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Enhancement of Nitric Oxide Solubility Using Fe(II)EDTA and Its Removal by Green Algae *Scenedesmus* Sp.

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Abstract A photoautotrophic cultivation of green algae *Scenedesmus* cells was used for the removal of nitric oxide (NO) from a model flue gas mixture. In an attempt to improve the solubility of NO in the culture broth, the addition of Fe(II)EDTA to the cultivation was investigated. The addition of Fe(II)EDTA greatly enhanced NO-dissolution in the culture broth and subsequently increased the algal-uptake of NO. NO was assimilated as a source of nitrogen for the growth of *Scenedesmus* cells since there was a steady increase in cell density with no other nitrogen source in the culture except the incoming NO. 40~45% of NO removal was maintained for more than 12 days with the addition of 5 mM Fe(II)EDTA in a 1-L air-lift type photobioreactor system fed with 300 ppm of NO gas at a rate of 0.3 vvm. However, the NO-dissolution-enhancing capacity of Fe(II)EDTA did not reach its full potential due to its oxidation to Fe(III)EDTA, possibly induced by molecular oxygen that evolved from algal photosynthesis, and subsequent loss of chelating capabilities. © KSBB

Keywords: nitric oxide removal, *microalgae*, Scenedesmus, *Fe(II)EDTA*

INTRODUCTION

The nitrogen oxides (NOx) are greenhouse gases of important consequences since they are implicated as major contributors of air pollution, acid rain, and global warming. Nitric oxide (NO) is one of the major constituents of the NOx in anthropogenic emission, comprising about 90~95% of NOx [1]. Conventional end-of-pipe controls of NOx emission include chemical reduction and adsorption [2,3]. The major drawbacks of such processes are the requirement of expensive catalysts or adsorbents, high-energy consumption due to high operation temperatures, exposure of the environment to ammonia or urea, and generation of secondary wastes, which often requires further treatment. Hence, the need to develop a more efficient and low-cost NO-removal technology has arisen.

Biological NOx removal from contaminated emission gas streams is an alternative, cost-effective and environmentally sustainable technology. Ammonia and nitrate as well as aqueous NOx are assimilated to organic nitrogen or reduced to nitrogen gas by some bacteria through nitrification and denitrification processes.

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Recently, the utilization of microalgae has attracted attention because some species of microalgae are able to use CO_2 and NOx for their cultivation [4-8]. The resulting algal biomass can be used for other valuable purposes such as animal livestock and renewable energy sources [9-11].

For microalgal NO-elimination, NO gas is first dissolved in the aqueous phase. It is known that dissolved NO can be oxidized to nitrite and nitrate in water that is coupled with dissolved oxygen. NO is then taken up, further oxidized and assimilated by algal cells [6,12]. NO taken up by the algal cells is known to be preferentially utilized as a nitrogen source for cell growth [3,4]. Some research has reported that the rate-limiting step in most bioreactor systems is the dissolution of NO into the algal culture [3,13,14]. The poor water-solubility of NO implies that relatively long gas retention times and large reactor volumes are required for significant NOx removal.

Using an efficient complexing agent like metal-chelated EDTA can enhance the solubility of NO gas in liquids. The Fe(II)EDTA complex is especially known to possess the ability to chelate NO to the Fe center of the Fe(II)EDTA complex [15,16]. The use of Fe(II)EDTA for bacterial NO removal has been previously studied [17,18]. It was observed that some denitrifying bacteria were able to reduce NO from the Fe(II)EDTA-NO complex to N_2 gas at the expense of organic electron donors like ethanol or glucose.

Fe(II)EDTA is easily oxidized to Fe(III)EDTA in the presence of dissolved oxygen. This reaction is undesirable in an NO-removal system because Fe(III)EDTA is unable to chelate NO. Therefore, Fe(II)EDTA should be added periodically in order to maintain the continuity of NO removal. Otherwise, a reduction system to convert Fe(III) to Fe(II) using iron-reducing microorganisms must be employed [19,20].

In this study, the effect of Fe(II)EDTA addition to the microalgae cultivation was investigated for the purposes of enhancing NO-dissolution. A photoautotrophic cultivation of green algae *Scenedesmus* sp. was applied to remove the NO from a model flue gas mixture. The fate of dissolved NO is discussed and the NO removal performance and the resulting cell growth are presented.

