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1 **Oxidation product (NO_3^-) of NO pollutant in flue gas used as**
2 **a nitrogen source to improve microalgal biomass production**
3 **and CO_2 fixation**

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6 **Abstract**

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

21 **Keywords:** microalgae, nitrogen oxide, UV/ H_2O_2 , CO_2 fixation, biomass

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1. Introduction

Pollutants (including CO₂, NO_x, SO₂, 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 CO₂ concentrations in the atmosphere, this condition has caused problems in terms of environmental and energy aspects. Thus, CO₂ 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 CO₂ with efficiency (2-10%) ten times greater than that of terrestrial plants (<1%), one of the efficient CO₂ reduction methods involves the cultivation of microalgae in photobioreactors supplied with CO₂-enriched gas streams, such as those emitted from coal-fired power plant flue gases.¹⁻⁴ In addition, the CO₂ 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 CO₂-tolerant microalgae species have been isolated out.¹²⁻¹⁶ However the inhibitory effects of toxic compounds, such as NO_x and SO₂, in addition to high CO₂ 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

43 hinder their growth and CO₂ fixation.^{17-21, 23, 24}

44 In recent years, some studies have focused on the alleviation of the effect of NO
45 on microalgae growth. These studies have shown that the growth and survival of
46 *Synechococcus* sp. and *Chlorella* sp. have improved against exposure to intermittent
47 NO₂ by adding growth stimulators, such as triacontanol and sodium bicarbonate.²⁵
48 The tolerance of *Chlorella* KR-1 to continuous NO exposure can be enhanced by
49 maintaining the pH of the culture media at an adequate value (~7), which is achieved
50 by adding an alkaline solution (NaOH).¹⁹ However, this condition can be effective for
51 some specific microalgae only. A previous study also showed that the presence of NO
52 may lead to the formation of toxic nitrites in microalgae culture, therefore, its
53 inhibitory effect on microalgae growth was evaluated²⁴. It must take some techniques
54 making NO dissolve into less NO₂⁻ but to more usable substances, such as NO₃⁻.

55 Advanced oxidation process (AOP) can produce free radicals with strong
56 oxidation, such as hydroxyl free radicals (•OH). By a wet AOP using hydrogen
57 peroxide solution with ultraviolet lamp (UV/H₂O₂), the toxic NO was completely
58 converted into valuable NO₃⁻ without generating any other byproduct.²⁶⁻²⁹ The wet
59 AOP (UV/H₂O₂) has been used in coal-fired power plants to simultaneously remove
60 NO, SO₂ and Hg pollutants in flue gas. But how to deal with and reutilize the large
61 amount of byproducts (nitrate, sulfate and Hg²⁺) is a big problem. Whether the
62 oxidation byproduct (NO₃⁻) derived from the wet AOP can be consumed and used by
63 microalgae has not been reported in literatures till now. Whether the different
64 oxidation conditions (UV lamp power, H₂O₂ and NO concentrations) in wet AOP

65 (UV/H₂O₂) have important effects on microalgae growth has not been clarified. It was
66 first proposed to reutilize the oxidation byproduct (NO³⁻) derived from the wet AOP
67 by microalgae as a supplementary nitrogen source in this paper. This novel process
68 not only eliminated the effect of toxic NO on microalgal growth but also improved
69 microalgal biomass productivity and CO₂ fixation. The effects of different UV/H₂O₂
70 conditions on microalgal growth and CO₂ fixation efficiency were investigated.

71 **2. Materials and methods**

72 **2.1 Strains and media**

73 *Chlorella* PY-ZU1, a highly CO₂-tolerant and fast-growing microalgal species,
74 was used in this study. This strain was obtained by γ irradiation and high
75 concentrations of CO₂ domesticated from *Chlorella pyrenoidosa*.¹⁵ The cells were
76 maintained in Brostol's solution (also known as soil extract, SE),^{15,30} containing 0.25
77 g of NaNO₃, 0.075 g of K₂HPO₄•3H₂O, 0.075 g of MgSO₄•7H₂O, 0.025 g of
78 CaCl₂•2H₂O, 0.175 g of KH₂PO₄, 0.025 g of NaCl, 40 mL of soil extract, 0.005 g of
79 FeCl₃•6H₂O, 1 mL of Fe-EDTA, and 1 mL of A5 solution in 958 mL of de-ionized
80 water.

81 **2.2 System design by which the oxidation product of NO in flue gas** 82 **with UV/H₂O₂ is used as a nitrogen source for microalgal growth**

83 Because of its strong oxidation ability and environmentally friendly
84 characteristics, UV/H₂O₂ AOP has a wide range of studies in the gas purification field.
85 Experimental system in which the NO in flue gas was converted to NO₃⁻ as nitrogen
86 source for microalgal growth was performed in a bubble column reactor (Fig. 1). The

87 proposed system comprised the following: (1) 3000ppm of NO and pure N₂ (used as
88 balance gas); (2) Mass flow meter; (3) a bubble column reactor (height of 450 mm
89 and inner diameter of 75 mm); (4) cooling water cycle system; (5) sand chip gas
90 distributor (outer diameter of 45 mm, height of 30 mm, and average pore size of 0.105
91 mm to 0.18 mm); (6) UV lamps (UV lamp powers were changed by replacing and
92 using three sets of UV lamps with different powers (36 W, 55 W, and 75 W, Haining
93 Light Factory). All the lamps were of the same model (L-L) and of the same
94 wavelength of 253.7 nm); and (7) effluent NO scrubber (the residual NO in the mixed
95 gas was further scrubbed using 400 mL mixed solution containing KMnO₄ (0.05
96 mol/L) and NaOH (0.1 mol/L; Sinopharm Chemical Reagent, China) to avoid
97 environmental pollution).

