Fe(III)(EDTA) Biological Reduction under Thermophilic Conditions by Anaerobic Sludge

JUN CHEN¹, BINHUAN ZHU², AO CHENG² and JIANMENG CHEN^{1,*}

¹Engineering Research Center of the Ministry of Education for Bioconversion and Biopurification, Zhejiang University of Technology, Hangzhou 310032, P.R. China

²College of Biological and Environmental Engineering, Zhejiang University of Technology, Hangzhou 310032, P.R. China

*Corresponding author: Tel: +86 571 88320226; E-mail: jchen@zjut.edu.cn

(Received: 29 September 2012;

Accepted: 18 July 2013)

AJC-13818

A novel process for removal of NO using Fe(II)(EDTA) absorption and microbial reduction has been demonstrated, in which a portion of the Fe(II)(EDTA) was oxidized by oxygen in flue gas to form Fe(III)(EDTA). The biological reduction of Fe(III)(EDTA) was crucial for continuous removal of NO. The biological reduction of Fe(III)(EDTA) by anaerobic sludge under thermophilic conditions (55 °C) was investigated. The effects of key parameters such as the carbon source, pH and NO₂⁻, NO₃⁻ and SO₃²⁻ contents on the Fe(III)(EDTA) reduction rate were investigated in batch experiments. The results showed that the Fe(III)(EDTA) reduction rate depended on the carbon source, with the highest reduction rate (60.45 %) being achieved with glucose, followed by sodium acetate, ethanol, methanol and formic acid. The optimal pH was 7.5, which produced an Fe(III)(EDTA) reduction rate of 64 % after 96 h. In addition, the Fe(III)(EDTA) reduction rate was influenced by the NO₂⁻, NO₃⁻ and SO₃²⁻ contents and its inhibition was in proportion to the initial concentrations. This study demonstrated that Fe(III)(EDTA) could be effectively reduced by microorganisms under thermophilic conditions at 55 °C.

Key Words: Biological reduction, Fe(III)(EDTA), Thermophilic conditions.

INTRODUCTION

Flue gas from power plants is a major source of the discharge of nitrogen oxides (NO_x) into the atmosphere¹. NO_x can cause serious environmental problems, *e.g.*, acid rain, global warming and the depletion of the ozone layer. Selective catalytic reduction (SCR) and selective non-catalytic reduction (SNCR) are two conventional approaches to NO_x removal from gases that have already been utilized in industrial applications. However, these conventional methods suffer from high costs and the risk of causing secondary pollution^{2,3}. To address this issue, a novel integrated chemical absorption-biological reduction approach has recently been developed^{4,5}. In this process, Fe(II) ethylenediaminetetra acetic acid (EDTA) is used to enhance the removal of NO (the most prevalent species of NO_x) from the gas phase into the scrubbing liquid⁶:

The industrial flue gases generally contain 2-8 % oxygen, Fe(II)(EDTA) will be oxidized into the undesirable Fe(III)(EDTA), which is incapable of binding NO during simultaneous gas scrubbing⁷:

The removal efficiency of NO in this integrated process depends strongly on the concentration of Fe(II)(EDTA) in the

scrubber liquid. Therefore, the reduction of Fe(III)(EDTA) is one of the core steps in this process. Glucose is used as an electron donor for the reduction of Fe(III)(EDTA)⁸:

Previous studies have shown that Fe(III)(EDTA) can be reduced efficiently by microorganisms^{9,10} and some efforts have been made to improve the bio-reduction rate of Fe(III)(EDTA). Manconi *et al.*¹¹ suggested that the addition of small amounts of sulfide could increase the Fe(III)(EDTA) reduction rate. Mi *et al.*⁸ used a bio-electro reactor to promote the reduction of Fe(III)(EDTA). Moreover, the temperature of a flue gas after wet desulphuration ranges between 50 and 60 °C^{12,13}, but most studies have suggested that the optimum temperature for denitrification is in the range of 20-40 °C^{14,15}. There has been little research on the reduction of Fe(III)EDTA by microorganisms under thermophilic conditions and the existent studies have produced inconclusive results. Furthermore, Van der Maas *et al.*¹⁶ suggested that the reduction of NO in Fe(II)(EDTA) occurred about three times faster at 55 °C than at 30 °C. The denitrifying activity of the biomass and the availability of free NO(aq) were lower at 30 °C than at 55 °C. Therefore, a further exploration of the reduction of Fe(III)(EDTA) under thermophilic conditions is warranted.

