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The potential impact of replacing nitrate with ammonium hydroxide in microalgae production on the biomass productivity and CO_2 utilization efficiency

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ABSTRACT

Ammonium hydroxide has many advantages as a CO_2 absorbent compared with other amine solutions in Carbon Capture, Utilization and Storage. In this work, the use of ammonium hydroxide was suggested to replace the nitrate as an alternative nitrogen source and to enhance the CO_2 utilization and biomass production during microalgae cultivation. Abiotic absorption experiments showed that the CO_2 absorption capacity of culture medium increased with the increase of ammonium hydroxide concentration, and it kept consistent with different kind of CO_2 absorbents. Consumption curves for sodium nitrate and ammonium hydroxide were built from batch cultivation experimental data and it was found that the microalgal cells had faster growth rates when these nutrients concentrations were kept at low levels (2 mmol/L and 4 mmol/L, respectively). In the fed-batch cultures under both indoor and outdoor operations, the maximum biomass concentration and carbon utilization efficiency using ammonium hydroxide as a nitrogen source was much higher than that of using sodium nitrate as a nitrogen source. By using pH-regulated CO_2 supplementation, the pH of the microalgae culture medium was maintained within the neutral or slightly alkaline range. Thus, the volatilization of ammonium hydroxide was negligible. These results indicated that replacing nitrate with ammonium hydroxide could be a promising approach to increase CO_2 utilization efficiency and to reduce production costs in microalgae cultivation.

1. Introduction

With the development of the world economy and the continuous increase of the population, the global demands for energy (oil, coal, natural gas) have increased substantially, and non-renewable resources are being depleted as a result [1,2]. The CO_2 produced by the combustion of traditional energy is one of the main causes of the global greenhouse effect [3]. Also, other pollutants such as SOx, NOx, and dust are the key culprits of the deterioration of the natural environment [4,5]. In April 2022, the International Energy Agency (IEA) released the Global Energy Review 2021, reporting that since 1990 global CO_2 emissions have grown uninterruptedly [6]. As the global economy recovers from the Covid-19 pandemic, CO_2 emissions have soared, reaching a record high of 36.3 billion tons in 2021, an increase of 6 % over 2020. The current global pressure to reduce greenhouse gas emissions is increasing and the measures taken by the international community to deal with climate change are not effective enough [7]. Therefore, interest in developing new methods for the production of clean renewable energy and the reduction of CO_2 emissions is increasing worldwide [8,9].

Within the processes of Carbon Capture, Utilization, and Storage (CCUS), applications using amine solutions such as monoethanolamine (MEA) are commonly utilized to separate and purify CO_2 from large-emission sources [10]. Such sources include power generation or industrial facilities that use either fossil fuels or biomass for fuel. In recent years, ammonium hydroxide was proposed to replace conventional alkanolamine solutions, like MEA, as CO_2 absorbents. Concurrently, various studies have pointed out the advantages of the application of wet ammonia for removing CO_2 from flue gases, due to its high absorption capacity [11–13]. An experiment by Hamouda et al. (2020) showed that the CO_2 absorption capacity of ammonia was >1.0 kg CO_2/kg NH_3, while MEA was only able to absorb 0.40 kg CO_2/kg MEA [11].

On the other hand, it is known that the –NH_2 functional group of amines can carry on the reversible reaction of the absorption and desorption of CO_2, which can enhance CO_2 absorption's rate and capacity. Many previous studies have shown that MEA and Tris

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(hydroxymethyl)aminomethane (TRIS) could notably increase CO_2 utilization efficiency and microalgal biomass productivity in microalgal cultures, although high dosages (>150 mg/L) would cause cell injury [14–17]. Therefore, there is a need to explore new gas carriers with high carrying capacity and low bio-toxicity. According to the two-film theory, the rate of CO_2 mass transfer from gas to liquid would be enhanced as the absorption rate of CO_2 by ammonium hydroxide increases. Alternatively, ammonium bicarbonate, as the main reaction product, can be directly used by microalgae cells as a carbon source.

Nitrogen is the second most important nutrient required for the growth of microalgae [18]. In large-scale microalgal cultures, nitrate is a preferred nitrogen source over nitrite, as the latter has higher costs [19–21]. However, the price of sodium nitrate is relatively high (US$ 380 per ton) and this increases the cost of microalgae production. Moreover, if ammonium hydroxide replaces sodium nitrate as the nitrogen source, the costs for nutrients in microalgae cultivation may be significantly reduced since ammonium hydroxide is relatively cheap (US $ 80 per ton) and will not introduce other metal ions into the microalgae culture medium. However, there are also problems when ammonium is used as a nitrogen source, as high concentration of ammonium may inhibit the growth of microalgae cells [22,23], and ammonia volatilization may cause loss of nitrogen.

This study proposes a new strategy to replace nitrate with ammonium hydroxide in microalgae cultivation, focusing on the effect of ammonium hydroxide on microalgae cell growth and CO_2 fixation. A gas absorption experiment is first carried out to investigate how ammonium hydroxide enhances the absorption of CO_2. Then, two culture modes, i.e., batch mode and fed-batch mode, are adopted to compare the impact of different forms of nitrogen sources on microalgae growth and carbon source utilization. Lastly, an application of the proposed strategy to replace nitrate with ammonium is carried-out on a small-scale, outdoor open, cultivation of microalgae.

2. Materials and methods

2.1. Abiotic absorption experiments

CO_2 absorption experiments were conducted in batch mode. The gas mixture, consisting of 90 % N_2 and 10 % CO_2, was sparged into a well-mixed airlift column photobioreactor at a flow rate of 0.2 L/min through a sintered glass plate (40–60 μm pores) positioned 3 cm above the bottom of the column. The 3 L photobioreactor was operated with 2.5 L (working volume) of BG-11 medium and the depth of the liquid phase was 46.5 cm. On the upper side, the bioreactor was equipped with two opening holes for gas exhaust and a pH electrode. A thermostatic jacket was coupled to a water bath to maintain the water temperature at 25 ± 1 °C. The effluent gas from the photobioreactor was continuously analyzed, to assess the CO_2 volumetric fraction, by quadruple mass spectrometry (GAM200, IPI). The pH of the liquid during this absorption process was also monitored using appropriate electrodes (Mettler-Toledo, CH). All electrical signals were recorded by a data acquisition system in situ, utilizing LabVIEW software.

The dose of ammonium hydroxide (0–8 mmol/L), as a variable affecting the efficiency of CO_2 absorption, was investigated, and the absorption capacity of different CO_2 absorbents was compared.

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. The microorganism was grown in a modified BG-11 medium [24], which contained (mg/L): $NaNO_3$, 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; H_3BO_3, 2.86.

