

# Evaluation of Microbial Reduction of Fe(III)EDTA in a Chemical Absorption-Biological Reduction Integrated NO<sub>x</sub> Removal System

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A chemical absorption-biological reduction integrated process can be used to remove nitrogen oxides (NO<sub>x</sub>) from flue gas. In such a process, nitric oxide (NO) can be effectively absorbed by the ferrous chelate of ethylenediaminetetraacetate (Fe(II)EDTA) to form Fe(II)EDTA-NO, which can be biologically regenerated by denitrifying bacteria. However, in the course of these processes, part of the Fe(II)EDTA is also oxidized to Fe(III)EDTA. The reduction of Fe(III)EDTA to Fe(II)EDTA depends on the activity of iron-reducing bacteria in the system. Therefore, the effectiveness of the system relies on how to effectively bioreduce Fe(III)EDTA and Fe(II)EDTA-NO in the system. In this paper, a strain identified as *Escherichia coli* FR-2 (iron-reducing bacterium) was used to investigate the reduction rate of Fe(III)EDTA. The experimental results indicate that Fe(II)EDTA-NO and Fe(II)EDTA in the system can inhibit both the FR-2 cell growth and thus affect the Fe(III)EDTA reduction. The FR-2 cell growth rate and Fe(III)EDTA reduction rate decreased with increasing Fe(II)EDTA-NO and Fe(II)EDTA concentration in the solution. When the concentration of Fe(II)EDTA-NO reached 3.7 mM, the FR-2 cell growth almost stopped. A mathematical model was developed to explain the cell growth and inhibition kinetics. The predicted results are close to the experimental data and provide a preliminary evaluation of the kinetics of the biologically mediated reactions necessary to regenerate the spent scrubber solution.

## Introduction

Nitrogen oxide is one of the major air pollutants emitted from various anthropogenic sources (e.g., flue gas from coal-fired power plants) (1, 2). However, due to its low solubility in aqueous solution, it is difficult to remove NO (the primary component of NO<sub>x</sub>) through traditional control systems such as the wet flue gas desulfurization scrubbers. One promising approach of removing NO from flue gas is the use of metal chelate additives (3, 4). Fe(II)EDTA is one of the most promising additives for the removal of NO from flue gas due to the rapid absorption rate and moderate cost (5–7). However, there are two major drawbacks associated with this approach. One is the difficulty in the regeneration of Fe(II)EDTA-NO in the scrubber solution, and the other is the easy oxidation of Fe(II)EDTA by oxygen in the flue gas to

form Fe(III)EDTA, which is not capable of binding NO (8, 9). As a result, the concentration of the active Fe(II)EDTA in the system is low, and thus an adequate NO removal efficiency cannot be sustained. Past studies demonstrated that chemical or electrochemical reduction techniques to regenerate ferrous chelates are not effective due to the high costs, production of unwanted byproducts, and/or low reduction rate (10–12). Recently, a new method by using the chemical absorption-biological reduction integrated system was studied to convert NO to N<sub>2</sub> and regenerate Fe(II)EDTA-NO and Fe(III)EDTA to Fe(II)EDTA (13–17). In this integrated process, two bacteria were isolated from the mixed cultures. One has the ability to convert Fe(II)EDTA-NO to N<sub>2</sub> and Fe(II)EDTA. Another one can reduce Fe(III)EDTA to Fe(II)EDTA (13). The main steps associated with this integrated system are illustrated in Figure 1.

Since only Fe(II)EDTA can absorb NO, the NO removal in this integrated process strongly depends on the Fe(III)EDTA reduction rate. Our previous report and other investigations have demonstrated that the biological reduction of Fe(III)EDTA is a key step in this integrated system (13, 16). Several dissimilatory iron-reducing bacteria that are capable of reducing Fe(III) have been isolated from a variety of anoxic environments and are widely distributed among bacteria, as evidenced by 16S rRNA gene sequences (18–23). Kieft (24) reported that a thermophilic bacterium SA-01 could use O<sub>2</sub>, NO<sub>3</sub><sup>−</sup>, Fe(III), and S<sup>0</sup> as terminal electron acceptors for growth. *Escherichia coli* (25) was also reported to have ability to reduce Fe(III) at 30 °C. However, those investigations were focused on the reduction of crystalline iron (e.g., crystalline iron oxides). Very limited information is available on biological reduction of Fe(II)EDTA. Lovley (26) reported that soluble chelated iron, e.g., Fe(III)EDTA, was suitable for microbial reduction. Since Fe(II)EDTA-NO, Fe(III)EDTA, and Fe(II)EDTA coexist in the chemical absorption-biological reduction integrated system, understanding the interdependency between various chemical species is important.