98 The prepared H₂O₂ solution with the required concentration (1%, 3%, 6%, and
99 9%) was placed in the bubble column reactor. Temperature was maintained at 25 °C
100 by recycling the cooling water. NO concentration (75, 150, 300, and 500 ppm,
101 balanced with N₂) was regulated using a mass flow meter (SevenstarCS200, China).
102 The NO gas passed uniformly across the sand chip gas distributor into the H₂O₂
103 solution at a rate of 600 ml/min. After the UV lamp was turned on, H₂O₂ was released,
104 forming hydroxyl free radicals (•OH). These free radicals exhibit an extremely strong
105 oxidation ability that can convert NO into HNO₃ without generating any other
106 byproduct via the following reactions (2)–(3).^{26, 31}





110 The reaction solution was collected after 6 h and the remaining H_2O_2 was
111 removed by ultrasonic wave (SK5210HP, China). The solution was then used to make
112 the medium for *Chlorella* PY-ZU1 by adding the same quantities of nutrients as those
113 present in the SE medium. The initial pH of the medium was adjusted to 6.5 with 0.1
114 M NaOH. The SE medium was used as the control condition. For the final AOP runs,
115 NO_x in the reactor was 500 ppm, H_2O_2 concentration was 6% (v/v), and UV power
116 was 55 W. The medium prepared with the 15 h oxidation solution was used as the
117 CO_2 fixation medium and labeled as SE#.

118 **2.3 NO_3^- produced from NO oxidation used as supplement nitrogen**
119 **source to improve *Chlorella* PY-ZU1 growth and CO_2 fixation**

120 All of the cultivation experiments were performed in an artificial greenhouse at
121 27 °C. Approximately 270 mL SE medium was inoculated with 30 mL of *Chlorella*
122 PY-ZU1 pre-culture in the bioreactor (BR, 160 mm × Φ 56 mm, 300 ml of working
123 volume). For the verification experiments of using NO_3^- (derived from NO oxidation
124 by UV/ H_2O_2) as a nitrogen source for *Chlorella* PY-ZU1, continuous light of 52
125 $\mu\text{mol}/\text{m}^2/\text{s}$ at the surface of BR was supplied by four cool white lights combined with
126 two plant lights (Philips, TLD 36W) that were fixed above the BR. For the other
127 experiments in this study, 68 $\mu\text{mol}/\text{m}^2/\text{s}$ of light was supplied by six cool white lights
128 (Philips, TLD 36 W) at the surface of BR. The mixed gas of 15% (v/v) CO_2
129 containing different NO concentrations was bubbled at a rate of 30 ml/min through a
130 long steel pipe (180 mm × Φ 3 mm). The NO concentrations were controlled at 0, 75,

131 150, 300, and 500 ppm by a mass flow meter (Sevenstar CS200, China).

132 *Chlorella* PY-ZU1 was cultured in SE# and aerated continuously with 15% (v/v)
133 CO₂ in nine-stage sequential bioreactors³⁰ to investigate the effect of NO₃⁻ produced
134 from NO on CO₂ fixation. For comparison, *Chlorella* PY-ZU1 was cultured with SE
135 medium and aerated continuously with 15% (v/v) CO₂ or with 15% (v/v) CO₂ gas
136 containing 500 ppm NO. The influent and effluent CO₂ concentrations were
137 monitored online by a CO₂ analyzer (Servomex4100, UK). CO₂ fixation efficiency
138 was calculated according to the carbon dioxide difference between influent and
139 effluent as described in a previous study.³⁰

$$140 \quad CO_2 \text{ fixation efficiency} = \left(1 - \frac{\text{total output } CO_2}{\text{total input } CO_2}\right) \times 100\% \quad (4)$$

141 where the total input CO₂ = influent CO₂ concentration × influent flow rate, and the
142 total output CO₂ = effluent CO₂ concentration × effluent flow rate.

143 **2.4 Analysis of microalgal productivity and biomass compositions**

144 During cultivation, 10 mL of the samples was dewatered by centrifugation
145 (Beckman Avanti J26-XP, USA) at 8,500 rpm for 10 min and dried at 70 °C for 24 h
146 to obtain the weight of the dried biomass. Biomass concentration (g/L) was calculated
147 from the microalgal dry weight produced per liter. Growth productivity (AGP, g/L/d)
148 was calculated using Eq. (5):

$$149 \quad AGP = \frac{M_1 - M_2}{t_1 - t_2} \quad (5)$$

150 where M_1 is the biomass concentration at time t_1 and M_2 is the biomass concentration
151 at time t_2 . Total carbohydrate quantity was determined using the anthrone method

152 (with glucose as the standard).⁸ The lipid of the biomass was extracted as described in
153 a previous study.⁶ Fatty acid compositions were determined by gas chromatography
154 (Agilent 7890A, USA).