The indoor cultivations were carried out in a 3 L airlift photobioreactor system with 2.5 L culture medium at 25 ± 1 °C. Continuous light intensity was kept at 150 μmol/(m^2·s) by means of eight 30 W fluorescent lamps. Sterilized air was introduced into the photobioreactor at a rate of 0.2 L/min. CO_2 was provided from a commercial cylinder and mixed with ambient air for a volumetric percentage of 10 % CO_2 in air, which was filtered through a membrane (0.22 μm) and supplied to the photobioreactor on demand controlled by the pH-feedback system. In the batch cultivations, different nitrogen species ($NaNO_3$ and NH_4HCO_3) were added into the BG11 nitrogen-free medium at different concentrations. Under the fed-batch cultivation mode, the nitrogen source was added every 12 h, and the added amount was calculated from the measured biomass concentration and the nitrogen source consumption curve.

The outdoor cultivations were conducted under the natural temperature and light irradiation conditions prevailing in the Lingcheng district of Dezhou, Shandong province, China (latitude 37° 13′ N, longitude 116° 21′ E) using two 2 m^2 raceway ponds. The images of bioreactors are shown in Supplementary material-Fig. SM1. The open pond was made of stainless steel with a length of 2.4 m, bend diameter of 1.0 m, and wall height of 0.45 m. A 1.4 m long and 0.45 m high steel sheet partition was installed in the middle of the pond to form a raceway pattern. A paddle wheel was equipped to drive the fluid and it was operated at a flow velocity of 20 cm/s. The total volume of the culture broth was 200 L, which corresponded to a fluid depth of 10 cm. During the cultivation process, CO_2 was supplemented as the carbon source at the rate needed to maintain the pH of the culture medium at 7.8. The microalgae biomass concentration was measured daily, and the quantities of carbon source and nitrogen source to be added were calculated accordingly.

2.3. Analytical methods

Biomass concentration was determined by measuring the optical density at 680 nm (OD_{680}) and correlating it with the predetermined biomass concentration standard curve: Biomass (g/L) = 0.4938 × OD_{680} (R^2 = 0. 998). Any optical density >1.0 was first diluted to give an absorbance in the range 0.1–1.0 before applying the equation.

The total lipid content in the microalgae cells was determined by the modified Bligh and Dyer method [25]. Samples of microalgae culture medium were taken and centrifuged for 5 min at 5000 r/min. Afterwards, each sample was washed twice with ultra-pure water, and the supernatant was discarded. The algae cells were collected and freeze-dried and 30 mg of algae powder were weighed and placed in a 10 mL centrifuge tube. 4 mL of a chloroform/methanol mixture (v/v, 2:1) were then added to the tube. The tube was placed in an ultrasonic cell disruptor in an ice bath for 10 min (Power: 600 W, ultrasound treatment time: 2 s, interval: 4 s) to disrupt the cells. 1 mL of distilled water was added to the tube and the disrupted sample was centrifuged (5000 r/min, 5 min). The chloroform layer was separated, weighed, transferred to a new centrifugal tube, and then an equal amount of distilled water was added. The tube was well-shaken and centrifuged again (5000 r/min, 5 min). The chloroform was volatilized under a nitrogen blanket at 60 °C, and then dried in a vacuum drying oven. The total lipid content of the algae cells was calculated by dividing the weight difference of the test tube with and without the algal lipids, by the dry weight of the algae powder. Fatty acid methyl esters were prepared by acidic transesterification of the liquid extracts as described by Liang et al. [26]. Then, the sample was redissolved in hexane and a 0.5 μL-volume aliquot was injected and analyzed in a gas chromatographer (Agilent, 7890A, America) equipped with an FID detector and an Agilent DB-17 column (30 m × 0.25 mm).

The nutritive salt concentrations in the cultivation process were determined by first taking 5 mL of microalgae culture medium and filtering them through a filter membrane (0.22 μm) to obtain a filtrate.

The nitrate ion concentration was determined by ion chromatography, and the ammonium ion concentration was determined using a gas-sensitive ammonia electrode.

2.4. Parameter calculation

In abiotic absorption experiments, the amount and rate of CO_2 absorbed by different absorbents can be calculated based on the CO_2 absorption curve. Specifically, CO_2 absorption rate refers to the real-time reduction of CO_2 in the mixed gas after contact with the absorbing solution. The calculation method is shown in the following equation [27]:

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

where η_{CO2} (%) represents the CO_2 gas absorptivity, $C_{CO2,\ in}$ (%) represents the inlet volume concentration of CO_2 and $C_{CO2,\ out}$ is the outlet volume concentration of CO_2.

The absorption rate represents the total amount of CO_2 absorbed by a unit volume of absorbing solution per unit time. Its unit is mmol/(L·min), and calculated as follows:

$$r = \frac{v_{cO2,in} - v_{cO2,out}}{22.4 \times V}, \tag{2}$$

where v (mL/min) represents the real-time volume flow rate of CO_2; V (L) is the absorbent volume, and 22.4 is the volume (L) per mole of ideal gas under standard temperature and pressure. The capacity of CO_2 (mmol/L) absorbed could be obtained by integrating the absorption rate vs time curve.

The specific growth rate at logarithmic growth phase μ (d^{-1}) can be calculated by the following equation:

$$\mu = \frac{lnX_2 - lnX_1}{t_2 - t_1} \tag{3}$$

where t_1 (d) and t_2 (d) represent the time points when the logarithmic growth phase started and ended respectively. X_1 (g/L) and X_2 (g/L) were the microalgae biomass concentrations corresponding to t_1 and t_2, respectively.

The biomass productivity P_V (g/(L·d)) was expressed as the increment of biomass concentration over a given time period, as shown in the following equation:

$$P_V = \frac{X_t - X_0}{t} \tag{4}$$

where X_0 (g/L) and X_t (g/L) are the microalgae biomass concentrations at the beginning of culture and at culture time t respectively, and t (d) is the culture time.

Eq. (5) gives the calculation of the microalgae biomass area productivity, P_A (g/(m^2·d)):

$$P_A = \frac{P_V \times V}{A} \tag{5}$$

where P_V (g/(L·d)) is the calculate volume productivity, V (L) is the volume of the culture medium. P_A (g/(m^2·d)) is the calculate area productivity, and A $(m)^2$ is the area of the raceway pond.

The microalgae lipid productivity, P_L (mg/(L·d)), can be calculated according to the microalgae biomass productivity in Eq. (6):

$$P_L = 1000 \times P_V \times F_L \tag{6}$$

where P_V (g/(L·d)) is the biomass volume productivity, and F_L (%) is the total lipid percentage in the microalgae cells.

The consumption of nutrients (nitrogen sources) in the culture medium was measured according to ion chromatography, and the specific nutrient consumption rate μQ (mmol/(g·d)) was calculated according to Eq. (7) [18]:

$$\mu Q = \frac{C_2\text{-}C_1}{(X_2 - X_1)(t_2 - t_1)} \tag{7}$$

where t_1 (d) and t_2 (d) refer to the different culture time of a growth phase, respectively, and C_1 (mmol/L), C_2 (mmol/L) denote the corresponding nutrient concentration in the liquid medium at time t_1 and t_2; X_1 (g/L) and X_2 (g/L) refer to the biomass concentration.