In this study, the effect of Fe(II)EDTA-NO or Fe(II)EDTA on the biological reduction of Fe(III)EDTA was investigated. Specifically, the inhibition kinetic of Fe(II)EDTA-NO or Fe(II)EDTA on the *Escherichia coli* cell growth and the associated biological reduction of Fe(III)EDTA was evaluated. A kinetic model was developed to explain the experimental findings. The results of this investigation provide insight on the system design, configuration, and operation.

## Materials and Methods

**Chemicals.** Na<sub>2</sub>EDTA (99.95%), FeCl<sub>2</sub>·4H<sub>2</sub>O (99.5%), FeCl<sub>3</sub>·6H<sub>2</sub>O (99.5%), and D-glucose (99.5%, cell culture tested) were from Shanghai Chemical Reagent Co. (Shanghai, China). 1% NO in N<sub>2</sub> and N<sub>2</sub> (99.99%) were obtained from Zhejiang Jingong Gas Co. (Hangzhou, China). All other chemicals were analytical grade reagents.

**Bacterial Strain and Media.** The basal media for the bacteria growth include the following components per liter of solution: 1000 mg of glucose, 1000 mg of NH<sub>4</sub>Cl, 625 mg of KH<sub>2</sub>PO<sub>4</sub>, 1000 mg of K<sub>2</sub>HPO<sub>4</sub>·3H<sub>2</sub>O, 70 mg of Na<sub>2</sub>SO<sub>3</sub>, 100 mg of MgSO<sub>4</sub>, 2 mg of CaCl<sub>2</sub>, 0.5 mg of MnSO<sub>4</sub>, 0.1 mg of Na<sub>2</sub>MoO<sub>4</sub>, and 0.1 mg of CuSO<sub>4</sub>·5H<sub>2</sub>O.

Strain FR-2 was isolated from the mixed culture in a lab-scale bioreactor. Taxonomical identification was performed by 16S rRNA amplification and sequencing at Shanghai Sangon Biological Engineering & Technology and Service Co., Ltd. (Shanghai, China). The 16S rRNA gene sequences similarity searches were conducted with BLAST program in the GenBank (NCBI), which exhibited high levels of 99%

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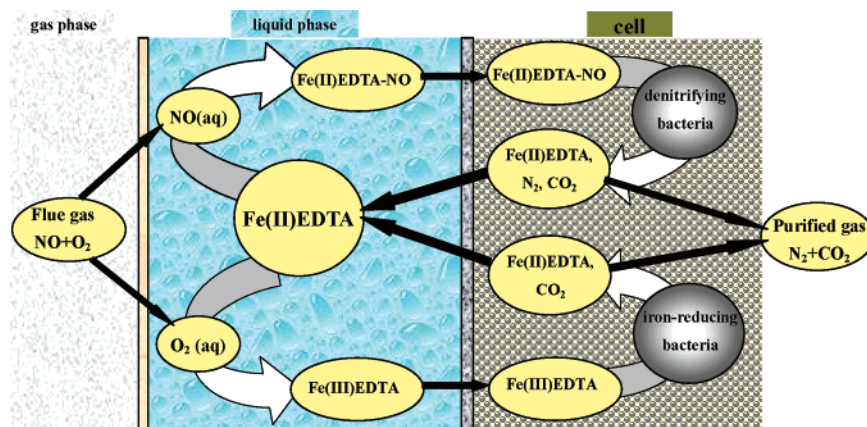


FIGURE 1. Schematic principle of the chemical absorption-biological reduction integrated NO<sub>x</sub> removal system.

similarity with *Escherichia coli*. Therefore, FR-2 was tentatively identified as *Escherichia coli*. Cultivation was done in 250 mL conical flasks containing 100 mL of basal medium at 40 °C and 140 r min<sup>-1</sup> in a rotary shaker. Cells in the medium were harvested by centrifugation at 2500 × g for 15 min, washed twice with the saline solution, and suspended in 0.1 mM phosphate buffer at a desired concentration for use.

