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Dissimilatory reduction of Fe^{III}(EDTA) with microorganisms in the system of nitric oxide removal from the flue gas by metal chelate absorption

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Abstract: In the system of nitric oxide removal from the flue gas by metal chelate absorption, it is an obstacle that ferrous absorbents are easily oxidized by oxygen in the flue gas to ferric counterparts, which are not capable of binding NO. By adding iron metal or electrochemical method, Fe^{III}(EDTA) can be reduced to Fe^{II}(EDTA). However, there are various drawbacks associated with these techniques. The dissimilatory reduction of Fe^{III}(EDTA) with microorganisms in the system of nitric oxide removal by metal chelate absorption was investigated. Ammonium salt instead of nitrate was used as the nitrogen source, as nitrates inhibited the reduction of Fe^{III} due to the competition between the two electron acceptors. Supplemental glucose and lactate stimulated the formation of Fe^{II} more than ethanol as the carbon sources. The microorganisms cultured at 50℃ were not very sensitive to the other experimental temperature, the reduction percentage of Fe^{III} varied little with the temperature range of 30—50℃. Concentrated Na_2CO_3 solution was added to adjust the solution pH to an optimal pH range of 6—7. The overall results revealed that the dissimilatory ferric reducing microorganisms present in the mix-culture are probably neutrophilic, moderately thermophilic Fe^{III} reducers.

Keywords: dissimilatory ferric reducing microorganisms; Fe^{III}(EDTA); microbial reduction; mix-culture

Introduction

The combustion of fossil fuels generates SO_2 and NOx pollutants which cause air pollution and acid rain. Existing flue gas desulfurization (FGD) scrubbers involve limestone processes, which are efficient for controlling SO_2 emission, but are incapable of removing almost water-insoluble nitric oxide. Several authors have reported the use of metal chelate additives in wet FGD systems for combined removal of NOx and SO_2 (Shi, 1996; Littlejohn, 1990; Harriott, 1993). The Fe^{II}(EDTA) addictive has been extensively studied for the removal of NO from flue gas by the rapid formation of [Fe^{II}(EDTA)(NO)]. However, this type chelate suffers several drawbacks. One of the major drawbacks is the easy oxidation of Fe^{II}(EDTA) by flue gas oxygen into Fe^{III}(EDTA), which is not capable of binding NO. Reducing agents such as sulfite/bisulfite, dithionate, sulfide, ascorbic acid, glyoxal, iron metal, etc. and electrolysis have been investigated for the regeneration of ferric chelates. However, none of these approaches has produced promising results, either because of their high costs and low reduction rates, or because of the formation of unwanted by-products (Shi, 1996).

An approach is being developed that utilizes microorganisms to reduce ferric chelates to their reactive ferrous counterparts. It is reported that certain microorganisms can couple the oxidation of hydrogen or organic compounds with the reduction of Fe^{III} and thus gain energy for growth (Boone, 1995; Slobodkin, 1997; Küsel, 1999; Fredrickson, 2000). Microbial Fe^{III} reduction in subsurface environments has become a hot topic. The mechanisms for microbial Fe^{III} reduction is that organic compounds or hydrogen serving as electron donors can be catalytically oxidized to carbon dioxide by microorganisms with Fe^{III} serving as the sole electron acceptor, during which microorganisms gain energy for metabolism (Lovley, 1997). However, little is known about the factors on the reduction of Fe^{III}(EDTA) in the system of nitric oxide removal from the flue gas by metal chelate absorption and very limited work has been carried out on iron reduction by microorganisms around 50℃ (the temperature of conventional wet FGD scrubbing), apart from some thermophilic bacteria such as *Sulfobacillus* sp.

The aim of this study was to determine the reduction efficiency of dissimilatory reducing microorganisms selectively enriched from the activated sludge in the anoxic zone of Hangzhou Sibao Municipal Wastewater Treatment Plant, with different carbon sources and nitrogen sources, and to study the effects of cell concentration, temperature and the solution pH in batch tests. Based on the test results and the abundance of microorganism sources, we anticipate the application of this method in industry is feasible.