In the process of microalgae cultivation, the yield of nitrogen source, $Y_{X/N}$ (g/g), refers to the dry weight of algal biomass produced per unit mass of nitrogen added to the culture medium, which is calculated by Eq. (8):

$$Y_{X/N} = \frac{(X_t - X_0) \times V}{N_t} \tag{8}$$

where X_0 (g/L) and X_t (g/L) represent the microalgae biomass concentrations at the beginning of the culture and at culture time t, respectively. V (L) is the volume of the culture medium, and N_t (g) is the amount of nitrogen to be added.

Eq. (8) presents the calculation of the carbon source utilization efficiency (E_C, %):

$$E_C = \frac{F_C \times \Delta X/12}{\sum_{i=0}^{t} C_{CO_2} + C_{NH_4HCO_3}} \tag{9}$$

where F_C (%) is the proportion of carbon to the dry weight of microalgae cells, 12 is the relative mass of carbon, ΔX (g/L) is the increment change of microalgae biomass concentration, and $\sum_{i=0}^{t} C_{CO_2} + C_{NH_4HCO_3}$ is the accumulated amount of CO_2 and ammonium bicarbonate added per unit culture volume.

2.5. Data analysis and statistics

For each experimental measurement, the relevant tests were repeated in triplicate, and all results were expressed as mean ± standard deviation (SD). SPSS software was used for variance analysis of the data, and significant difference was found if $P < 0.05$.

3. Results and discussion

3.1. Effect of chemical absorbent on CO_2 mass transfer

Different concentrations of ammonium hydroxide were added to the BG11 medium and the total CO_2 absorption amount over time was obtained (Eqs. (1) and (2)). According to Fig. 1A, the CO_2 absorption rates increased significantly as the ammonium hydroxide concentration increased. Specifically, when 2 mmol/L, 4 mmol/L and 8 mmol/L of ammonium hydroxide were added to the microalgae culture medium, the average absorption rates were 0.252 mmol/(L·min), 0.293 mmol/(L·min) and 0.361 mmol/(L·min), respectively, representing an increase of 54 %, 79 % and 120 % as compared with the average absorption rate of the control group (0.164 mmol/(L·min)). Similarly, the amount of CO_2 absorbed also increased as the concentration of ammonium hydroxide increased. When 8 mmol/L of ammonium hydroxide was added, the amount of CO_2 absorbed increased to 14.43 mmol/L, which was 2.2 times that of the control group (6.56 mmol/L).

Equal concentrations (8 mmol/L) of MEA, TRIS and ammonium hydroxide were added to the medium, and the corresponding amounts of CO_2 absorbed are shown in Fig. 1B. As evident, the amount of CO_2 absorbed when an absorbent was used was significantly higher compared to the control group (BG11 culture medium), indicating that chemical absorption strengthened the gas-liquid mass transfer process. Furthermore, the rate of CO_2 absorption in the medium with ammonium hydroxide was similar to that of the medium with MEA and TRIS, but the amount of CO_2 absorbed in the medium was slightly lower than that of

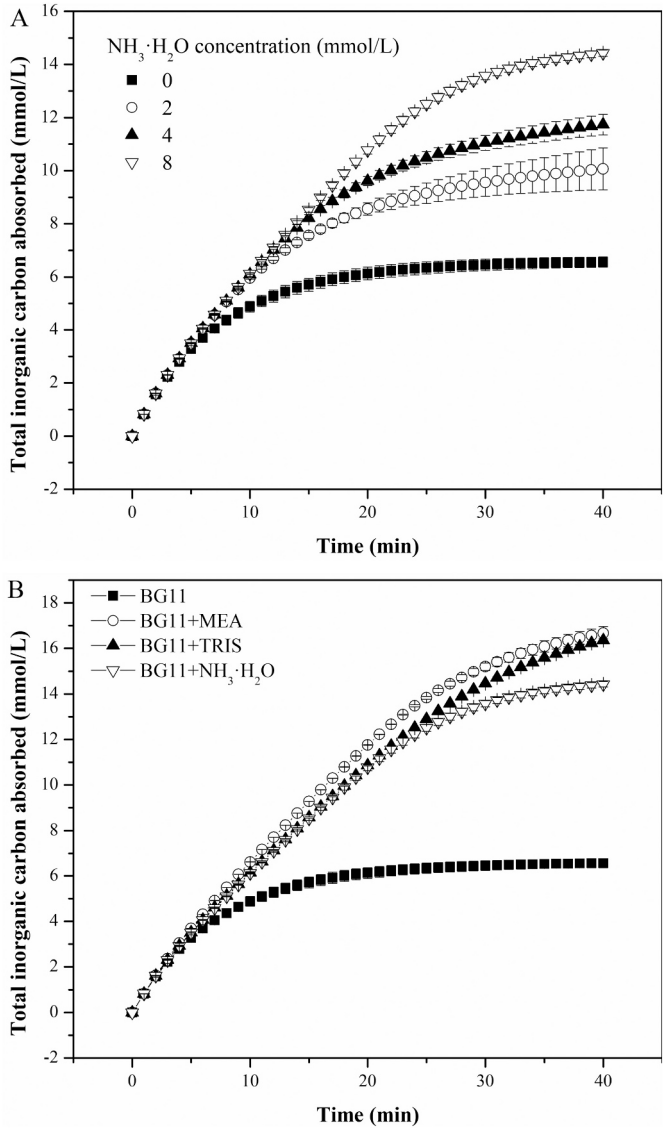

Fig. 1. Effect of chemical absorbent on CO_2 mass transfer (A, Effect of time and concentration of $NH_3 \cdot H_2O$ on the total inorganic carbon in the liquor; B, Comparison of the amount of CO_2 absorbed by the medium with three absorbents (MEA, TRIS, ammonium hydroxide)).

MEA and TRIS. The reason for this result might be that partial volatilization of ammonium hydroxide that resulted in the loss of absorbent in the bubbling column [28,29]. Ammonia loss during microalgae cultivation, can be reduced by controlling the pH of the culture medium, or by implementing the fed-batch cultivation mode to reduce the concentration of ammonia in the culture medium and improve the nitrogen source yield of microalgae.

The process by which a medium containing ammonium hydroxide absorbs CO_2 is a typical chemical absorption process, and the main reactions are shown in Eqs. (10)–(13):

$$CO_2 + 2NH_3 \rightarrow NH_2COONH_4 \quad (10)$$

$$NH_2COONH_4 + H_2O \rightarrow NH_3 + NH_4HCO_3 \quad (11)$$

$$NH_3 + NH_4HCO_3 \rightarrow (NH_4)_2CO_3 \quad (12)$$

$$(NH_4)_2CO_3 + CO_2 + H_2O \rightarrow 2NH_4HCO_3 \quad (13)$$

As expected, the main product of the reaction between ammonium hydroxide and CO_2 was ammonium bicarbonate. According to the two-film theory [30,31], when the gas reacts with the absorbent in the solution, the absorbed component diffuses and reacts in the liquid film, thus decreasing the resistance of liquid mass transfer and greatly increasing the absorption rate. Therefore, for the same absorption time, the absorption amount, and consequently, the absorption rate was higher as compared with pure physical absorption. In addition, when NH_3 dissolved and diffused in the solution as it approached the gas-liquid interface, it would enter the gas phase through physical dissociation from the equilibrium partial pressure of the gas phase. This process was accelerated by bubbling, resulting in the partial loss of absorbent.

