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Oxidation product (NO$_3^-$) of NO pollutant in flue gas used as a nitrogen source to improve microalgal biomass production and CO$_2$ fixation

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Abstract

In order to eliminate the inhibition of the toxic nitric oxide (NO) in flue gas on microalgal growth and CO$_2$ fixation, NO was converted by a wet UV/H$_2$O$_2$ 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$_2$O$_2$, and NO concentrations increased, resulting in an improved microalgal growth. The concentration of NO$_3^-$ from 500 ppm NO wet-oxidized by 6% (v/v) H$_2$O$_2$ and 55 W UV light was up to 8.8 mM. When the produced nitrate used as supplementary nitrogen source, the maximum growth productivity of Chlorella PY-ZU1 at 15% (v/v) CO$_2$ reached 1.18 g/L/d (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).

Keywords: microalgae, nitrogen oxide, UV/H$_2$O$_2$, CO$_2$ fixation, biomass

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1. Introduction

Pollutants (including CO\textsubscript{2}, NO\textsubscript{X}, SO\textsubscript{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, greenhouse effect occurs because of excessive CO\textsubscript{2} concentrations in the atmosphere, this condition has caused problems in terms of environmental and energy aspects. Thus, CO\textsubscript{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\textsubscript{2} with efficiency (2-10%) ten times greater than that of terrestrial plants (<1%), one of the efficient CO\textsubscript{2} reduction methods involves the cultivation of microalgae in photobioreactors supplied with CO\textsubscript{2}-enriched gas streams, such as those emitted from coal-fired power plant flue gases.\textsuperscript{1-4} In addition, the CO\textsubscript{2} capture process using microalgae has the following advantages: (i) co-producing high value materials based on biomass, such as biofuel and biogas;\textsuperscript{5-10} (ii) being an environmental sustainable method that can be connected to urban and industrial sewage cleaning.\textsuperscript{11}

Some high CO\textsubscript{2}-tolerant microalgae species have been isolated out.\textsuperscript{12-16} However, the inhibitory effects of toxic compounds, such as NO\textsubscript{X} and SO\textsubscript{2}, in addition to high CO\textsubscript{2} concentrations, on microalgae can be critical.\textsuperscript{17-21} It was reported that NO in fossil fuel flue gas can be removed and used by the microalgae, Dunaliella tertiolecta.\textsuperscript{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 CO₂ fixation.\textsuperscript{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 \textit{Synechococcus} sp. and \textit{Chlorella} sp. have improved against exposure to intermittent NO\textsubscript{2} by adding growth stimulators, such as triacontanol and sodium bicarbonate.\textsuperscript{25}

The tolerance of \textit{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).\textsuperscript{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.\textsuperscript{24} It must take some techniques making NO dissolve into less NO\textsubscript{2}⁻ but to more usable substances, such as NO\textsubscript{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\textsubscript{2}O\textsubscript{2}), the toxic NO was completely converted into valuable NO\textsubscript{3}⁻ without generating any other byproduct.\textsuperscript{26-29} The wet AOP (UV/H\textsubscript{2}O\textsubscript{2}) has been used in coal-fired power plants to simultaneously remove NO, SO\textsubscript{2} and Hg pollutants in flue gas. But how to deal with and reutilize the large amount of byproducts (nitrate, sulfate and Hg\textsuperscript{2+}) is a big problem. Whether the oxidation byproduct (NO\textsuperscript{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\textsubscript{2}O\textsubscript{2} and NO concentrations) in wet AOP
(UV/H₂O₂) have important effects on microalgae growth has not been clarified. It was first proposed to reutilize the oxidation byproduct (NO³⁻) 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 CO₂ fixation. The effects of different UV/H₂O₂ conditions on microalgal growth and CO₂ fixation efficiency were investigated.

2. Materials and methods

2.1 Strains and media

Chlorella PY-ZU1, a highly CO₂-tolerant and fast-growing microalgal species, was used in this study. This strain was obtained by γ irradiation and high concentrations of CO₂ 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 NaNO₃, 0.075 g of K₂HPO₄•3H₂O, 0.075 g of MgSO₄•7H₂O, 0.025 g of CaCl₂•2H₂O, 0.175 g of KH₂PO₄, 0.025 g of NaCl, 40 mL of soil extract, 0.005 g of FeCl₃•6H₂O, 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/H₂O₂ is used as a nitrogen source for microalgal growth

Because of its strong oxidation ability and environmentally friendly characteristics, UV/H₂O₂ AOP has a wide range of studies in the gas purification field. Experimental system in which the NO in flue gas was converted to NO₃⁻ as nitrogen source for microalgal growth was performed in a bubble column reactor (Fig. 1). The
The proposed system comprised the following: (1) 3000 ppm of NO and pure N\textsubscript{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\textsubscript{4} (0.05 mol/L) and NaOH (0.1 mol/L; Sinopharm Chemical Reagent, China) to avoid environmental pollution).