As mentioned above, the bio-reduction of Fe(III)(EDTA) and the characteristics of microorganisms at relatively low temperatures were studied intensively. It is, however, unknown whether Fe(III)(EDTA) can be efficiently reduced by bacteria at higher temperatures. In this study, the experiment was performed using batch experiments ($\text{pH} = 7.2 \pm 0.1$; 55°C) to gain better insight into the biological reduction of Fe(III)(EDTA) under thermophilic conditions. The influences of the carbon source, pH and nitrate, nitrite and sulphite concentrations were investigated.

EXPERIMENTAL

The ethylenediaminetetra acetic acid disodium salt (Na_2EDTA , 99.95 %) and D-glucose (99.5 %, cell culture tested) used herein were from the Guangdong Guang hua Chemical Factory Co., Ltd., China. Our $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ was from Sinopharm Chemical Reagent Co., (Shanghai, China). All other chemicals used in this study were analytical-grade reagents.

Microorganisms and media: The organisms used in this study were taken from the anaerobic sludge of a sewage treatment plant and enrichment-acclimated for a long time using Fe(III)(EDTA) as a terminal electron acceptor. The enrichment was conducted in a 1 L flask with 500 mL of a basal medium at 55°C on a rotary shaker (160 r/min) under anaerobic conditions (by replacing the air above the solution surface with N_2). The basal medium contained the following: 2000 mg L^{-1} glucose; 1000 mg L^{-1} NH_4Cl ; 500 mg L^{-1} KH_2PO_4 ; 500 mg L^{-1} $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$; 100 mg L^{-1} MgSO_4 ; 50 mg L^{-1} CaCl_2 ; 100 mg L^{-1} ZnCl_2 ; 1 mg L^{-1} $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$; 1 mg L^{-1} $\text{MnSO}_4 \cdot \text{H}_2\text{O}$; 1 mg L^{-1} $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and 1 mg L^{-1} $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$. After the completion of the enrichment, the microorganisms were harvested by centrifugation at 12000 r/min for 5 min and washed twice with phosphate buffer ($\text{pH} 7.0$). Next, the harvested bacteria were transferred to 500 mL of a fresh medium containing 1 mmol/L Fe(III)(EDTA). This acclimatization procedure was repeated several times while gradually increasing the Fe(III)(EDTA) concentration from 1-5 mmol/L in 1 mmol/L increments. When the acclimatization was completed, the microorganisms were harvested and suspended in a 0.1 mmol/L phosphate buffer at the necessary concentrations.

Fe(III)(EDTA) reduction experiments: The Fe(III)(EDTA) solution was prepared with equal concentrations of Na_2EDTA and $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$. Fe(III)(EDTA) reduction experiments were conducted in 250 mL glass serum vials in a gyrating shaker at 160 r/min and 55°C in anaerobic conditions (again, by replacing the air above the solution's surface with N_2). The volume of the basal medium was 100 mL, containing 10 mmol/L Fe(III)(EDTA) (except for the experiments on the selection of the carbon source, where the concentration of Fe(III)(EDTA) was 5 mmol/L) and the initial organisms' inoculum was 0.75 g (DCW) L^{-1} . The pH of the media was adjusted to 7.2 ± 0.1 with 0.1 mol/L HCl or NaOH. Each sample included a carbon source of either glucose, ethanol, methanol, sodium acetate or formic acid at initial concentrations of 2000, 1827, 2560, 2320 and 2480 mg L^{-1} , respectively, to identify the most suitable carbon source for Fe(III)(EDTA) reduction. To investigate the

influence of pH on Fe(III)(EDTA) reduction, the initial pH values of the media were 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5 and 9. To evaluate the effects of NO_2^- , NO_3^- and SO_3^{2-} on Fe(III)(EDTA) reduction, different concentrations of NaNO_2 (0, 1, 2, 4 and 8 mmol/L), NaNO_3 (0, 1, 2, 4 and 8 mmol/L) and Na_2SO_3 (0, 0.05, 0.1, 0.3, 0.5, 1, 3, 5 and 10 mmol/L) were added to the media. Samples were taken at regular intervals to measure the ferrous iron and total iron concentrations. All data reported here were the averages of duplicate or triplicate experiments.

