RSC Advances

PAPER

View Article Online
View Journal | View Issue

Cite this: *RSC Adv.*, 2014, **4**, 42147

The oxidation product (NO_3^-) of NO pollutant in flue gas used as a nitrogen source to improve microalgal biomass production and CO_2 fixation†

Jun Cheng,* Yun Huang, Hongxiang Lu, Rui Huang, Junhu Zhou and Kefa Cen

Received 9th June 2014
Accepted 29th August 2014

DOI: 10.1039/c4ra05491a

www.rsc.org/advances

In order to eliminate the inhibition effect of the toxic nitric oxide (NO) in flue gas on microalgal growth and CO_2 fixation, NO was converted by a wet UV/H_2O_2 method to produce nitrate (NO_3^-), which then be used as a nitrogen source for microalgae to improve its growth. The growth ability and biomass compositions of the microalgae cultivated with the produced NO_3^- from NO gas were similar to those of the microalgae cultivated with equivalent moles of commercial $NaNO_3$. The NO_3^- concentration produced from NO increased with UV lamp power, H_2O_2, and NO concentrations, resulting in an improved microalgal growth. The concentration of NO_3^- from 500 ppm NO wet-oxidized by 6% (v/v) H_2O_2 and 55 W UV light was up to 8.8 mM. When the produced nitrate was used as supplementary nitrogen source, the maximum growth productivity of *Chlorella* PY-ZU1 at 15% (v/v) CO_2 reached 1.18 g L^{-1} per day (0.97 times higher than that cultivated with the standard medium). The peak fixation efficiency of 15% (v/v) CO_2 was 69.6% (1.13 times higher than that cultivated with the standard medium).

1. Introduction

Pollutants (including CO_2, NO_x, SO_2, and fine particles) are released into the atmosphere when fossil fuels are burned. As a result, environment and human health are seriously harmed. For example, the greenhouse effect occurs because of excessive CO_2 concentrations in the atmosphere, and this condition has caused problems in terms of environmental and energy aspects. Thus, CO_2 emissions should be reduced using efficient and economical methods. For microalgae has a higher growth rate (1 to 3-fold increases in biomass per day), and can fix CO_2 with efficiency (2–10%) ten times greater than that of terrestrial plants (<1%), one of the efficient CO_2 reduction methods involves the cultivation of microalgae in photobioreactors supplied with CO_2-enriched gas streams, such as those emitted from coal-fired power plant flue gases.[1–4] In addition, the CO_2 capture process using microalgae has the following advantages: (i) co-producing high value materials based on biomass, such as biofuel and biogas;[5–10] (ii) being an environmental sustainable method that can be connected to urban and industrial sewage cleaning.[11]

Some high CO_2-tolerant microalgae species have been isolated out.[12–16] However the inhibitory effects of toxic compounds, such as NO_x and SO_2, in addition to high CO_2 concentrations, on microalgae can be critical.[17–21] It was reported that NO in fossil fuel flue gas can be removed and used by the microalgae, *Dunaliella tertiolecta*.[22] However, for almost all of the other microalgal species, the presence of NO will lead to the formation of toxic nitrites or pH decrease in their culture, therefore, it will hinder their growth and CO_2 fixation.[17–21,23,24]

In recent years, some studies have focused on the alleviation of the effect of NO on microalgae growth. These studies have shown that the growth and survival of *Synechococcus* sp. and *Chlorella* sp. have improved against exposure to intermittent NO_2 by adding growth stimulators, such as triacontanol and sodium bicarbonate.[25] The tolerance of *Chlorella* KR-1 to continuous NO exposure can be enhanced by maintaining the pH of the culture media at an adequate value (~7), which is achieved by adding an alkaline solution (NaOH).[19] However, this condition can be effective for some specific microalgae only. A previous study also showed that the presence of NO may lead to the formation of toxic nitrites in microalgae culture, therefore, its inhibitory effect on microalgae growth was evaluated.[24] It must take some techniques making NO dissolve into less NO_2^- but to more usable substances, such as NO_3^-.

Advanced oxidation process (AOP) can produce free radicals with strong oxidation, such as hydroxyl free radicals (·OH). By a wet AOP using hydrogen peroxide solution with ultraviolet lamp (UV/H_2O_2), the toxic NO was completely converted into valuable NO_3^- without generating any other byproduct.[26–29] The wet AOP (UV/H_2O_2) has been used in coal-fired power plants to simultaneously remove NO, SO_2 and Hg pollutants in flue gas. But how to deal with and reutilize the large amount of byproducts (nitrate, sulfate and Hg^{2+}) is a big problem. Whether the

State Key Laboratory of Clean Energy Utilization, Zhejiang University, Hangzhou 310027, China. E-mail: juncheng@zju.edu.cn; Fax: +86 571 87951616; Tel: +86 571 87952889

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c4ra05491a

oxidation byproduct (NO_3^-) derived from the wet AOP can be consumed and used by microalgae has not been reported in literatures till now. Whether the different oxidation conditions (UV lamp power, H_2O_2 and NO concentrations) in wet AOP (UV/H_2O_2) have important effects on microalgae growth has not been clarified. It was first proposed to reutilize the oxidation byproduct (NO_3^-) derived from the wet AOP by microalgae as a supplementary nitrogen source in this paper. This novel process not only eliminated the effect of toxic NO on microalgal growth but also improved microalgal biomass productivity and CO_2 fixation. The effects of different UV/H_2O_2 conditions on microalgal growth and CO_2 fixation efficiency were investigated.

