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Reductive Dissolution of Iron (III) from Ilmenite Ore ($FeTiO_3$) by Iron Reducing Bacteria

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Abstract. Extracting titanium from ilmenite ore ($FeTiO_3$) by using the conventional methods require usage of hash chemicals and excessive heating, which are not environmentally friendly. Iron oxides are the most common impurities contained in ilmenite ores. Separating iron impurities from ilmenite ore remains as a challenging part in titanium extraction proses. In this study, we explored the application of iron-reducing bacterium, *Shewanella oneidensis* MR-1 to leach out iron from ilmenite ore. *S. oneidensis* MR-1 is known for its capability to reduce various metal oxides during anaerobic respiration. Reduction of iron oxides contained in ilmenite ore by respiration activity of *S. oneidensis* MR-1 transform insoluble iron(III) to soluble iron(II), which can leach out from the ilmenite ore. Different concentrations of ilmenite were incubated with *S. oneidensis* MR-1 under anaerobic conditions for 30 days. Reductive dissolutions of iron(III) from ilmenite ore were determined by monitoring iron(II) accumulation in the growth media every 3-4 days. Transformations of ilmenite ore after 23 days of *S. oneidensis* MR-1 treatment were further analysis by X-Ray diffraction (XRD) analysis. Incubation of *S. oneidensis* MR-1 with 0.10 g/ml of ilmenite ore showed the highest iron(III) reductive dissolution activity, with 1.4mM of iron(II) was leached out from ilmenite ore after 23 days. The XRD analyses indicated chemical composition and structural changes in the ilmenite incubated with *S. oneidensis* MR-1. The findings of this study show that reductive dissolution of iron(III) by *S. oneidensis* MR-1 is a promising environmentally friendly and inexpensive bioleaching strategy in removing iron impurities from ilmenite.

INTRODUCTION

Ilmenite($FeTiO_3$) is the most common reserve of titanium in nature [1]. Ilmenite composed of 40-60% of TiO_2, while remaining component is mainly composed of iron oxides [2, 3]. Removing iron impurities is the major step in ilmenite processing to produce high purity TiO_2. There is no physical method for separation of iron from TiO_2 in ilmenite currently available, thus, industrial scale separation of iron impurities is conducted via chemical processes, which required applications of hash chemicals and excessive heating [4]. With the increasing demand for titanium metal and titania (TiO_2) pigment from the industry all over the world, ilmenite processing could leave devastating impacts to the environments, therefore, more environmentally friendly and sustainable ilmenite processing methods are more favorable.

Application of microorganism in metal processing or bioleaching is an alternative method for removal of impurities from its metal ores. Bioleaching is applicable in the recovery of metals from low-grade ores, mineral concentrates, or mineral waste. Through bioleaching processes, metals can be processed without the requirements of excessive heating,

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hash chemicals, extensive infrastructure and bulky hardware. Thus, bioleaching strategy can be applied under resource-limited set ups. This environmental-friendly metal extraction technology is an intriguing sustainable technology worth for further investigations. However, the application of microorganisms for bioleaching of ilmenite ore is still poorly investigated. Previous study investigated ilmenite bioleaching by iron-oxidizing bacteria, *Acidithiobacillus* ferrooxidans and iron-scavenging bacteria, *Pseudomonas mendocina* showed a mixed results [5].

The potential application of iron-reducing bacteria (IRB) in bioleaching of ilmenite has not been investigated. IRB play important roles in a variety of environmentally important processes, including the biogeochemical cycling of elements [6, 7], immobilization of radionuclides contaminants [8], and the electricity production of in microbial fuel cells [9-11]. IRB is a group of bacteria that are capable of utilizing both soluble and insoluble iron(III) oxides as electron acceptors for anaerobic respiration. During anaerobic respiratory process of IRB, electrons originated from the cells are transferred to insoluble iron(III) oxides, which resulting in reductive dissolution of iron(III) oxides to soluble iron(II), that could be leached out from metal ore into the solution. This unique iron(III) reductive dissolution capability of IRB suggests that IRB could be applied in bioleaching application for removal of iron(III) oxides impurities from ilmenite ores.