The prepared H\textsubscript{2}O\textsubscript{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\textsubscript{2}) was regulated using a mass flow meter (SevenstarCS200, China). The NO gas passed uniformly across the sand chip gas distributor into the H\textsubscript{2}O\textsubscript{2} solution at a rate of 600 ml/min. After the UV lamp was turned on, H\textsubscript{2}O\textsubscript{2} was released, forming hydroxyl free radicals (•OH). These free radicals exhibit an extremely strong oxidation ability that can convert NO into HNO\textsubscript{3} without generating any other byproduct via the following reactions (2)–(3).\textsuperscript{26,31}

\[
\begin{align*}
\text{H}_2\text{O}_2 + h\nu &\rightarrow 2\cdot\text{OH} & (1) \\
\text{NO} + \cdot\text{OH} &\rightarrow \text{HNO}_2 & \text{HNO}_2 + \cdot\text{OH} \rightarrow \text{HNO}_3 + \cdot\text{H} & (2)
\end{align*}
\]
The reaction solution was collected after 6 h and the remaining H\textsubscript{2}O\textsubscript{2} was removed by ultrasonic wave (SK5210HP, China). The solution was then used to make the medium for \textit{Chlorella} PY-ZU1 by adding the same quantities of nutrients as those present in the SE medium. The initial pH of the medium 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\textsubscript{x} in the reactor was 500 ppm, H\textsubscript{2}O\textsubscript{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\textsubscript{2} fixation medium and labeled as SE#.

2.3 NO\textsubscript{3}\textsuperscript{-} produced from NO oxidation used as supplement nitrogen source to improve \textit{Chlorella} PY-ZU1 growth and CO\textsubscript{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 \textit{Chlorella} PY-ZU1 pre-culture in the bioreactor (BR, 160 mm × Ф56 mm, 300 ml of working volume). For the verification experiments of using NO\textsubscript{3}\textsuperscript{-} (derived from NO oxidation by UV/H\textsubscript{2}O\textsubscript{2}) as a nitrogen source for \textit{Chlorella} PY-ZU1, continuous light of 52 \textmu mol/m\textsuperscript{2}/s 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 \textmu mol/m\textsuperscript{2}/s of light was supplied by six cool white lights (Philips, TLD 36 W) at the surface of BR. The mixed gas of 15\% (v/v) CO\textsubscript{2} containing different NO concentrations was bubbled at a rate of 30 ml/min through a long steel pipe (180 mm × Ф3 mm). The NO concentrations were controlled at 0, 75,
131 150, 300, and 500 ppm by a mass flow meter (Sevenstar CS200, China).
132 
133 *Chlorella* PY-ZU1 was cultured in SE# and aerated continuously with 15% (v/v) CO₂ in nine-stage sequential bioreactors" to investigate the effect of NO₃⁻ produced from NO on CO₂ fixation. For comparison, *Chlorella* PY-ZU1 was cultured with SE medium and aerated continuously with 15% (v/v) CO₂ or with 15% (v/v) CO₂ gas containing 500 ppm NO. The influent and effluent CO₂ concentrations were monitored online by a CO₂ analyzer (Servomex4100, UK). CO₂ fixation efficiency was calculated according to the carbon dioxide difference between influent and effluent as described in a previous study.³⁰

\[
\text{CO}_2 \text{ fixation efficiency} = (1 - \frac{\text{total output CO}_2}{\text{total input CO}_2}) \times 100\% \tag{4}
\]

where the total input CO₂ = influent CO₂ concentration × influent flow rate, and the total output CO₂ = effluent CO₂ 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 8,500 rpm for 10 min and dried at 70 °C for 24 h to obtain the weight of the dried biomass. Biomass concentration (g/L) was calculated from the microalgal dry weight produced per liter. Growth productivity (AGP, g/L/d) was calculated using Eq. (5):

\[
AGP = \frac{M_1 - M_2}{t_1 - t_2} \tag{5}
\]

