Bioresource Technology 128 (2013) 359–364

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

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.

ARTICLE INFO

Article history:
Received 27 April 2012
Received in revised form 3 September 2012
Accepted 8 October 2012
Available online 2 November 2012

Keywords:
Flue gas
$CaCO_3$ addition
Intermittent sparging
Scenedesmus dimorphus

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.

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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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http://dx.doi.org/10.1016/j.biortech.2012.10.119

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

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