}$ with 8 $mmol \cdot L^{-1}$ THAM, which was 3.5 times greater than that of the blank medium. Regarding the pH, it was high at the beginning but decreased with continuous CO_2 supply until a minimum range of 6.0 to 6.6 was

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achieved (Fig. 2c).

The absorption of CO_2 and the resulting increase in amount of TIC absorbed by the liquid phase in the presence of THAM occur through the following two-step reactions [37, 38]:

$$2RNH_2 + CO_2 \leftrightarrow RNHCOO^- + RNH_3^+ \quad (5)$$

$$RNHCOO^- + H_2O \leftrightarrow RNH_2 + HCO_3^- \quad (6)$$

The alkanolamine (RNH_2) reacts with CO_2, producing a carbamate intermediate ($RNHCOO^-$) through the reaction in Eq. (5). The carbamate intermediate is then hydrolysed by the reaction in Eq. (6), resulting in bicarbonate ion production. Upon dissolution in a liquid phase containing THAM, four carbon chemical species would be formed: carbamate, CO_2(aq), HCO_3^-, and CO_3^{2-}. The results in Fig. 2 showed that the presence of THAM enhanced the amount of TIC absorption by the culture medium while the CO_2 gas absorptivity remained above 60%, especially under a neutral pH range. It seemed that carbon dioxide was fixed by THAM temporarily as carbamate during the absorption process, which resulted in the increase of the driving forces of gas mass transfer. Afterwards, the carbamate was hydrolysed to bicarbonate, which was the preferred form of inorganic carbon for microalgal uptake [15], therefore THAM was regenerated naturally. This idea of THAM as gas carrier has also been verified through comparison with other researches and our culture experiments [21, 37, 38].

Aishvarya reported an improved CO_2 sequestration method that fixes the dissolved CO_2 in the form of bicarbonate using sodium hydroxide [15]. Besides, the use of carbonate was also applied to absorb diluted CO_2 sources and then regenerate it by microalgal growth [17]. To compare the CO_2 absorption efficiency with three absorbents, similar abiotic experiments were performed within our carbonator and the results were shown in Fig. 3. Under the same concentrations of absorbents and within the same absorption time, the amount of CO_2 absorbed by THAM was most, followed by sodium hydroxide, and sodium bicarbonate (Fig. 3a). The same tendency was also

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observed in CO_2 gas absorptivities (Fig. 3b). Therefore, we concluded that the use of THAM might be a better option to enhance the CO_2 mass transfer in algal cultivation.

Fig. 3 (a) The increment of TIC due to THAM, NaOH, and Na_2CO_3 addition in blank culture medium; (b) the average CO_2 gas absorptivity during the whole absorption process in the presence of different concentrations of three absorbents

3.2 Cell growth and CO_2 fixation in the presence of THAM

3.2.1 Indoor culture experiments. The growth-promoting effect of the different concentrations of THAM in the shake flask cultures is shown in Fig. 4a. The growth rate and final biomass concentration were both higher than in the control case. Such a increase in growth was obtained with THAM concentrations from 2 $mmol \cdot L^{-1}$ to 6 $mmol \cdot L^{-1}$, showing a maximum at 6 $mmol \cdot L^{-1}$, and higher THAM concentration (8 $mmol \cdot L^{-1}$) did not increase the growth rate any further. Biomass concentration, at 6 $mmol \cdot L^{-1}$ THAM, increased to 1.4 $g \cdot L^{-1}$ over 8 days, with an overall biomass productivity of 0.161 $g \cdot L^{-1} \cdot d^{-1}$ correspondingly, which was a 66 % increment compared to the case of no added THAM (Table. 1). The loss of THAM due to microalgal degradation was negligible in all experiments (Fig. 4b).

Fig. 4 (a) The growth curves of *S. dimorphus* at different THAM concentrations cultured indoors and (b) THAM changes during cultivation

Table 1 The final biomass concentration, lipid content, and biomass productivities of *S.dimorphus* cultured indoors with different THAM concentrations

The increment of microalgal growth in the presence of THAM can be attributed to the enhancement of TIC absorption in the liquid phase. The course of TIC concentration and pH of the culture broth along with the culture time on the third day are recorded in Fig. 5. During the culture process, the assimilation of CO_2 or bicarbonate by rapidly growing microalgal cells caused a shift of the chemical equilibrium, resulting in elevated pH values after the every carbonation process. As seen from Fig. 5, the pH of the medium with 6 $mmol \cdot L^{-1}$ THAM increased from 6.7 to 8.0 over four hours, which was a slower rise than that of the control case (6.3 to 8.8). Simultaneously, the TIC concentration in the liquid phase with 6 $mmol \cdot L^{-1}$ THAM decreased from 130 to 93 $mg \cdot L^{-1}$, while in the control case the decrease was

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from 46 to 39 mg·L^{-1}. It appeared that the total inorganic carbon absorbed by the basic culture medium was too low to neutralise the excretion of OH^- ions by the algae and could not meet the carbon requirement for higher biomass productivity.