**Fe(III) Reduction Experiments.** The Fe(III)EDTA and Fe(II)EDTA complex stock solution were prepared with equal concentration (40 mM) of Na<sub>2</sub>EDTA and FeCl<sub>2</sub> or FeCl<sub>3</sub> under anoxic condition. The solution pH was adjusted with 0.1 mM phosphate buffer (pH 7.0). The Fe(II)EDTA-NO complex solution was prepared by bubbling NO through the ferrous EDTA solution until full breakthrough of NO was observed (The inlet and outlet concentration of NO was measured to be equal with a chemiluminescent NO<sub>x</sub> analyzer.) in the sparging vessel effluent. The prepared solution were kept in glass serum vials under N<sub>2</sub> positive pressure in order to avoid the oxidation of the ferrous EDTA in the solution. The Fe(II)EDTA-NO saturated solution exhibited negligible break down over a period of 1 week under a blanket of inert gas.

Fe(III) reduction experiments were conducted in 100 mL glass serum vials in a gyrating shaker at 140 r min<sup>-1</sup> and temperature of 40 °C. The basal medium was added, and cells were inoculated to an initial concentration of 0.1 g (DCW) L<sup>-1</sup>. The total volume of liquid was 80 mL, and the anaerobic condition was obtained by replacing the air above the solution surface with nitrogen gas. For the evaluation of Fe(II)EDTA-NO inhibition on the cell growth and Fe(III)EDTA reduction, the initial concentration of Fe(III)EDTA was at 12 mM with different concentrations of Fe(II)EDTA-NO in each serum vial. Similarly, the inhibition of Fe(II)EDTA on the cell growth and Fe(III)EDTA reduction was evaluated at an initial concentration of 6 mM Fe(III)EDTA with different concentrations of Fe(II)EDTA in each serum vial. As control experiments, the effect of initial Fe(III)EDTA concentration on the Fe(III)EDTA reduction rate was also investigated.

**Analytical Methods.** The concentration of ferrous ions and total iron in solution was determined by a modified 1,10-phenanthroline colorimetric method at 510 nm. Fe(III) concentrations were calculated from the difference between total Fe and Fe(II) (15, 27). The concentration of Fe(II)EDTA-NO was measured by a model UV-2000 spectrophotometer (UNICO (Shanghai) Instruments Co., Ltd.) at 420 nm. The concentration of cells was determined from the linear relationship between the optical density at 610 nm and dry cell weight (DCW). The data shown in this work were the mean values of the duplicate or triplicate experiments.

**Model Development.** The equations describing cell growth, production formation, and substrate consumption are as follows:

The logistic equation (28) for a description of cell growth is shown in eq 1

$$\frac{dX}{dt} = \mu_{\max} \left( 1 - \frac{X}{X_{\max}} \right) X \quad (1)$$

where  $dX/dt$  is the rate of cell growth;  $\mu_{\max}$  is the maximum specific growth rate of the microorganisms;  $X$  is the concentration of cells;  $X_{\max}$  is the maximum value of the cells concentration; and  $t$  is the cultivation time.

The Luedeking–Piret equation (29) for Fe(II)EDTA production is shown in eq 2

$$\frac{dP}{dt} = \alpha \frac{dX}{dt} + \beta X \quad (2)$$

where  $dP/dt$  is Fe(II)EDTA formation rate;  $\alpha$  is the production formation coefficient; and  $\beta$  is a nongrowth correlation coefficient.

Since Fe(III)EDTA only served as electric acceptor, the consumption of substrate (Fe(III)EDTA) was equal to the production of Fe(II)EDTA, that is  $-dS/dt = dP/dt$ . As such, the following relationship is obtained

$$-\frac{dS}{dt} = \alpha \frac{dX}{dt} + \beta X \quad (3)$$

where  $dS/dt$  is the consumption rate of Fe(III)EDTA.

When there are external variables that influence microbial growth, for example, some inhibitor present in the solution, eq 1 is rewritten as follows

$$\frac{dX}{dt} = \mu_{\max} \left( 1 - \frac{X}{X_{\max}} \right) X \gamma_i \quad (4)$$

where  $\gamma_i$  is the inhibition factor.