2. Materials and methods

2.1 Strains and media

Chlorella PY-ZU1, a highly CO_2-tolerant and fast-growing microalgal species, was used in this study. This strain was obtained by γ irradiation and high concentrations of CO_2 domesticated from *Chlorella pyrenoidosa*.[15] The cells were maintained in Brostol's solution (also known as soil extract, SE),[15,30] containing 0.25 g of $NaNO_3$, 0.075 g of $K_2HPO_4 \cdot 3H_2O$, 0.075 g of $MgSO_4 \cdot 7H_2O$, 0.025 g of $CaCl_2 \cdot 2H_2O$, 0.175 g of KH_2PO_4, 0.025 g of NaCl, 40 mL of soil extract, 0.005 g of $FeCl_3 \cdot 6H_2O$, 1 mL of Fe–EDTA, and 1 mL of A5 solution in 958 mL of de-ionized water.

2.2 System design by which the oxidation product of NO in flue gas with UV/H_2O_2 is used as a nitrogen source for microalgal growth

Because of its strong oxidation ability and environmentally friendly characteristics, UV/H_2O_2 AOP has a wide range of studies in the gas purification field. Experimental system in which the NO in flue gas was converted to NO_3^- as nitrogen source for microalgal growth was performed in a bubble column reactor (Fig. 1). The proposed system comprised the following: (1) 3000 ppm of NO and pure N_2 (used as balance gas); (2) mass flow meter; (3) a bubble column reactor (height of 450 mm and inner diameter of 75 mm); (4) cooling water cycle system; (5) sand chip gas distributor (outer diameter of 45 mm, height of 30 mm, and average pore size of 0.105 mm to 0.18 mm); (6) UV lamps (UV lamp powers were changed by replacing and using three sets of UV lamps with different powers (36 W, 55 W, and 75 W, Haining Light Factory). All the lamps were of the same model (L-L) and of the same wavelength of 253.7 nm); and (7) effluent NO scrubber (the residual NO in the mixed gas was further scrubbed using 400 mL mixed solution containing $KMnO_4$ (0.05 mol L^{-1}) and NaOH (0.1 mol L^{-1}; Sinopharm Chemical Reagent, China) to avoid environmental pollution).

The prepared H_2O_2 solution with the required concentration (1%, 3%, 6%, and 9%) was placed in the bubble column reactor. Temperature was maintained at 25 °C by recycling the cooling water. NO concentration (75, 150, 300, and 500 ppm, balanced with N_2) was regulated using a mass flow meter (SevenstarCS200, China). The NO gas passed uniformly across the sand chip gas distributor into the H_2O_2 solution at a rate of 600 mL min^{-1}. After the UV lamp was turned on, H_2O_2 was released, forming hydroxyl free radicals (·OH). These free radicals exhibit an extremely strong oxidation ability that can convert NO into HNO_3 without generating any other byproduct *via* the following reactions (2)–(3).[26,31]

$$H_2O_2 + h\nu \rightarrow 2{\cdot}OH \quad (1)$$

$$NO + {\cdot}OH \rightarrow HNO_2,\ HNO_2 + {\cdot}OH \rightarrow HNO_3 + {\cdot}H \quad (2)$$

$$NO + {\cdot}OH \rightarrow {\cdot}H,\ NO_2 + {\cdot}OH \rightarrow HNO_3 \quad (3)$$

The reaction solution was collected after 6 h and the remaining H_2O_2 was removed by ultrasonic wave (SK5210HP, China). The solution was then used to make the medium for *Chlorella* PY-ZU1 by adding the same quantities of nutrients as those present in the SE medium. The initial pH of the medium

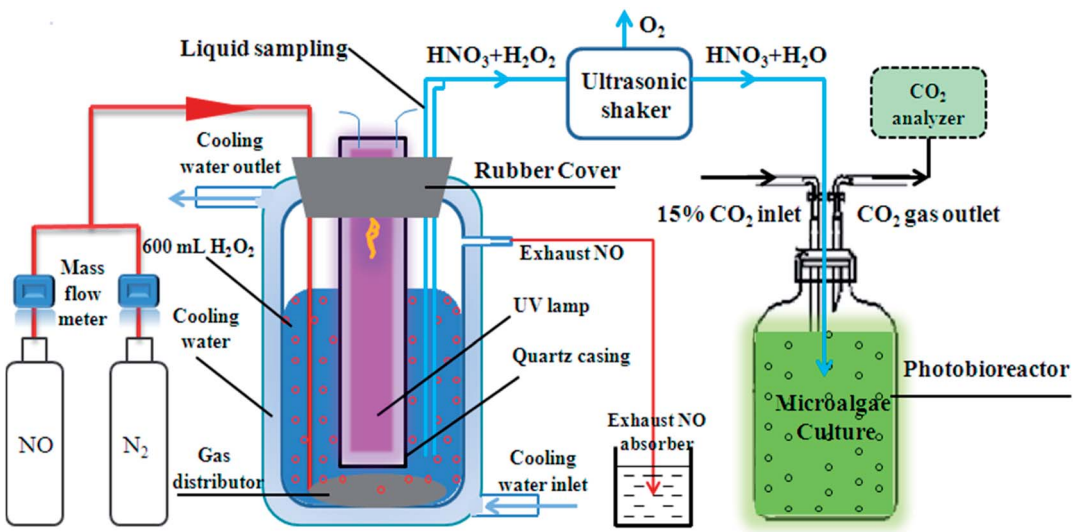


Fig. 1 Experimental system in which the NO in flue gas was converted to NO_3^- as nitrogen source for microalgal growth.

was adjusted to 6.5 with 0.1 M NaOH. The SE medium was used as the control condition. For the final AOP runs, NO in the reactor was 500 ppm, H_2O_2 concentration was 6% (v/v), and UV power was 55 W. The medium prepared with the 15 h oxidation solution was used as the CO_2 fixation medium and labeled as SE#.

