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Utilization of simulated flue gas for cultivation of *Scenedesmus dimorphus*

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HIGHLIGHTS

- *Scenedesmus dimorphus* showed excellent tolerance to high CO_2 (2–20%) and NO concentrations (150–500 ppm).
- The maximum SO_2 concentration *S. dimorphus* could tolerant was 100 ppm.
- The extremely low pH as well as the accumulation of bisulfite caused by SO_2 inhibited algae growth.
- By neutralization with $CaCO_3$, *S. dimorphus* could grow well on flue gas.
- The toxicity of flue gas could be overcome by intermittent sparging and CO_2 utilization efficiency was enhanced.

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ABSTRACT

Effects of flue gas components on growth of *Scenedesmus dimorphus* were investigated and two methods were carried out to eliminate the inhibitory effects of flue gas on microalgae. *S. dimorphus* could tolerate CO_2 concentrations of 10–20% and NO concentrations of 100–500 ppm, while the maximum SO_2 concentration tolerated by *S. dimorphus* was 100 ppm. Addition of $CaCO_3$ during sparging with simulated flue gas (15% CO_2, 400 ppm SO_2, 300 ppm NO, balance N_2) maintained the pH at about 7.0 and the algal cells grew well (3.20 g L^{-1}). By intermittent sparging with flue gas controlled by pH feedback, the maximum biomass concentration and highest CO_2 utilization efficiency were 3.63 g L^{-1} and 75.61%, respectively. These results indicated that *S. dimorphus* could tolerate high concentrations of CO_2 and NO, and the methods of $CaCO_3$ addition and intermittent sparging have great potential to overcome the inhibition of flue gas on microalgae.

1. Introduction

CO_2 fixation by microalgae is seen as an economically feasible and environmentally sustainable way to mitigate CO_2 emissions and to generate biomass for the productions of biofuels and other chemicals. At least 1.83 tons of CO_2 are needed for obtaining 1 ton of algal biomass (Ho et al., 2010a). Consequently, coupling the cultivation of microalgae with bio-fixation of the CO_2 in flue gas from combustion sources has the potential not only to reduce the cost of microalgae production on an industrial scale but also to offset carbon emissions (de Morais and Costa, 2007a; Hughes and Benemann, 1997). Typical flue gas emitted from combustion sources contains 10–15% CO_2, and 100–300 ppm NO_X and SO_X (Lee et al., 2002). Some species of microalgae showed little growth inhibition at the typical CO_2 percentages in flue gas (Hanagata et al., 1992; Ho et al., 2010b; Tang et al., 2010), but some other studies showed that growth of microalgae was inhibited at CO_2 concentrations above 5% (Chiu et al., 2008; de Morais and Costa, 2007a,b). In contrast, the SO_X and NO_X in flue gas, especially from coal-fired power plants, impose more serious inhibition on microalgae (Lee et al., 2002; Negoro et al., 1991). When sparged with a gas mixture containing 300 ppm NO, the growth of *Chlorella* KR-1 was suppressed (Lee et al., 2002), and *Nannochloris* sp. (NANNO02) showed some growth only after a considerable lag time (Negoro et al., 1991). Sulfur oxides, particularly SO_2, cause a dramatic decline in pH of the culture medium (Lee et al., 2002; Meada et al., 1995). When the SO_2 concentration reaches 400 ppm, the medium pH decreases to below 4 within 24 h, significantly reducing the growth rate of microalgae (Matsumoto et al., 1997). Growth of *Nannochloris* sp. (NANNO02) was strictly inhibited within 20 h at SO_2 concentration of 300 ppm (Negoro et al., 1991).

Several attempts have been made to overcome the toxic effects, more specifically, the acidification of the medium when using flue gas for microalgae cultivation. Some researchers tried to screen NO_X- and SO_X-tolerant microalgae or acidophilic algae, but such algae grew consistently only at concentrations of 50 ppm SO_2 or below (Hauck et al., 1996; Kurano et al., 1995). Some researchers

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have reported that controlling the pH of medium by adding NaOH solution was an effective method to overcome the acid inhibition of flue gas (Lee et al., 2000; Westerhoff et al., 2010); however, neutralization with NaOH not only results in undesirably high ionic strengths which may inhibit growth of microalgae but also makes the cultivation process more complicated and costly.