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.
The lipid of the biomass was extracted as described in a previous study. 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 Eq. (6):

$$\text{NO oxidation efficiency} = \frac{M_{\text{NO}_3} \times V}{\sum M_{\text{NOin}}} \quad (6)$$

where $M_{\text{NO}_3}$ is the molar concentration of NO$_3^-$ in volume $V$ (L) of the oxidized solution and $\sum M_{\text{NOin}}$ 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 Eq. (7):

$$C_{\text{NOout}} = C_{\text{NOin}} \times (1 - \text{NO oxidation efficiency}) \quad (7)$$

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. When aerated with 15% CO$_2$ gas containing 150ppm 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 and decreased by 24.3% to that of microalgae cultivated without NO aeration (2.68 g/L). 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.\(^ \text{19, 20} \) 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,\(^ \text{20} \) whose growth was completely suppressed when aerated with 15% CO\(_2\) gas containing 300ppm NO. This verified that microalgae tolerance to NO depends on the microalgae species but with a decrease in biomass productivity.\(^ \text{19} \) Some methods were used to alleviate microalgae growth inhibition caused by NO, such as controlling culture pH and adding some growth stimulators to culture.\(^ \text{25} \) Although *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\(_3^-\).\(^ \text{22} \) 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$_2$O$_2$) as a nitrogen source for Chlorella PY-ZU1

During UV/H$_2$O$_2$ AOP process, the remaining H$_2$O$_2$ 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$_2$O$_2$, the NO$_3^-$ production rate was stabilized at 0.427 mM/h 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$_2$O$_2$ 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$_2$.\textsuperscript{30} Chlorella PY-ZU1 cultivated in the SE# medium under 52 µmol/m$^2$/s of continuous light and 15% CO$_2$ for 11 d exhibited a peak growth productivity and maximum biomass concentration of 0.76 g/L/d and 5.48 g/L, respectively. These values were almost equal to those of Chlorella PY-ZU1 (0.73 g/L/d and 5.31 g/L, 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/H₂O₂) can be used as a nitrogen source for  
Chlorella PY-ZU1 instead of the commercial NaNO₃.

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

The NO₃⁻ concentration produced from NO increased with increase of lamp  
power, H₂O₂, and NO concentration. As a result, microalgae growth was improved.  
Under UV light irradiation, H₂O₂ can release •OH free radicals. •OH free radicals  
exhibit strong oxidation ability to convert NO to NO₃⁻.²⁶,²⁹ A high concentration of  
produced NO₃⁻ in AOPs results in a high biomass yield during microalgae  
cultivation.³⁰,³²  
NO₃⁻, the oxidation product derived from 300 ppm NO with 6% H₂O₂ for 6 h,  
could increase the biomass productivity of Chlorella PY-ZU1 under 15% CO₂ as UV  
lamp power was increased (Fig.4). The maximum biomass concentration of  
microalgae was evidently increased from 3.45 g/L to 3.85 g/L [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 75W, the growth rate of maximum biomass concentration gradually  
decreased. Two main reasons could explain the results. On one hand, under UV light  
irradiation, H₂O₂ can release •OH free radicals by Eq. (1) reaction.²⁶ The •OH free
radicals have extremely strong oxidation ability to convert NO into NO\(_3^−\) according to Eq. (2–3). 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 •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 \textit{Chlorella} PY-ZU1 was increased. On the other hand, once the power of UV lamp exceeds a certain value, some side reactions, such as Eq. (8–9), may occur in the solution, leading to a great loss of •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 \textit{Chlorella} PY-ZU1.\

\[
\text{H}_2\text{O}_2 + \cdot\text{OH} \rightarrow \text{HO}_2^- + \text{H}_2\text{O}
\]

(8)

\[
\cdot\text{OH} + \cdot\text{OH} \rightarrow \text{H}_2\text{O}_2
\]

(9)

Similarly, the NO\(_3^−\) production efficiency derived from NO (300 ppm) by UV/H\(_2\)O\(_2\) (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\(_2\)O\(_2\) 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 to 3.85 g/L [Fig.4(d)]. However, a further increase in H\(_2\)O\(_2\) concentration from 6% to 9% did not increase the maximum biomass concentration (stabilized at 3.91 g/L). This is mainly because appropriate H\(_2\)O\(_2\) concentration may cause a reaction such as Eq.(1) in the solution. Therefore, within a certain range, the increase in H\(_2\)O\(_2\) concentration can
improve the yield of $\text{NO}_3^-$, and then increased the biomass growth of *Chlorella* PY-ZU1. Once $\text{H}_2\text{O}_2$ concentration exceeding a certain value, any further increase may cause side reactions as Eq. (8–9) which lead to a decrease in the oxidation ability of free radicals. Therefore, further increase in $\text{H}_2\text{O}_2$ concentration only had little effect on the yield of $\text{NO}_3^-$ and a slight impact on biomass production of *Chlorella* PY-ZU1.