2.3 NO_3^- produced from NO oxidation used as supplement nitrogen source to improve *Chlorella* PY-ZU1 growth and CO_2 fixation

All of the cultivation experiments were performed in an artificial greenhouse at 27 °C. Approximately 270 mL SE medium was inoculated with 30 mL of *Chlorella* PY-ZU1 pre-culture in the bioreactor (BR, 160 mm × Φ56 mm, 300 mL of working volume). For the verification experiments of using NO_3^- (derived from NO oxidation by UV/H_2O_2) as a nitrogen source for *Chlorella* PY-ZU1, continuous light of 52 μmol m^{-2} s^{-1} at the surface of BR was supplied by four cool white lights combined with two plant lights (Philips, TLD 36W) that were fixed above the BR. For the other experiments in this study, 68 μmol m^{-2} s^{-1} of light was supplied by six cool white lights (Philips, TLD 36W) at the surface of BR. The mixed gas of 15% (v/v) CO_2 containing different NO concentrations was bubbled at a rate of 30 mL min^{-1} through a long steel pipe (180 mm × Φ3 mm). The NO concentrations were controlled at 0, 75, 150, 300, and 500 ppm by a mass flow meter (Sevenstar CS200, China).

Chlorella PY-ZU1 was cultured in SE# and aerated continuously with 15% (v/v) CO_2 in nine-stage sequential bioreactors[30] to investigate the effect of NO_3^- produced from NO on CO_2 fixation. For comparison, *Chlorella* PY-ZU1 was cultured with SE medium and aerated continuously with 15% (v/v) CO_2 or with 15% (v/v) CO_2 gas containing 500 ppm NO. The influent and effluent CO_2 concentrations were monitored online by a CO_2 analyzer (Servomex4100, UK). CO_2 fixation efficiency was calculated according to the carbon dioxide difference between influent and effluent as described in a previous study.[30]

$$CO_2\text{fixation efficiency} = \left(1 - \frac{\text{total output } CO_2}{\text{total input } CO_2}\right) \times 100\% \quad (4)$$

where the total input CO_2 = influent CO_2 concentration × influent flow rate, and the total output CO_2 = effluent CO_2 concentration × effluent flow rate.

2.4 Analysis of microalgal productivity and biomass compositions

During cultivation, 10 mL of the samples was dewatered by centrifugation (Beckman Avanti J26-XP, USA) at 8500 rpm for 10 min and dried at 70 °C for 24 h to obtain the weight of the dried biomass. Biomass concentration (g L^{-1}) was calculated from the microalgal dry weight produced per liter. Growth productivity (AGP, g L^{-1} per day) was calculated using eqn (5):

$$\text{AGP} = \frac{M_1 - M_2}{t_1 - t_2} \quad (5)$$

where M_1 is the biomass concentration at time t_1 and M_2 is the biomass concentration at time t_2. Total carbohydrate quantity was determined using the anthrone method (with glucose as the standard).[8] The lipid of the biomass was extracted as described in a previous study.[6] Fatty acid compositions were determined by gas chromatography (Agilent 7890A, USA).

2.5 Calculation of NO oxidation efficiency and residual NO concentration

The NO_3^- concentrations in the collected solution as prepared in Section 2.2 were analyzed with ion chromatography (MagIC, Metrohm, Switzerland). The NO oxidation efficiency (mean value) was calculated according to NO_3^- in the solution using eqn (6):

$$\text{NO oxidation efficiency} = \frac{M_{NO_3^-} \times V}{\sum M_{NO_{in}}} \quad (6)$$

where $M_{NO_3^-}$ is the molar concentration of NO_3^- in volume V (L) of the oxidized solution and $\sum M_{NO_{in}}$ is the total number of moles of NO flowing into the oxidation reactor. In this study, NO_3^- was the only product of NO oxidation; thus, NO oxidation efficiency also corresponded to NO_3^- production efficiency. The remaining NO concentration (mean value) was calculated using eqn (7):

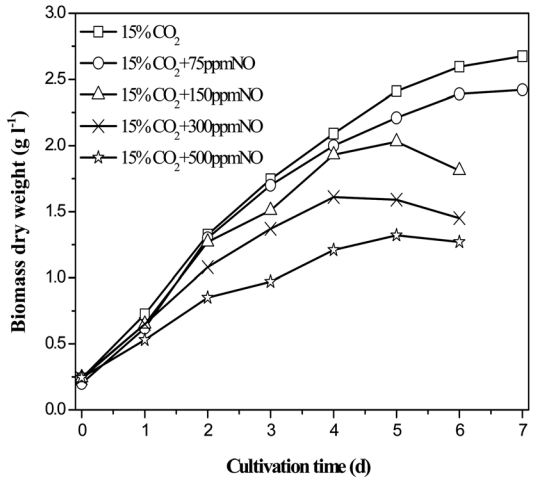
$$C_{NO_{out}} = C_{NO_{in}} \times (1 - \text{NO oxidation efficiency} \quad (7)$$

3. Results and discussion

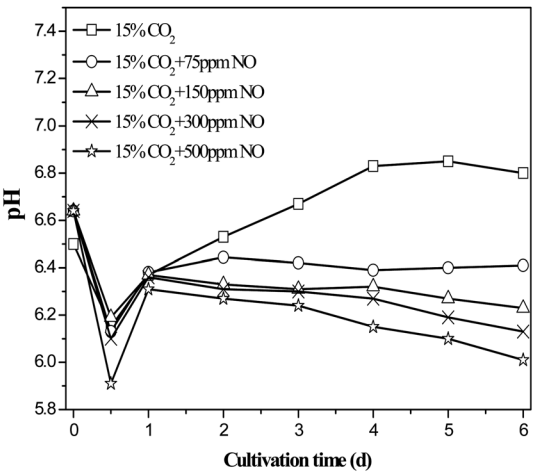
3.1 Effects of NO on the growth of *Chlorella* PY-ZU1