Analytical methods: The concentrations of Fe(II)(EDTA) and the total amount of iron in solution were determined by a modified 1,10-phenanthroline colorimetric method at 510 nm¹⁷. The Fe(III)(EDTA) concentration was calculated by taking the difference between the total Fe and Fe(II)EDTA. The biomass was measured by dry weight.

RESULTS AND DISCUSSION

Carbon source selection: Carbon sources are needed during Fe(III)(EDTA) reduction to act as electron donors and energy sources. Different microorganisms have different selectivities for carbon sources and different carbon sources will influence the growth of microbe and bio-reduction rates immediately upon addition-therefore, carbon sources should be selected before acclimatizing microorganisms. Screening tests were conducted with a number of different carbon sources (2000 mg L^{-1} glucose, 1827 mg L^{-1} ethanol, 2560 mg L^{-1} methanol, 2320 mg L^{-1} sodium acetate or 2480 mg L^{-1} formic acid). The different concentrations served to achieve the same carbon:nitrogen ratio. Microorganisms cultivated with NaNO_3 were inoculated in media containing 5 mmol/L Fe(III)(EDTA) and the Fe(III)(EDTA) reduction rate was measured every 12 h for 4 days.

As shown in Fig. 1, the Fe(III)(EDTA) reduction rate was highest when glucose was used as the carbon source and the reduction rate was 60.45 % after 96 h. Sodium acetate, ethanol, methanol and formic acid followed, with reduction rates of 51.01, 48.99, 33.8 and 10.91 %, respectively.

Fig. 1. Effect of kind of carbon source on reduction of Fe(III)(EDTA) at 55°C and $\text{pH} 7.2 \pm 0.1$. The initial concentration of glucose, ethanol, methanol, sodium acetate and formic acid was 2000, 1827, 2560, 2320 and 2480 mg L^{-1} , respectively. (●) Glucose, (▲) ethanol, (▼) methanol, (◆) sodium acetate, (★) formic acid

In general, easily degradable organic substances are suitable electron donors. This study shows that at the same carbon:nitrogen ratio, glucose was the most suitable carbon source for the biological reduction of Fe(III)(EDTA), when compared with sodium acetate, ethanol, methanol or formic acid under thermophilic conditions. Organic substances of low molecular weight, such as ethanol and methanol, were previously considered to be better carbon sources than macromolecular organic substances, such as glucose, in the denitrification process¹⁸. However, Fredrickson *et al.*¹⁹ argued that ethanol is ill-suited for ferric iron reduction. In this project, ethanol and methanol displayed a lower reduction rate for Fe(III)(EDTA) than did glucose. This behaviour may be caused by ethanol and methanol acting as bactericidal agents. Furthermore, the amount of methanol in the medium was more than that of glucose at the same carbon:nitrogen ratio, increasing the toxicity of methanol²⁰, which inhibits microorganisms' growth and reductive properties.

Effect of pH on Fe(III)(EDTA) reduction: The effect of pH on Fe(III)(EDTA) reduction was investigated under a wide range of pH values and the results are shown in Fig. 2. It is obvious that higher initial pH values corresponded to increased Fe(III)(EDTA) reduction efficiency for initial pH < 7.5. For the initial pH > 7.5, the Fe(III)(EDTA) reduction efficiency declined with increasing initial pH. The optimum pH in these cases appeared to be 7.5, for which the Fe(III)(EDTA) reduction efficiency was 64 % after 96 h with a final pH of 5.04. Moreover, although Fe(III)(EDTA) could also be effectively reduced in alkaline conditions, acidic, weakly acidic and alkaline conditions were not well suited for Fe(III)(EDTA) reduction at 55 °C.

Fig. 2. Effect of pH on reduction of Fe(III)(EDTA) at 55 °C with glucose as carbon source. The initial concentration of Fe(III)(EDTA) was 10 mmol/L, the microbial inoculum was 0.75 g DCW L⁻¹.

This study shows that the Fe(III)(EDTA) reduction rate was highest when the pH was 7.5. The pH is an important factor in the growth of microorganisms and can affect enzymatic activity during metabolism. Champine *et al.*²¹ demonstrated that many enzymes, such as isocitrate dehydrogenase, coenzyme A-dependent 2-oxoglutarate, methyl viologen oxidoreductase, succinate dehydrogenase and malate dehydrogenase,

are present in these dissimilar iron-reducing processes. Further, the stability of these enzymes in cells and the rate of enzyme-catalyzed reactions can easily be affected by environmental conditions, such as pH. Under acidic or alkaline conditions the activities of these enzymes were low, leading to a low reduction rate of Fe(III)(EDTA). The optimum pH for many iron-reducing bacteria was neutral or near neutral^{9,22}. When in acidic, weakly acidic or alkaline conditions, the microbes could not grow well. Furthermore, during the Fe(III)(EDTA) reduction process, the final pH became acidic or weakly acidic due to the consumption of OH⁻, as can be explained by the following reaction:

This pH decrease may be one of the reasons for the low reduction rate of Fe(III)(EDTA) at 55 °C.