3.2. *Effect of nitrogen source and concentration on the growth and lipid accumulation of S. dimorphus*

In the 3 L airlift photobioreactor, CO_2 was added as the carbon source based on the pH-feedback system, and the pH of the medium was controlled at 7.5–7.8 (Fig. SM3). The effects of different initial concentrations of sodium nitrate and ammonium bicarbonate on the growth of *S. dimorphus* were investigated by holding other conditions constant. The results are shown in Fig. 2.

When sodium nitrate was used as the nitrogen source, increasing the nitrogen source concentration resulted in a higher concentration of microalgae biomass, as it can be seen in Fig. 2A. Specifically, when the initial nitrogen source concentration ranged from 2 to 8 mmol/L, the highest biomass concentrations obtained at the end of the cultivation process were 1.21 g/L, 1.79 g/L, 2.28 g/L and 2.33 g/L. However, in terms of the specific growth rate during the logarithmic growth phase, the growth potential of microalgae cells under high nitrogen source concentrations was significantly lower than that under low nitrogen source concentrations (Table 1), which was consistent with previously reported studies [32–34]. The results showed that the microalgae cell growth activity was more easily stimulated by the low initial concentration of nutritive salts, which also provided a theoretical basis for the fed-batch cultivation mode.

When the initial concentration of ammonium bicarbonate was 2 mmol/L, no significant growth of *S. dimorphus* was observed after the 4th day of the cultivation, due to insufficient amount of nitrogen (Fig. 2B). When the initial concentration of ammonium bicarbonate was 4–6 mmol/L, *S. dimorphus* grew well and the maximum biomass concentration was >2.0 g/L. However, in the culture with an initial ammonium bicarbonate concentration of 8 mmol/L, the growth of *S. dimorphus* was initially delayed by the high concentration of ammonium. However, as the nitrogen source was consumed, the ammonium concentration was gradually reduced and the microalgae cells grew rapidly, reaching a maximum biomass concentration of 2.30 g/L, which was slightly less than the level reached with 6 mmol/L of ammonium bicarbonate (2.42 g/L). These results were consistent with the results when sodium nitrate was used as the nitrogen source. Specifically, the growth rate in the logarithmic growth phase was higher when low concentrations of ammonium bicarbonate were used as the nitrogen source (Table 1).

The lipid content and lipid productivity of *S. dimorphus* grown at different initial nitrogen concentrations is shown in Table 1. An increase in nitrogen concentration led to a gradual decrease in the total lipid content in the *S. dimorphus* cells. When the initial nitrogen concentration was 2 mmol/L, the microalgae cells entered in a state of nitrogen limitation after 4 days culture, and the total lipid content was at maximum at the end of the cultivation. When sodium nitrate was used as the nitrogen source, the total lipid content reached a maximum of 29.07 %, and when ammonium bicarbonate was used as the nitrogen source, the total lipid content reached a maximum of 30.12 %. These results indicate that the lack of nitrogen could induce a significant increase in the lipid content of oleaginous microalgae, which is consistent with the reports of many studies [18,35]. However, the high lipid content observed under low nitrogen concentrations was at the expense of the biomass productivity, and thus, the overall lipid productivity was not high. Specifically, when