Fig. 5 The course of TIC concentration and pH of the culture broth along with the changes of the oscillation time on the third day, (a) control case and (b) THAM

As an alternative way of enhancing CO_2 input, THAM acted as a gas carrier during microalgal cultivation, which fixed CO_2 temporarily as carbamate to increase the carbon storage capacity of the liquid phase during the absorption process (Fig. 2b). Thereafter, the carbamate was hydrolysed to bicarbonate, which was the preferred form of carbon source for cell growth (Fig. 4a). Therefore, the application of THAM may reduce the number of carbonation stations required for an open pond of specified size, depth, and liquid velocity, or it would enlarge the pond size serviceable with one CO_2 station.

3.2.2 Outdoor culture experiments. The growth kinetic data of *S. dimorphus* in two batches culture under outdoors with different culture media are shown in Figs 6a and 6b, respectively.

Fig. 6 The growth curves of *S. dimorphus* cultured outdoors with different concentration THAM (0 and 6 mmol·L^{-1}), (a) 12 Aug. to 20 Aug. and (b) 26 Aug. to 3 Sep.

After a one day lag phase in the first batch cultivation (Fig. 6a), the algal cells adapted to the outdoor conditions in the 2-m^2 open raceway pond. Following the lag, the cells entered the rapid growth period, and remained at a high growth rate until the end of the cultivation (day 8). However, the cell densities and productivities showed differences between 6 mmol·L^{-1} THAM and the control case. As illustrated in Table 2, the maximum biomass concentration with 6 mmol·L^{-1} THAM (1.16 g·L^{-1}) was higher than that of the control case (0.81 g·L^{-1}): this was accompanied by a higher net biomass increment (252 g for the 6 mmol·L^{-1} THAM versus 190 g for the control case). This resulted in an overall biomass areal productivity as high as 15 g·m^{-2}·d^{-1} with 6 mmol·L^{-1} THAM, while the productivity of the control case reached only 11 g·m^{-2}·d^{-1}. According to material balance theory, the CO_2 utilisation efficiency was calculated using net biomass production and the total mass of input CO_2. It can be

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seen from Table 2 that the value with 6 mmol·L^{-1} THAM was much higher than that of the control case (47% versus 35%). Similar results were also obtained in the second batch cultivation (Fig. 6b and Table 2); however, there were differences in growth rate, and other parameters between the two batches, due to the variation of outdoor conditions. As illustrated in Supplementary material-Fig. SM2, the weather conditions (including the light intensity and temperature) between 12 Aug. and 22 Aug. were much better than those between 25 Aug. and 5 Sep.

Table 2 The biomass production data, cell lipid content, and CO_2 utilisation efficiency of *S.dimorphus* in twice outdoor cultivations

Seen from the results of the twice outdoor experiments, the biomass productivities obtained with 6 mmol·L^{-1} THAM were much higher than those obtained with the routine culture (32-33%), and the CO_2 utilisation efficiency also increased by 6-12%. These differences were consistent with the results of the indoor experiments. When *S. dimorphus* was cultured outdoors with 6 mmol·L^{-1} THAM in the 2 m^2 raceway pond, more inorganic carbon source was serviceable for a higher cell growth rate. Meanwhile, the CO_2 absorption efficiency in the carbonation area was elevated, due to the enhancement of the chemical reaction during the gas/liquid mass transfer process.

Since pure CO_2 is expensive, alternative and cheaper carbon sources, such as flue gases from industrial combustion processes, have been suggested as the only sustainable way of supplying carbon in biofuel production [39]. However, when flue gas is used directly, the mass transfer rate might be insufficient to support the production of fast-growing microorganisms unless more carbonation stations per unit pond size are included [9, 17]. Malcata et al. have suggested that the poor solubility of CO_2 in water might be alleviated by mixing an organic solvent (or even an ionic liquid) with the aqueous medium [14]. One method proposed to increase microbial growth and CO_2 fixation by Nonomura et al. was the addition of methanol to the growth medium [40]. When a dilute mixture of methanol (3%, v/v) was introduced to algae *Botryococcus braunii*, the biomass productivity doubled. However, overuse of methanol could be