In the present study, a series of experiments were conducted to investigate the effects of CO_2, NO, SO_2 on growth of *Scenedesmus dimorphus*, and neutralization by $CaCO_3$ addition and intermittent sparging of flue gas by pH feedback control were tested to overcome the inhibition of flue gas on microalgal growth.

2. Methods

2.1. Microalgae cultures

S. dimorphus (Chlorophyta, Chlorophyceae), a highly CO_2-tolerant and fast-growing microalgae, was selected from stock cultures kept in our laboratory. Modified BG-11medium (Stanier et al., 1971) was used for cultivation of the strain. The medium contained (mg 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; $CuSO_4{\cdot}7H_2O$, 0.079; $MnCl_2{\cdot}4H_2O$, 1.81; $NaMoO_4{\cdot}2H_2O$, 0.39; $Co(NO_3)_2{\cdot}6H_2O$, 0.0494; H_3BO_3, 2.86.

2.2. Experimental system with photobioreactors

The photobioreactors (PBRs) were glass columns (30 mm or 50 mm in diameter; 58 cm in length), which were sparged continuously and kept at 25 ± 1 °C during cultivations. Different column sizes were used over the course of the two-year study for logistical reasons. The columns were illuminated by fluorescent lamps with an intensity of about 100 μmol m^{-2} s^{-1}, as measured by a Basic Quantum meter (Li-250A light meter, USA). The pH of culture medium was determined by a pH meter (Sartorius PB-10, Germany). For intermittent sparging of flue gas under pH feedback control, the pH meter (405-DAPS-SC-k8s/325, Switzerland) was immersed into the medium and connected to a pH controller (Apure RP-100, China). Flow meters (LZB, China) were utilized to control the flow rates of CO_2, house air (0.038% CO_2) and other gases.

In the experiment of intermittent sparging of flue gas, compressed air was continuously supplied to make the culture solution mixed at the aeration rate of 0.25 vvm. The pH value of culture solution was set in a certain range, and regulated by switch on-off mode of flue gas using a pH controller. When the pH reached the minimum point, the aeration of flue gas was switched off automatically, and when the pH reached the maximum point because of the photosynthesis and metabolism of microalgae, the flue gas was automatically sparged again.

2.3. Gas mixtures

In the CO_2 experiments, pure CO_2 and house air were mixed to prepare CO_2 concentrations of 0.038%, 2%, 10% and 20%. Since the main constituents of SO_X and NO_X are SO_2 and NO, respectively, gas mixtures of 2% CO_2 and different NO concentrations (150, 300, 500 ppm, respectively) were used to evaluate the effect of NO on *S. dimorphus*, while the similar gas mixtures with 2% CO_2 and different SO_2 concentrations (100, 150, 200 ppm, respectively) were used in the SO_2 experiments. Simulated flue gas, with the composition of 15% (v/v) CO_2, 400 ppm SO_2, 300 ppm NO and balance N_2, was adopted in the experiments of $CaCO_3$ addition and intermittent sparging of flue gas. All the aeration rates were fixed at 0.25 vvm (volume gas/volume liquid/min).

2.4. Assay

Optical density of microalgae biomass was measured with a UV/Visible spectrophotometer (UNICO7200, USA) at 730 nm (OD_{730}). When necessary, the sample was diluted to give an absorbance in the range of 0.1–1.0. The relationship between optical density and biomass concentration of *S. dimorphus* could be obtained as following:

$$Y = 0.4209X - 0.1189 (R^2 = 0.9987) \quad (1)$$

where Y refers to the biomass concentration (g L^{-1}) and X refers to the optical density (OD_{730}).

The concentration of total nitrogen concentration in the medium was determined by the method of alkaline potassium persulfate digestion method (Yang et al., 2005).