155 **2.5 Calculation of NO oxidation efficiency and residual NO**

156 **concentration**

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

$$161 \quad \text{NO oxidation efficiency} = \frac{M_{\text{NO}_3^-} \times V}{\sum M_{\text{NOin}}} \quad (6)$$

162 where $M_{\text{NO}_3^-}$ is the molar concentration of NO_3^- in volume V (L) of the oxidized
163 solution and $\sum M_{\text{NOin}}$ is the total number of moles of NO flowing into the oxidation
164 reactor. In this study, NO_3^- was the only product of NO oxidation; thus, NO oxidation
165 efficiency also corresponded to NO_3^- production efficiency. The remaining NO
166 concentration (mean value) was calculated using Eq. (7):

$$167 \quad C_{\text{NOout}} = C_{\text{NOin}} \times (1 - \text{NO oxidation efficiency}) \quad (7)$$

168 **3. Results and discussion**

169 **3.1 Effects of NO on the growth of *Chlorella* PY-ZU1**

170 The effects of NO concentrations on the growth of *Chlorella* PY-ZU1 and the pH
171 of the culture were examined in the BR (Fig.2). *Chlorella* PY-ZU1 showed a higher
172 tolerance to NO than other NO-tolerant algal strains, which could not grow under 150
173 ppm NO.²⁰ When aerated with 15% CO_2 gas containing 150ppm NO, biomass

174 concentration of *Chlorella* PY-ZU1 decreased after 5 days of cultivation, and the pH
175 of culture decreased to 6.27. The maximum biomass concentration was 2.03 g/L and
176 decreased by 24.3% to that of microalgae cultivated without NO aeration (2.68 g/L).
177 When NO concentration was further increased to 500 ppm, microalgae could grow but
178 with a 50.7% decrease in the maximum biomass concentration to that of microalgae
179 cultivated without NO. The decrease in biomass yield was due to pH decrease in the
180 culture caused by NO aeration.^{19, 20} The pH of the culture decreased with the
181 increasing cultivation time. Once the pH of the culture decreased beyond the adequate
182 range (6.5~7.5 for *Chlorella*), the microalgae growth was inhibited. This was why the
183 biomass concentration of *Chlorella* PY-ZU1 decreased after 5 days cultivation
184 with >150 ppm of NO. However, *Chlorella* PY-ZU1 showed a higher tolerance to NO
185 than *Chlorella* KR-1,²⁰ whose growth was completely suppressed when aerated with
186 15% CO₂ gas containing 300ppm NO. This verified that microalgae tolerance to NO
187 depends on the microalgae species but with a decrease in biomass productivity.¹⁹

188 Some methods were used to alleviate microalgae growth inhibition caused by
189 NO, such as controlling culture pH and adding some growth stimulators to culture.²⁵
190 Although *Dunaliella tertiolecta* could use NO dissolved in microalgae culture as a
191 nitrogen source, NO absorbed in the medium could be converted to NO₂⁻ and then
192 oxidized to NO₃⁻.²² This oxidation process was extremely slow. The improvement
193 effect of little NO₃⁻ produced from NO on *Chlorella* PY-ZU1 did not overcome the
194 toxic effect of NO. Thus, a much faster NO oxidation method will be needed.

195 **3.2 Confirmation of using NO₃⁻ (derived from NO oxidation by**

196 **UV/H₂O₂) as a nitrogen source for *Chlorella* PY-ZU1**

197 During UV/H₂O₂ AOP process, the remaining H₂O₂ concentration in the solution
198 was decreased with the oxidation time, resulting in a decrease in NO₃⁻ production
199 efficiency.²⁶ In the process of 500 ppm NO oxidized by 55 W UV/6% H₂O₂, the NO₃⁻
200 production rate was stabilized at 0.427 mM/h and 53% of NO was converted into
201 NO₃⁻ in the first 6 h [Fig.3(a)]. In the next 6 h, the NO₃⁻ production rate gradually
202 decreased to 10.65% with H₂O₂ digestion. After 15 h, NO₃⁻ concentration in the
203 solution reached to 8.8 mM. The total NO₃⁻ concentration in the medium prepared
204 with this oxidation solution was 11.8 mM, which could satisfy the NO₃⁻ requirement
205 of *Chlorella* PY-ZU1 under 15% CO₂.³⁰ *Chlorella* PY-ZU1 cultivated in the SE#
206 medium under 52 μmol/m²/s of continuous light and 15% CO₂ for 11 d exhibited a
207 peak growth productivity and maximum biomass concentration of 0.76 g/L/d and 5.48
208 g/L, respectively. These values were almost equal to those of *Chlorella* PY-ZU1 (0.73
209 g/L/d and 5.31 g/L, respectively) cultivated in the SE medium with 11.8 mM
210 commercial NaNO₃. In addition, the growth curve of *Chlorella* PY-ZU1 cultivated
211 with NO₃⁻ produced from NO is consistent with that of the *Chlorella* PY-ZU1
212 cultivated with commercial NaNO₃ [Fig.3(b)].