The effects of NO concentrations on the growth of *Chlorella* PY-ZU1 and the pH of the culture were examined in the BR (Fig. 2). *Chlorella* PY-ZU1 showed a higher tolerance to NO than other NO-tolerant algal strains, which could not grow under 150 ppm NO.[20] When aerated with 15% CO_2 gas containing 150 ppm NO, biomass concentration of *Chlorella* PY-ZU1 decreased after 5 days of cultivation, and the pH of culture decreased to 6.27. The maximum biomass concentration was 2.03 g L^{-1} and decreased by 24.3% to that of microalgae cultivated without NO aeration (2.68 g L^{-1}). When NO concentration was further increased to 500 ppm, microalgae could grow but with a 50.7% decrease in the maximum biomass concentration to that of microalgae cultivated without NO. The decrease in biomass yield was due to pH decrease in the culture caused by NO aeration.[19,20] The pH of the culture decreased with the increasing cultivation time. Once the pH of the culture decreased beyond the adequate range (6.5–7.5 for *Chlorella*), the microalgae growth was inhibited. This was why the biomass concentration of *Chlorella* PY-ZU1 decreased after 5 days cultivation with >150 ppm of NO. However, *Chlorella* PY-ZU1 showed a higher tolerance to NO than *Chlorella* KR-1,[20] whose growth was completely suppressed when aerated with 15% CO_2 gas containing 300 ppm NO. This verified that microalgae tolerance to NO depends on the microalgae species but with a decrease in biomass productivity.[19]

Some methods were used to alleviate microalgae growth inhibition caused by NO, such as controlling culture pH and adding some growth stimulators to culture.[25] Although

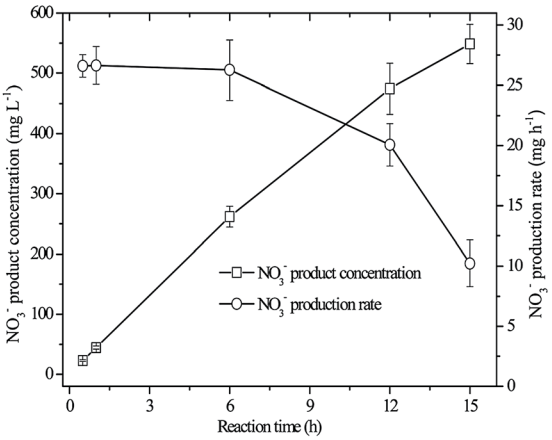


(a) Effects on biomass dry weight

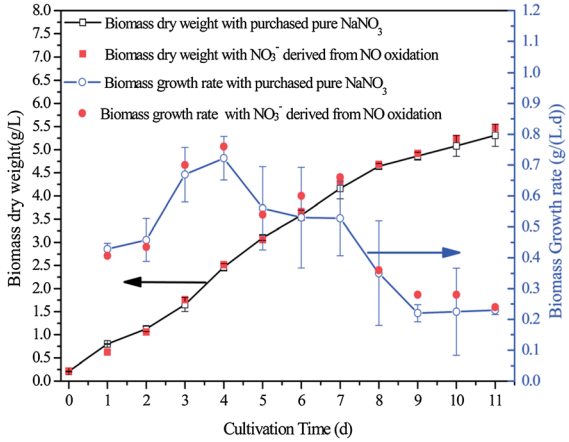


(b) Effects on pH value.

Fig. 2 Effects of NO on *Chlorella* PY-ZU1 growth and pH of the cultures.



(a) NO_3^- production from NO oxidation with UV/H_2O_2



(b) Microalgal growth in SE medium with derived NO_3^- from NO and commercial $NaNO_3$

Fig. 3 Microalgal growth with NO_3^- derived from NO oxidation and commercial $NaNO_3$.

Dunaliella tertiolecta could use NO dissolved in microalgae culture as a nitrogen source, NO absorbed in the medium could be converted to NO_2^- and then oxidized to NO_3^-.[22] This oxidation process was extremely slow. The improvement effect of little NO_3^- produced from NO on *Chlorella* PY-ZU1 did not overcome the toxic effect of NO. Thus, a much faster NO oxidation method will be needed.

3.2 Confirmation of using NO_3^- (derived from NO oxidation by UV/H_2O_2) as a nitrogen source for *Chlorella* PY-ZU1

During UV/H_2O_2 AOP process, the remaining H_2O_2 concentration in the solution was decreased with the oxidation time, resulting in a decrease in NO_3^- production efficiency.[26] In the process of 500 ppm NO oxidized by 55 W UV/6% H_2O_2, the NO_3^- production rate was stabilized at 0.427 mM h^{-1} and 53% of NO was converted into NO_3^- in the first 6 h [Fig. 3(a)]. In the next 6 h, the NO_3^- production rate gradually decreased to 10.65% with H_2O_2 digestion. After 15 h, NO_3^- concentration in the solution reached to 8.8 mM. The total NO_3^- concentration in the medium prepared with this oxidation solution was 11.8 mM, which could satisfy the NO_3^- requirement of *Chlorella* PY-ZU1 under 15% CO_2.[30] *Chlorella* PY-ZU1 cultivated in the SE# medium under 52 μmol m^{-2} s^{-1} of continuous light and 15% CO_2 for 11 d exhibited a peak growth productivity and maximum biomass concentration of 0.76 g L^{-1} per day and 5.48 g L^{-1}, respectively. These values were almost equal to those of *Chlorella* PY-ZU1 (0.73 g L^{-1} per day and 5.31 g L^{-1}, respectively) cultivated in the SE medium with 11.8 mM commercial $NaNO_3$. In addition, the growth curve of *Chlorella* PY-ZU1 cultivated with NO_3^- produced from NO is consistent with that of the *Chlorella* PY-ZU1 cultivated with commercial $NaNO_3$ [Fig. 3(b)].