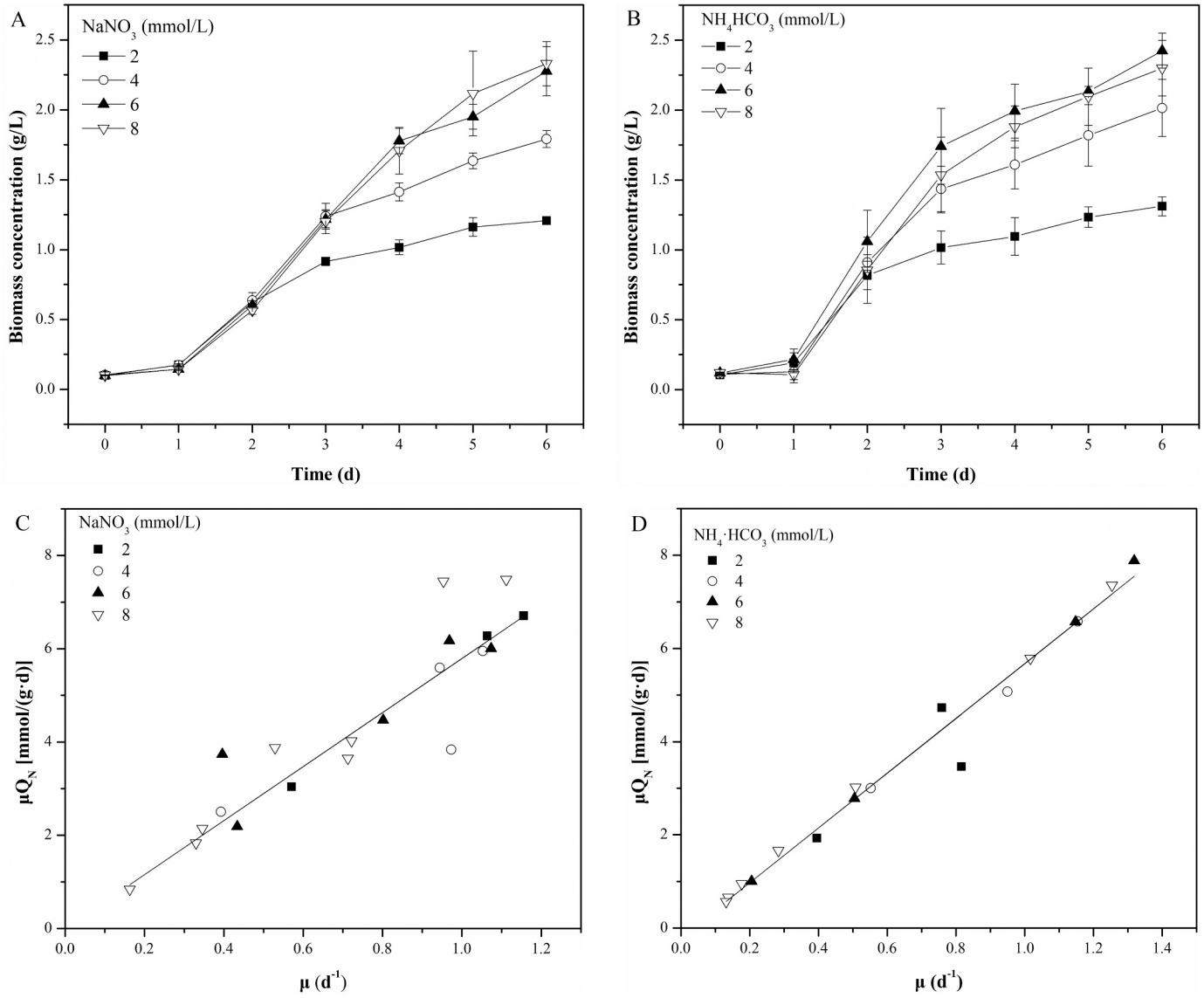

Fig. 2. Effect of nitrogen source at different concentration on the growth of *S. dimorphus* (A, The growth curve of *S. dimorphus* with 2–12 mmol/L $NaNO_3$; B, The growth curve of *S. dimorphus* with 2–12 mmol/L NH_4HCO_3; C, The specific consumption rate of nitrate as an indicator of *S. dimorphus* the growth rate; D, The specific consumption rate of ammonium as an indicator of *S. dimorphus* the growth rate).

8 mmol/L of sodium nitrate and 6 mmol/L of ammonium bicarbonate were used as the nitrogen sources, the lipid productivity of *S. dimorphus* reached maximum values of 87.40 mg/(L·d) and 85.47 mg/(L·d), respectively.

During the cultivation experiments of *S. dimorphus* with different nitrogen concentrations, the biomass growth trend was essentially consistent with the nitrogen consumption trend in the culture medium. *S. dimorphus* showed the greatest growth potential during the logarithmic growth phase, and the absorbed nitrogen source was mainly used for cell synthesis. Thus, the specific nitrogen consumption rate in the culture medium was approximate to the specific nitrogen absorption rate of *S. dimorphus*. Based on the *S. dimorphus* growth data of batch cultivation, the nitrogen consumption curves of *S. dimorphus* under different conditions may be obtained when the nitrogen concentration in the culture medium is measured in the logarithmic growth phase. The relationship between the specific consumption rate of nitrogen (μQ_N) and the specific growth rate (μ) is shown in Fig. 2C, D.

According to Fig. 2C, the relationship between nitrogen consumption (ΔN, mmol/L) and *S. dimorphus* biomass concentration increment (ΔX, g/L) when sodium nitrate was used as the nitrogen source was $\Delta N = 5.789\Delta X$. Similarly, according to Fig. 2D, when ammonium bicarbonate (or ammonium hydroxide) was used as the nitrogen source, the relationship between nitrogen consumption (ΔN, mmol/L) and *S. dimorphus* biomass concentration increment (ΔX, g/L) is $\Delta N = 5.649\Delta X$. Based on the nitrogen consumption curve of *S. dimorphus*, the nitrogen consumption rate in the culture medium can be derived by detecting the growth of microalgae cells during cultivation, and thus, the nitrogen concentration can be maintained at a stable level by supplementing with nutritive salt.

It should be noted that ammonium bicarbonate was the main product of the reaction between ammonium hydroxide and CO_2. The ammonium generated by dissociation can be used as a nitrogen source, while the dissociated bicarbonate may be used as a carbon source. Test results of batch culture have revealed that the specific growth rate was high when the initial nitrogen concentration was low. In addition, high concentrations of ammonia nitrogen may inhibit the growth of *S. dimorphus*. Therefore, based on the results obtained from the metabolic kinetics study of microalgae with different nitrogen sources, subsequent cultivation experiments investigated the microalgae cell growth with ammonium hydroxide were used directly as the nitrogen source. Some

Table 1
The specific growth rate, maximum biomass concentration, and lipid accumulation of *S. dimorphus* cells in batch cultures with different nitrogen sources.

Nitrogen source (mmol/L)		Specific growth rate μ (d^{-1})	Maximum biomass concentration (g/L)	Lipid content (%, w/w)	Lipid productivity (mg/(L·d))
$NaNO_3$	2	1.27 ± 0.02 a	1.21 ± 0.02 c	29.07 ± 1.32 a	53.69 ± 2.44 c
	4	0.98 ± 0.01 b	1.79 ± 0.06 b	25.32 ± 2.35 b	71.23 ± 6.61 b
	6	0.84 ± 0.01 c	2.28 ± 0.18 a	21.20 ± 1.09 c	76.98 ± 3.96 b
	8	0.83 ± 0.04 c	2.33 ± 0.16 a	23.52 ± 3.96 c	87.40 ± 14.71 a
NH_4HCO_3	2	1.48 ± 0.26 a	1.31 ± 0.07 c	30.12 ± 3.44 a	60.62 ± 6.93 c
	4	1.26 ± 0.39 b	2.01 ± 0.20 b	24.03 ± 2.20 b	76.33 ± 6.97 b
	6	1.06 ± 0.10 c	2.42 ± 0.13 a	22.24 ± 0.54 c	85.47 ± 2.06 a
	8	1.35 ± 0.08 a	2.30 ± 0.20 a	20.11 ± 0.47 c	73.03 ± 1.69 b

Values in a column with different letters are significantly different according to one-way analysis of variance (ANOVA) ($P < 0.05$).

factors such as lipid accumulation and carbon source utilization will be further investigated.

3.3. Effect of replacing nitrate with ammonium on growth and carbon source utilization under limited nitrogen source conditions

Ammonium hydroxide could be used as a CO_2 absorbent for strengthening the CO_2 supplementation in microalgae cultivation, and it also serves as a nitrogen source for microalgae growth. However, there are two drawbacks in this nitrogen source replacement strategy. First, high ammonia content may inhibit microalgae growth [22], and second ammonia volatilization may cause loss of nutritive salt during cultivation, thereby increasing the costs of cultivation. Based on the nitrogen consumption curve of *S. dimorphus*, a fed-batch cultivation mode was adopted to maintain a low nitrogen concentration in the microalgae culture medium, and to control the pH of the culture medium between 7.5 and 7.8 to reduce losses caused by ammonia volatilization. Fig. 3 shows the growth curve of *S. dimorphus* when the initial nitrogen concentrations were 2 mmol/L and 4 mmol/L, respectively.

As shown in Fig. 3, when ammonium bicarbonate, ammonium hydroxide and sodium nitrate were used as the nitrogen sources, *S. dimorphus* grew well. After a 12 h lag, the microalgae growth entered the logarithmic growth phase. When there were no limitations of the higher nitrogen supply, the logarithmic growth phase of the fed-batch cultivation mode was significantly longer than that of the batch culture mode. After 96 h of growth, the addition of nitrogen was discontinued. Another 24 h of culture was carried out continuously with a nitrogen concentration of 2 mmol/L. Then, the cultivation was carried out for 72 h continuously with a nitrogen concentration of 4 mmol/L, so as to consume the remaining nitrogen in the medium and save the costs of the nutritive salt. When the microalgae biomass concentration reached a high level, there was not enough nitrogen, and thus, the synthesis of lipids may have been induced. Under the fed-batch cultivation mode, the different nitrogen sources promoting microalgae growth can be ranked as follows: ammonium bicarbonate > ammonium hydroxide > sodium nitrate. Ammonium nitrogen was substantially more effective than nitrate nitrogen as the nitrogen source in microalgae cultivation, which was consistent with the experimental results under the batch culture mode (Table 1). The reason for this result may be that NH_4^+ can be directly absorbed by microalgae, while NO_3^- needs to be converted to NH_4^+ through a series of enzymatic reactions before it can be absorbed by microalgae [36,37].