213 The total carbohydrate quantity of the dried biomass of *Chlorella* PY-ZU1
214 cultivated with NO₃⁻ produced from NO (41.57%, w/w biomass) was almost equal to
215 that of the *Chlorella* PY-ZU1 cultivated with commercial NaNO₃ (43.57%; data not
216 shown). The lipid contents in the two biomasses were 18.11% and 17.92%,
217 respectively. The biodiesel compositions from these two kinds of biomasses were

218 analyzed (Table 1). The fatty acid profiles indicated the presence of C16:0, C16:1,
219 C16:2, C16:3, C18:0, C18:1, C18:2, and C18:3. Palmitic acid, oleic acid, linoleic
220 acid, and linolenic acid were considered as the main components, which ranged from
221 12% to 24% of the total fatty acids. These results indicated that oxidation product of
222 NO (derived from NO in flue gas by UV/H₂O₂) can be used as a nitrogen source for
223 *Chlorella* PY-ZU1 instead of the commercial NaNO₃.

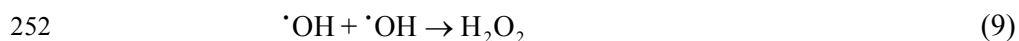
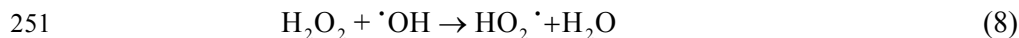
224 **3.3 Effects of different NO conversion conditions on the growth of**

225 ***Chlorella* PY-ZU1**

226 The NO₃⁻ concentration produced from NO increased with increase of lamp
227 power, H₂O₂, and NO concentration. As a result, microalgae growth was improved.
228 Under UV light irradiation, H₂O₂ can release •OH free radicals. •OH free radicals
229 exhibit strong oxidation ability to convert NO to NO₃⁻.^{26, 29} A high concentration of
230 produced NO₃⁻ in AOPs results in a high biomass yield during microalgae
231 cultivation.^{30, 32}

232 NO₃⁻, the oxidation product derived from 300 ppm NO with 6% H₂O₂ for 6 h,
233 could increase the biomass productivity of *Chlorella* PY-ZU1 under 15% CO₂ as UV
234 lamp power was increased (Fig.4). The maximum biomass concentration of
235 microalgae was evidently increased from 3.45 g/L to 3.85 g/L [Fig.4(b)] as UV lamp
236 power increased from 36 W to 55 W. However, with further increasing the UV lamp
237 power from 55 to 75W, the growth rate of maximum biomass concentration gradually
238 decreased. Two main reasons could explain the results. On one hand, under UV light
239 irradiation, H₂O₂ can release •OH free radicals by Eq. (1) reaction.²⁶ The •OH free

240 radicals have extremely strong oxidation ability to convert NO into NO₃⁻ according to
241 Eq. (2–3). Therefore, compared with the reaction system without UV light, addition of
242 UV light can greatly enhance NO conversion into NO₃⁻. Furthermore, increasing UV
243 lamp power can improve the energy density per unit in solution, thus produce more
244 effective photons and •OH free radicals. Therefore, the NO₃⁻ produced rate increased
245 with an increase in UV lamp power.^{26, 31} Consequently, the maximum biomass
246 concentration of *Chlorella* PY-ZU1 was increased. On the other hand, once the power
247 of UV lamp exceeds a certain value, some side reactions, such as Eq. (8–9), may
248 occur in the solution, leading to a great loss of •OH free radicals.²⁷ Therefore, a
249 further increase in UV lamp power only has a little impact on NO₃⁻ production and
250 thus a little effect on the growth of *Chlorella* PY-ZU1.



253 Similarly, the NO₃⁻ production efficiency derived from NO (300 ppm) by
254 UV/H₂O₂ (55 W of UV for 6 h) increased from 56.60% to 79.33% and the derived
255 NO₃⁻ concentration increased from 2.70 mM to 3.79 mM [Fig.4(c)] when H₂O₂
256 concentration increased from 3% to 6%. This finding resulted in an evident increase
257 in the maximum biomass concentration of microalgae from 3.43 g/L to 3.85 g/L
258 [Fig.4(d)]. However, a further increase in H₂O₂ concentration from 6% to 9% did not
259 increase the maximum biomass concentration (stabilized at 3.91 g/L). This is mainly
260 because appropriate H₂O₂ concentration may cause a reaction such as Eq.(1) in the
261 solution. Therefore, within a certain range, the increase in H₂O₂ concentration can

262 improve the yield of NO_3^- ,²⁶ and then increased the biomass growth of
263 *Chlorella*.PY-ZU1.²⁵ Once H_2O_2 concentration exceeding a certain value, any further
264 increase may cause side reactions as Eq. (8–9) which lead to a decrease in the
265 oxidation ability of free radicals.²⁷ Therefore, further increase in H_2O_2 concentration
266 only had little effect on the yield of NO_3^- and a slight impact on biomass production
267 of *Chlorella*.PY-ZU1.