2.5. Determination of CO_2 utilization efficiency

The initial biomass concentration of inoculum and maximum biomass concentration achieved in the PBRs were designated as X_0 and X_{max} (g L^{-1}), respectively. The biomass concentration ΔX (g L^{-1}) over cultivation time of Δt was calculated as $\Delta X = X_{max} - X_0$. The overall biomass productivity $P_{overall}$ (g L^{-1} d^{-1}) was calculated using Eq. (2):

$$P_{overall} = \frac{\Delta X}{\Delta t} \quad (2)$$

Thus the CO_2 fixation rate F_C (g CO_2 L^{-1} d^{-1}) was calculated according to Eq. (3):

$$F_c = \frac{P_{overall} \times 50\%}{12} \times 44 \quad (3)$$

where 50% is designated as carbon content of microalgae dry biomass; 12 (g/mol) and 44 (g/mol) present the molecular weights of carbon and CO_2, respectively.

The total CO_2 amount injected into the culture medium included all the CO_2 introduced into the bubbling column, including CO_2 in mixed gas (pure CO_2-air or flue gas) and air for stirring. For each pH feedback control experiment with flue gas, the aeration time and total time per cycle each day were measured, and the total CO_2 amount from the flue gas could be calculated (M_{c1}, g); the compressed house air (0.038% CO_2) was continuously injected (0.25 vvm) to keep the culture medium mixed, so the total CO_2 from the air could be obtained (M_{c2}, g). The CO_2 utilization efficiency E_C (%) was derived from Eq. (4):

$$E_c = \frac{F_c}{M_{c1} + M_{c2}} \times 100\% \quad (4)$$

3. Results and discussion

3.1. Effect of CO_2 concentration on microalgae growth

Industrial exhaust gases such as flue gases contain 10–20% CO_2, providing a CO_2-rich source for microalgae cultivation and a potentially more efficient route for CO_2 bio-fixation (Wang et al., 2008). To demonstrate the tolerance of *S. dimorphus* for high CO_2 concentration, growth experiments were carried out under CO_2 concentrations of 0.038%, 2%, 10%, 20% CO_2 (v/v). The PBR was a 300-ml bubble column (30 mm in diameter) with a 200-ml working volume. The initial inoculum concentration was about 0.2 g L^{-1} and the pH was unregulated (7.0 ± 0.2). As shown in Fig. 1, growth of *S. dimorphus* sparged with 0.038% CO_2 was slow, while the cells grew well with CO_2 concentrations ranging from 2% to 20%. The pH changes of culture media with 2% to 20% CO_2 tended to be stable at around 6–8 after 1 day of cultivation. In the culture aerated

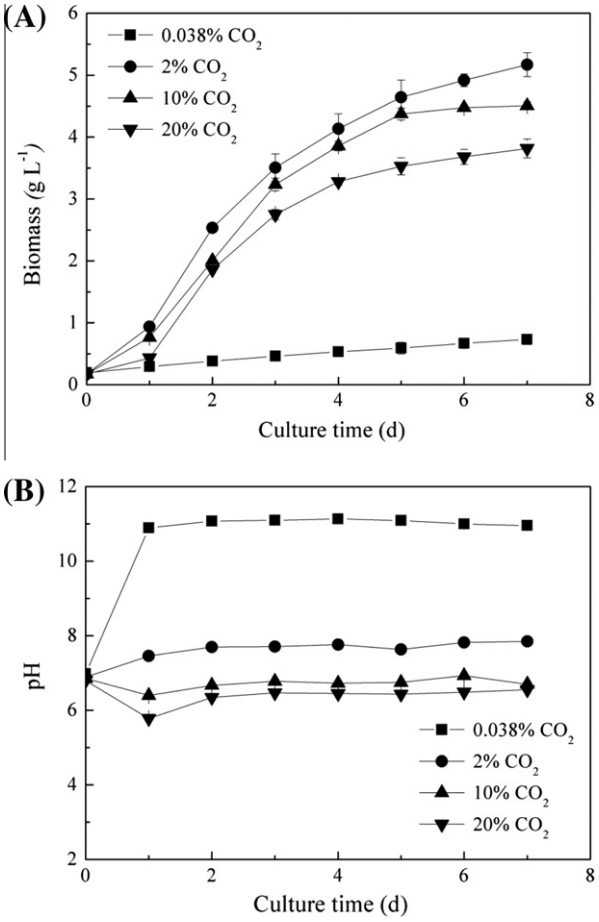

Fig. 1. Growth (A) and pH (B) of cultures of *S. dimorphus* sparged with 0.038%, 2%, 10%, or 20% CO_2 (column diameter = 30 mm). Values are means ± SD of three repeated experiments.