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toxic to the shaded cells. Moreover, methanol is a volatile liquid, and larger amounts of loss would elevate the culture costs. In recent studies, Peng et al. developed a methodology which involved gradually supplying CO_2 as well as removing the accumulated oxygen through the use of perfluorocarbons (PFCs) [41]. In PFCs, CO_2/O_2 solubility improved greatly. With the aid of PFC emulsions, the oxygen concentration in tubular photobioreactor was reduced from 47% to 4% after 9 days of their cultures. However, the biomass productivity appeared little difference between the culture with 2.5% PFC emulsions and routine culture. Moreover, PFCs are insoluble in water; thus they had to be well-dispersed in the culture medium in the form of emulsions by using a surfactant. Also, adding PFC emulsions to microalgal culture would diminish the depth to which light could penetrate and therefore decreased cell growth. In spite of ionic liquids (ILs) being a class of novel green chemicals, and being designed to replace traditional volatile organic solvents in industrial processes, few articles have reported their use in such microalgal culture systems [42]. Kulacki et al. evaluated the potential effects of three imidazolium ionic liquids on aquatic ecosystems [43]. The results of their studies showed that ILs could elicit a range of responses to both *Scenedesmus quadricauda* (EC_{50} values of 0.005-13.23 $mg \cdot L^{-1}$) and *Chlamydomonas reinhardtii* (EC_{50} values of 4.07-2138 $mg \cdot L^{-1}$). Although these ILs has higher CO_2 solubility, none of these liquid phases can be used in algal cultivations because they are more likely to be toxic. To summarise, CO_2 absorbed using methanol, PFCs, or ICs was expensive and generally impractical for industrial biomass production, and these methods cannot be the promising solutions to the problem of low CO_2 mass transfer and low microalgal growth rate.

3.3 Effects of THAM on the lipid content and fatty acid profiles

The total lipid content and fatty acid profiles of the microalgal biomass cultured indoors or outdoors under different concentrations of THAM was analysed and the results are presented in Tables 3 and 4.

Table 3 The fatty acid composition of algal cells cultured indoors with different THAM concentrations

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Table 4 The fatty acid composition of algal cells cultured outdoors with different THAM concentrations

When the THAM concentration was between 0 mmol·L^{-1} and 8 mmol·L^{-1}, the total lipid content was around 20%. GC analysis showed that the major fatty acid components of *S. dimorphus* cultured indoors under different concentrations of THAM were C16 and C18 groups ranging from 76.38 % to 80.68 %. These fatty acids are favourable for biodiesel production [32]. As shown in Table 3, the C16 and C18 contents decreased gradually with increasing THAM concentration. However, the contents of other fatty acids that were not included in our standard substances, such as C20 and C22 groups, increased from 18.97 % to 23.35 %, as the THAM concentration increased from 0 mmol·L^{-1} to 8 mmol·L^{-1}. It seemed that the greater amount of TIC in the culture medium stimulated the algae to produce longer-chain fatty acids. Similar trends were found in Tang's research [44]. In addition, higher levels of polyunsaturated fatty acids were found in culture with THAM. A possible reason was that the increase of THAM in the medium could lead to a greater availability of carbon to the cells, which resulted in a relative decrease in O_2 concentration. It is known that O_2 concentration affects enzymatic desaturation during fatty acid synthesis. Therefore, the addition of THAM to the microalgal culture medium would enhance the production of polyunsaturated fatty acids.

Although there was no appreciable difference in total lipid content, the fatty acid composition of the outdoor culture was distinct from that cultured indoors (Table 4). The most striking divergence occurred in the proportion of polyunsaturated fatty acids in algal cells, which reached higher values in outdoor cultures than in those from indoors. Similar results have been reported by Zhu et al. [45]. It has been previously demonstrated that a higher temperature and irradiance level will result in more saturated fatty acids [46]. During the shake cultures, the light source used for cell growth was a fluorescent lamp, and the light intensity was constant (150 μE·m^{-2}·s^{-1}); in outdoor cultivation, the light source was sunlight that varied from 0 to 2500 μE·m^{-2}·s^{-1}, and was therefore much higher at noon outdoors than at any time indoors: outdoor irradiance also fluctuated significantly. In addition, the indoor temperature

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was constant at 25 ℃, while the outdoor temperature fluctuated from 20 to 30 ℃ during the day and fell to below 20 ℃ at night. The high polyunsaturated fatty acid content in outdoor cultures was a likely response to the lower temperature. Besides, that THAM in the culture medium promoted PUFA synthesis in two outdoor batch cultures was consistent with data from indoor experiments.

4 Conclusions

Supply of CO_2 in shallow suspensions of open raceway ponds at near neutral pH is hardly effective, especially when flue gases are used as a carbon source. Better efficiencies are attained in the present study by the addition of THAM to the culture medium as a gas carrier. The proposed method allows CO_2 gas absorptivity to remain greater than 60 % over a large pH range (6.5 to 10.0) in a bench-scale carbonation trap, and achieved a much higher TIC concentration in the liquid phase. With the aid of THAM, the overall biomass productivity of *S. dimorphus* in shake cultures increased (at most) by 66 % compared to the culture without the addition of THAM. On the other hand, the results of two outdoor experiments illustrated that the biomass productivity and CO_2 utilisation efficiency both increased. Meanwhile, the addition of THAM could supply more carbon source to microalgal cells to promote the accumulation of polyunsaturated fatty acids. In short, since THAM cannot be consumed by the microalgal cells and could be reused along with the recycle of medium, the use of THAM as a gas carrier would be a cost-effective approach for an open culture system that normally cannot use CO_2 effectively.