At the beginning of cultivation ( $t = 0$ ), the cell concentration is given by the initial concentration value ( $X = X_0$ ). After integration, eq 4 becomes

$$X = \frac{X_0 X_{\max} e^{\mu_{\max} t \gamma_i}}{X_{\max} - X_0 + X_0 e^{\mu_{\max} t \gamma_i}} \quad (5)$$

Substituting  $X$  by eq 5 into eq 3, the following expression (eq 6) for the Fe(III)EDTA concentration was obtained

$$S = S_0 - \alpha \frac{X_0 X_{\max} e^{\mu_{\max} t \gamma_i}}{X_{\max} - X_0 + X_0 e^{\mu_{\max} t \gamma_i}} + \alpha X_0 - \beta \frac{X_{\max}}{\mu_{\max}} \ln \frac{X_{\max} - X_0 + X_0 e^{\mu_{\max} t \gamma_i}}{X_{\max}} \quad (6)$$

where  $S_0$  is the initial Fe(III)EDTA concentration.

TABLE 1. Values of Model Parameters

inhibitor	initial concn (mM)	$X_0$ (g L <sup>-1</sup> )	$X_{\max}$ (g L <sup>-1</sup> )	$\mu_{\max}$ (h <sup>-1</sup> )	$\gamma_i$	$\alpha$ (mmol g <sup>-1</sup> )
Fe(II)EDTA-NO	0	0.09362	0.4484	0.9390	1	16.15
	0.8	0.09391	0.3393	0.9390	0.3163	26.17
	1.9	0.08962	0.3357	0.9390	0.2652	34.71
	3.7	0.1077	0.1077	0.9390	tend to 0	0
Fe(II)EDTA	0	0.1120	0.3597	0.9246	1	11.56
	3.2	0.1039	0.3320	0.9246	0.8877	16.48
	5.7	0.09205	0.2919	0.9246	0.6944	20.56
	8.8	0.1034	0.2105	0.9246	0.6762	8.66

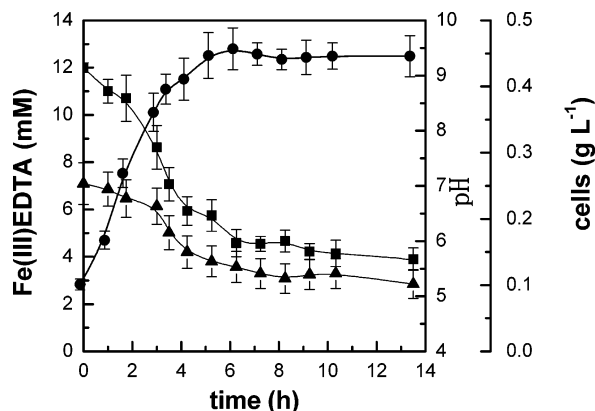


FIGURE 2. Characterization of strain FR-2: (■) the concentration of Fe(III)(EDTA); (▲) the value of pH; and (●) the concentration of cells ( $T = 313$  K,  $[\text{Fe(III)(EDTA)}]_0 = 12$  mM).

Parameter values were obtained by fitting between the models used and the experimental data. Table 1 lists the values of the parameters used in the above mathematical model.

## Results

**Characterization of *Escherichia coli* FR-2.** The time course of Fe(III)(EDTA) reduction and the growth of strain FR-2 are shown in Figure 2. The growth of cells has no distinct lag phase at the beginning of cultivation. When the cell growth reached the exponential phase, the concentration of cells increases quickly (from 0.1 g (DCW) L<sup>-1</sup> to 0.450 g (DCW) L<sup>-1</sup>), and the concentration of Fe(III)EDTA declines sharply (from 12 mM to 4.6 mM) within 6 h. While the concentration of Fe(III)EDTA has no remarkable change during stationary phase, the concentration of cells maintains at about 0.45 g (DCW) L<sup>-1</sup>. Moreover, the value of pH declines from 7 to 5.5 at the exponential phase, but there is no significant change during the stationary phase.

The action of strain FR-2 on the Fe(III)(EDTA) or Fe(II)-EDTA-NO is shown in Figure 3. FR-2 had a good performance in the reduction of Fe(III)EDTA. After 9 h of cultivation, Fe(III)EDTA concentration decreases from about 6 to 0.8 mM, about 87% was reduced to Fe(II)EDTA. However, strain FR-2 could not metabolize Fe(II)EDTA-NO at an initial concentration of 5.7 mM.