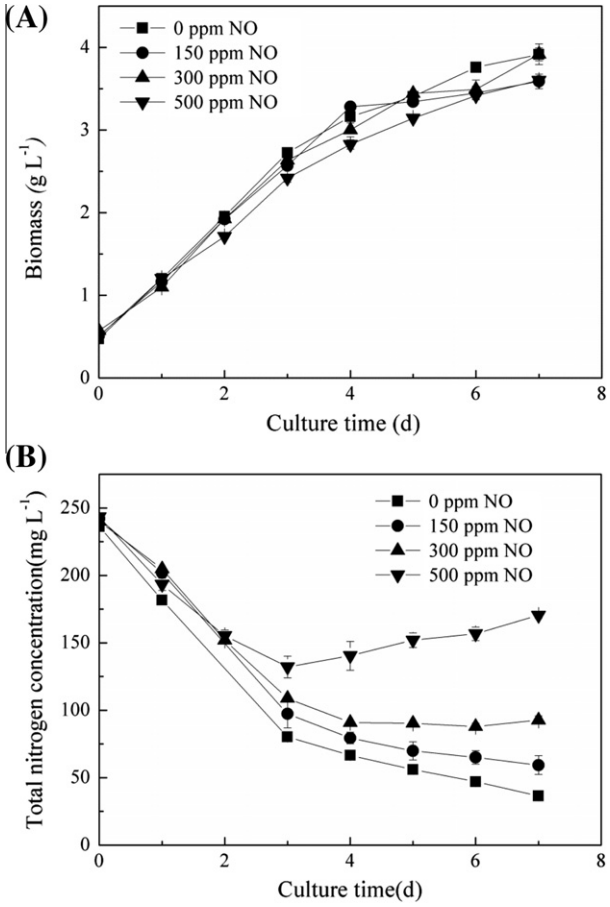

Fig. 2. Growth of *S. dimorphus* (A) and total nitrogen concentration (B) in medium supplied with 0, 100, 150, 300, 500 ppm NO (column diameter = 50 mm). Values are means ± SD of three repeated experiments.

with house air, the pH value increased from 7 to about 11 within one day. It appears that the concentration of 0.038% CO_2 in house air was too low to neutralize alkaline substances produced by the micro-algae and carbon limitation may also have occurred.

Chiu et al. reported that *Chlorella* sp. (Chiu et al., 2008) and *Nannochloropsis oculata* (Chiu et al., 2009) showed optimal growth potential with 2% CO_2, while growths of both algal strains were completed inhibited at 5–15% CO_2. In the present study, *S. dimorphus* grew well with 2–20% CO_2, and the maximum biomass concentration of 5.17 g $L^{-1}$was observed with 2% CO_2. *S. dimorphus* exhibited a longer lag phase when sparged with 10 and 20% CO_2, and the biomass concentrations after 7 days of cultivation were 4.51 and 3.82 g L^{-1}, respectively. Tang et al. (2010) reported that *Scenedesmus obliquus* SJTU-3 and *Chlorella pyrenoidosa* SJTU-2 grew well at CO_2 concentrations ranging from 5% to 30%, and the biomass concentrations of *S. obliquus* SJTU-3 and *C. pyrenoidosa* SJTU-2 at 20% CO_2 after 14 days of cultivation were 1.65 g L^{-1} and 1.22 g L^{-1},respectively, which were much lower than that of *S. dimorphus* in our study. For most flue gas sources, the CO_2 concentrations were generally below 20% (Lee et al., 2002; Westerhoff et al., 2010), under which *S. dimorphus* screened in our study showed excellent growth potential.

3.2. Effects of NO and SO_2 concentration on microalgae growth

Effects of different NO concentrations on *S. dimorphus* were showed in Fig. 2A. *S. dimorphus* grew well at NO concentrations of 150–500 ppm, similarly as with control cultures with 2% CO_2 alone. Changes of total nitrogen concentrations in the medium during microalgae growth were showed in Fig. 2B. The results showed that at higher NO concentration, the consumption rate of total nitrogen concentration was slower, indicating that NO might be used as nitrogen source for algal cells (Kumar et al., 2010).