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Sun et al., Table captions

Table 1 The final biomass concentration, lipid content, and biomass productivities of *S.dimorphus* cultured indoors with different THAM concentrations

Table 2 The biomass production data, cell lipid content, and CO_2 utilisation efficiency of *S.dimorphus* in twice outdoor cultivations

Table 3 The fatty acid composition of algal cells cultured indoors with different THAM concentrations

Table 4 The fatty acid composition of algal cells cultured outdoors with different THAM concentrations

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Sun et al., Figure captions

Fig. 1 Sketch of (a) the gas-carbonator and (b) a schematic diagram of the abiotic experiments

Fig. 2 Effect of time and concentration of THAM on (a) CO_2 gas absorptivity, (b) total inorganic carbon absorbed and (c) pH of the liquid phase

Fig. 3 (a) The increment of TIC due to THAM, NaOH, and Na_2CO_3 addition in blank culture medium; (b) the average CO_2 gas absorptivity during the whole absorption process in the presence of different concentrations of three absorbents

Fig. 4 (a) The growth curves of *S. dimorphus* at different THAM concentrations cultured indoors and (b) THAM changes during cultivation

Fig. 5 The course of TIC concentration and pH of the culture broth along with the changes of the oscillation time on the third day, (a) control case and (b) THAM

Fig. 6 The growth curves of *S. dimorphus* cultured outdoors with different concentration THAM (0 and 6 $mmol \cdot L^{-1}$), (a) 12 Aug. to 20 Aug. and (b) 26 Aug. to 3 Sep.

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Sun et al., Table 1

Table 1 The final biomass concentration, lipid content, and biomass productivities of *S.dimorphus* cultured indoors with different THAM concentrations

Parameters	THAM concentration (mmol·L^{-1})				
	0	2	4	6	8
Biomass concentration (g·L^{-1})	0.87 ± 0.01	1.12 ± 0.02	1.30 ± 0.03	1.40 ± 0.01	1.24 ± 0.04
Lipid content (%, w/w)	19.27 ± 3.86	17.14 ± 1.17	19.19 ± 2.82	22.33 ± 2.10	20.03 ± 1.31
Biomass productivity (mg·L^{-1}·d^{-1})	97.03 ± 2.13	127.32 ± 2.96	150.11 ± 4.28	161.59 ± 1.34	143.47 ± 6.51

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Sun et al., Table 2

Table 2 The biomass production data, cell lipid content, and CO_2 utilisation efficiency of *S.dimorphus* in twice outdoor cultivations

Test		Max biomass concentration (g/L)	Net biomass increment (g)	CO_2 utilization efficiency (%)	Overall biomass productivity ($g/m^2/d$)	Lipid content (%)
0812-0820	Control	0.81	189.09	34.93	11.23	26.68
	THAM	1.16	251.95	46.55	14.96	20.96
0826-0903	Control	0.56	98.63	18.32	5.89	19.33
	THAM	0.77	130.47	24.23	7.79	20.68

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Sun et al., Table 3

Table 3 The fatty acid composition of algal cells cultured indoors with different THAM concentrations

FA composition	FA content (% of total FA)				
	0 mmol/L THAM	2 mmol/L THAM	4 mmol/L THAM	6 mmol/L THAM	8 mmol/L THAM
14:0	0.35 ± 0.03	0.32 ± 0.00	0.33 ± 0.01	0.33 ± 0.00	0.27 ± 0.04
16:3	2.04 ± 0.16	2.27 ± 0.01	2.72 ± 0.05	2.81 ± 0.29	2.75 ± 0.40
16:2	3.66 ± 0.42	3.08 ± 0.21	3.72 ± 0.17	3.76 ± 0.57	3.01 ± 0.72
16:1	4.18 ± 0.23	4.39 ± 0.09	4.23 ± 0.15	4.65 ± 0.35	4.70 ± 0.22
16:0	23.76 ± 0.91	23.30 ± 0.45	22.18 ± 0.83	21.88 ± 1.07	21.37 ± 0.08
18:3	1.53 ± 0.02	1.50 ± 0.01	1.39 ± 0.03	1.33 ± 0.12	1.31 ± 0.01
18:2	13.54 ± 0.21	14.98 ± 0.76	16.31 ± 0.60	16.80 ± 0.45	17.22 ± 0.59
18:1	30.69 ± 0.60	28.71 ± 1.22	26.74 ± 1.35	24.88 ± 0.67	25.22 ± 0.05
18:0	1.29 ± 0.05	1.24 ± 0.50	0.88 ± 0.04	0.89 ± 0.15	0.80 ± 0.00
Others	18.97 ± 0.24	20.20 ± 0.63	21.50 ± 0.04	22.67 ± 0.29	23.35 ± 0.32
C16-C18	80.68 ± 0.83	79.48 ± 0.94	78.17 ± 0.82	77.00 ± 0.85	76.38 ± 0.85
SFA	25.39 ± 0.89	24.86 ± 0.05	23.38 ± 0.78	23.10 ± 1.22	22.43 ± 0.04
MUFA	34.87 ± 0.37	33.10 ± 1.13	30.97 ± 1.21	29.53 ± 0.32	29.92 ± 0.17
PUFA	20.77 ± 0.77	21.84 ± 0.55	24.15 ± 0.46	24.70 ± 1.19	24.29 ± 0.53

SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated acid. $n = 3$, values are means ± S.D.

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Sun et al., Table 4

Table 4 The fatty acid composition of algal cells cultured outdoors with different THAM concentrations

FA composition	FA content (% of total FA)			
	Control (0812-0820)	THAM (0812-0820)	Control (0826-0903)	THAM (0826-0903)
14:0	0.40 ± 0.00	0.33 ± 0.00	0.35 ± 0.00	0.34 ± 0.00
16:3	7.44 ± 0.01	7.65 ± 0.02	7.15 ± 0.01	8.05 ± 0.04
16:2	0.85 ± 0.01	1.23 ± 0.01	1.28 ± 0.00	1.35 ± 0.03
16:1	2.82 ± 0.09	2.60 ± 0.02	2.47 ± 0.07	2.45 ± 0.14
16:0	21.94 ± 0.22	20.22 ± 0.81	18.93 ± 0.14	17.62 ± 0.70
18:3	12.83 ± 0.13	14.3 ± 0.83	11.09 ± 0.08	14.43 ± 0.64
18:2	15.78 ± 0.03	16.40 ± 0.27	16.29 ± 0.12	16.48 ± 0.29
18:1	21.09 ± 0.02	19.51 ± 0.24	24.52 ± 0.34	21.6 ± 0.13
18:0	4.61 ± 0.05	4.16 ± 0.11	4.28 ± 0.14	4.27 ± 0.25
Others	12.23 ± 0.23	13.60 ± 0.22	13.64 ± 0.28	13.42 ± 0.55
C16-C18	87.36 ± 0.10	86.07 ± 0.61	86.01 ± 0.36	86.25 ± 0.09
SFA	26.95 ± 0.26	24.71 ± 0.71	23.56 ± 0.00	22.23 ± 0.45
MUFA	23.91 ± 0.07	22.11 ± 0.22	26.99 ± 0.41	24.05 ± 0.26
PUFA	36.90 ± 0.03	39.58 ± 0.27	35.81 ± 0.13	40.31 ± 0.36

SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated acid. $n = 3$, values are means ± S.D.

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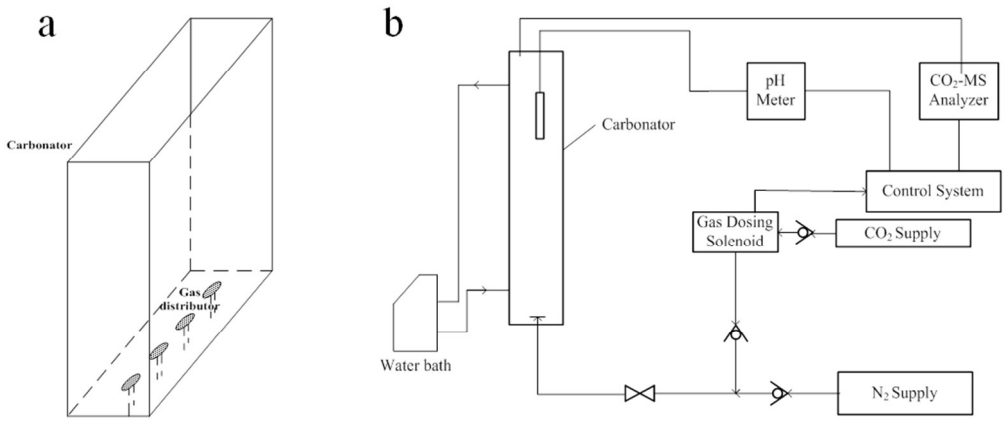

