Bioresource Technology 101 (2010) 6035–6038

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Direct current stimulation of *Thiobacillus ferrooxidans* bacterial metabolism in a bioelectrical reactor without cation-specific membrane

Xin Li [a,b,*], Yunguo Liu [a,b], Guangming Zeng [a,b], Yile Niu [b], Xin Xiao [a,b], Weihua Xu [a,b], Wenbing Xia [a], Yun Zhu [b], Jiadong Liu [a]

[a] College of Environmental Science and Engineering, Hunan University, Changsha 410082, PR China
[b] Key Laboratory of Environmental Biology and Pollution Control, Hunan University, Ministry of Education, Changsha 410082, PR China

ARTICLE INFO

Article history:
Received 20 November 2009
Received in revised form 23 February 2010
Accepted 23 February 2010
Available online 12 March 2010

Keywords:
Bioelectrical reactor
Thiobacillus ferrooxidans bacteria
Current stimulation

ABSTRACT

A bioelectrical reactor without cation-specific membrane was designed to test effects of direct electrical current on growth of *Thiobacillus ferrooxidans* bacterium. The results indicated that the cell significantly enhanced the growth of *T. ferrooxidans*. At a current of 30 mA, the maximum cells density reached 1.39×10^9 cells/mL within 84 h, which was 10 times faster than under a conventional cultivation method, in which electrical current is not used. A lag phase during the growth of *T. ferrooxidans* was observed when direct electrical current was applied, and the lag phase became longer under higher current intensity.

1. Introduction

Electrokinetic remediation has been proved to be an effective way to remove heavy metals from fine porous solids such as soil, clay, fly ash and sludge (Henrik and Adrián, 2007). The key of this technique is to dissolve heavy metals into a solution which can be further treated by electromigration and electroosmosis (Acar and Alshawabkeh, 1993; Probstein and Hicks, 1993). Metals, which exist in forms as hydroxides or oxides, can be dissolved by the electrokinetic acidification, but metals exist as insoluble sulfides, a common speciation in former gasworks sites and mining wastes, will not be extracted by this method (Kim et al., 2002; Adrián and Luis, 2009). However, bacteria involved in bioleaching processes can convert metal sulfides to sulfates, enabling their solubilization and subsequently being transported by electromigration. In addition, the directional transport of metal ions by electrokinetics is a useful complement to bioleaching as solubilized metals can be removed at the cathode for straightforward downstream processing (Giacomo et al., 2000). The research of direct electrical current effects on bacteria is a prelude for combining bioremediation and electrokinetics in soil remediation.

Since the first report of using anodic oxygen generated from electrolysis for media oxygenation in 1956 (Sadoff et al., 1956), the behavior of bacteria in electric fields has been intensively studied in the last several decades with focuses on bacterial viability, metabolism, and movement in electrical fields (Gregory and Lovley, 2005; She et al., 2006; Simon et al., 1999). With increased need for more environmentally conscious solutions to waste-treatment and remediation, systems that supply energy to microorganisms without chemical addition are becoming increasingly attractive (Thrash and Coates, 2008). The electrochemical apparatus system used in these studies is now named bioelectrical reactors (BER) (Thrash et al., 2007). The structure and composition of BER can vary greatly although BER is typically designed with two main configurations: single- or dual-chamber. The primary part of bioelectrical reactors consist of direct current power, electrode, reference electrode, liquid culture and cation-specific membrane. The BERs are closely related microbial fuel cell (in terms of MFC). MFC systems and BER share similar characteristics from standpoints of circuitry, chamber construction, and basic electrochemical parameters (Logan et al., 2006; Lovley, 2006). The key to MFC design is to choose a suitable membrane that allows protons to pass between the chambers (Logan et al., 2006). Also, many BER systems had been used to stimulate bacterial metabolism with aid of cation-specific membranes (Satoshi et al., 1997; Norio et al., 2002; Thrash et al., 2007). The primary structure and composition of BER is also similar with that of electrokinetics remediation cell, and the only different is that there is a cation-specific membrane in BER systems. However, in order to combine bioleaching with electrokinetics remediation, it is necessary to explore the bacterial viability and metabolism in a BER system without using a cation-specific membrane.

* Corresponding author. Address: College of Environmental Science and Engineering, Hunan University, Changsha 410082, PR China. Tel.: +86 731 8882 1838; fax: +86 731 8882 2829.
E-mail address: hgxlixin@yahoo.com.cn (X. Li).