MATERIALS AND METHODS

Green Algae

A green algae *Scenedesmus* sp. NIER-10060 was obtained from the National Institute of Environment Research (NIER) of Korea and cultured in C medium [21]. This media contained no organic or inorganic carbons except a trace amount of vitamins. The initial pH of the media was 7.5. The cells were cultivated photoautotrophically in a bioreactor in which 15% CO_2 and 200 μmol/m^2/s of light energy were supplied. Once the cell density reached 1.4 g/L and the nitrogen source in the medium was almost completely depleted, the gas mixture containing NO was supplied to the culture so that the dissolved NO in the gas mixture could serve as the sole nitrogen source.

Model Flue Gas

A model flue gas was prepared by mixing CO_2, N_2, and nitric oxide (NO). The gas mixture with 15% (v/v) CO_2 and 300 ppm (v/v) NO was balanced by the N_2 gas. Mixed gas was supplied to the reactor at a flow rate of 0.3 vvm via bubbling through a diffuser with a 10-μm-pore size installed at the conical bottom of the reactor.

Fe(II)-Complexed EDTA

Ferrous iron-complexed ethylenediaminetetraacetic acid (EDTA) solution was prepared by mixing $FeSO_4$ and Na_4EDTA in deionized, distilled water in which all the dissolved oxygen was eliminated by purging with N_2 gas. In order to observe the influence of iron-complexed EDTA on algal NO removal, 5 mM Fe(II)EDTA was supplied to the algal culture.

Photobioreactor

A conical-ended cylindrical glass reactor (35 cm length, 7 cm diameter) with a 1-L working volume was used for algal cultivation [22] and NO removal experiments. The reactor was operated in air-life type manner by introducing the gas stream to be treated through an inlet at the conical bottom. A 10 μm pore-sized diffuser was installed at the conical entrance for efficient gas bubbling and mass transfer. The top of the reactor was tightly sealed except a port for effluent gas outlet. External illumination by fluorescent lamps was applied so that the light intensity at the center of the medium-filled reactor was around 200 μmol/m^2/s.

NO-Removal Experiment

The photobioreactor filled with 1 L of C medium was inoculated with seed culture of *Scenedesmus* cells. Once the cell density achieved levels in the range of 1.3~1.5 g/L and the nitrogen source in the medium was almost depleted, the supply of the NO-containing gas mixture was initiated so that the introduced and dissolved NO could function as the sole source of nitrogen. The gas mixture consisted of 15% (v/v) of CO_2 and 300 ppm of NO, balanced by N_2 gas. In order to investigate the effects of iron-complexed EDTA on algal NO-removal, Fe(II)EDTA or Fe(III)EDTA was added to the algal culture at a concentration of 5 mM. The removal efficiency of NO was estimated by analyzing the NO-content in the effluent gas stream and comparing it to the NO-content in the entering gas mixture.

Analyses

The algal cell density was expressed as dry cell weight (DCW) per liter of culture. DCW was evaluated by drying cells at 105°C for 4 h after filtration through a 0.45 μm filter. The nitric oxide (NO) concentration in the gas stream was measured using the ECA 450 Gas Analyzer (Bacharach, Inc.). The light intensity at the center of the medium-filled reactor was measured using a LI-250A light sensor (LI-COR, Inc.). Nitrate ion (NO_3^-) was determined by UV spectrophotometry according to Standard Methods of Water Quality published by The Ministry of Environment, Korea [23]. The concentration of nitrite ion (NO_2^-) was analyzed based upon colorimetric methods after diazotization with sulfanilamide [23].

RESULTS AND DISCUSSION

NO-Removal through the Reactor without Microalgae Cells

The effect of 5 mM Fe(II)EDTA in the culture media without *Scenedesmus* cells, on NO-removal, is shown in Fig. 1. The NO-contents in the effluent stream, which was passed through a blank culture medium and through the medium containing Na_4EDTA only, were also compared. It was confirmed that the NO-dissolving capability was both negligible in the culture medium itself and in the medium containing non-iron-chelated EDTA.

While in the culture media with Fe(II)EDTA, the NO content in the effluent stream was almost zero at the beginning

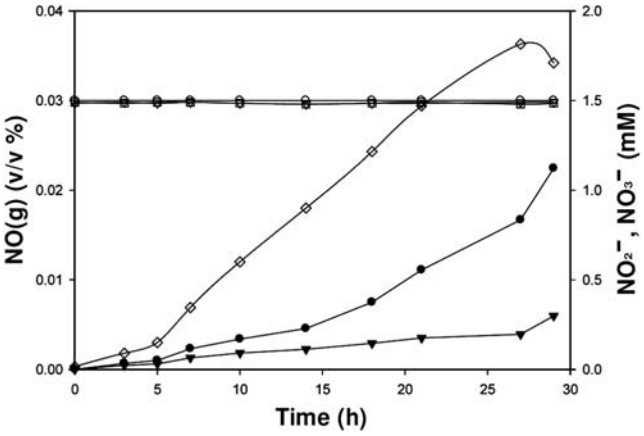

Fig. 1. NO-removal through various media without algae cells and the concentrations of dissolved nitrate and nitrite. ○, NO in influent gas; ▽, NO in effluent gas through blank culture medium; □, NO in effluent gas through medium with Na_4EDTA; ◇, NO in effluent gas through medium with Fe(II)EDTA; ●, NO_3^- concentration in the medium with Fe(II)EDTA; ▼, NO_2^- concentration in the medium with Fe(II)EDTA.