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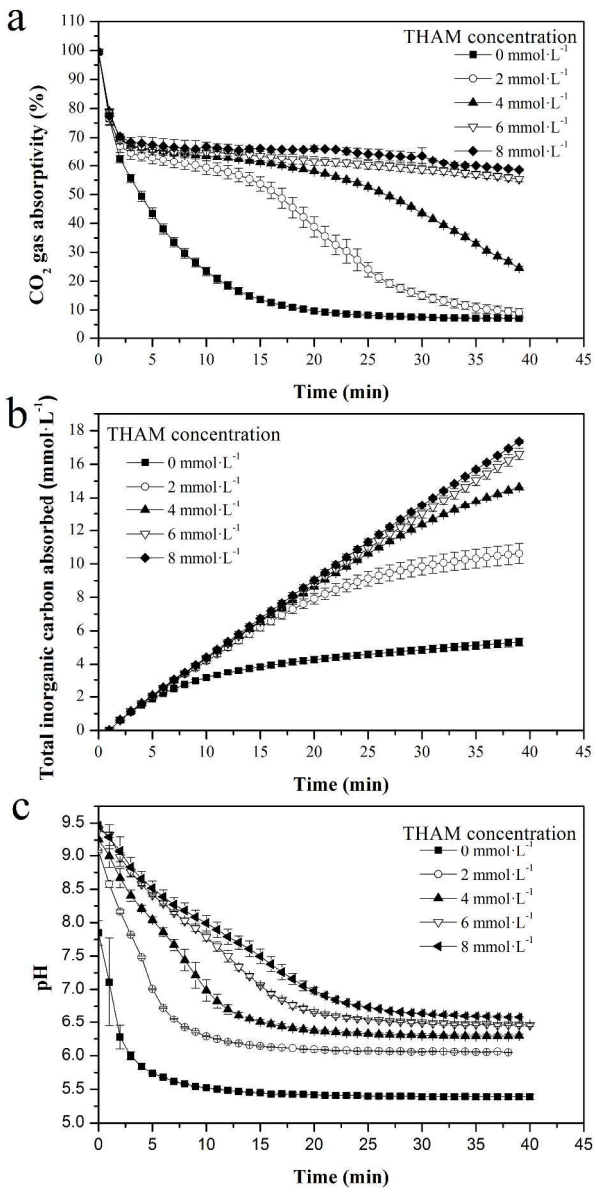

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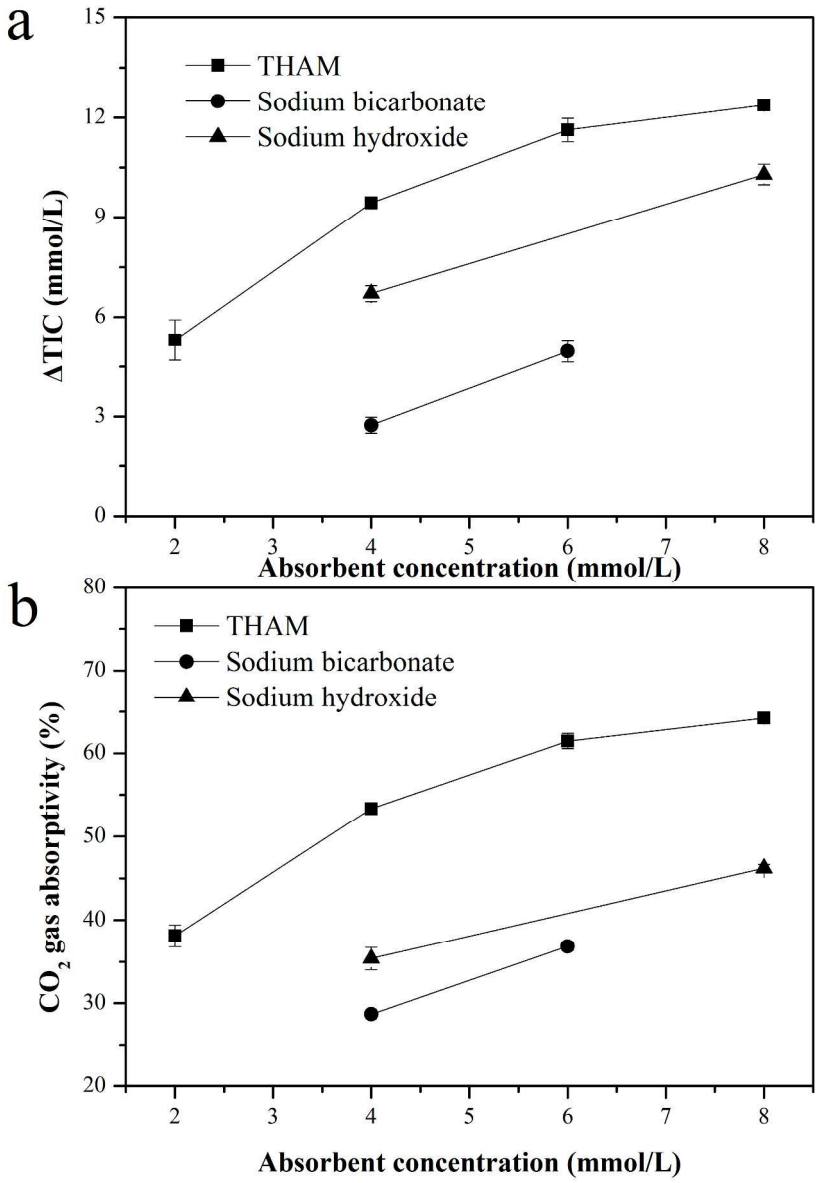