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

273 **3.4 CO_2 fixation by *Chlorella* PY-ZU1 cultivated with NO_3^- derived**

274 **from NO oxidation**

275 When 500 ppm NO was directly aerated into microalgal culture, biomass
276 production was decreased by 50.7% to that of 2.68 g/L of microalgae cultivated
277 without aerated NO (Fig.2[a]). By contrast, biomass production increased when 500
278 ppm NO was converted into nitrate by UV/ H_2O_2 as a supplement nitrogen source for
279 microalgae under continuous light of $68 \mu\text{mol}/\text{m}^2/\text{s}$. Overall, the maximum biomass
280 concentration and peak growth productivity of *Chlorella* PY-ZU1 were 5.40 g/L and
281 1.18 g/L/d. These dependent parameters increased by 107.7% and 96.7%, respectively,
282 compared with those of the microalgae cultured in the SE medium (2.68 g/L and 0.60
283 g/L/d, respectively) (Fig.6).

284 Although *Chlorella* can tolerate up to 50 % concentration of CO₂, the biomass
285 concentration does not reach a higher value (almost < 1 g/L).³³ That makes CO₂
286 mitigation by microalgae difficult. The appropriate concentration of CO₂ for
287 microalgae growth is always below 10%. Anjos et al. optimized CO₂-mitigation by
288 *Chlorella vulgaris* P12 under different CO₂ concentrations (ranging from 2% to 10%).
289 Results showed that 6.5% was the most appropriate CO₂ concentration for *Chlorella*
290 P12.³⁴ When *Chlorella pyrenoidosa* was cultivated with SE medium, experiments also
291 showed that 6% was the most appropriate CO₂ concentration.¹⁵ In order to increase
292 the ability of *Chlorella* to grow under higher CO₂ concentrations, *Chlorella*
293 *pyrenoidosa* was mutated by nuclear irradiation and domesticated with high
294 concentrations of CO₂ in our previous study. The most appropriate CO₂ concentration
295 for the mutant *Chlorella* PY-ZU1 was up to 12 % (v/v).^{15,30}

296 CO₂ fixation experiments were performed in a nine-stage sequential bioreactor
297 described in the previous studies.^{15, 30} The sequential bioreactor was filled with SE#
298 medium and operated for 2 days without microalgae to determine the abiotic removal
299 of CO₂. Hence, the abiotic removal of CO₂ should be eliminated in the calculation of
300 CO₂ fixation efficiency by microalgae.

301 In the nine-stage sequential bioreactor, the CO₂ fixation efficiency of the
302 microalgae cultivated at 500 ppm NO was lower than that of the microalgae cultivated
303 without NO (Fig.6). The peak CO₂ fixation efficiency of 26.2% was decreased by
304 19.9%, whereas the mean CO₂ fixation efficiency of 17.3% was decreased by 33.2%.
305 However, when 500 ppm NO was converted into NO₃⁻ by UV/H₂O₂ as a supplement

306 nitrogen source for *Chlorella* PY-ZU1, CO₂ fixation efficiency was higher than that of
307 microalgae cultured in the SE medium without NO. The peak and mean CO₂ fixation
308 efficiency were 69.6% and 52.3%, respectively, increased by 112.8% and 101.9%
309 compared with those of the microalgae cultivated in the SE medium without aerated
310 NO (32.7% of the peak CO₂ fixation efficiency and 25.9% of the mean CO₂ fixation
311 efficiency).

312 Ramanan et al. has demonstrated an increase in CO₂ fixation efficiency by
313 maneuvering chemically aided biological sequestration of CO₂. *Chlorella sp.* showed
314 the peak CO₂ fixation efficiency of 46 % at input CO₂ concentration of 10 %.³⁵ Chiu
315 et al. replaced a half of the culture broth with fresh medium every day to enhance
316 growth rate of *Chlorella sp.* and CO₂ reduction. The CO₂ fixation efficiency of
317 *Chlorella sp.* was 16% at input CO₂ concentration of 15 %.³⁶ In this study, the
318 produced NO₃⁻ from the oxidation of 500 ppm NO was used as supplementary
319 nitrogen source. The peak CO₂ fixation efficiency of *Chlorella* PY-ZU1 was 69.6% at
320 input CO₂ concentration of 15 %. These results indicated that NO₃⁻ derived from NO
321 oxidation as a nitrogen source for microalgae growth can overcome the toxic effect of
322 NO and improve microalgal biomass production and CO₂ fixation.

323 **4. Conclusions**

324 NO pollutant in flue gas could be converted into useful NO₃⁻ by UV/H₂O₂
325 oxidation. The NO₃⁻ product can be used as a nitrogen source to improve microalgal
326 growth and CO₂ fixation ability. When NO₃⁻ derived from 500 ppm NO oxidation
327 was used as a nitrogen source, the peak growth productivity and CO₂ fixation

328 efficiency of *Chlorella* PY-ZU1 were increased by 96.67% (1.18 g/L/d) and 112.8%
329 (69.6%), respectively. This finding provided information regarding environmental and
330 economical benefits to culture microalgae with waste carbon and nitrogen sources
331 (exhaust CO₂ gas and NO oxidation products) in flue gas.