As shown in Fig. 3A, *S. dimorphus* only grew well when SO_2 concentrations were lower than 100 ppm. When the SO_2 concentration exceeded 100 ppm, the pH of culture medium dropped to 3 within one day (Fig. 3B). Experiments were then conducted with addition of sodium sulfite and sodium bisulfite, respectively, instead of gaseous SO_2. As shown in Fig. 4,*S. dimorphus* could grow well at sulfite concentrations of 0–60 mmol/L, while the maximum bisulfite concentration *S. dimorphus* could tolerate was only 1.0 mmol/L. Similar results were reported by Yang et al. (2004), bisulfite was toxic to *Botryococcus braunii* when the concentration was above 1.0 mmol/L. Wodzinski et al. (1978) reported that the toxicity of bisulfite–sulfite increased as the pH decreased, and as the pH decreased from 7.7 to 6.0, the bisulfite–sulfite ratio changed from 0.16:1 to 8.1:1. At the extremely low pH caused by large amount of dissolved SO_2 gas, there was a higher proportion of bisulfite than that of sulfite in the solution. Therefore, limiting the accumulation of bisulfite in solution is essential to prevent growth inhibition on microalgae.

Negoro et al. (1991) reported that growth of *Nannochloris* sp. ($NANNO_2$) and *Nannochloropsis* sp. (NANNP2) was not influenced by SO_2 at a concentration of 50 ppm, while growth ceased after 20 h cultivation when the SO_2 concentration was increased to 400 ppm. Lee et al. (2002) reported that growth of *Chlorella* KR-1

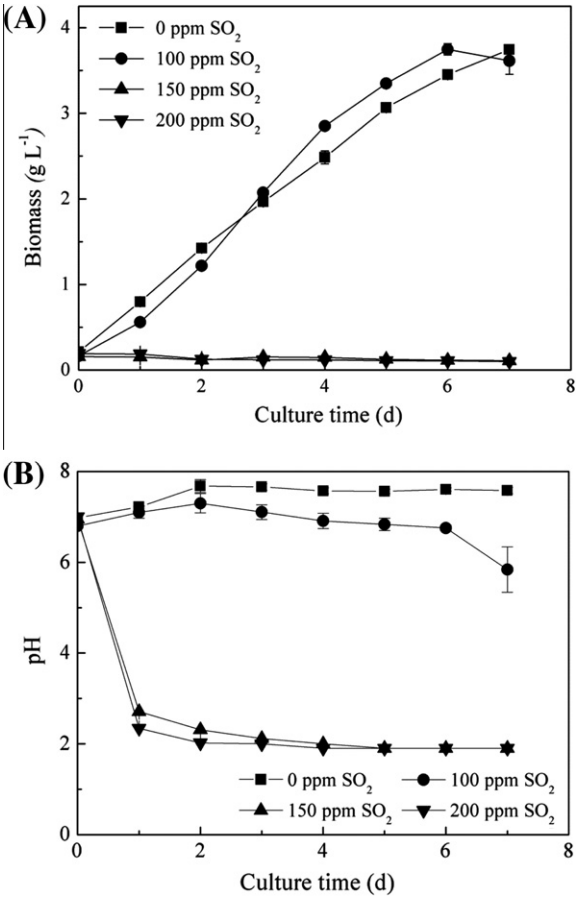

Fig. 3. Growth (A) and pH (B) of cultures of *S. dimorphus* supplied with 0, 100, 150, 200 ppm SO_2 (column diameter = 50 mm). Values are means ± SD of three repeated experiments.