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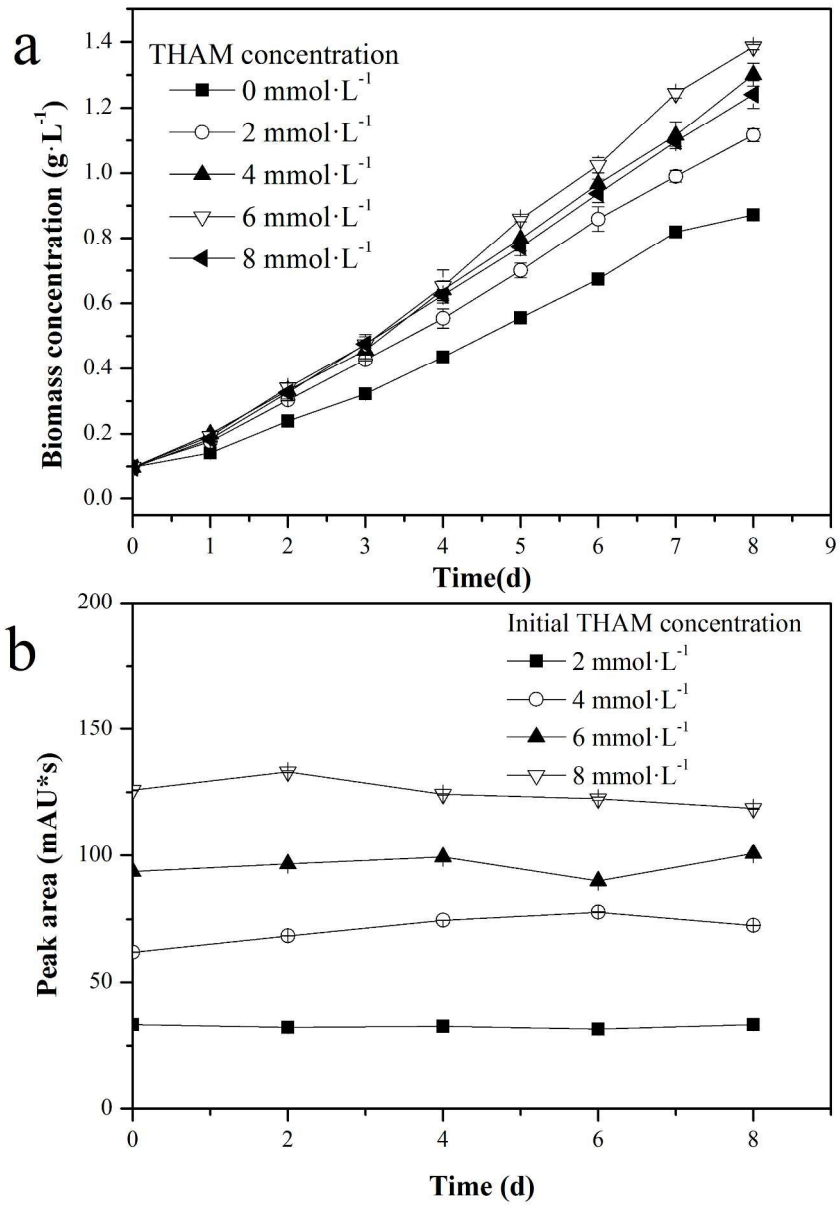