The total carbohydrate quantity of the dried biomass of *Chlorella* PY-ZU1 cultivated with NO_3^- produced from NO (41.57%, w/w biomass) was almost equal to that of the *Chlorella* PY-ZU1 cultivated with commercial $NaNO_3$ (43.57%; data not shown). The lipid contents in the two biomasses were 18.11% and 17.92%, respectively. The biodiesel compositions from these two kinds of biomasses were analyzed (Table 1). The fatty

acid profiles indicated the presence of C16: 0, C16: 1, C16: 2, C16: 3, C18: 0, C18: 1, C18: 2, and C18: 3. Palmitic acid, oleic acid, linoleic acid, and linolenic acid were considered as the main components, which ranged from 12% to 24% of the total fatty acids. These results indicated that oxidation product of NO (derived from NO in flue gas by UV/H_2O_2) can be used as a nitrogen source for *Chlorella* PY-ZU1 instead of the commercial $NaNO_3$.

3.3 Effects of different NO conversion conditions on the growth of *Chlorella* PY-ZU1

The NO_3^- concentration produced from NO increased with increase of lamp power, H_2O_2, and NO concentration. As a result, microalgae growth was improved. Under UV light irradiation, H_2O_2 can release $^{\cdot}OH$ free radicals. $^{\cdot}OH$ free radicals exhibit strong oxidation ability to convert NO to NO_3^-.[26,29] A high concentration of produced NO_3^- in AOPs results in a high biomass yield during microalgae cultivation.[30,32]

NO_3^-, the oxidation product derived from 300 ppm NO with 6% H_2O_2 for 6 h, could increase the biomass productivity of *Chlorella* PY-ZU1 under 15% CO_2 as UV lamp power was increased (Fig. 4). The maximum biomass concentration of microalgae was evidently increased from 3.45 g L^{-1} to 3.85 g L^{-1} [Fig. 4(b)] as UV lamp power increased from 36 W to 55 W. However, with further increasing the UV lamp power from 55 to 75 W, the growth rate of maximum biomass concentration gradually decreased. Two main reasons could explain the results. On one hand, under UV light irradiation, H_2O_2 can release $^{\cdot}OH$ free radicals by eqn (1) reaction.[26] The $^{\cdot}OH$ free radicals have extremely strong oxidation ability to convert NO into NO_3^- according to eqn (2) and (3). Therefore, compared with the reaction system without UV light, addition of UV light can greatly enhance NO conversion into NO_3^-. Furthermore, increasing UV lamp power can improve the energy density per unit in solution, thus produce more effective photons and $^{\cdot}OH$ free radicals. Therefore, the NO_3^- produced rate increased with an increase in UV lamp power.[26,31] Consequently, the maximum biomass concentration of *Chlorella* PY-ZU1 was increased. On the other hand, once the power of UV lamp exceeds a certain value, some side reactions, such as eqn (8) and (9), may occur in the solution, leading to a great loss of $^{\cdot}OH$ free radicals.[27] Therefore, a further increase in UV lamp power only has a little impact on NO_3^- production and thus a little effect on the growth of *Chlorella* PY-ZU1.

$$H_2O_2 + {}^{\cdot}OH \rightarrow HO_2^{\cdot} + H_2O \quad (8)$$

$$^{\cdot}OH + {}^{\cdot}OH \rightarrow H_2O_2 \quad (9)$$

Similarly, the NO_3^- production efficiency derived from NO (300 ppm) by UV/H_2O_2 (55 W of UV for 6 h) increased from 56.60% to 79.33% and the derived NO_3^- concentration increased from 2.70 mM to 3.79 mM [Fig. 4(c)] when H_2O_2 concentration increased from 3% to 6%. This finding resulted in an evident increase in the maximum biomass concentration of microalgae from 3.43 g L^{-1} to 3.85 g L^{-1} [Fig. 4(d)]. However, a further increase in H_2O_2 concentration from 6% to 9% did not increase the maximum biomass concentration (stabilized at 3.91 g L^{-1}). This is mainly because appropriate H_2O_2 concentration may cause a reaction such as eqn (1) in the solution. Therefore, within a certain range, the increase in H_2O_2 concentration can improve the yield of NO_3^-,[26] and then increased the biomass growth of *Chlorella* PY-ZU1.[25] Once H_2O_2 concentration exceeding a certain value, any further increase may cause side reactions as eqn (8) and (9) which lead to a decrease in the oxidation ability of free radicals.[27] Therefore, further increase in H_2O_2 concentration only had little effect on the yield of NO_3^- and a slight impact on biomass production of *Chlorella* PY-ZU1.

NO_3^- production efficiency decreased from 91.26% to 53.00% [Fig. 5(a)] as NO concentration increased from 75 ppm to 500 ppm because of the limitation of NO residence time and $^{\cdot}OH$ free radicals.[26,31] However, the derived NO_3^- concentration from NO increased from 1.09 mM to 4.22 mM; thus, the maximum biomass concentration of *Chlorella* PY-ZU1 increased from 3.05 g L^{-1} to 4.15 g L^{-1} [Fig. 5(b)].

3.4 CO_2 fixation by *Chlorella* PY-ZU1 cultivated with NO_3^- derived from NO oxidation

When 500 ppm NO was directly aerated into microalgal culture, biomass production was decreased by 50.7% to that of 2.68 g L^{-1} of microalgae cultivated without aerated NO

Table 1 Compositions of lipids in microalgae cultivated with commercial $NaNO_3$ and NO_3^- derived from NO oxidation

Conditions		Commercial $NaNO_3$	NO_3^- derived from NO oxidation
Lipid content (% of dry biomass)		17.92	18.11
Lipids composition (% of total lipid)	C16: 0	23.85 ± 0.29	22.37 ± 0.10
	C16: 3	7.02 ± 0.34	6.80 ± 0.29
	C18: 0	3.15 ± 0.26	3.17 ± 0.01
	C18: 1	15.88 ± 0.75	14.82 ± 0.76
	C18: 2	15.52 ± 0.83	14.76 ± 0.57
	C18: 3	12.77 ± 0.34	12.65 ± 0.46
	Others (C16–C24)	21.8 ± 0.63	25.4 ± 0.45
	Total	100	100