$\text{NO}_3^-$ production efficiency decreased from 91.26% to 53.00% [Figure 5(a)] as NO concentration increased from 75 ppm to 500 ppm because of the limitation of NO residence time and •OH free radicals. However, the derived $\text{NO}_3^-$ 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 to 4.15 g/L [Fig.5(b)].

### 3.4 CO$_2$ fixation by *Chlorella* PY-ZU1 cultivated with $\text{NO}_3^-$ 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 of microalgae cultivated without aerated NO (Fig.2[a]). By contrast, biomass production increased when 500 ppm NO was converted into nitrate by UV/$\text{H}_2\text{O}_2$ as a supplement nitrogen source for microalgae under continuous light of 68 μmol/m$^2$/s. Overall, the maximum biomass concentration and peak growth productivity of *Chlorella* PY-ZU1 were 5.40 g/L and 1.18 g/L/d. 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 and 0.60 g/L/d, 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).\textsuperscript{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.\textsuperscript{34} When *Chlorella pyrenoidosa* was cultivated with SE medium, experiments also showed that 6% was the most appropriate CO$_2$ concentration.\textsuperscript{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).\textsuperscript{15,30} CO$_2$ fixation experiments were performed in a nine-stage sequential bioreactor described in the previous studies.\textsuperscript{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$_2$O$_2$ 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).

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%. 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%. 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.

4. Conclusions

NO pollutant in flue gas could be converted into useful NO$_3^-$ by UV/H$_2$O$_2$ 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/d) 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 CO₂ gas and NO oxidation products) in flue gas.

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Fig.6. CO$_2$ fixation and biomass growth of *Chlorella* PY-ZU1 cultivated with NO$_3^-$ derived from NO oxidation.

Table 1. Compositions of lipids in microalgae cultivated with commercial NaNO$_3$ and NO$_3^-$ derived from NO oxidation.
Fig. 1. Experimental system in which the NO in flue gas was converted to NO$_3^-$ as nitrogen source for microalgal growth.
(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) $\text{NO}_3^-$ production from NO oxidation with UV/H$_2$O$_2$

(b) Microalgal growth in SE medium with derived $\text{NO}_3^-$ from NO and commercial NaNO$_3$

Fig. 3. Microalgal growth with $\text{NO}_3^-$ derived from NO oxidation and commercial NaNO$_3$. 
Fig. 4. Effects of UV lamp power and H$_2$O$_2$ concentration on NO$_3^-$ production and microalgal growth.

(a) Effects of UV power on NO$_3^-$ production

(b) Effects of UV power on microalgal growth.

(c) Effects of H$_2$O$_2$ on NO$_3^-$ production

(d) Effects of H$_2$O$_2$ on microalgal growth.
(a) Effects of NO concentration on $\text{NO}_3^-$ production

(b) Effects of NO concentration on microalgal growth

Fig. 5. Effects of NO concentration on $\text{NO}_3^-$ production and microalgal growth.
**Fig. 6.** CO\textsubscript{2} fixation and biomass growth of *Chlorella* PY-ZU1 cultivated with NO\textsubscript{3}\textsuperscript{−} derived from NO oxidation.
Table 1. Compositions of lipids in microalgae cultivated with commercial NaNO$_3$ and NO$_3^-$ derived from NO oxidation.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Commercial NaNO$_3$</th>
<th>NO$_3^-$ derived from NO oxidation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid content (% of dry biomass)</td>
<td>17.92</td>
<td>18.11</td>
</tr>
<tr>
<td>lipids composition (% of total lipid)</td>
<td></td>
<td></td>
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<tr>
<td>C16:0</td>
<td>23.85±0.29</td>
<td>22.37±0.10</td>
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<tr>
<td>C16:3</td>
<td>7.02±0.34</td>
<td>6.80±0.29</td>
</tr>
<tr>
<td>C18:0</td>
<td>3.15±0.26</td>
<td>3.17±0.01</td>
</tr>
<tr>
<td>C18:1</td>
<td>15.88±0.75</td>
<td>14.82±0.76</td>
</tr>
<tr>
<td>C18:2</td>
<td>15.52±0.83</td>
<td>14.76±0.57</td>
</tr>
<tr>
<td>C18:3</td>
<td>12.77±0.34</td>
<td>12.65±0.46</td>
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<tr>
<td>Others(C16-C24)</td>
<td>21.8±0.63</td>
<td>25.4±0.45</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>