Journal of Zhejiang University SCIENCE
ISSN 1009-3095
http://www.zju.edu.cn/jzus
E-mail: jzus@zju.edu.cn

Science Letters:

Regeneration of nitric oxide chelate absorption solution by two heterotrophic bacterial strains*

JING Guo-hua (荆国华), LI Wei (李 伟)†,
SHI Yao (施 耀), MA Bi-yao (马碧瑶), TAN Tian-en (谭天恩)

(Institute of Environmental Engineering, Zhejiang University, Hangzhou 310027, China)

†E-mail: w_li@zju.edu.cn

Received Dec. 24, 2003; revision accepted Jan. 30, 2004

Abstract: Ferrous chelate absorption is deemed a promising method for NO removal from flue gas, but the key problem is the difficulty to regenerate the absorption solution, i.e. the complexes of Fe^{II}(EDTA)NO and Fe^{III}(EDTA) in the solution. Two bacterial strains isolated recently from the sludge of the denitrification step of a municipal wastewater treatment plant could be applied effectively to regenerate the absorbent were *Pseudomonas sp.* and *klebsiella trevisan sp*. *Pseudomonas sp.* exhibited high reduction ability on Fe^{II}(EDTA)NO and the *klebsiella trevisan sp*. was more suitable for Fe^{III}(EDTA) reduction.

Key words: microorganism, NO_x, Fe^{II}(EDTA)NO, Fe^{III}(EDTA)
Document code: A **CLC number:** X701; X511

INTRODUCTION

The combustion of fossil fuels generates SO_2 and NO_x pollutants causing air pollution and acid rain. Existing flue gas desulfurization (FGD) scrubbers are efficient for controlling SO_2 emission, but are incapable of removing water-insoluble nitric oxide. We and other researches had reported the use of metal chelate additives in FGD systems for combined removal of NO_x and SO_2 (Shi *et al*., 1996a; 1996b; 1996c; 1997; Littlejohn and Chang, 1990; Tsai *et al*., 1989; Harriott *et al*., 1993). Fe^{II}(EDTA, ethylenediaminetetraacetate) is one of the most promising additives for the removal of NO from flue gas by the rapid formation of Fe^{II}(EDTA)NO. However, this type of chelate has two deadly drawbacks. One is the difficulty in regeneration of Fe^{II}(EDTA)NO and the other is the easy oxidation of Fe^{II}(EDTA) by flue gas oxygen to form Fe^{III}(EDTA), which is not capable of binding NO. To circumvent this problem, reducing agents such as sulfite/bisulfite, dithionate, sulfide, ascorbic acid, glyoxal, iron metal, etc. and electrochemical method have been researched to regenerate ferrous chelates. However, none of these approaches produced promising results due to high costs, the production of unwanted by-products, and/or low reduction rate (Shi *et al*., 1996a; 1996b; 1996c).

Our researchers recently proposed a new approach for reducing Fe^{II}(EDTA)NO and Fe^{III}(EDTA) using cultivated active sludge containing denitrifying bacteria and iron-reducing bacteria (Li *et al*., 2003; Jing *et al*., 2003). It is reported that many bacteria are capable of growing anaerobically by

*Project supported by the National Natural Science Foundation of China (No. 20176052) and the Scientific Research Foundation for Returned Overseas Chinese Scholars, Ministry of Education, China

reducing ionic nitrogenous oxides to gaseous product (Jeter and Ingraham, 1981) and several microorganisms can couple oxidation hydrogen or organic compounds to the reduction of Fe^{III} and gain energy for growth (Boone *et al*., 1995; Fredrickson *et al*., 2000). In the mechanisms for microbial nitrates/nitrites or Fe^{III} reduction, organic compounds serving as electron donors can be catalyzed-oxidized to carbon dioxide by microorganisms with nitrates/nitrites or Fe^{III} serving as the sole electron acceptor, during which microorganisms gain energy for metabolism. However, little is known about the reduction of Fe^{II}(EDTA)NO or Fe^{III}(EDTA) in the system of nitric oxide removal from the flue gas by metal chelate absorption.