0960-8524/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2010.02.094

The purpose of this study was to investigate the growth characteristics of iron oxidizing bacterium, *Thiobacillus ferrooxidans*, in an electrochemical system. *T. ferrooxidans*, obtains energy for its growth by oxidation of ferrous [Fe(II)] ions, which is usually utilized in bioleaching studies and hydrometallurgy (Giacomo et al., 2000; Liu et al., 2008).

2. Methods

2.1. Microorganism

The iron oxidizing bacteria *T. ferrooxidans*, used throughout this study, was enriched and acclimatized from acid mine waste water in a way previously described by Liu et al. (2008). The bacteria was precultured at 28 °C for 72 h in a modified 9K medium, which contained (per liter):3.0 g $(NH_4)_2SO_4$, 0.1 g KCl, 0.5 g K_2HPO_4, 0.01 g $Ca(NO_3)_2$, 1.0 mL H_2SO_4(5 mol/L), 300 mL $FeSO_4{\cdot}7H_2O$ 14.78%(w/v) (Matsumoto et al., 1999). The medium was sterilized in an autoclave (HVE-50, HIRAYAMA) at 1.2 bar, at 120 °C, for 15 min. The chemical reagents used in this study were analytical reagent.

2.2. Bioelectrical reactor

The experiments were carried out in an opened PVC cell with a rectangular base of 250 × 100 (mm) and 150 (mm) height. An aerator (SP-780, Shanghai) was placed in the middle of the cell. Proximity to both sides of the cell, two graphite electrodes (Φ = 20 mm, L = 200 mm, Sichuan) were inserted (Fig. 1.). And the electrodes were connected with a direct current power outputting constant current. (WYK10001, EKSI). Then 1000 mL of 9K medium was poured into the bioelectrical reactor. The bioelectrical reactors were sterilized with UV sterilizer (SJ/CX-Y, Henan). The experiment was processed in a hyperpurificatory biosafety cabinet (EBC-4, Chongqing).

2.3. Experimental plan

Two experiments were presented. The experiment A was to test and verify the feasibility of this electrochemical apparatus for cultivation of the *T. ferrooxidans*. The details were: four bioelectrical reactors were poured with 1 L 9K medium, and two cells were applied 40 mA constant direct current (one cell was added 100 ml *T.f.* bacterial suspension precultured in 9K medium, the other was not added), the other two cells (one cell was added 100 ml *T.f.* bacterial suspension precultured in 9K medium, the other was not added) were not applied electrical field.

The main objective of the experiment B was to explore the best current intensity for cultivation of the *T. ferrooxidans*. The experiment B was carried out under the same operational conditions except of that different current intensity of 20, 30, 40, 80 and 120 mA were tested to find out the best growing conditions.

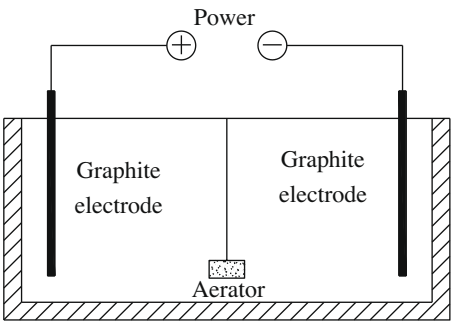

Fig. 1. Diagram of bioelectrical reactor system.

2.4. Analysis

During the process of experiment, pH, Fe^{2+} and *T. ferrooxidans* cells concentration were monitored, and the monitoring frequency was 12 h. In order to avoid the error caused by sampling, the same amount of 9K medium with sample was added to BER system. The cell concentration was determined five times with a haemocytometer (XB-K-25, Zhejiang). The standard error was below 20% of the mean (Satoshi et al., 1997). Ferrous ion (II) concentration was determined with titration using potassium dichromate.

3. Results and discussion

3.1. Feasibility of bioelectrical reactors for cultivation of T. ferrooxidans

The max *T.f.* cells concentration cultivated under direct electrical field reached approx. 2.8×10^8 cells/mL, and it was more than 55% of conventional cultivation, which only reached approx. 1.8×10^8 cells/mL (Fig. 2A). Previous researches reported the bacterium could not grow to a high intensity similar to heterotrophic bacteria in common batch cultures (Norio et al., 2002). For example, the final cell density of iron oxidizing bacteria in a conventional cultivation with adequate concentration of Fe(II) could hardly reach up to 10^8 cells/mL, which was one percent of the density of other heterotrophic bacteria (Sugio et al., 1981). Some researchers reported the electrochemical cultivation of *T.f.* based on electrochemical regeneration of Fe(II) by control of voltage or current, and the cells density as high as 10^9–10^{10} cells/mL had been found in previous studies (Satoshi et al., 1997; Norio et al., 2002). In those studies, cation-specific membranes were used in the BER systems. The membranes prevented the Fe(II) ions from diffusing to anode area and then being oxidized by anode, hence, the bacterial growth energy was supplied by the electrochemical regeneration of Fe(II). In contract, in this study, the Fe(II) ions could diffuse to anode surface and then be oxidized by anode because there was no cation-specific membrane to separate the anode electrolyte from the cathode electrolyte.