Effects of NO₃⁻ and NO₂⁻ contents on Fe(III)(EDTA) reduction: To illustrate the effects of NO₃⁻ and NO₂⁻ on Fe(III)(EDTA) reduction, different initial concentrations (0, 1, 2, 4 and 8 mmol/L) of NO₃⁻ and NO₂⁻ were added to the basal medium (containing 10 mmol/L Fe(III)(EDTA)). The effects of NO₃⁻ and NO₂⁻ on Fe(III)(EDTA) reduction are shown in Figs. 3 and 4, respectively. It is apparent that the Fe(III)(EDTA) reduction rate decreased with the presence of NO₃⁻ and NO₂⁻. Moreover, the inhibitory effect was proportional to the concentrations of both NO₃⁻ and NO₂⁻ in the media. After 96 h of cultivation, the respective Fe(III)(EDTA) reduction rates were 62.4, 52.9, 41.8, 38.5 and 35.9 % for 0, 1, 2, 4 and 8 mmol/L NO₃⁻ (Fig. 3) and 62.4, 28.1, 22.3, 14.5 and 6.2 % for 0, 1, 2, 4 and 8 mmol/L NO₂⁻ (Fig. 4). From these results, it is concluded that the inhibitory effect of NO₂⁻ is stronger than that of NO₃⁻ at the same concentrations at 55 °C. The Fe(III)(EDTA) reduction activity was almost completely suppressed when 8 mmol/L NO₂⁻ was added.

Fig. 3. Effect of NO₃⁻ on reduction of Fe(III)(EDTA) at 55 °C and pH 7.2 ± 0.1 with glucose as carbon source. The initial concentration of Fe(III)(EDTA) was 10 mmol/L, the microbial inoculum was 0.75 g DCW L⁻¹. (■) Control, (●) 0 mmol/L, (▲) 1 mmol/L, (▼) 2 mmol/L, (◆) 4 mmol/L, (★) 8 mmol/L.

Figs. 3 and 4 showed that NO₃⁻ and NO₂⁻ will constrain the reduction of Fe(III)(EDTA) by activated sludge under

Fig. 4. Effect of NO_2^- on reduction of Fe(III)(EDTA) at $55\text{ }^\circ\text{C}$ and $\text{pH } 7.2 \pm 0.1$ with glucose as carbon source. The initial concentration of Fe(III)(EDTA) was 10 mmol/L , the microbial inoculum was 0.75 g DCW L^{-1} . (■) Control, (●) 0 mmol/L , (▲) 1 mmol/L , (▼) 2 mmol/L , (◆) 4 mmol/L , (★) 8 mmol/L

thermophilic conditions and that the inhibition of NO_3^- and NO_2^- was linearly related to the amounts of these species added. The inhibition of Fe(III)(EDTA) reduction by NO_3^- and NO_2^- can be explained as follows. The redox potentials between NO_2^-/NO ($+0.99\text{ V}$)¹⁰ and $\text{NO}_3^-/\text{NO}_2^-$ ($+0.433\text{ V}$)²³ are higher than those for Fe(III)/Fe(II) ($+0.34\text{ V}$)¹⁰; this difference in the potentials caused electrons to be transferred to NO_3^- and NO_2^- ²⁴. Consequently, the inhibition by NO_3^- and NO_2^- on Fe(III)(EDTA) reduction might be the result of the difference in the electron transport rates among NO_3^- , NO_2^- and Fe(III)(EDTA) , which had evolved to allow preferential use of the most favorable oxidant²⁵. The reactions of NO_3^- to NO_2^- and Fe(III)(EDTA) to Fe(II)(EDTA) occurred simultaneously; therefore, NO_2^- and Fe(II)(EDTA) were present in the medium at the same time and Fe(II)(EDTA) can chemically react with NO_2^- to form Fe(II)(EDTA)-NO ¹⁶:

Proof can be found in the medium's colour change from orange to black-green. Because the flask colour remained black-green, it is evident that the nitrosyl-complex, Fe(II)(EDTA)-NO , is relatively stable under sterile conditions for prolonged periods of time (96 h). It has previously been illustrated that Fe(II)(EDTA)-NO inhibited Fe(III)(EDTA) reduction²⁶. In addition, the higher inhibitory effect of NO_2^- on Fe(III)(EDTA) reduction may be due to its toxicity to microorganisms.