**Effect of Fe(II)EDTA-NO on the Cell Growth and Fe(III)EDTA Reduction.** The effect of different initial concentration of Fe(II)EDTA-NO addition in the solution on strain FR-2 growth is shown in Figure 4. The results indicate that the cell growth period changes, and the stationary phase of cell growth is obviously delayed when Fe(II)EDTA-NO is present. The cell growth rate decreases with the increase of the Fe(II)EDTA-NO concentration. The increased biomass was 0.13 g (DCW) L<sup>-1</sup> and 0.09 g (DCW) L<sup>-1</sup> (within 6 h) when 0.8 mM and 1.9 mM Fe(II)EDTA-NO were added, respectively,

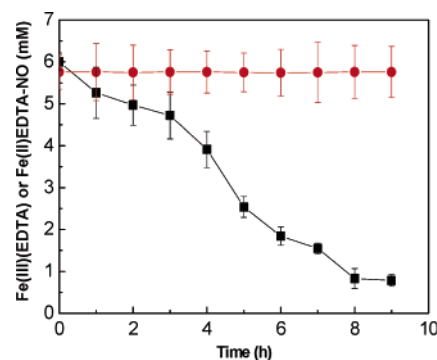


FIGURE 3. Reduction property of strain FR-2 on the Fe(III)(EDTA) or Fe(II)EDTA-NO, respectively: (■) the concentration of Fe(III)(EDTA) and (●) the concentration of Fe(II)EDTA-NO ( $T = 313$  K,  $[\text{Fe(III)(EDTA)}]_0 = 6$  mM or  $[\text{Fe(II)EDTA-NO}]_0 = 5.7$  mM).

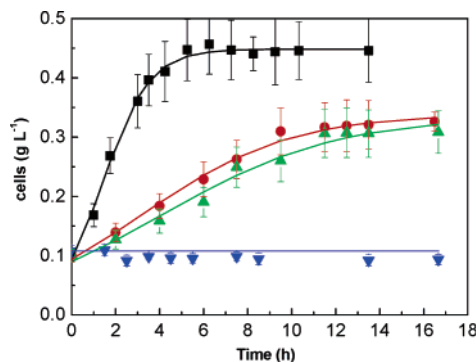


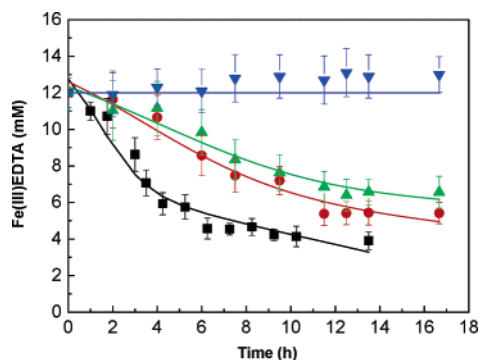
FIGURE 4. Curves of cells growth containing different initial concentration of Fe(II)EDTA-NO: (■) 0 mM; (●) 0.8 mM; (▲) 1.9 mM; (▼) 3.7 mM; and (—) the predicting data ( $T = 313$  K,  $[\text{Fe(III)(EDTA)}]_0 = 12$  mM).

compared to the 0.35 g (DCW) L<sup>-1</sup> biomass when Fe(II)-EDTA-NO was absent. The cell growth was inhibited and almost stopped when 3.7 mM Fe(II)EDTA-NO was added. The final cell concentration was also low compared to no Fe(II)EDTA-NO in the solution.

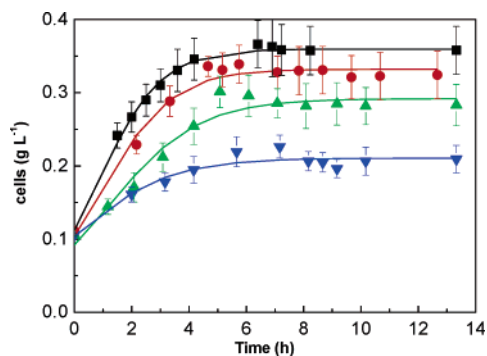
Figure 5 shows the effect of a different initial concentration of Fe(II)EDTA-NO addition on Fe(III)EDTA reduction. During the first 6 h reaction, the average reduction rate of Fe(III)-EDTA was about 1.23 mM h<sup>-1</sup> when Fe(II)EDTA-NO was absent. However, it decreased to 0.57 mM h<sup>-1</sup> and 0.37 mM h<sup>-1</sup> when 0.8 mM and 1.9 mM Fe(II)EDTA-NO were added, respectively. The total Fe(III)EDTA reduced was 8.1 mM when there was no Fe(II)EDTA-NO present, while it was only 6.6 mM and 5.6 mM when there were 0.8 mM and 1.9 mM Fe(II)EDTA-NO present. Strangely, a little more Fe(III)EDTA was increased compared with its initial concentration when Fe(II)EDTA-NO reached 3.7 mM.