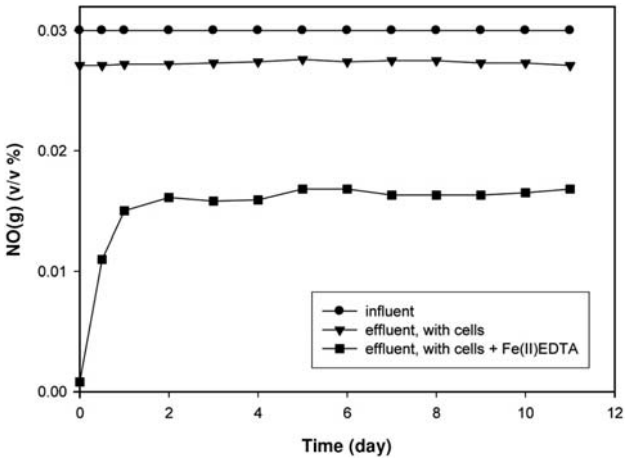

Fig. 2. NO-removal with and without Fe(II)EDTA in photoautotrophic cultivation of *Scenedesmus* cells.

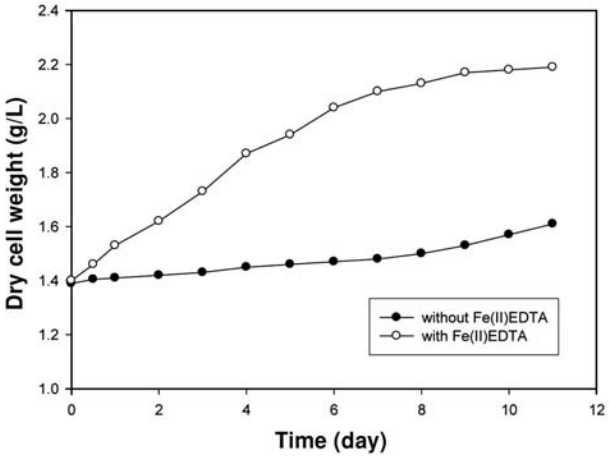

Fig. 3. Cell growth with and without Fe(II)EDTA in photoautotrophic cultivation of *Scenedesmus* cells.

and below 30% of influent concentration for the first 8 h. This result verified that Fe(II)EDTA was responsible for NO-dissolution. The area above the curve below inlet concentration (0.03%) was proportional to the total mass of dissolved NO. The concentration of NO in the effluent gas stream increased as time passed and NO-dissolution stopped after 21 h probably because all Fe(II)EDTA in the reactor was saturated with NO. The dissolved NO was converted to Fe(II)EDTA-NO complex or to ionic forms like NO_3^- and NO_2^-. From the observation that the NO_3^- and NO_2^- concentrations were very small while the NO-removal was almost perfect during 0~5 h, it was concluded that the dissolved NO preferentially existed as Fe(II)EDTA-NO complex rather than as NO_3^- or NO_2^-.

The NO concentration above 0.03% (300 ppm) after 21 h indicated that the NO in the effluent gas stream surpassed the entering concentration. It is possible that there was insufficient free Fe(II)EDTA available for NO-chelation after 21 h and the captured NO may have been stripped away by the action of incoming gas bubbles.

Influences of Fe(II)EDTA on Algal NO-Removal and Cell Growth

The concentration of NO in the effluent gas stream of the regular culture medium was compared to that of the medium supplemented with 5 mM Fe(II)EDTA (Fig. 2). In the cultivation of microalgae with regular medium, the concentration of NO in the effluent gas stream was in the range of 0.026~0.027% (260~270 ppm). Only 8~10% of NO was removed for more than 10 days, although the cell density increased steadily over time (Fig. 3). The average cell growth rate was 0.024 g/L/d. Dissolved NO (aq) or oxidized forms like nitrate and nitrite are taken up by microalgae. Since the only nitrogen source in the reactor is the incoming NO gas, the steady growth of cells implied that the dissolved NO served as a source of nitrogen for the growth of *Scenedesmus* cells.