1 Material and methods

1.1 Chemicals

Na_2(EDTA) (99%), $FeCl_3 \cdot 6H_2O$ (99.5%), ethanol (95%), glucose (99.5%), lactate (95%), and all other chemicals were of analytical grade. All chemicals were obtained commercially and used as received.

1.2 Media

The mineral basal medium used for all media contained the following (in mg/L of distilled water): glucose or other carbon sources 2500, $K_2HPO_4 \cdot 3H_2O$ 1000, KH_2PO_4 625, NH_4Cl 500 or $NaNO_3$ 1000, Na_2SO_3 70, $MgSO_4$ 100 and 10 ml/L of a trace element solution containing (in mg/L): $CaCl_2$ 200, $MnSO_4$ 50, Na_2MoO_4 10, $CuSO_4 \cdot 5H_2O$ 10.

1.3 Cell concentration

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For investigation of the effect of cell concentration on the Fe^{III} (EDTA) reduction rate, cells were collected and suspended in cell-free medium. The cell-free medium was 1625 mg/L $K_2HPO_4 \cdot 3H_2O - KH_2PO_4$ buffer solution. The cell concentration based on the dry weight of the mix-culture, which cultivated from activated sludge of the anoxic zone at the municipal wastewater treatment plant. Cells collected from a 100 ml mix-culture by centrifugation were dried in an oven at 100℃ for 5 h and their weight was measured.

1.4 Microbial Fe^{III}(EDTA) reduction

The batch tests were carried out in the 250 ml erlynmeyer flasks each containing basal media and trace element solution, mix-culture and Fe^{III}(EDTA) solution. The total volume of solution in each flask was 100 ml. The anaerobic condition was obtained by replacing the air above the solution surface with oxygen-free nitrogen gas. The rotation velocity of the shaking apparatus was 140 r/min and the temperature in the shaking apparatus was kept at 50℃ except in the tests of studying the effect of temperature on the reduction efficiency. The concentrations of Fe^{III}(EDTA) were measured every day until the concentrations almost arrived steady values.

1.5 Analytical techniques

The concentration of ferrous ions and total iron in solution were determined by the 1, 10-phenanthroline-colorimetry method at 510 nm with a spectrophotometer. In order to determine the total amount of iron in solution, hydroxylamine hydrochloride ($NH_2OH \cdot HCl$) was used to reduce ferric into ferrous at pH < 2. Fe^{III} concentrations were calculated from the difference between total Fe and Fe^{II}. Preliminary experiments showed a difference between $Fe^{II}SO_4$ and Fe^{II}(EDTA) during the Fe^{II} phenanthroline colorimetric assay in the presence of EDTA, most likely due to the competition between EDTA and phenanthroline for Fe^{II}. Therefore, standard curves were prepared with Fe^{II} standard solutions having EDTA concentrations equal to those used in the reduction experiments. The calibration curves are $C = 0.08998A$ ($R = 0.99995$), here C is the concentration of Fe^{II}(EDTA) (mmol/L), A is the corresponding absorbency.

2 Results and discussion

2.1 Effect of nitrogen source

During the cultivation test, Fe^{II} was detected in the solution with NH_4Cl as the nitrogen source after 24 h, and the reduction efficiency of Fe^{III}(EDTA) increased gradually in the subsequent days. However, no Fe^{II} was found in the solution with $NaNO_3$ as the nitrogen source after 24 h, and only trace Fe^{II} was found in the following days. The results showed that nitrate inhibited the reduction of Fe^{III} due to the competition between the two electron acceptors, which agreed with the report of Wang *et al*. (Wang, 1997). Thus ammonium salt was used as the nitrogen source instead of nitrate.