The kinetic growth parameters and lipid productivity of *S. dimorphus* under the fed-batch cultivation mode are shown in Table 2. After 96 h of continuous culture, the addition of nitrogen was discontinued, and the microalgae cells did not have sufficient nitrogen source. Thus, the conversion of components like polysaccharides to lipids was stimulated [35]. When the nitrogen concentration in the culture medium was controlled to 2 mmol/L, the total lipid content was 32.86 % (ammonium bicarbonate), 27.47 % (ammonium hydroxide) and 25.61 % (sodium nitrate). When the nitrogen source concentration in the culture medium was controlled to 4 mmol/L, the total lipid content was 26.61 % (ammonium bicarbonate), 23.78 % (ammonium hydroxide) and 24.20 % (sodium nitrate). Thus, lipid productivity increased significantly at both concentration levels in fed-batch culture mode because that was not at the expense of lower microalgae biomass (Tables 1 and 2).

In the neutral or slightly alkaline pH range, ammonium hydroxide or ammonium bicarbonate dissolves in water, mainly in the form of NH_4^+ (Fig. SM2). In the cultivation experiments of this study, the pH value was stabilized by adding CO_2 based on a feedback loop to reduce ammonium hydroxide volatilization. In terms of the nitrogen utilization, when ammonium bicarbonate and ammonium hydroxide were used as the nitrogen sources, the microalgae biomass achieved by per unit of nitrogen element was not significantly different from that when sodium nitrate was used as the nitrogen source ($P < 0.05$). Specifically, when the nitrogen concentration was 2 mmol/L, the Biomass/Nitrogen was 11.46 g/g (ammonium bicarbonate), 11.91 g/g (ammonium hydroxide) and 11.46 g/g (sodium nitrate). When the nitrogen concentration was 4 mmol/L, the Biomass/Nitrogen was 12.95 g/g (ammonium bicarbonate), 12.54 g/g (ammonium hydroxide) and 12.89 g/g (sodium nitrate). Based on these results, ammonium bicarbonate and ammonium hydroxide can be widely used as cheap sources of nitrogen for microalgae cultivation within reasonable ranges of concentration.

As shown in Table 2, when ammonium bicarbonate, ammonium hydroxide and sodium nitrate were used as the nitrogen sources for fed-batch culture mode of *S. dimorphus*, the carbon source utilization efficiency in the cultivation process was significantly different. They can be ranked from highest to lowest as follows: ammonium hydroxide > ammonium bicarbonate > sodium nitrate. Specifically, when the nitrogen concentration was 2 mmol/L, the carbon source utilization efficiency was 64.61 % (ammonium bicarbonate), 87.84 % (ammonium hydroxide) and 24.95 % (sodium nitrate). When the nitrogen source concentration was 4 mmol/L, the carbon source utilization efficiency was 47.75 % (ammonium bicarbonate), 114.93 % (ammonium hydroxide) and 40.34 % (sodium nitrate). In the airlift photobioreactor system, the addition of CO_2 was mainly dependent on the growth of microalgae. When sodium nitrate was used as a nitrogen source, the utilization of nitrate and carbon sources in the culture medium by microalgae cells may produce OH^-, resulting in increase of the pH value [38]. When the pH value of the culture medium was higher than 7.8, CO_2 was introduced to neutralize OH^-. When ammonium bicarbonate and ammonium hydroxide were used as the nitrogen sources, the utilization of carbon by microalgae cells in the culture medium may produce OH^- but the utilization of ammonium may produce H^+. Thus, changes in the pH value of the culture medium were reduced, and the frequency of CO_2 addition was decreased. Furthermore, because ammonium hydroxide could enhance the mass transfer of mixed gases with low concentrations of CO_2 (Fig. 1A) and improve the absorption capacity of CO_2, the carbon utilization efficiency when ammonium hydroxide was used as the nitrogen source was higher than when ammonium bicarbonate was used as the nitrogen source.

The above experimental results indicate that ammonium hydroxide could be used as a CO_2 absorbent to enhance the carbon supplement in microalgae cultivation and increase the carbon utilization efficiency. At the same time, as a cheaper and more suitable nitrogen source, ammonium hydroxide could increase the biomass concentration and lipid productivity of microalgae significantly.