The major limiting factor for NO-removal here is the low solubility of NO in microalgal culture. The addition of Fe(II)EDTA was investigated in order to enhance the amount of NO dissolved in the culture broth and to increase the availability of NO to microalgal uptake. Fe(II)EDTA was proved to be capable of forming chelates with NO in Fig. 1 and the chelated form of NO was found to be highly soluble in the aqueous phase.

The result of NO-removal in the presence of 5 mM Fe(II)EDTA in the culture broth is shown in Fig. 2. The content of NO in the effluent gas stream was almost zero at the beginning of NO-supply but this soon increased to 0.017~0.018% which corresponded to about 40~45% re-

moval. This level was maintained for more than 10 days. Compared to the regular culture media, the addition of Fe(II)EDTA greatly improved the removal of NO by *Scenedesmus* cells. This could be attributable to the increased bioavailability of NO by chelating with Fe(II)EDTA. As can be seen in Fig. 3, the average cell growth rate was calculated to be 0.082 g/L/d, which was 3-fold higher than that obtained in the regular culture medium without Fe(II)EDTA. These results implied that Fe(II)EDTA enhanced the NO-dissolution and increased the uptake of NO by the microalgae.

The removal of NOx by Fe(II)EDTA chelation was previously referred to as the BioDeNOx process [20,24]. This process consists of two stages; chemical absorption of NO in Fe(II)EDTA solution and bacterial denitrification in a bioreactor inoculated with a denitrifying sludge [25]. The biological reduction of NO to N_2 takes place under thermophilic conditions and an electron donor like ethanol is required for this process. NO-removal with an efficiency of 40~70% was obtained under a continuous flue gas flow of 650 L/h with 300 ppm NO and 1% O_2, and could be raised up to 80% with bioaugmentation. In this process, however, the Fe(III)EDTA that was formed due to the presence of oxygen had to be reduced back to Fe(II)EDTA with the help of sulfur-reducing bacteria or iron-reducing bacteria and with the expense of an electron donor.

Loss of Chelating Capability of Fe(II)EDTA

The microalgal NO-removal system developed in this study is simple and straightforward in its process constituents, compared to the bacterial NO-removal described in the BioDeNOx process. However, the 40~45% algal NO-removal by Fe(II)EDTA, shown in Fig. 2, was unsatisfactory under conditions of instantaneous NO-dissolution and quick algal uptake of NO [4,14]. In addition, the initial concentration of Fe(II)EDTA (5 mM) was sufficient to chelate all the incoming NO at 0.03% (v/v), based on the reaction shown in Eq. (1) [15,16,26].

$$NO(aq) + Fe(II)EDTA \leftrightarrow Fe(II)EDTA\text{-}NO \quad (1)$$

Therefore, the NO-dissolving capability of Fe(II)EDTA in this system did not reach its full potential. The possibility of EDTA-consumption by microalgal degradation was little because of the sufficient supply of CO_2 as a carbon source. So, the probable explanation for the low NO removal was the oxidation of Fe(II) to Fe(III) as shown in Eq. (2) [18,27].

$$4\ Fe(II)EDTA + O_2 + 4H^+ \rightarrow 4\ Fe(III)EDTA + 2\ H_2O \quad (2)$$

Although the photobioreactor was operated in an anaerobically closed system and the influent gas stream did not contain oxygen, molecular oxygen may have evolved from algal photosynthesis and dissolved in water to a certain extent [28]. Therefore, Fe(II)EDTA could have lost its chelating capability when oxidized to Fe(III)EDTA. Although initial DO value was zero (Fig. 1), the actual DO value in the culture during the middle of NO-removal was about 0.05~0.08 mg/L.

CONCLUSION

The addition of Fe(II)EDTA to the culture of *Scenedesmus* cells greatly improved the NO-removal. The existence of Fe(II)EDTA enhanced NO-dissolution in culture broth through chelate-formation with NO and subsequently increased microalgal-uptake of NO. NO was assimilated as a source of nitrogen as there was no other nitrogen source in the culture except the incoming NO and the growth of *Scenedesmus* cells increased steadily with time. The NO-dissolving capability of Fe(II)EDTA did not reach its full potential because some fraction of Fe(II)EDTA lost their chelating capability when oxidized to Fe(III)EDTA, probably due to the molecular oxygen evolved from algal photosynthesis. However, this microalgal NO-removal system has unique advantages in that CO_2 and NOx are eliminated simultaneously, being assimilated and fixed to algal biomass and that there is no need of an extra source of carbon and nitrogen. This system warrants further studies for improving the NO-removal performance, minimizing the oxidation of Fe(II) and increasing the gas residence time in a given reactor.

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