The concentrations of Fe(II) in EA2 and EA4 were relatively stable compares to that in EA1 and EA3 (Fig. 2B), which indicates that aeration and direct current had no significant effects on the concentration of Fe(II) in the 9K medium; *T.f.* was the main factor that resulted in the decrease of Fe(II) concentration. Interestingly, the variation tendency of the concentration of in EA1 was similar with that of EA3, but the bacterial concentration of EA1 is more than that of EA3 (Fig. 2A).

Culturing of *T.f.* microorganisms has involved providing the energy source, which usually are Fe(II) ions (Norio et al., 2002). In previous electrical cultivation *T.f.* studies, the BER system has been explored in a multitude of ways to improve the growth yield further by making use of single-chamber systems, separate growth and iron-reduction chambers, combined cathodic iron-reduction and anodic oxygen production, potentiostatic control, and bubbled air in a separate growth chamber (Thrash and Coates, 2008). However, the basic principles of them are utilizing cathodic reductive regeneration of Fe(II) to stimulate higher cell numbers and better growth rates, namely, in those studies, electron donor and electron acceptor are still O_2 and Fe(II), respectively. In this experiment (EA1), the bioelectrochemical process was more involved than that of present researches, which included electrode reactions, chemical reactions and biochemical reactions, etc. The reactions that might occur in EP1 are listed followed:

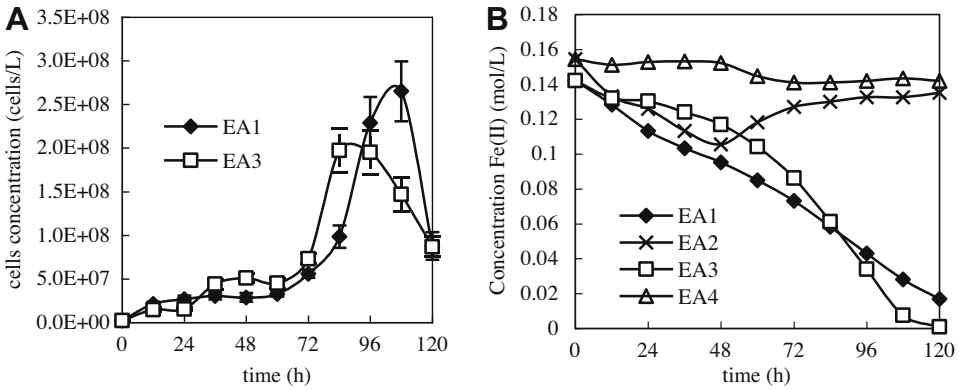

Fig. 2. (A) The growth of *T.f.* bacteria at 28 °C in bioelectrical reactors under different condition. (B) The change of concentration of Fe(II) in medium during cultivation. ♦ (EA1): applied 40 mA current, added 100 ml *T.f.* bacterial suspension; × (EA2): applied 40 mA current, did not added *T.f.* bacterial suspension. spension; □ (EA3): applied 40 mA current, did not added 100 ml *T.f.* bacterial suspension; △ (EA4): no current and no bacterial.

$$\text{Anode}: 2H_2O \rightarrow O_2 + 4H^+ + 4e \quad (E^\circ = +1.23\ \text{V}) \tag{1}$$

$$Fe^{2+} \rightarrow Fe^{3+} + e \quad (E^\circ = +0.77\ \text{V}) \tag{2}$$

$$\text{Cathode}: 4H_2O + 4e \rightarrow 2H_2 + 4OH^- \quad (E^\circ = -0.83\ \text{V}) \tag{3}$$

$$Fe^{3+} + e \rightarrow Fe^{2+} \quad (E^\circ = -0.77\ \text{V}) \tag{4}$$

$$\text{Chemical reaction}: 14Fe^{2+} + 3.5O_2 + 14H^+ \rightarrow 14Fe^{3+} + 7H_2O \tag{5}$$

$$\text{Biochemical reaction}: 14Fe^{2+} + 3.5O_2 + 14H^+ \xrightarrow{T.f} 14Fe^{3+} + 7H_2O \tag{6}$$