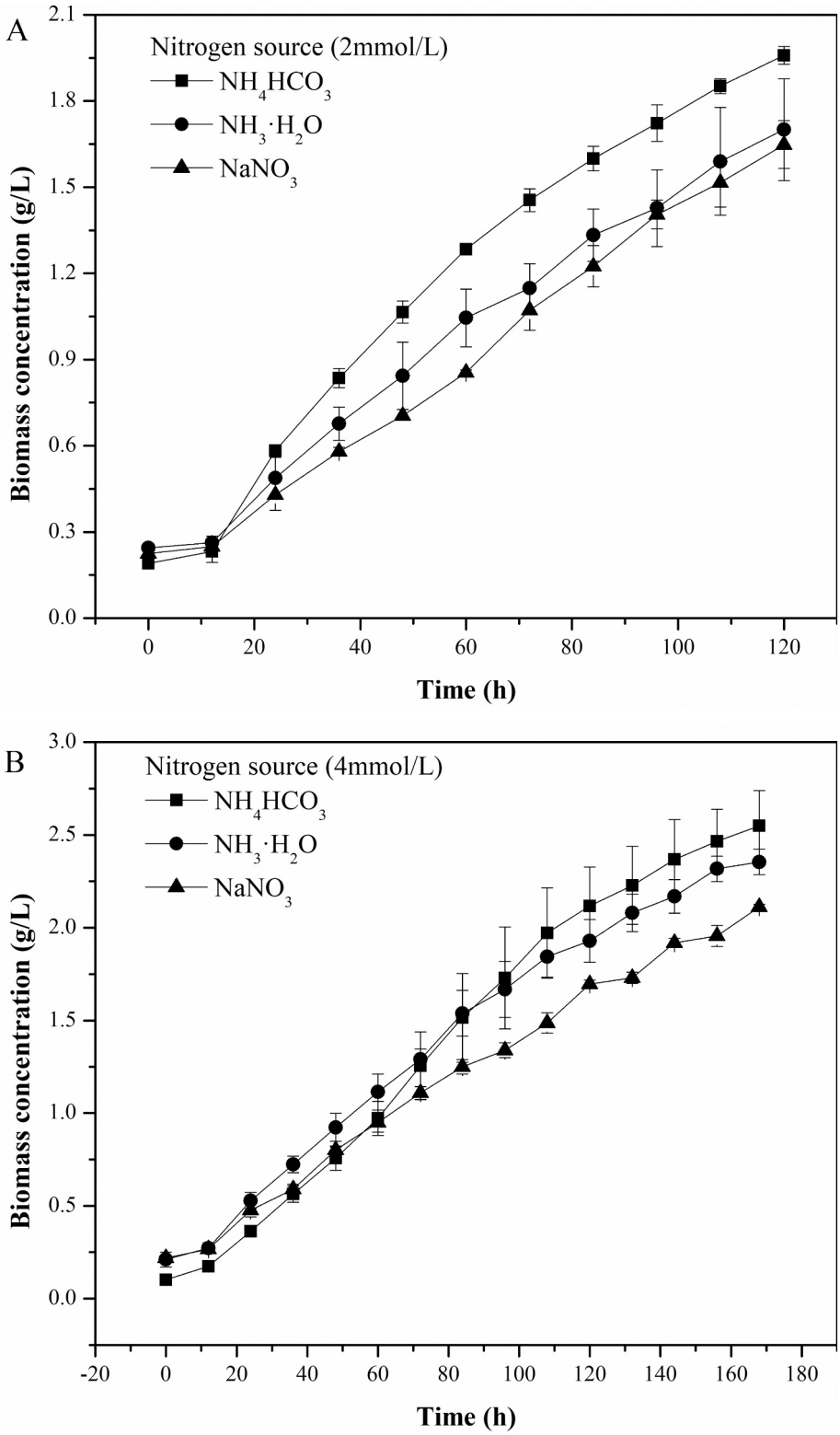

Fig. 3. Effect of different nitrogen source on the growth of *S. dimorphus* in fed-batch cultures (A, C_N = 2 mmol/L; B, C_N = 4 mmol/L).

3.4. Application of replacing nitrate with ammonium hydroxide in outdoor open ponds

Ammonium hydroxide and sodium nitrate were used as the nitrogen sources for fed-batch cultivation experiments in an outdoor 2 m^2 raceway pond. Based on the nitrogen consumption curve of *S. dimorphus* in Section 3.2, the nitrogen concentrations in the culture medium were controlled at 2 mmol/L and 4 mmol/L. The results are shown in Fig. 4.

When the nitrogen concentration in the culture medium was 2 mmol/L (Fig. 4A), the microalgae cells gradually adapted to the outdoor environment after 1 day of lag phase, and then entered the rapid growth phase. The addition of nitrogen was discontinued after 6 days of continuous cultivation. As shown in Fig. 4A, the growth of *S. dimorphus* with different nitrogen source was significantly different. Specifically,

Table 2
The biomass production data, lipid productivity, nitrogen, and carbon source utilization of *S. dimorphus* in fed-batch cultures using different nitrogen sources.

Nitrogen source (mmol/L)		Specific growth rate μ (d^{-1})	Maximum biomass concentration (g/L)	Lipid content (%)	Lipid productivity (mg/(L·d))	Biomass/nitrogen $Y_{X/N}$ (g/g)	CO_2 utilization efficiency (%)
2	NH_4HCO_3	0.55 ± 0.04 a	1.96 ± 0.03 a	32.86 ± 3.18 a	116.22 ± 11.24 a	11.46 ± 0.45 a	64.41 ± 2.35 b
	$NH_3·H_2O$	0.44 ± 0.02 b	1.70 ± 0.18 b	27.47 ± 2.46 b	80.00 ± 7.16 b	11.91 ± 0.41 a	87.84 ± 13.82 a
	$NaNO_3$	0.46 ± 0.02 b	1.65 ± 0.08 b	25.61 ± 4.08 b	72.94 ± 11.63 c	11.46 ± 0.25 a	24.95 ± 0.10 c
4	NH_4HCO_3	0.71 ± 0.04 a	2.55 ± 0.19 a	26.61 ± 2.67 a	93.02 ± 9.32 a	12.95 ± 0.15 a	47.45 ± 11.53 b
	$NH_3·H_2O$	0.52 ± 0.02 b	2.35 ± 0.07 a	23.78 ± 4.50 b	72.82 ± 13.78 b	12.54 ± 0.42 a	114.93 ± 0.20 a
	$NaNO_3$	0.45 ± 0.01 c	2.11 ± 0.01 b	24.20 ± 4.89 b	65.42 ± 13.23 c	12.89 ± 0.36 a	40.34 ± 2.68 c

Values in a column with different letters are significantly different according to one-way analysis of variance (ANOVA) ($P < 0.05$).