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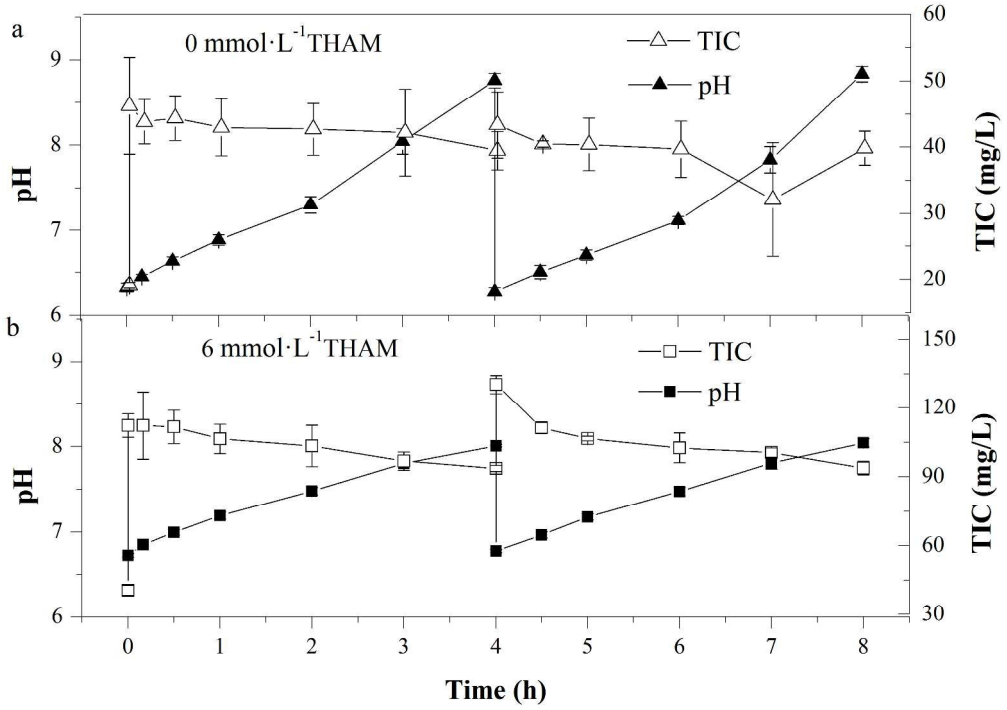

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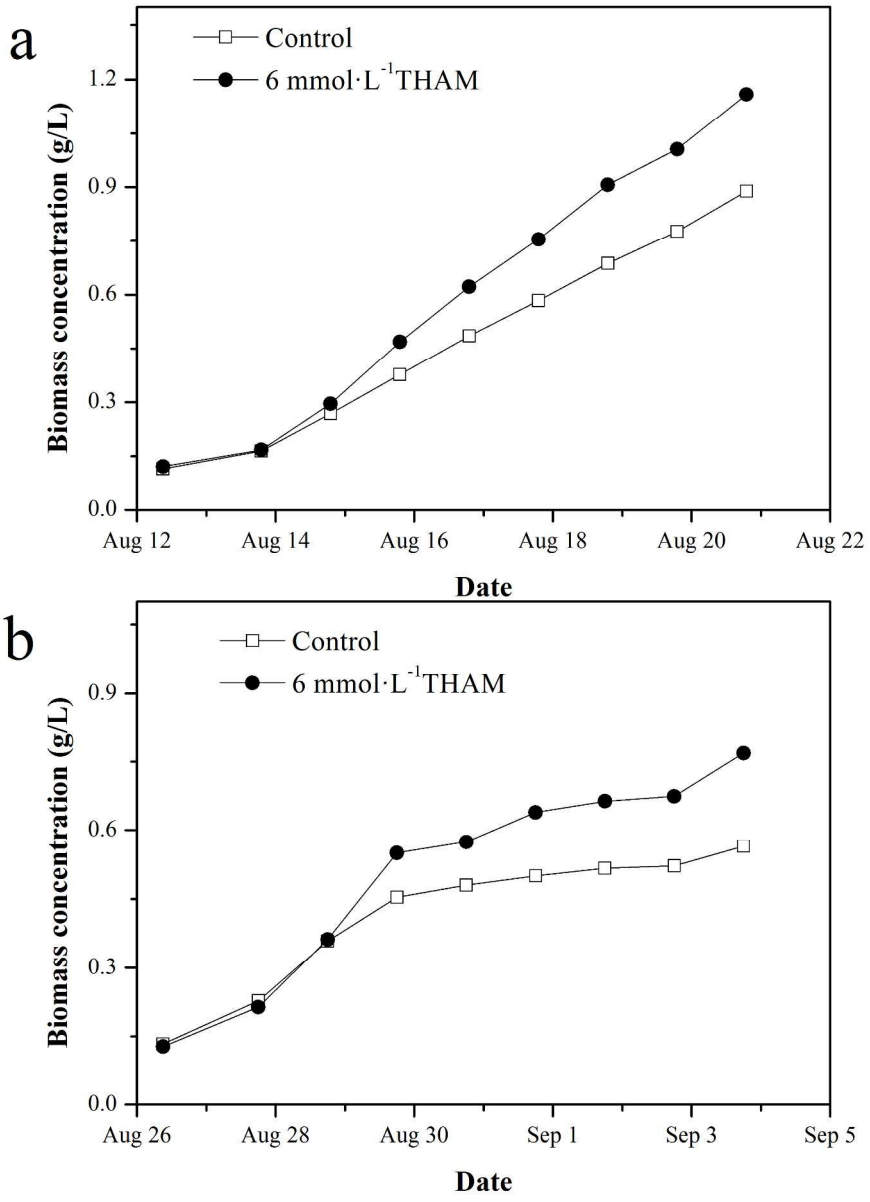

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