**Effect of Fe(II)EDTA on the Cell Growth and Fe(III)-EDTA Reduction.** The effect of different initial concentration

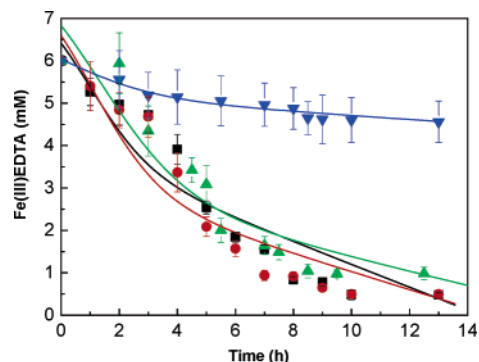




**FIGURE 5.** Effect of different initial concentration of Fe(II)EDTA-NO on Fe(III)EDTA reduction: (■) 0 mM; (●) 0.8 mM; (▲) 1.9 mM; (▼) 3.7 mM; and (—) the predicting data ( $T = 313$  K,  $[\text{Fe(III)EDTA}]_0 = 12$  mM).



**FIGURE 6.** Curves of cell growth with different initial concentrations of Fe(II)EDTA: (■) 0 mM; (●) 3.2 mM; (▲) 5.7 mM; (▼) 8.8 mM; and (—) the predicting data ( $T = 313$  K,  $[\text{Fe(III)EDTA}]_0 = 6$  mM).



**FIGURE 7.** Effect of the initial concentration of Fe(II)EDTA on Fe(III)EDTA reduction: (■) 0 mM; (●) 3.2 mM; (▲) 5.7 mM; (▼) 8.8 mM; and (—) the predicting data ( $T = 313$  K,  $[\text{Fe(III)EDTA}]_0 = 6$  mM).

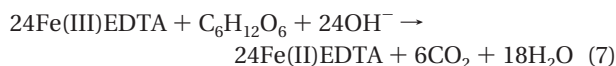
of Fe(II)EDTA addition in the solution on strain FR-2 growth is shown in Figure 6. Similar findings were observed as for the Fe(II)EDTA-NO. Cells growth rate decreases with the increase of Fe(II)EDTA concentration. In the first 4 h, the increased biomass was 0.20, 0.15, and 0.09 g (DCW)  $\text{L}^{-1}$  when 3.2, 5.7, and 8.8 mM Fe(II)EDTA were added, respectively. But it was 0.24 g (DCW)  $\text{L}^{-1}$  when no Fe(II)EDTA was added. The final concentration of cells also decreases with the increase of the Fe(II)EDTA concentration.

Figure 7 shows the inhibition effect on Fe(III)EDTA reduction with a different initial concentration of Fe(II)EDTA added to the solution. The average Fe(III)EDTA reduction rate (within 10 h) has no obvious change ( $0.55 \text{ mM h}^{-1}$ ) when the addition of Fe(II)EDTA is less than 5.7 mM, but it decreases sharply (about  $0.15 \text{ mM h}^{-1}$ ) as the Fe(II)EDTA concentration was increased to as high as 8.8 mM.

## Discussion

Although *Escherichia coli* was reported to be incapable of reducing crystalline Fe(III) (30), our experimental results indicate that *Escherichia coli* FR-2 could reduce Fe(III)EDTA efficiently with glucose as electron donor. This finding is consistent with other studies (25, 31, 32). Vartivarian (33) demonstrated that the enzymes from *Escherichia coli* were capable of efficiently reducing iron that was coordinated to ligands with different geometries and found that the enzymes could reduce a variety of ferric chelates with activities ranging from approximately 3 to 390 mmol Fe(II)  $\text{g}^{-1}$  protein  $\text{min}^{-1}$ . van der Maas (31) indicated the direct enzymatic reduction of Fe(III)EDTA cannot be excluded in the chemical absorption-biological reduction integrated system.

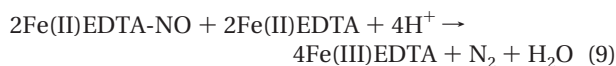
In Figure 2, the experimental results show that the value of pH gradually declines with the reduction of Fe(III)EDTA. This could be explained by the following reaction.