In this work, two bacterial strains DN-1 and FR-1 isolated from the above mixed cultures were employed effectively to reduce Fe^{II} (EDTA)NO and Fe^{III}(EDTA). The chelated NO or ferric iron served as a terminal electron acceptor, and was reduced to the environmentally benign gaseous product of N_2 or ferrous iron and thus Fe^{II}(EDTA) was regenerated.

MATERIAL AND METHODES

Chemicals

Sodium ethylenediaminetetraacetate (Na_2EDTA, 98%), $FeSO_4·7H_2O$ (99.5%), D-glucose (99.5%, cell culture tested), NO (1%, with N_2 balanced). The complex of Fe^{II}(EDTA)NO was prepared by the absorption of NO with Fe^{II}(EDTA) solution under oxygen free conditions. The Fe^{II}(EDTA) solution was prepared with equal mol $FeSO_4·7H_2O$ and Na_2EDTA with oxygen free distilled water. The complex of Fe^{III}(EDTA) was obtained by stirring Fe^{II}(EDTA) solution with oxygen until all the ferrous complex changed to ferric complex.

Bacterial strains and media

The basal medium contained the following components (in mg/L): Glucose 1500; KH_2PO_4 625; $K_2HPO_4·3H_2O$ 1000; Na_2SO_3 70; $MgSO_4$ 100; $CaCl_2$ 2; $MnSO_4$ 0.5; Na_2MoO_4 0.1; $CuSO_4·5H_2O$ 0.1.

Cultivation and isolation: Bacterial strains were isolated from the mixed culture cultivated for a long time with terminal electron acceptors of Fe^{II}(EDTA)NO and Fe^{III}(EDTA). Isolation of pure microbial species was carried out anaerobically on agar plates at 40 ºC with 50 μl of enriched mixed culture preliminarily diluted 10^8 times in sterile 200 mg/L phosphate buffer. Pure colonies were then inoculated into sterile basal medium containing 7 mmol/L Fe^{II}(EDTA)NO or Fe^{III}(EDTA) solution. Selection of strain DN-1 and FR-1 was based on their ability to reduce Fe^{II}(EDTA)NO and Fe^{III}(EDTA).

Cell enrichment: Enrichment of bacterial strains was performed in 250 ml conical flasks containing 100 ml basal medium at 40 ºC and subjected to 140 r/min in a rotary shaker. Strain DN-1 grew slowly in Fe^{II}(EDTA)NO solution. Instead of Fe^{II}(EDTA)NO, 1000 mg/L $NaNO_3$ served as nitrogen source and 0.1 mg/L yeast extract were added to the basal medium to promote the growth of strain DN-1. In the cultivation of strain FR-1, 100 mg/L NH_4Cl and 10 mmol/L Fe^{III}(EDTA) was added as nitrogen source and electron acceptor. After 24 hrs of cultivation, the cells were harvested by centrifugation at 5000 r/min for 15 min and washed twice with 0.1 mol/L phosphate buffer (pH 7.0), and then suspended in the phosphate buffer at a desired concentration for use.

Reduction experiment

Reduction experiment was conducted in 250 ml glass serum bottles sealed with teflon-coated rubber septa in a 140 r/min gyrating shaker at 40 ºC. The anaerobic condition was obtained by replacing the air above the solution surface with oxygen-free nitrogen gas. Glucose (1500 mg/L) was added to supply electron acceptor for bio-reduction. The total volume of the solution was 100 ml. In the reduction experiments, the single chelate complex by strain DN-1 or FR-1, the concentration of Fe^{II}(EDTA)NO or Fe^{III}(EDTA) was about 7 mmol/L, and cell concentration was 0.25 mg/L in each flask. In the simultaneous reduction experiment on these two complexes by mixing of the two strains, the concentration of the two complexes and the two strains were the same as above, but the total concentration was doubled.

Analytical methods

The concentration of Fe^{II}(EDTA)NO was measured by a Model 723A spectrophotometer at 420 nm. The concentration of ferrous ions and total irons in the solution were determined by the 1,10-phenanthroline colorimetric method at 510 nm. The biomass was measured by dry weight.