Effect of SO_3^{2-} on Fe(III)(EDTA) reduction: It is well known that the industrial flue gas contains both NO and sulphurous pollutants, *e.g.*, sulphur dioxide (SO_2). The dissolved SO_2 in the scrubber liquid becomes sulphite or sulfate. Because of its low toxicity and inability to compete for electrons, sulfate had no influence on the Fe(III)EDTA reduction¹⁰. Thus, the effects of sulphite (SO_3^{2-}) on Fe(III)EDTA reduction should be taken into account.

The effect of different initial concentrations (0 – 10 mmol/L) of SO_3^{2-} on Fe(III)(EDTA) reduction is shown in Fig. 5. The addition of 0.05 mmol/L SO_3^{2-} produced a noticeable

Fig. 5. Effect of SO_3^{2-} on reduction of Fe(III)(EDTA) at $55\text{ }^\circ\text{C}$ and $\text{pH } 7.2 \pm 0.1$ with glucose as carbon source. The initial concentration of Fe(III)(EDTA) was 10 mmol/L , the microbial inoculum was 0.75 g DCW L^{-1} . (■) Control, (●) 0.05 mmol/L , (▲) 0.1 mmol/L , (▼) 0.3 mmol/L , (○) 0.5 mmol/L , (□) 1 mmol/L , (◆) 3 mmol/L , (★) 5 mmol/L , (Δ) 10 mmol/L

slowing of the reduction of Fe(III)(EDTA) as compared to when no SO_3^{2-} was added. After 96 h, the Fe(III)(EDTA) reduction rate was 62.6, 61.9, 61.4, 60.2, 59.8, 56.9, 44.3, 34.4 and 22.9 % when 0, 0.05, 0.1, 0.3, 0.5, 1, 3, 5 and 10 mmol/L SO_3^{2-} were added, respectively. It is concluded that the sulphite inhibited the reduction of Fe(III)(EDTA) at $55\text{ }^\circ\text{C}$. Furthermore, the inhibitory effect increased with increasing sulphite concentration.

Fig. 5 showed that SO_3^{2-} inhibited the reduction of Fe(III)(EDTA) . The inhibitory effect of SO_3^{2-} on Fe(III)(EDTA) reduction may be due to sulphite having a directly toxic effect on the microorganisms' growth. Maas *et al.*²⁷ found that the strong inhibition of Fe(III)(EDTA) reduction by calcium sulphite may similarly be due to sulphite toxicity for the bacterial population. Balderston and Payne reported that 0.9 mmol/L sodium sulphite completely inhibited the methanogenic activity of *methanobacterium formicicum*²⁸. Another possible explanation is that the standard redox potential of $\text{SO}_3^{2-}/\text{S}^{2-}$ ($+0.342\text{ V}$) was close to that of Fe(III)/Fe(II) ($+0.34\text{ V}$)¹⁰. Thus, electrons could potentially be transported to both sulphite and Fe(III)(EDTA) such that sulphite is a competitor for electrons.

Conclusion

This study demonstrated that Fe(III)(EDTA) could be effectively reduced by microorganisms at a high temperature ($55\text{ }^\circ\text{C}$). Compared with ethanol, methanol, sodium acetate and formic acid, glucose was the most suitable carbon source for the reduction of Fe(III)(EDTA) to Fe(II)(EDTA) under thermophilic conditions at an optimal pH of 7.5, producing an Fe(III)(EDTA) reduction rate of 64 % after 96 h. In addition, experimental results indicated that the rate of Fe(III)(EDTA) reduction was influenced by potential competitive electron acceptors in the medium, such as NO_2^- , NO_3^- and SO_3^{2-} and the inhibition was in proportion to their initial concentrations. This study lays a theoretical foundation for an integrated chemical absorption/biological reduction NO_x removal process at high temperature.

ACKNOWLEDGEMENTS

The research is supported by the National Natural Science Foundation of China (No. 51008280, No. 21277125), the Environmental Science and Technology Project of Zhejiang Province (No. 2010A10) and Zhejiang Provincial Engineering Research Center of Industrial Bioler & Furnace Flue Gas Pollution Control (No. 2011B01).