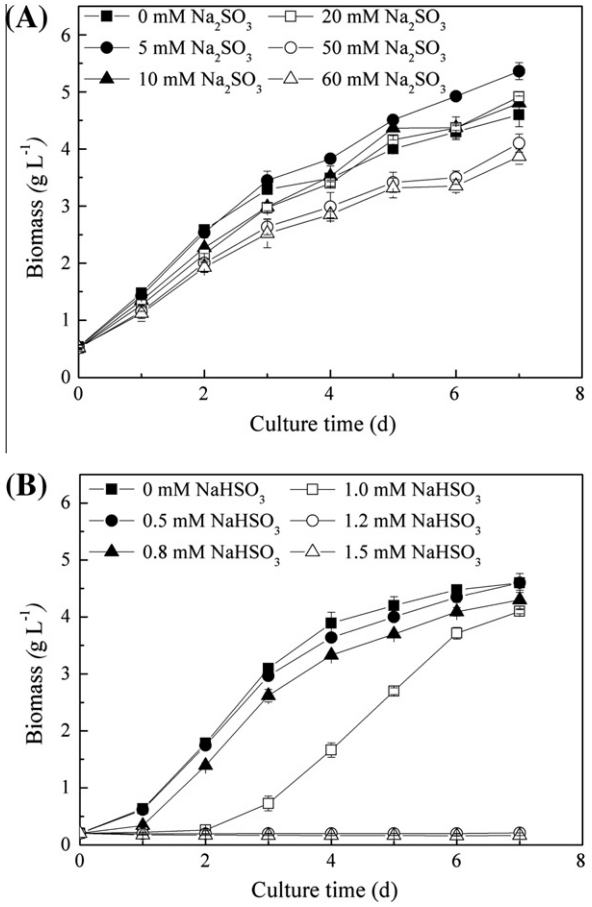

Fig. 4. Effects of Na_2SO_3 (A) and $NaHSO_3$ (B) on growth of *S. dimorphus* supplied with 2% CO_2 (column diameter = 30 mm). Values are means ± SD of three repeated experiments.

was totally inhibited by 150 ppm SO_2.Westerhoff et al. (2010) reported that *Scenedesmus*, *Chlorella* or a mixture of the two cultures died almost immediately upon gaseous addition of 313 ppm SO_2 in 20% CO_2. Nitrous oxides were also present in the flue gas. When the NO concentration was 300 ppm, NANNP2 did not grow and NANNO2 grew only after a prolonged lag period (Negoro et al., 1991), and growth of *Chlorella* KR-1 was also completely suppressed (Lee et al., 2002). In the present study, the maximum NO and SO_2 concentrations *S. dimorphus* could tolerant were 500 ppm and 100 ppm, respectively, which are much higher than those reported in previous studies (Lee et al., 2002; Negoro et al., 1991; Westerhoff et al., 2010). However, considering that typical industrial flue gases usually contain 100–300 ppm SO_X and NO_X, it is necessary to develop strategies to overcome the adverse effects of flue gas on microalgae growth.

3.3. Methods to overcome the toxic effect of flue gas on S. dimorphus

3.3.1. Neutralization of the medium with $CaCO_3$

Though complete removal of SO_X and NO_X in flue gas by washing with an alkaline solution prior to injecting into the culture medium would be feasible, the consumption and recycling of absorbing agents would result in high costs. Inspired by the process of wet limestone flue gas desulfurization, adding $CaCO_3$ to culture medium was tested as a means of neutralizing the acidification caused by SO_2 in flue gas. For each column with 700 ml culture broth, 3.15 g $CaCO_3$ was added. The amount of $CaCO_3$ was determined by the stoichiometric ratio of 1:1 with the entire SO_2 amount in flue gas for 7 d cultivation. Upon addition of $CaCO_3$, the pH of media sparged with simulated flue gas stabilized at above 6 and the cells grew very well (Fig. 5). Interestingly, with $CaCO_3$ addition, the biomass concentration of *S. dimorphus* with flue gas was 3.20 g L^{-1}, 27% higher than that of the control culture with pure CO_2-air gas sparging. Meada et al. (1995) found that the pH could be maintained neutral with $CaCO_3$ addition when cultivating *Chlorella* sp. T-1 with flue gas containing 50 or 80 ppm SO_2; however, the aeration time was only 70 h. Limestone powder, whose main component is $CaCO_3$ can be adopted instead of pure $CaCO_3$ because of its rich source and lower price. Putt et al. (2011) observed a low CO_2 utilization efficiency when CO_2-enriched air was continuously supplied into algae ponds; however, with measures to increase the CO_2 utilization efficiency, such as decreasing the aeration rate and CO_2 concentration (Ryu et al., 2008), intermittent aeration (Chiu et al., 2011), the required stoichiometric amount of $CaCO_3$ to neutralize the SO_2 would be greatly reduced. The produced $CaSO_4$ precipitate could be easily separated by centrifugation, with little influence on the growth of algal cells and the quality of microalgae product.