RESULTS AND DISCUSSIONS

Isolation of strains DN-1 and FR-1

Colony purification of the reduction bacteria from the cultivated mixed cultures was carried out on agar plates containing basal medium. Among the 8 strains were isolated, strain DN-1 showed highest ability to reduce Fe^{II}(EDTA)NO and strain FR-1 was more suitable for Fe^{III}(EDTA) reduction. Strain DN-1 was in the form of a rod, 0.6 μm in diameter and 2.5 μm in length, arranged singly, had no vagina, protuberance, or gemma, was Gram negative and was motile (by means of 2 to 3 polar bunchy flagella). Colonies grown on agar plates were milky white, glutinous, lustrous, opaque, round, and had orderly, wet, slippery edge. Strain FR-1 was also in the form of a rod and Gram negative, was 1.0 μm in diameter and 3.0 μm in length, had mono, binary or short catenary arrangement, was nonmotile and without gemma. Colonies of strain FR-1 were similar to those of strain DN-1. Detailed physiological properties are given in Table 1. Strain DN-1 was identified as *Pseudomonas sp* and strain FR-1 was identified as *klebsiella trevisan sp*.

Reduction properties of these two strains

Fig.1 shows that strain DN-1 had good performance in reduction of Fe^{II}(EDTA)NO solution. After 15 hrs of reaction, Fe^{II}(EDTA)NO concentration decreased from 6.76 to 0.58 mmol/L, with reduction rate being 91.4%. During the following hours, Fe^{II}(EDTA)NO could be reduced completely. In the reduction of Fe^{III}(EDTA) solution, strain DN-1 had a lower reduction rate. After 48 hrs of reduction, Fe^{III}(EDTA) concentration decreased from 7.20 to 3.47 mmol/L (reduction rate of 51.8%) and reduced no more in the following days.

Table 1 The physiological properties of bacterial strains

Taxonomic properties	Test results	
	Strain DN-1	Strain FR-1
Catalase	+	+
Oxidase	+	−
V.P.	−	+
Benzazole	−	−
Oxidation-fermentation of sugar and mellow	−	−
Oxidation-fermentation of sugar and mellow	Without gas production	With gas production
Utilization of citrate	+	+
Utilization of acrylate	+	−
Utilization of NO_3^--N	−	−
Utilization of NH_4^+-N	−	+
Production of pyocyanin	−	
Fluorescene A	Without pigment production	
Fluorescene B	−	
Solubility in gelatin		−
Methyl red		−
Sulfureted hydrogen		−

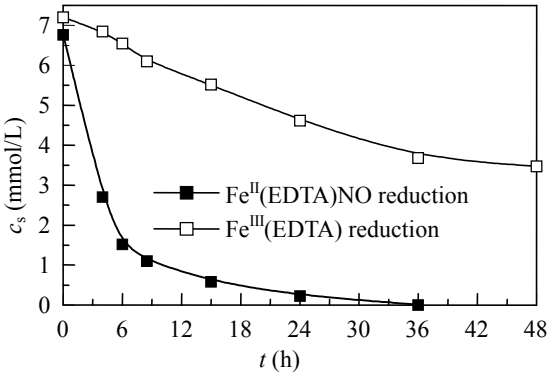

Fig.1 Reduction property of strain DN-1

Fig.2 shows the reduction results of strain FR-1. The reduction ability of this strain was opposite to that of strain DN-1 in reduction of Fe^{II}(EDTA)NO and Fe^{III}(EDTA). Strain FR-1 had a better performance in Fe^{III}(EDTA) reduction. After 48 hrs of cultivation, Fe^{III}(EDTA) concentration decreased from 7.20 to 0.87 mmol/L, about 87.8% reduced. However, strain DN-1 just reduced 22.5% of Fe^{II}(EDTA)NO during the same time.

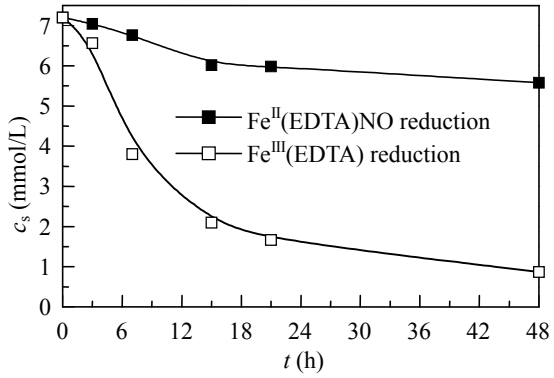

Fig.2 Reduction property of strain FR-1

Fig.3 shows the results of mixed strains simultaneously reducing two complexes. In the combined function of the two strains, Fe^{II}(EDTA)NO and Fe^{III}(EDTA) were both reduced rapidly. These two strains did not have much effect on each other. The reduction of Fe^{II}(EDTA)NO by the mixed strains being a little slower than reduction by strain DN-1 may be due to the change of pH value during Fe^{III}(EDTA) reduction. The pH of the solution was decreased from 6.98 to 5.76 after 48 hrs.