2.2 Effect of carbon source

In the pre-cultivation tests, varied concentrations of Fe^{II} were detected in the solutions with different carbon sources: glucose, ethanol and lactate after 24 h. The amount of Fe^{II} increased with time and almost ceased to grow after 5 d, although it showed some little variation during the next few days that indicated the reduction process might have become "product-inhibited". As shown in Table 1, it was feasible for mix-culture to couple the reduction of Fe^{III} to oxidation of these three carbon sources in the experiment conditions. The reduction efficiency of Fe^{III} with glucose or lactate was higher than that with ethanol serving as the carbon source. The difference in the reduction percentages probably indicated that most of the microorganisms in this mix-culture are fermentative iron reducers, which can hardly use ethanol as the carbon source. And in the subsequent experiments glucose has been used as the carbon source, which is commercially available at a relatively low cost.

Table 1 Effect of carbon sources on the reduction percentage of Fe^{III}(EDTA) during the pre-cultivation of microorganisms

Carbon sources	Lactate	Ethanol	Glucose
Reduction percentage, %	46	20	49

Notes: All values represent the means of three replicates. The reproducibility of analysis was within 10 %

2.3 Effect of cell and glucose concentrations

The effect of the mix-culture concentration on Fe^{III}(EDTA) reduction is shown in Fig. 1. It can be indicated that no Fe^{III}(EDTA) was reduced in the solution without mix-culture. Fe^{III}(EDTA) reduced increased with an increase of cell concentration and time, and became constant at 50% when cell concentration was higher than 120 mg/L after 5 d. Thus 200 mg/L mix-culture were used in the following Fe^{III}(EDTA) reduction tests.

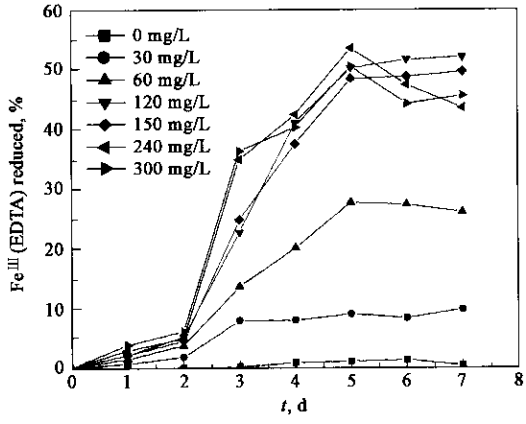

Fig. 1 Effect of cell concentration on the reduction of Fe^{III}(EDTA)
[Fe^{III}(EDTA)] = 8.0 mmol/L, [glucose] = 8.0 mmol/L, pH = 6.2

During the Fe^{III}(EDTA) reduction by dissimilatory ferric reducing microorganisms, carbon source served as the electron donor and the energy source for the growth of the microorganisms. Consequently, the amount of the carbon source was an important factor on the reaction rate.

As shown in Fig. 2, the average reduction rate γ_{av} (mmol/(L·d)) in 5 d increased sharply with the glucose concentration and stopped growing at 0.75 mmol/(L·d) when glucose concentration was higher than 8.0 mmol/L. Extra glucose could not stimulate higher reduction rate, which indicated it was useless and uneconomical to add more glucose than enough amount for the growth of the microorganisms.

2.4 Effect of temperature and solution pH

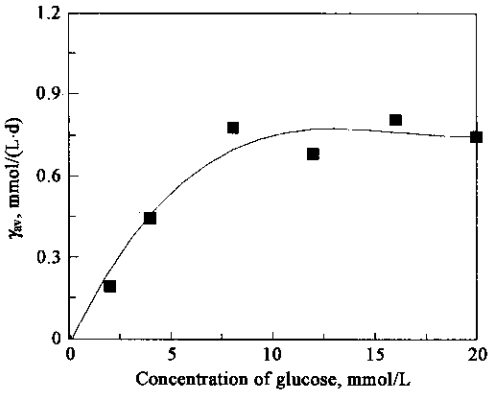

Fig.2 Effect of glucose concentration on the reduction rate of Fe^{III}(EDTA)
[Fe^{III}(EDTA)] = 8.0 mmol/L, [cell] = 200 mg/L, pH = 6.2