Based on the above reaction,  $\text{OH}^-$  is consumed with Fe(III)EDTA reduction. The pH in the solution declines with Fe(III)EDTA reduction. Therefore, the pH value has no obvious change when Fe(III)EDTA is not reduced during the stationary phase.

The model simulation was performed by using the origin 7.0 software. The simulation results show that the model matches the experimental data satisfactorily. The average deviation is less than 8.2%. From Table 1, it can be seen that the value of  $\gamma_i$  decrease with the increase of Fe(II)EDTA-NO or Fe(II)EDTA concentration. However,  $\gamma_i$  for Fe(II)EDTA was larger than those in the Fe(II)EDTA-NO inhibition experiments, indicating that the inhibition of Fe(II)EDTA on the cell growth of FR-2 was weaker than that of Fe(II)EDTA-NO. The Fe(III)EDTA reduction rate had no obvious change when the initial concentration of Fe(II)EDTA was less than 5.7 mM, but the reduction rate would decrease quickly if the Fe(II)EDTA concentration in the solution were further increased (Figure 7). We also found that the parameter  $\beta$  was nearly equal to zero (data not shown), and the item  $\beta(X_{\text{max}}/\mu_{\text{max}}) \ln[(X_{\text{max}} - X_0 + X_0 e^{\mu_{\text{max}} t/\gamma_i})X_{\text{max}}]$  was too small to be included. This finding indicates that Fe(II)EDTA is referred to as a growth-associated product, as its rate of production parallels the growth of the cell population. To our best knowledge, the inhibition of growth by end-products of metabolism is a universal phenomenon in the biologically reducing process. Therefore, Fe(II)EDTA, as a product of cellular metabolism in this system, would inhibit cell growth and hence slow the rate of its production.

When the concentration of Fe(II)EDTA-NO was up to 3.7 mM, about 1 mM Fe(III)EDTA was increased after the cultivation. This might be due to the following two reactions in the solution.



Another study also reported that Fe(II)EDTA could serve as electron donor for the biological reduction of NO to  $\text{N}_2$  (17).

As shown in Table 2, the addition of an inhibitor (e.g., Fe(II)EDTA-NO or Fe(II)EDTA) results in an obvious decline in the specific Fe(III)EDTA reduction rate. Fe(III)EDTA reduction stops at 3.7 mM Fe(II)EDTA-NO. The reduction rate declines about 74% at 8.8 mM Fe(II)EDTA. However, the specific Fe(III)EDTA reduction rate increases gradually with

**TABLE 2. Effect of the Initial Fe(III)EDTA Concentration and the Amount of Inhibitor (Fe(II)EDTA-NO or Fe(II)EDTA) on the Specific Fe(III)EDTA Reduction Rate<sup>a</sup>**

Fe(III)EDTA (mM)	addition of inhibitor		specific Fe(III)EDTA reduction rate (mmol g DCW <sup>-1</sup> h <sup>-1</sup> )
	Fe(II)EDTA-NO (mM)	Fe(II)EDTA (mM)	
6	— <sup>b</sup>	—	4.251
	—	3.2	4.234
	—	5.7	4.016
	—	8.8	1.107
10	—	—	5.621
12	—	—	6.032
	0.8	—	4.861
	1.9	—	4.025
	3.7	—	0
15	—	—	6.242
22	—	—	6.372
30	—	—	6.428
40	—	—	6.512

<sup>a</sup> Initial biomass concentration: ~0.1 g DCW L<sup>-1</sup>. <sup>b</sup> "—": no inhibitor was added.

the increase of Fe(II)EDTA concentration (from 6 to 40 mM) when the inhibitor is absent. The above results indicate that EDTA alone is not inhibiting reduction rather than Fe(II)-EDTA-NO or Fe(II)EDTA.

In the chemical absorption-biological reduction integrated NO<sub>x</sub> removal system, the concentration of the active Fe(II)-EDTA in solutions was a key factor for NO<sub>x</sub> removal efficiency (13, 16). A proper concentration of Fe(II)EDTA should be chosen in the chemical absorption-biological reduction integrated system due to the inhibition of cell growth by Fe(II)EDTA. Since Fe(II)EDTA-NO present in the absorption solution could strongly inhibit the regeneration of Fe(II)-EDTA, another bacterium (13), which has the ability to convert Fe(II)EDTA-NO to N<sub>2</sub>, was also needed for the continual NO<sub>x</sub> removal. In short, further studies on the denitrifying bacterium should be performed in the future.

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