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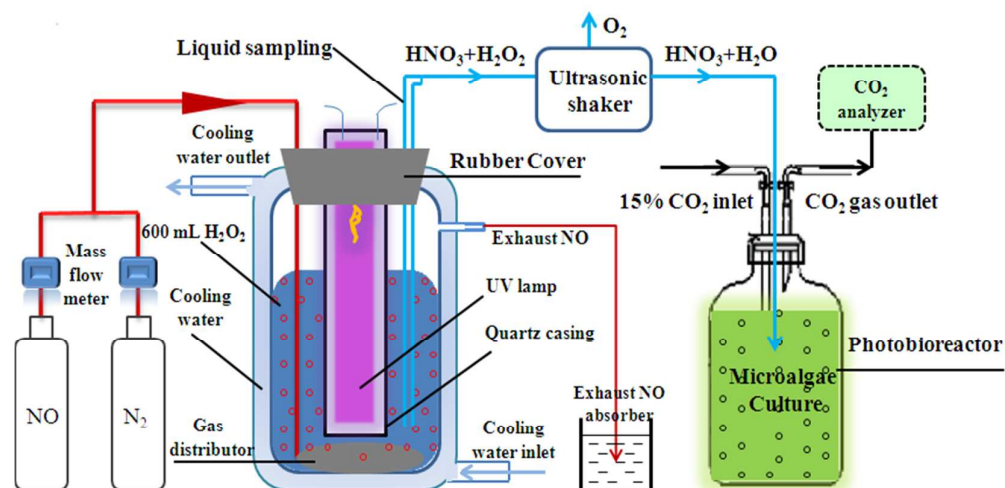
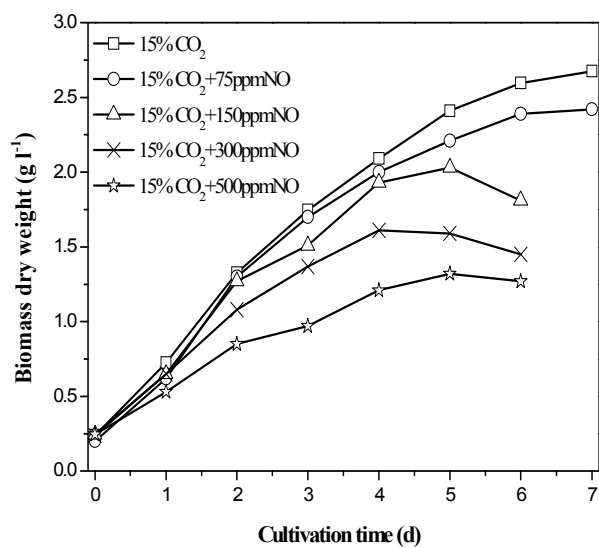
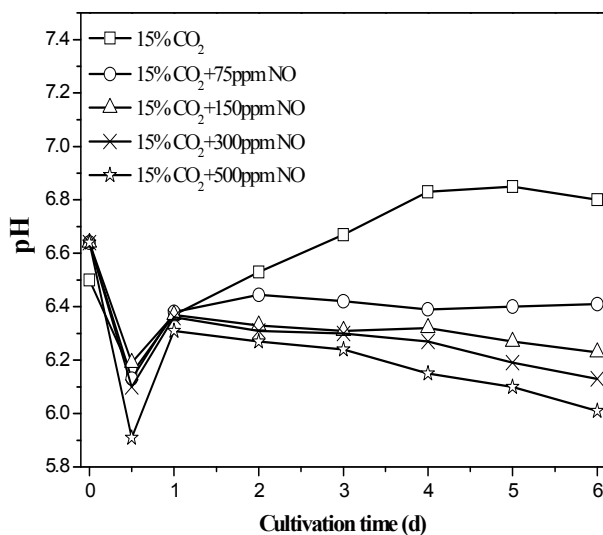


Fig.1. Experimental system in which the NO in flue gas was converted to NO_3^- as nitrogen source for microalgal growth.