According to the contrast experiments' results of EA2 and EA4 (Fig. 2B), electrode reactions and chemical reactions could hardly affect the concentration of Fe(II). However, the max concentration of *T.f.* in EP1 was more than that in EP3, it demonstrated that the *T.f.* in EP1 utilized more Fe(II) ions than that in EP3. The initial amount Fe(II) in culture medium was 0.154 mol. Assumed that Eqs. (2), (3), and (5) would not occur, the amount Fe(II) regenerated by cathode reduction was 0.179 mol according to Faraday's law. However, this deduction was contradictory to the results of experiment B (Fig. 3A), in which obtained a greater concentration of bacteria at a smaller current within a shorter time. The possible explanation of this result is that the *T.f.* not only utilizes the Fe(II) ions reduced by cathode reaction as energy source, but also directly utilizes the graphite cathode as electron donor. The pathway and mechanism extracellular electron transfer between bacteria with electrode is not clear. However, it has been demonstrated that microorganisms in the *Geobacteraceae* family can oxidize organic compounds with electrodes serving as the sole electron acceptor (Bond et al., 2002; Bond and Lovley, 2003), and a pure culture of *Geobacter metallireducens* can reduce nitrate to nitrite using electrodes serving as the sole electron donor with the expected stoichiometry of electron consumption (Gregory et al., 2004).

3.2. Effect of current intensity on cultivation

The bacterial cells concentration reached up to 10^9 cells/mL at the current intensity of 20, 30 and 40 mA, respectively, and the maximum reached 1.39×10^9 cells/mL at the current of 30 mA after 84 h. The maximum bacterial cells concentration at the current intensity of 0, 80 and 120 mA was 1.98×10^8 cells/mL at 60 h, 2.65×10^8 cells/mL at 96 h and 1.65×10^8 cells/mL at 96 h, respectively (Fig. 3A).

There was an obvious lag phase of *T. ferrooxidans* bacteria under the stimulation of direct electrical current, and the lag phase extended with increase of the current intensity (Fig. 3A). Fig. 3B indicates that the concentration of Fe(II) decreased with the inoculation time. From 72 to 108 h the concentration of Fe(II) dropped sharply at the current intensity of 20 and 30 mA . This suggests that the rapid growth of *T. ferrooxidans* in the period quickly consumed iron ions.

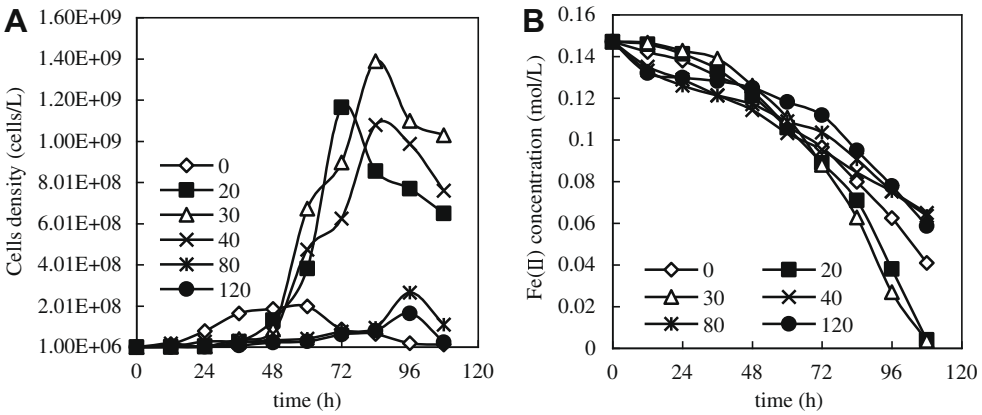

Fig. 3. (A) The growth of *T f.* bacteria at 28 °C in bioelectrical reactors with 0, 20, 30, 40, 80 and 120 mA current. (B) The change of concentration of Fe(II) in medium during cultivation.

4. Conclusion

This study demonstrates that the direct electrical current is able to enhance the growth of *T. ferrooxidans* bacteria in a bioelectrical reactor without using a cation-specific membrane. At a current intensity of 30 mA, the bacterial cells concentration reached up to 1.39×10^9 cells/mL. However, further research is required to determine the mechanism of the electron transfer process among electrodes, bacteria and ferrous ions.

Acknowledgements

The study was funded by Central Colleges of basic scientific research and operating expenses of special funds (No. 531107040079); the National 863 High Technologies Research Foundation of China (No. 2004AA649370), the National Basic Research Program (973 Program) (No. 2005CB724203), the National Foundation for Distinguished YoungScholars (Nos. 5042596 and 50225926), the Program for ChangjiangScholars and Innovative Research Team in University (IRT0719).