3.3.2. Intermittent sparging of flue gas by pH feedback

Since *Scenedesmus* sp. prefers a slightly alkaline medium (Hodaifa et al., 2009), three pH ranges were adopted in this study: pH 8.0–8.5, pH 8.5–9.0 and pH 9.0–9.5. Growth of *S. dimorphus* at different pH regimes was compared with control cultures continuously sparged with simulated flue gas or 15% CO_2. Fig. 6A shows the pH variations of *S. dimorphus* cultures supplied with different

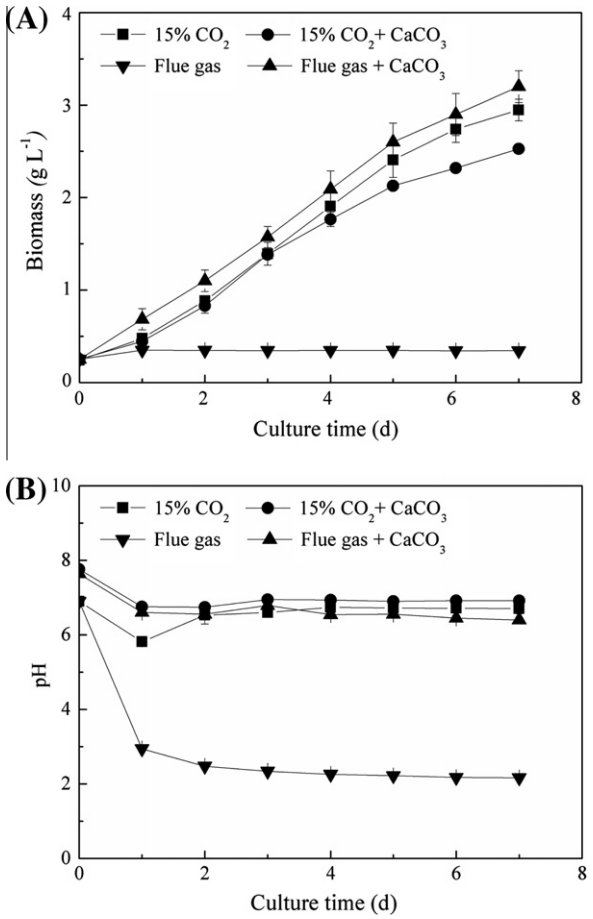

Fig. 5. Biomass concentration (A) and pH (B) of cultures of *S. dimorphus* exposed to simulated flue gas (15% CO_2, 400 ppm SO_2, 300 ppm NO, balance N_2) with or without $CaCO_3$ addition (column diameter = 50 mm). Values are means ± SD of three repeated experiments.