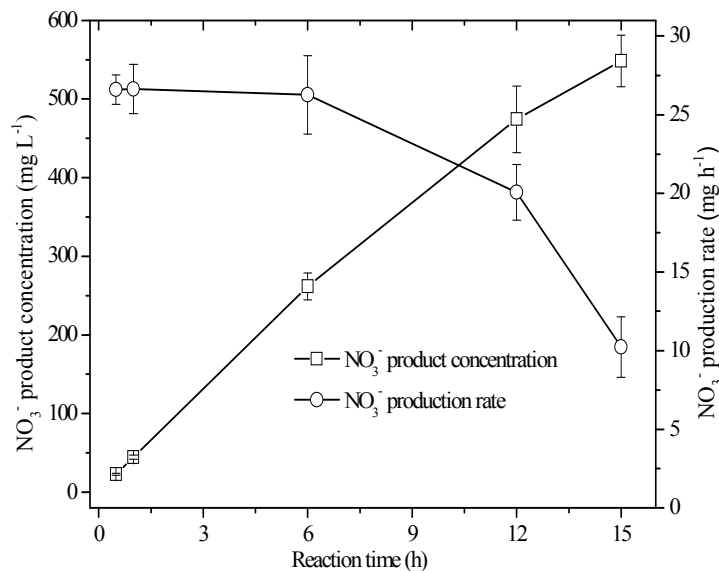


(a) Effects on biomass dry weight

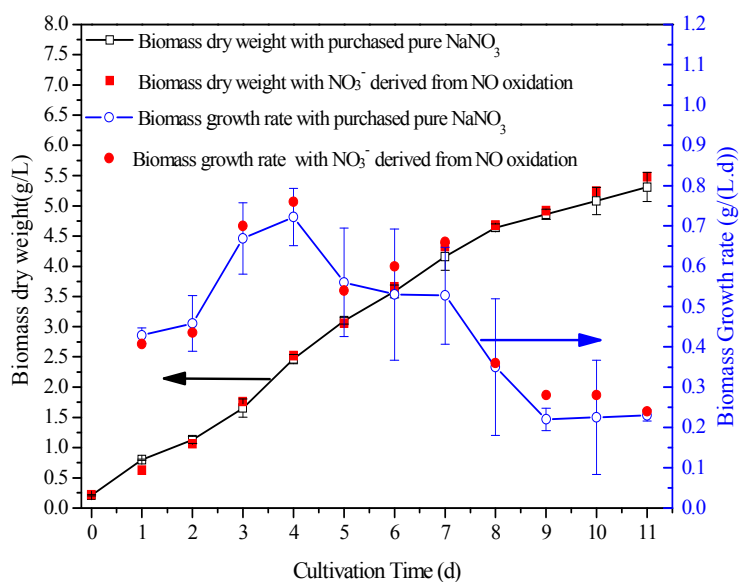


(b) Effects on pH value.

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



(a) 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 .

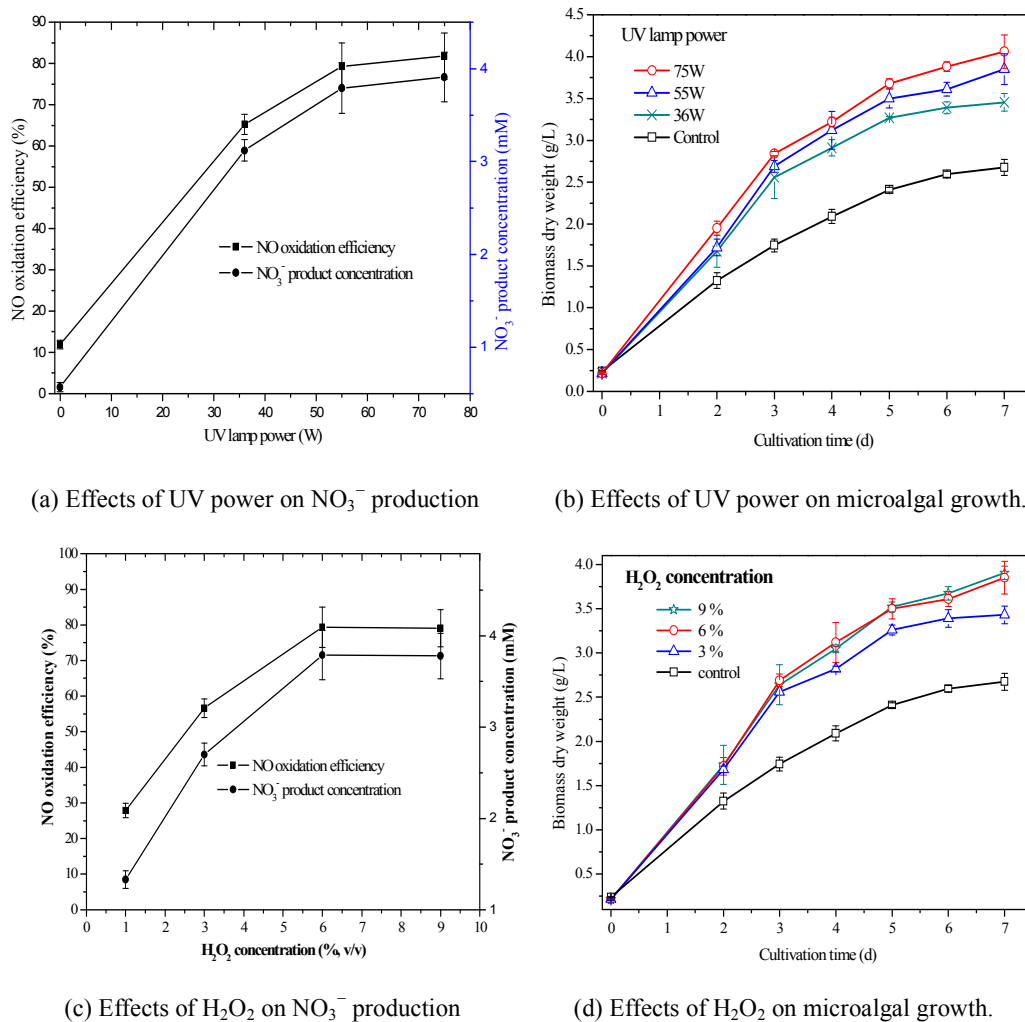
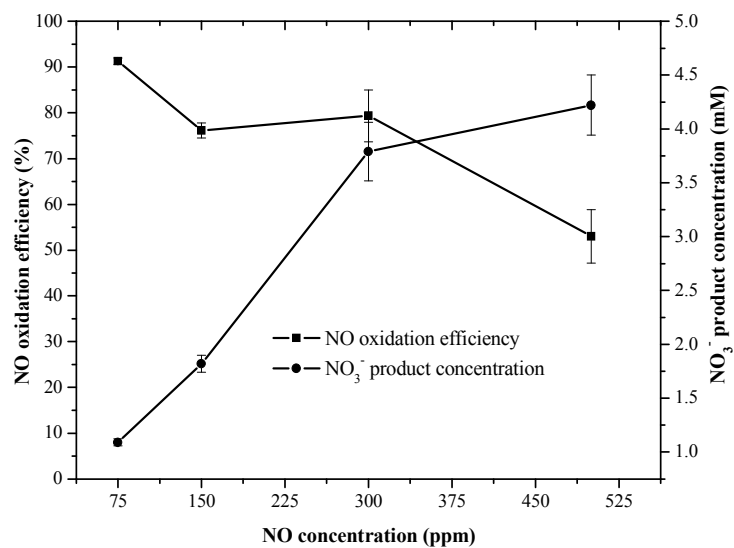
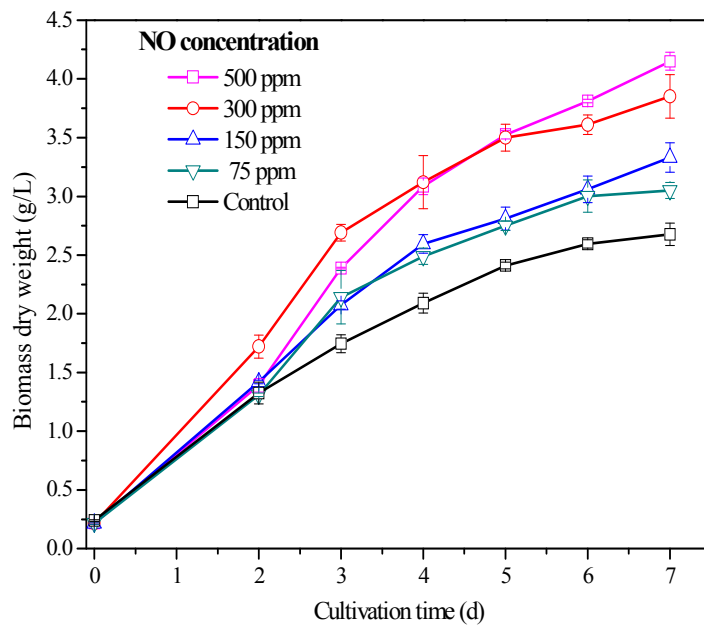