References

Acar, Y.B., Alshawabkeh, A.N., 1993. Principles of electrokinetic remediation. Environ. Sci. Technol. 27, 2638–2647.

Adrián, R., Luis, C., 2009. Electrodialytic remediation of copper mine tailings using bipolar electrodes. J. Hazard. Mater. 168, 1177–1183.

Bond, D.R., Lovley, D.R., 2003. Electricity production by *Geobacter sulfurreducens* attached to electrodes. Appl. Environ. Microbiol. 69, 1548–1555.

Bond, D.R., Holmes, D.E., Tender, L.M., Lovley, D.R., 2002. Electrode-reducing microorganisms that harvest energy from marine sediments. Science 295, 483–485.

Giacomo, M., Ajay, K.S., Garry, S., Christopher, J.K., Simona, J., 2000. An integrated method incorporating sulfur-oxidizing bacteria and electrokinetics to enhance removal of copper from contaminated soil. Environ. Sci. Technol. 34, 1081–1087.

Gregory, K.B., Lovley, D.R., 2005. Remediation and recovery of uranium from contaminated subsurface environments with electrodes. Environ. Sci. Technol. 39, 8943–8947.

Gregory, K.B., Bond, D.R., Lovley, D.R., 2004. Graphite electrodes as electron donors for anaerobic respiration. Environ. Microbiol. 6, 596–604.

Henrik, K.H., Adrián, R., 2007. Testing pulsed electric fields in electroremediation of copper mine tailings. Electrochim. Acta 52, 3399–3405.

Kim, S.O., Kim, K.W., Doris, S., 2002. Evaluation of electrokinetic removal of heavy metals from tailing soils. J. Environ. Eng. 128, 705–715.

Logan, B.E., Hamelers, B., Rozendal, R., Schroder, U., Keller, J., Freguia, S., Aelterman, P., Verstraete, W., Rabaey, K., 2006. Microbial fuel cells: methodology and technology. Environ. Sci. Technol. 40, 5181–5192.

Lovley, D.R., 2006. Bug juice: harvesting electricity with microorganisms. Nat. Rev. Microbiol. 4, 497–508.

Matsumoto, N., Nakasono, S., Ohmuta, N., Saiki, H., 1999. Extension of logarithmic of *Thibacillus ferrouxidans* by potential controlled electrochemical reduction of Fe(III). Biotechnol. Bioeng. 64, 716–721.

Norio, M., Hisao, Y., Naoya, O., Aikazu, A., Hiroshi, S., 2002. Numerical simulation for electrochemical cultivation of iron oxidizing bacteria. Biotechnol. Bioeng. 78, 17–23.

Probstein, R.F., Hicks, R.E., 1993. Removal of contaminants from soil by electric fields. Science 260, 498–503.

Sadoff, H.L., Halvorson, H.O., Finn, R.K., 1956. Electrolysis as a means of aerating submerged cultures of microorganisms. Appl. Environ. Microbiol. 4, 164–170.

Satoshi, N., Norio, M., Hiroshi, S., 1997. Electrochemical cultivation of *Thiobacillus ferrooxidans* by potential control. Bioelectrochem. Bioenerg. 43, 61–66.

She, P., Song, B., Xing, X.-H., Loosdrecht, M.V., Liu, Z., 2006. Electrolytic stimulation of bacteria *Enterobacter dissolvens* by a direct current. Biochem. Eng. J. 28, 23–29.

Simon, A.J., Giacomo, M., Ajay, K.S., Christopher, J.K., 1999. The effects of direct electric current on the viability and metabolism of acidophilic bacteria. Enzyme Microbial Technol. 24, 316–324.

Sugio, T., Tano, T., Imai, K., 1981. The purification and some properties of a factor having a stimulating effect on iron-oxidizing activity of *Thiobacillus ferrooxidans*. Agric. Biol. Chem. 45, 405–412.

Thrash, J.C., Coates, J.D., 2008. Review: direct and indirect electrical stimulation of microbial metabolism. Environ. Sci. Technol. 42, 3921–3931.

Thrash, J.C., VanTrump, J.I., Weber, K.A., Miller, E., Achenbach, L.A., Coates, J.D., 2007. Electrochemical stimulation of microbial perchlorate reduction. Environ. Sci. Technol. 41, 1740–1746.

Liu, Y.G., Zhou, M., Zheng, G.M., Wang, X., Li, X., Fan, T., Xu, W.H., 2008. Bioleaching of heavy metals from mine tailings by indigenous sulfur-oxidizing bacteria: effects of substrate concentration. Bioresource Technol. 99, 4124–4129.