The effects of temperature on growth and Fe^{III}(EDTA) reduction by mix-culture were measured with basal medium containing 8.0 mmol/L glucose, 8.0 mmol/L Fe^{III}(EDTA), and 200 mg/L mix-culture. As shown in Fig. 3, the cultures incubated at 50℃ could reduce almost equivalent amount of Fe^{III}(EDTA) in the temperature range of 30—50℃ with the same other conditions. However, the reduction efficiency decreased remarkably when the temperature exceeded 50℃. This investigation indicated the mix-culture cultivated at the simulated flue gas temperature (45—50℃) in this study almost comprised of moderate thermophiles.

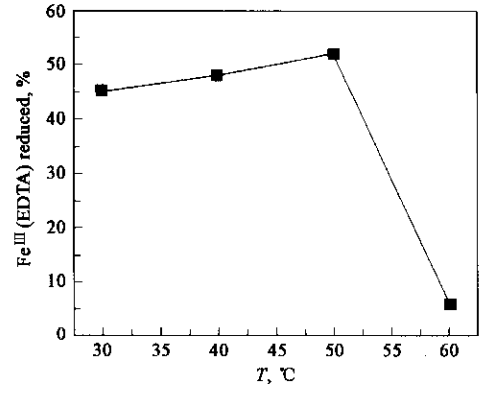

Fig.3 Effect of the temperature on Fe^{III}(EDTA) reduction

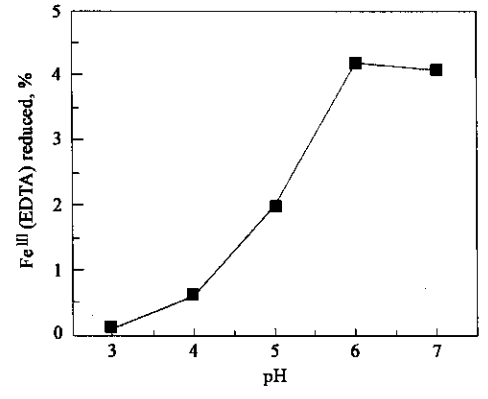

Fig.4 Effect of solution pH on Fe^{III}(EDTA) reduction
[Fe^{III}(EDTA)] = 8.0 mmol/L, [cell] = 200 mg/L, [glucose] = 8.0 mmol/L

The effect of solution pH on Fe^{III}(EDTA) reduction by microorganisms was investigated with pH range of 3—7 (Fig. 4). The experimental results showed that microorganisms in this system were sensitive to pH and almost ceased to reduce Fe^{III}(EDTA) when pH less than 4, which demonstrated that the neutrophilic bacteria most likely dominated in this mix-culture. The suitable pH range was around 6—7. Concentrated Na_2CO_3 was used to adjust the solution pH around 6.2 in the test.

3 Conclusions

The results of our experiments showed that the mix-culture selectively enriched from activated sludge has the ability to reduce Fe^{III}(EDTA) at a temperature of 50℃.

As the carbon source, glucose and lactate stimulated the formation of Fe^{II} more than ethanol. And the pH value of solution decreased during the dissimilatory reduction. When pH value was less than 4, the microorganisms could hardly reduce Fe^{III}. The optimal pH range was around 6—7. The reduction percentage of Fe^{III} with the microorganisms cultured at 50℃ varied little with temperature range of 30—50℃. These collective results reveal that the dissimilatory ferric reducing microorganisms present in the mix-culture are probably neutrophilic, moderately thermophilic Fe^{III} reducers that remain to be isolated and identified in the following study.

When the initial concentration of Fe^{III}(EDTA) was not more than 8 mmol/L, 8 mmol/L glucose and 200 mg/L(dry weight) mix-culture were enough to reduce more than 50% Fe^{III}(EDTA). No more Fe^{II} could be detected after 5 d of operation, which indicated the reduction process might have become product limited. The rate of Fe^{III} reduction did not remarkably increase with adding extra amount of glucose or mix-culture.

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