Fig.4. Effects of UV lamp power and H₂O₂ concentration on NO₃⁻ production and microalgal growth.



(a) Effects of NO concentration on NO₃⁻ production



(b) Effects of NO concentration on microalgal growth

Fig.5. Effects of NO concentration on NO₃⁻ production and microalgal growth.

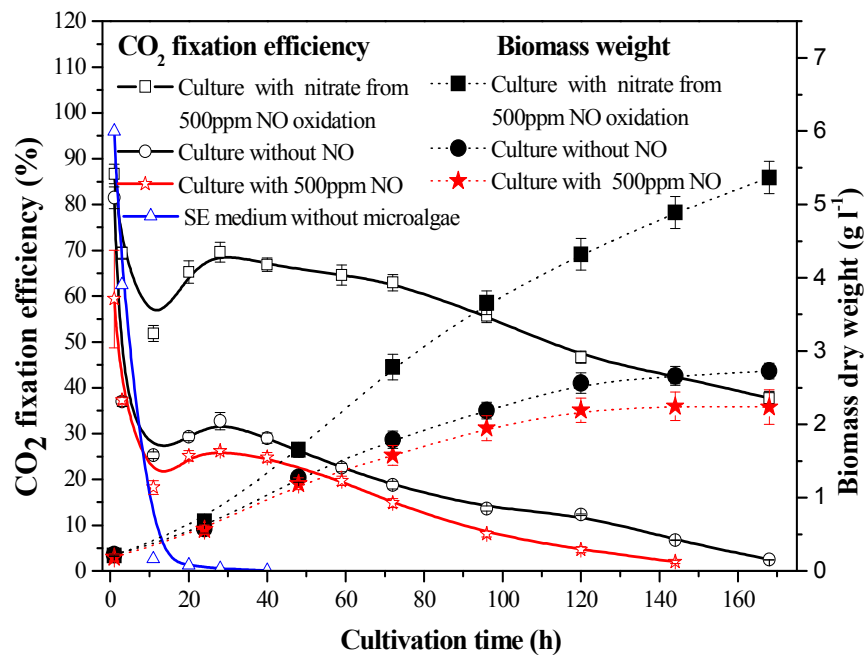


Fig.6. CO₂ fixation and biomass growth of *Chlorella.PY-ZU1* cultivated with NO₃⁻ derived from NO oxidation.

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