REFERENCES

1. K.B. Schnelle and C.A. Brown, Air Pollution Control Technology Handbook, Handbook Series for Mechanical Engineering, Boca Raton, FL: CRC Press, Vol. 8 (2002).
2. L. Gao, X.H. Mi, Y. Zhou and W. Li, *Bioresour. Technol.*, **102**, 2605 (2011).
3. Y.M. Jin, M.C. Veiga and C.J. Kennes, *Chem. Technol. Biotechnol.*, **80**, 483 (2005).
4. R. Dilmore, R.D. Neufeld and R.W. Hammack, *Environ. Eng. Sci.*, **23**, 788 (2006).
5. W. Li, C.Z. Wu and Y. Shi, *J. Chem. Technol. Biotechnol.*, **81**, 306 (2006).
6. J.F. Demmink, I.C.F. Van Gils and A.A.C.M. Beenackers, *Ind. Eng. Chem. Res.*, **36**, 4914 (1997).
7. H.J. Wubs and A.A.C.M. Beenackers, *AIChE J.*, **32**, 2580 (1993).
8. X.H. Mi, L. Gao, S.H. Zhang, L.L. Cai and W. Li, *Bioresour. Technol.*, **100**, 2940 (2009).
9. G.H. Jing, J. Zhou, Z.M. Zhou and T.M. Lin, *Bioresour. Technol.*, **108**, 169 (2012).
10. W. Li, N. Liu, L.L. Cai, J.L. Jiang and J.M. Chen, *Bioresour. Technol.*, **102**, 3049 (2011).
11. I. Manconi, P. van der Maas and P.N. Lens, *Nitric Oxide*, **15**, 40 (2006).
12. B.D. Lee, W.A. Apel and W.A. Smith, *Environ. Prog.*, **20**, 157 (2001).
13. Y.L. Yang, S.B. Huang, W. Liang, Y.Q. Zhang, H.X. Huang and F.Q.J. Xu, *Hazard. Mater.*, **203**, 326 (2012).
14. J.K. Kim, K.J. Park, K.S. Cho, S.W. Nam, T.J. Park and R. Bajpai, *Bioresour. Technol.*, **96**, 1897 (2005).
15. T. Lukow and H. Diekmann, *Biotechnol. Lett.*, **19**, 1157 (1997).
16. P. Van der Maas, L. Harmsen, S. Weelink, B. Klapwijk and P. Lens, *J. Chem. Technol. Biotechnol.*, **79**, 835 (2004).
17. S.H. Zhang, W. Li, C.Z. Wu, H. Chen and Y. Shi, *Appl. Microbiol. Biotechnol.*, **76**, 1181 (2007).
18. P.A. Wilderer, W.L. Jones and U. Dau, *Water Res.*, **21**, 239 (1987).
19. J.K. Fredrickson, H.M. Kostandarithes, S.W. Li, A.E. Plymale and M.J. Daly, *Appl. Environ. Microbiol.*, **66**, 2006 (2000).
20. J. Chen, J.J. Yu, Y.F. Jiang and J.M. Chen, *Acta Scient. Circumst.*, **31**, 948 (2011).
21. J.E. Champine, B. Underhill, J.M. Johnston, W.W. Lilly and S. Goodwin, *Anaerobe*, **6**, 187 (2000).
22. P. van der Maas, S. Peng, B. Klapwijk and P. Lens, *Sci. Technol.*, **39**, 2616 (2005).
23. G.E. Jenneman, M.J. McInerney and R.M.E. Knapp, *Appl. Environ. Microbiol.*, **51**, 1205 (1986).
24. S.H. Zhang, L.L. Cai, Y. Liu, Y. Shi and W. Li, *Appl. Microbiol. Biotechnol.*, **82**, 557 (2009).
25. T.J. DiChristina, *J. Bacteriol.*, **174**, 1891 (1992).
26. W. Li, C.Z. Wu, S.H. Zhang, K. Shao and Y. Shi, *Environ. Sci. Technol.*, **41**, 639 (2007).
27. P. van der Maas, P. van den Brink, B. Klapwijk and P. Lens, *Chemosphere*, **75**, 243 (2009).
28. W.L. Balderston and W.J. Payne, *Appl. Environ. Microbiol.*, **32**, 264 (1976).