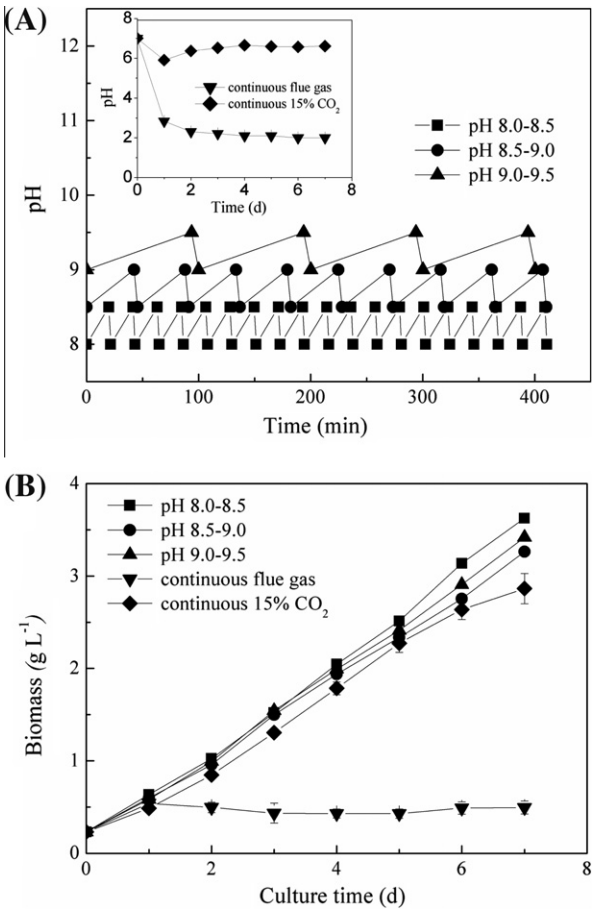

Fig. 6. (A) pH of culture medium under continuous and intermittent sparging with simulated flue gas on day 7. (B) Growth of *S. dimorphus* under different culture conditions (flue gas composition: 15% CO_2, 400 ppm SO_2, 300 ppm NO, balance N_2; column diameter = 50 mm).Values are means ± SD of three repeated experiments.

Table 1
Comparison of growth and CO_2 utilization of *S. dimorphus* supplied with different gas mixtures (flue gas composition: 15% CO_2, 400 ppm SO_2, 300 ppm NO, balance N_2; column diameter = 50 mm). Values are means ± SD of three repeated experiments.

Aeration modes		X_{max} (g L^{-1})	$P_{overall}$ (g L^{-1} d^{-1})	F_C (g CO_2 L^{-1} d^{-1})	E_C (%)
Intermittent sparging of flue gas by pH feedback	pH 8.0–8.5	3.63	0.485	0.889	75.61*
	pH 8.5–9.0	3.36	0.448	0.821	65.29*
	pH 9.0–9.5	3.42	0.455	0.835	67.71*
Continuous aeration	15% CO_2	2.77	0.362	0.664	1.60
	Flue gas	0.49	0.037	0.068	0.17

* The total carbon supplied was calculated from the aerated CO_2 amount from both flue gas and compressed air for stirring.

gas mixtures. The pH value with continuous 15% CO_2 stabilized at around 6.5, while the pH with continuous flue gas dropped to around 3 after 1 d of cultivation. For the three pH-controlled experiments, the fastest sparging frequency was observed when the feedback pH was set at pH 8.0–8.5, and the slowest sparging frequency was at pH 9.0–9.5. In the pH range of 8.0–9.5, the main carbon form in solution is HCO_3^-. At a higher pH, the culture has a higher buffering capacity which could explain the low sparging frequency at pH range 9.0–9.5. All the pH feedback-controlled cultures of pH controlling achieved higher growth rates than those with continuous 15% CO_2 sparging (Fig. 6B). As shown in Table 1, the maximum biomass concentration, CO_2 fixation rate and CO_2 utilization efficiency of 3.63 g L^{-1}, 0.889 g d^{-1} and 75.61%, respectively, were obtained at pH 8.0–8.5, while the corresponding values were only 2.77 g L^{-1}, 0.664 g d^{-1} and 1.6% with 15% CO_2 sparging. Bao et al. (2012) reported that, in the cultivation of *Spirulina platensis* with CO_2 aeration by pH feedback, the pH was maintained at the range of 9.7–9.9, and the maximum biomass concentration and average CO_2 utilization efficiency were 0.84 g L^{-1} and 79.18%, respectively. Currently, the major drawback of using CO_2 for large-scale microalgae cultivation is the low CO_2 utilization efficiency. It has been reported that 80–90% of the CO_2 would eventually be lost to the atmosphere (Putt et al., 2011), which makes microalgae production costly. In the present study, the strategy of intermittent sparging of flue gas by pH feedback not only relieved the acidic inhibition of flue gas on microalgae but also enhanced the CO_2 utilization efficiency.

4. Conclusions

In this work, *S. dimorphus* showed excellent tolerance to flue gas containing high concentrations of CO_2 (2–20%) and NO (150–500 ppm), and the maximum SO_2 concentration *S. dimorphus* could tolerant was 100 ppm. The extremely low pH as well as the accumulation of bisulfite caused by SO_2 inhibited algae growth. Upon addition of $CaCO_3$ and intermittent sparging of flue gas controlled by pH feedback, the inhibition of flue gas on *S. dimorphus* could be well overcome and algal cells grew well (>3 g L^{-1}), indicating that these two methods have great potential when using flue gas for microalgae cultivation.

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