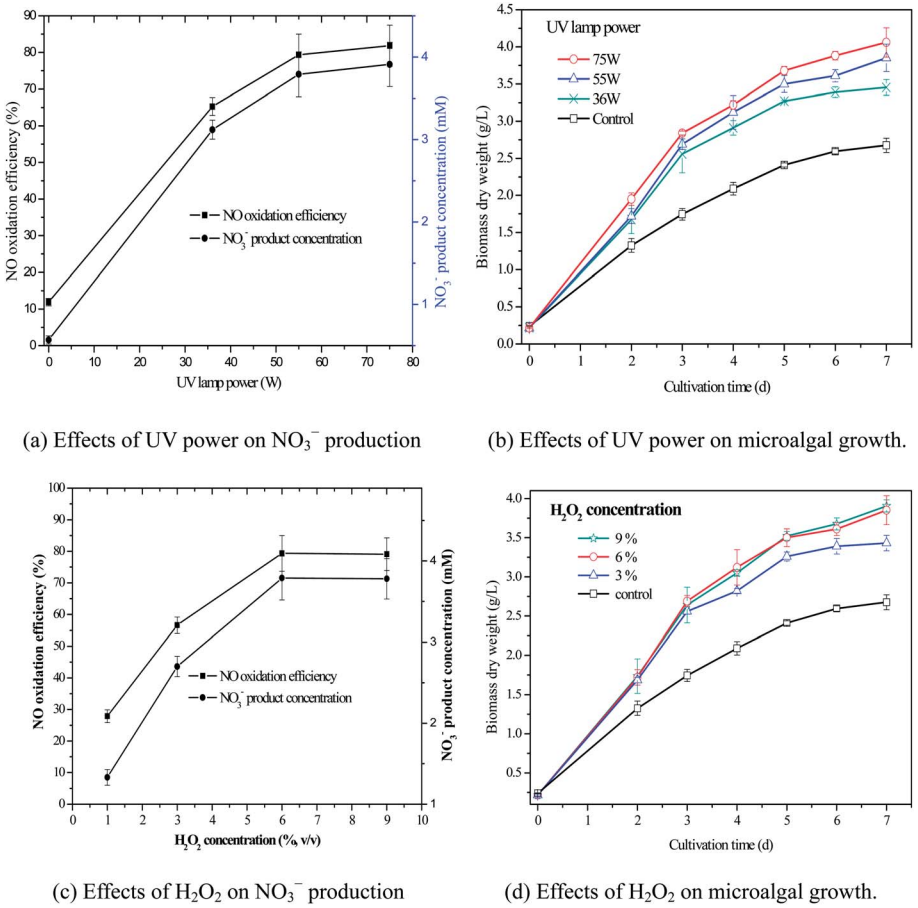


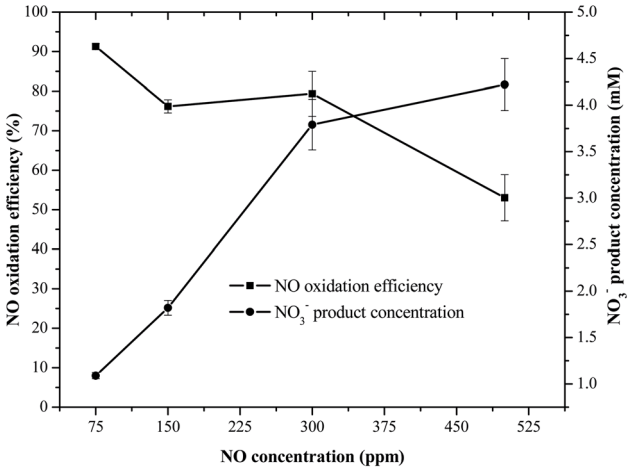
Fig. 4 Effects of UV lamp power and H_2O_2 concentration on NO_3^- production and microalgal growth.

(Fig. 2(a)). By contrast, biomass production increased when 500 ppm NO was converted into nitrate by UV/H_2O_2 as a supplement nitrogen source for microalgae under continuous light of 68 μmol m^{-2} s^{-1}. Overall, the maximum biomass concentration and peak growth productivity of *Chlorella* PY-ZU1 were 5.40 g L^{-1} and 1.18 g L^{-1} per day. These dependent parameters increased by 107.7% and 96.7%, respectively, compared with those of the microalgae cultured in the SE medium (2.68 g L^{-1} and 0.60 g L^{-1} per day, respectively) (Fig. 6).

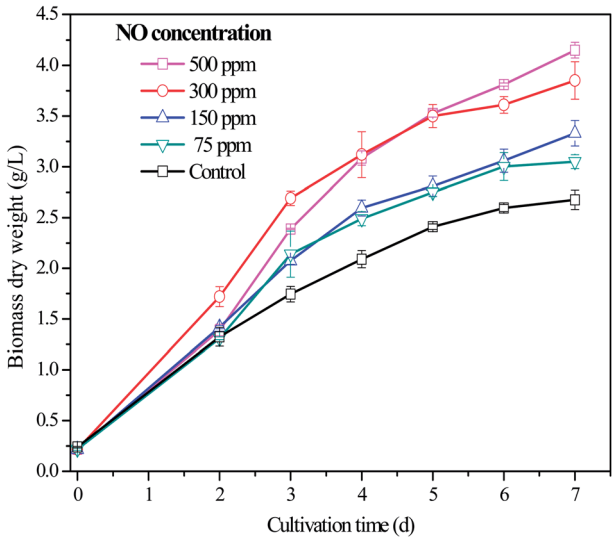
Although *Chlorella* can tolerate up to 50% concentration of CO_2, the biomass concentration does not reach a higher value (almost <1 g L^{-1}).[33] That makes CO_2 mitigation by microalgae difficult. The appropriate concentration of CO_2 for microalgae growth is always below 10%. Anjos *et al.* optimized CO_2-mitigation by *Chlorella vulgaris* P12 under different CO_2 concentrations (ranging from 2% to 10%). Results showed that 6.5% was the most appropriate CO_2 concentration for *Chlorella* P12.[34] When *Chlorella pyrenoidosa* was cultivated with SE medium, experiments also showed that 6% was the most appropriate CO_2 concentration.[15] In order to increase the ability of *Chlorella* to grow under higher CO_2 concentrations, *Chlorella pyrenoidosa* was mutated by nuclear irradiation and domesticated with high concentrations of CO_2 in our previous study. The most appropriate CO_2 concentration for the mutant *Chlorella* PY-ZU1 was up to 12% (v/v).[15,30]