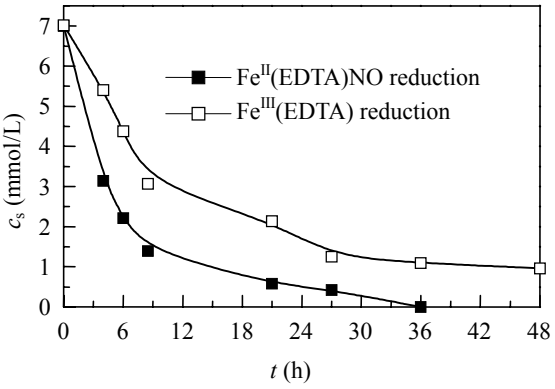

Fig.3 Reduction properties of mix strains

References

Boone, D.R., Liu, Y., Zhao, Z., Balkwill, D.L., Drake, G.R., Stevens, T.O., 1995. Terrestrial subsurface. *Int. J. Syst. Bacteriol.*, **45**:441-448.

Fredrickson, J.K., Kostandarithes, H.M., Li, S.W., Plymale, A.E., Daly, M.J., 2000. Reduction of Fe(III), Cr(VI), U(VI), and Tc(VII) by Deinococcus radiodurans R1. *Appl. Environ. Microbiol.*, **66**(5):2006-2011.

Harriott, P., Smith, K., Benson, L.B., 1993. Simultaneous removal of NO and SO_2 in packed scrubbers or spray towers. *Environ. Prog.*, **12**:110-113.

Jeter, R.M., Ingraham, J.L., 1981. A Handbook on Habitats. *In*: Isolation and Identification of Bacteria. Springer-Verlag, New York, p.913-925.

Jing, G.H., Li, W., Shi, Y., Ma, B.Y., Tan, T.E., 2003. Microbial Reduction of Fe^{II}(EDTA)NO in Nitric Oxide Removal with Ferrous Chelate Absorption. *In*: Proceeding of the First International Conference on Energy and Environment, China, p.655-659.

Littlejohn, D., Chang, S.G., 1990. Reaction of ferrous chelate nitrosyl complexes with sulfite and bisulfite ions. *Ind. Eng. Chem. Res.*, **29**:10-14.

Li, W., Shi, Y., Jing, G.H., Ma, B.Y., Chang, S.G., 2003. A novel approach for simultaneous reduction of Fe^{II}(EDTA)NO and Fe^{III}(EDTA) using microorganisms. *J. Chem. Eng.*(*China*), **54**(9):1340-1342.

Shi, Y., Littlejohn, D., Chang, S.G., 1996a. Kinetics of NO absorption in aqueous iron(II) bis(2,3-dimercapto-1-propanesulfonate) solutions using a stirred reactor. *Ind. Eng. Chem. Res.*, **35**(5):1668-1672.

Shi, Y., Littlejohn, D., Kettler, P.B., Chang, S.G., 1996b. Removal of nitric oxide from flue gas with iron thiochelate aqueous solution in a turbulent contact absorber. *Environ. Prog.*, **15**(3):153-158.

Shi, Y., Littlejohn, D., Chang, S.G., 1996c. Integrated tests for removal nitric oxide with iron thiochelate in wet flue gas desulfurization systems. *Environ. Sci. Technol.*, **30**(11):3371-3376.

Shi, Y., Wang, H., Chang, S.G., 1997. Kinetics of NO absorption in aqueous iron(II) thiochelate solutions. *Environ. Prog.*, **16**(4):301-306.

Tsai, S.S., Bedell, S.A., Kirby, L.H., Zabick, D.J., 1989. Field evaluation of nitric oxide abatement with ferrous chelates. *Environ. Prog.*, **8**:126-129.