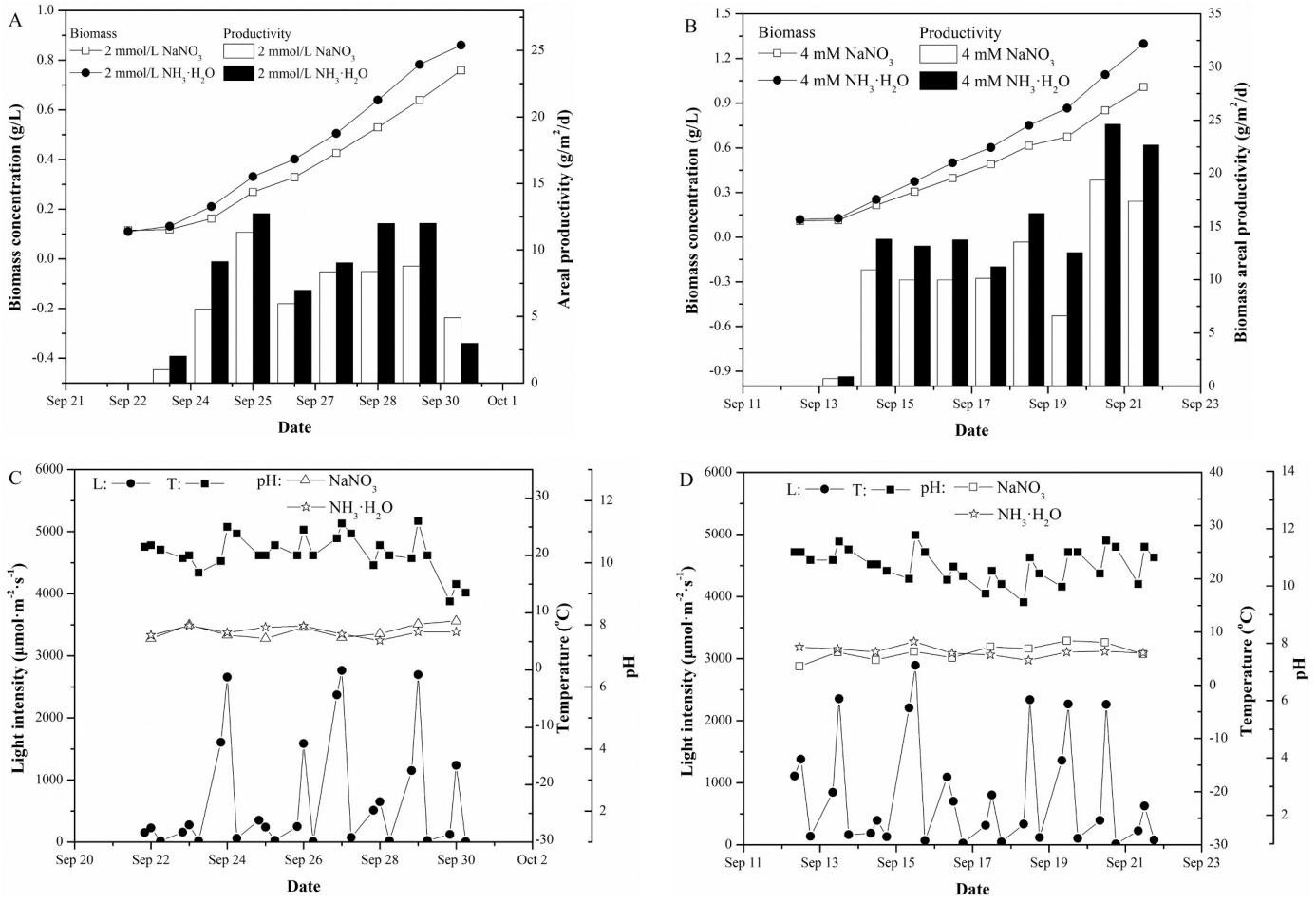

Fig. 4. The growth curves of *S. dimorphus* cultured outdoors in fed-batch mode and the culture environment (A, $C_N = 2$ mmol/L; B, $C_N = 4$ mmol/L; C, Light intensity, temperature and pH value versus time in Sep 22, 2021 to Oct 1, 2021; D: Light intensity, temperature and pH value versus time in Sep 10, 2021 to Sep 21, 2021).

when ammonium hydroxide was used as the nitrogen source, the microalgae biomass concentration reached 0.86 g/L after 8 days of cultivation, which was 13.2 % higher than that when sodium nitrate was used as the nitrogen source (0.76 g/L). The dry weight of microalgae cells obtained at the end of cultivation was 133.80 g and 108.62 g, for nitrogen sources of ammonium hydroxide and sodium nitrate, respectively. Correspondingly, when ammonium hydroxide was used as the nitrogen source for *S. dimorphus* culture, the average area productivity (7.43 g/(m^2·d)) increased by 23.2 % as compared with that when sodium nitrate was used as the nitrogen source (6.03 g/(m^2·d)). In addition, based on the principle of material balance, the CO_2 utilization efficiency in the cultivation process was calculated from the measured

Table 3
The biomass production data, nitrogen, and carbon utilization of *S. dimorphus* in the fed-batch outdoor cultures using different nitrogen sources.

Nitrogen source (mmol/L)		Max biomass concentration (g/L)	Net biomass increment (g)	Biomass/nitrogen $Y_{X/N}$ (g/g)	CO_2 utilization efficiency (%)	Overall biomass productivity (g/(m^2·d))
2	$NaNO_3$	0.76	108.62	9.31	21.12	6.03
	$NH_3·H_2O$	0.86	133.80	11.13	26.02	7.43
4	$NaNO_3$	1.01	171.96	9.58	29.72	8.71
	$NH_3·H_2O$	1.30	205.87	11.53	35.58	11.57

dry weight of microalgae cells and the cumulative amount of CO_2 sparged based on the pH-feedback. As shown in Table 3, the CO_2 utilization efficiency was 26.02 % when ammonium hydroxide was used as the nitrogen source, which was 20 % higher than when using sodium nitrate as the nitrogen source. In a small-scale open pond, there was no space for carbon supplementation devices. Therefore, in a large-scale cultivation process, the carbon utilization rate may be improved by increasing the gas-liquid contact time and contact area [39,40].

As shown in Fig. 4B, the growth curves of *S. dimorphus* for different sources of nitrogen were similar whether the nitrogen concentration in the microalgae culture medium was 4 mmol/L or 2 mmol/L. When the nitrogen source concentration was kept at 4 mmol/L, the average biomass productivity using ammonium hydroxide as nitrogen source was 32.8 % higher than when sodium nitrate was used. However, the biomass obtained after a period of fed-batch culture at 4 mmol/L of nitrogen source was much higher than that of 2 mmol/L (Table 3). The reason for this difference may be due to the changes of air temperature and sunlight: the fed-batch culture with 4 mmol/L nitrogen source was conducted in Sep 12 to Sep 21, 2021, and the culture with 2 mmol/L nitrogen source was conducted in Sep 22 to Oct 1, 2021 (Fig. SM3).

It should be noted that during the two batches of outdoor culture, the nitrogen yield using ammonium hydroxide was much higher that of using sodium nitrate (20 %), indicating that the volatilization of ammonium hydroxide was negligible. After analyzing the results of the outdoor cultivation experiment, replacing sodium nitrate with ammonium hydroxide as the nitrogen source not only promotes the growth of microalgae cells, but also absorbs CO_2 to strengthen the gas-liquid mass transfer process, thus improving the CO_2 utilization efficiency and reducing the costs of microalgae cultivation.

4. Conclusions

When 8 mmol/L of ammonium hydroxide was added into the medium, the amount of CO_2 absorbed by the liquid phase reached 2.2 times that of the control group. Based on the batch cultivation experiment, consumption curves may be obtained for sodium nitrate and ammonium hydroxide as nitrogen sources, i.e., $\Delta N = 5.789\Delta X$ and $\Delta N = 5.649\Delta X$. In the airlift photobioreactor system, the maximum biomass concentration, lipid productivity and carbon utilization efficiency using ammonium hydroxide as nitrogen sources were achieved by 2.35 g/L, 72.82 mg/(L·d) and 114.93 %, respectively. Under outdoor cultivation conditions, the use of ammonium hydroxide as the nitrogen source resulted in a 32.8 % increase in the biomass productivity compared to when sodium nitrate was used as the nitrogen source. Also, the CO_2 utilization efficiency was up to 1.2 times higher than the efficiency obtained using sodium nitrate. These results showed that ammonium hydroxide could be a cheaper source of nitrogen that can enhance CO_2 utilization by microalgae, and that replacing nitrate with ammonium hydroxide could be a cost-effective approach for the large-scale microalgae culture system.

CRediT authorship contribution statement

Yonghan Liu contributed to the data curation, formal data analysis, methodology, validation, and writing of the original draft. Liqin Sun contributed to the study design, manuscript writing, review, and editing. Zhongliang Sun contributed to the study conceptualization, manuscript writing, review, and editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could influence this work.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.algal.2022.102870.

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