CO_2 fixation experiments were performed in a nine-stage sequential bioreactor described in the previous studies.[15,30] The sequential bioreactor was filled with SE# medium and operated for 2 days without microalgae to determine the abiotic removal of CO_2. Hence, the abiotic removal of CO_2 should be eliminated in the calculation of CO_2 fixation efficiency by microalgae.

In the nine-stage sequential bioreactor, the CO_2 fixation efficiency of the microalgae cultivated at 500 ppm NO was lower than that of the microalgae cultivated without NO (Fig. 6). The peak CO_2 fixation efficiency of 26.2% was decreased by 19.9%, whereas the mean CO_2 fixation efficiency of 17.3% was decreased by 33.2%. However, when 500 ppm NO was converted into NO_3^- by UV/H_2O_2 as a supplement nitrogen source for *Chlorella* PY-ZU1, CO_2 fixation efficiency was higher than that of microalgae cultured in the SE medium without NO. The peak and mean CO_2 fixation efficiency were 69.6% and 52.3%, respectively, increased by 112.8% and 101.9% compared with those of the microalgae cultivated in the SE medium without aerated NO (32.7% of the peak CO_2 fixation efficiency and 25.9% of the mean CO_2 fixation efficiency).



(a) Effects of NO concentration on NO_3^- production



(b) Effects of NO concentration on microalgal growth

Fig. 5 Effects of NO concentration on NO_3^- production and microalgal growth.

Ramanan *et al.* has demonstrated an increase in CO_2 fixation efficiency by maneuvering chemically aided biological sequestration of CO_2. *Chlorella* sp. showed the peak CO_2 fixation efficiency of 46% at input CO_2 concentration of 10%.[35] Chiu *et al.* replaced a half of the culture broth with fresh medium every day to enhance growth rate of *Chlorella* sp. and CO_2 reduction. The CO_2 fixation efficiency of *Chlorella* sp. was 16% at input CO_2 concentration of 15%.[36] In this study, the produced NO_3^- from the oxidation of 500 ppm NO was used as supplementary nitrogen source. The peak CO_2 fixation efficiency of *Chlorella* PY-ZU1 was 69.6% at input CO_2 concentration of 15%. These results indicated that NO_3^- derived from NO oxidation as a nitrogen source for microalgae growth can overcome the toxic effect of NO and improve microalgal biomass production and CO_2 fixation.

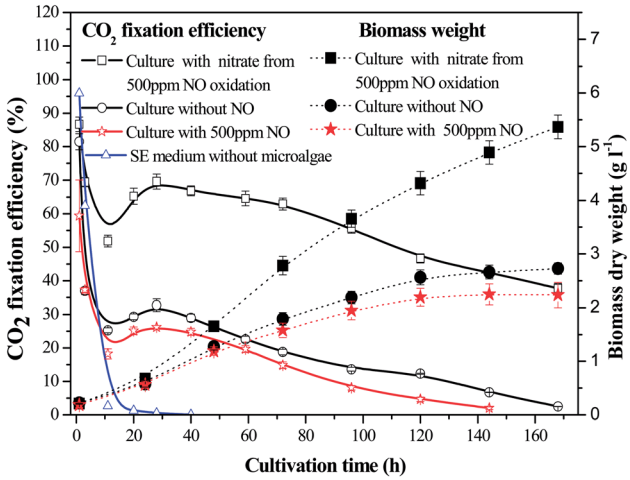


Fig. 6 CO_2 fixation and biomass growth of *Chlorella* PY-ZU1 cultivated with NO_3^- derived from NO oxidation.

4. Conclusions

NO pollutant in flue gas could be converted into useful NO_3^- by UV/H_2O_2 oxidation. The NO_3^- product can be used as a nitrogen source to improve microalgal growth and CO_2 fixation ability. When NO_3^- derived from 500 ppm NO oxidation was used as a nitrogen source, the peak growth productivity and CO_2 fixation efficiency of *Chlorella* PY-ZU1 were increased by 96.67% (1.18 g L^{-1} per day) and 112.8% (69.6%), respectively. This finding provided information regarding environmental and economical benefits to culture microalgae with waste carbon and nitrogen sources (exhaust CO_2 gas and NO oxidation products) in flue gas.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (51176163, 51476141), National High Technology R&D Program of China (2012AA050101), International Sci. & Tech. Cooperation Program of China (2012DFG61770), Zhejiang Provincial Natural Science Foundation of China (LR14E060002), Program for New Century Excellent Talents in University (NCET-11-0446), Specialized Research Fund for the Doctoral Program of Higher Education (20110101110021), Science and Technology Project of Guangxi Province (1346011-1).

References

1 C. Yoo, S.-Y. Jun, J.-Y. Lee, C.-Y. Ahn and H.-M. Oh, *Bioresour. Technol.*, 2010, **101**, S71–S74.
2 A. F. Clarens, E. P. Resurreccion, M. A. White and L. M. Colosi, *Environ. Sci. Technol.*, 2010, **44**, 1813–1819.
3 Y. Chisti, *Biotechnol. Adv.*, 2007, **25**, 294–306.
4 J. Cheng, A. Xia, W. Song, H. Su, J. Zhou and K. Cen, *Int. J. Hydrogen Energy*, 2012, **37**, 6536–6544.

5 A. Xia, J. Cheng, L. Ding, R. Lin, R. Huang, J. Zhou and K. Cen, *Bioresour. Technol.*, 2013, **146**, 436–443.
6 J. Cheng, T. Yu, T. Li, J. Zhou and K. Cen, *Bioresour. Technol.*, 2013, **131**, 531–535.
7 L. Jiang, S. Luo, X. Fan, Z. Yang and R. Guo, *Appl. Energy*, 2011, **88**, 3336–3341.
8 J. Cheng, A. Xia, Y. Liu, R. Lin, J. Zhou and K. Cen, *Int. J. Hydrogen Energy*, 2012, **37**, 13330–13337.
9 X. L. Zhang, S. Yan, R. D. Tyagi and R. Y. Surampalli, *Renewable Sustainable Energy Rev.*, 2013, **26**, 216–223.
10 Y. T. Huang, H. T. Lee and C. W. Lai, *J. Nanosci. Nanotechnol.*, 2013, **13**, 2117–2121.
11 O. Komolafe, S. B. V. Orta, I. Monje-Ramirez, I. Noguez, A. P. Harvey and M. O. Ledesma, *Bioresour. Technol.*, 2014, **154**, 297–304.
12 L. Yue and W. Chen, *Energy Convers. Manage.*, 2005, **46**, 1868–1876.
13 K. D. Sung, J. S. Lee, C. S. Shin and S. C. Park, *Renewable Energy*, 1999, **16**, 1019–1022.
14 M. G. de Morais and J. A. V. Costa, *Energy Convers. Manage.*, 2007, **48**, 2169–2173.
15 J. Cheng, Y. Huang, J. Feng, J. Sun, J. Zhou and K. Cen, *Bioresour. Technol.*, 2013, **136**, 496–501.
16 S. Basu, A. S. Roy, K. Mohanty and A. K. Ghoshal, *Bioresour. Technol.*, 2013, **143**, 369–377.
17 E. M. Radmann, F. V. Camerini, T. D. Santos and J. A. V. Costa, *Energy Convers. Manage.*, 2011, **52**, 3132–3136.
18 M. Negoro, N. Shioji, K. Miyamoto and Y. Micira, *Appl. Biochem. Biotechnol.*, 1991, **28–29**, 877–886.
19 J.-N. Lee, J.-S. Lee, C.-S. Shin, S.-C. Park and S.-W. Kim, in *Twenty-First Symposium on Biotechnology for Fuels and Chemicals*, ed. M. Finkelstein and B. Davison, Humana Press, 2000, pp. 329–342.
20 J.-S. Lee, D.-K. Kim, J.-P. Lee, S.-C. Park, J.-H. Koh, H.-S. Cho and S.-W. Kim, *Bioresour. Technol.*, 2002, **82**, 1–4.
21 J.-S. Lee and J.-P. Lee, *Biotechnol. Bioprocess Eng.*, 2003, **8**, 354–359.
22 H. Nagase, K.-i. Yoshihara, K. Eguchi, Y. Okamoto, S. Murasaki, R. Yamashita, K. Hirata and K. Miyamoto, *Biochem. Eng. J.*, 2001, **7**, 241–246.
23 S. Y. Chiu, C. Y. Kao, T. T. Huang, C. J. Lin, S. C. Ong, C. D. Chen, J. S. Chang and C. S. Lin, *Bioresour. Technol.*, 2011, **102**, 9135–9142.
24 P. Westerhoff, Q. Hu, M. Esparza-Soto and W. Vermaas, *Environ. Technol.*, 2010, **31**, 523–532.
25 B. E. Taştan, E. Duygu, O. Atakol and G. Dönmez, *Energy Convers. Manage.*, 2012, **64**, 28–34.
26 Y. Liu, J. Zhang, C. Sheng, Y. Zhang and L. Zhao, *Chem. Eng. J.*, 2010, **162**, 1006–1011.
27 Y. Liu, J. Zhang and C. Sheng, *Chem. Eng. J.*, 2011, **168**, 183–189.
28 A. M. El-Dein, J. Libra and U. Wiesmann, *J. Chem. Technol. Biotechnol.*, 2006, **81**, 1239–1245.
29 C. Cooper, C. Clausen, L. Pettey, M. Collins and M. Pozo de Fernandez, *J. Environ. Eng.*, 2002, **128**, 68–72.
30 J. Cheng, Y. Huang, J. Feng, J. Sun, J. Zhou and K. Cen, *Bioresour. Technol.*, 2013, **144**, 321–327.
31 M. Muruganandham and M. Swaminathan, *Dyes Pigm.*, 2004, **62**, 269–275.
32 W. Kim, J. Park, G. Gim, S.-H. Jeong, C. Kang, D.-J. Kim and S. Kim, *Bioprocess Biosyst. Eng.*, 2012, **35**, 19–27.
33 Y. T. Huang and C. P. Su, *Int. J. Environ. Sci. Tech.*, 2014, **11**, 703–710.
34 M. Anjos, B. D. Fernandes, A. A. Vicente, J. A. Teixeira and G. Dragone, *Bioresour. Technol.*, 2013, **139**, 149–154.
35 R. Ramanan, K. Kannan, A. Deshkar, R. Yadav and T. Chakrabarti, *Bioresour. Technol.*, 2010, **101**, 2616–2622.
36 S. Y. Chiu, C. Y. Kao, C. H. Chen, T. C. Kuan, S. C. Ong and C. S. Lin, *Bioresour. Technol.*, 2008, **99**, 3389–3396.