Among IRB, *Shewanella oneidensis* MR-1 is the most widely studied facultative IRB. *S. oneidensis* MR-1 capable of to respiring on a wide range of electron acceptors including manganese(IV) oxides, iron(III) oxides, fumarate, nitrate, trimethylamine N-oxide (TMAO), hematite, ferrihydrite and several other compounds [12, 13]. In this study, we investigated the application of reductive dissolution process of iron(III) oxides by *Shewanella oneidensis* MR-1 as a possible bioleaching strategy for removal of iron impurities from ilmenite ore.

MATERIALS AND METHODS

Bacterial Strain and Cultivation Conditions

S. oneidensis MR-1 was isolated from the metal-rich sediments of Oneida Lake (NY) [14]. Overnight seed culture was aerobically cultured in the test tubes containing 5 mL of Luria-Bertani (LB) broth (10 g L^{-1} tryptone, 10 g L^{-1} NaCl, 5 g L^{-1} yeast extract) at 30°C with agitation speed of 150 rpm for 24 hours. The overnight seed cultures were cultivated into 50 mL LB broth as fresh medium and cultured for 5 hours prior to inoculation into serum bottle for ilmenite bioleaching incubations.

Ilmenite Reductive Dissolution Bioleaching

The reductive dissolutions of iron(III) oxides contained in ilmenite ore were determined by monitoring accumulation of soluble iron(II) concentration in the growth media. 0.5 ml of *S. oneidensi*s MR-1 inoculums were added into sterile serum bottles that containing 50ml of LB broth and supplemented with different concentrations of ilmenite grains (0.05, 0.10, 0.20, 0.30 g/ml) under sterile conditions. The head spaces of the serum bottles were kept at minimum level. The serum bottles were then capped with rubber stoppers and sealed with aluminum rings to prevent atmospheric air from entering the serum bottles to create anaerobic growth condition in the serum bottle upon depletion of oxygen in the bottles. Abiotic controls were prepared in the identical set-ups without the addition of *S. oneidensis* inoculums. Accumulations of soluble iron(II) in the growth media were measured every 3-4 days by Ferrozine assay.

Ferrozine Assay

The accumulations of soluble iron(II) as the products of microbial reductive dissolution of iron(III) oxides contained in ilmenite ores were determined by Ferrozine assay. 200 µl of media was collected from serum bottle using syringe needle and immediately added into microcentrifuge tube containing 1 ml of 0.5 N hydrochloric acid (HCl). The microcentrifuge tube was left in dark for 30 minutes before 100 µl of the mixture were then added into 900 µl of Ferrozine reagent. The absorbance reading was then measured by spectrophotometer at 562 nm wavelength.

X-Ray Diffraction (XRD) Analysis

The mineral phase changes of microbial treated and untreated ilmenite ores were examined by using Bruker D2 Phaser X-ray diffraction (XRD) equipped with Cu Kα radiation and the samples were scanned from 20° to 90° with a step of 0.02° and a dwell time of 4 s similar to protocol from previous study [15]. The XRD raw data was interpreted by DIFFRAC.EVA version 3.2, by performing subsequent search and match scan from reference library derived from crystallography open database (COD) to trace the peak pattern.

RESULTS AND DISCUSSION

Reductive Dissolution of Iron(III) from Ilmenite

The ability of *S. oneidensis* MR-1 to reductively dissolve iron(III) was tested by incubation of *S. oneidensis* in serum bottles containing LB broth and supplemented with different concentrations (0.05, 0.10, 0.20, 0.30 g/ml) of ilmenite grains. The oxygen depleted condition in the serum bottle force *S. oneidensis* MR-1 to undergo anaerobic respiration by utilizing iron(III) oxides impurities from the ilmenite ore as the electron acceptor. The reduction of the iron oxides by *S. oneidensis* MR-1 transformed iron(III) oxides to soluble iron(II) that were leached out into the growth media. The incubation was performed for 23 days and the accumulations of iron(II) in the growth media were measured every 3-4 days using Ferrozine assay method (Fig. 1). After 3 days of incubation, iron(II) concentration in all incubations with *S. oneidensis* MR-1 in different concentrations of ilmenite showed noticeable increment of iron(II) concentration in the growth media compared to abiotic control supplied with 0.10 g/ml ilmenite, which only showed marginal change. This indicated that *S. oneidensis* MR-1 activity contributed to the dissolution of iron(II) from ilmenite ore into the growth media. Intriguingly, iron(II) accumulations at early stage of incubations showed that lower concentrations of ilmenite accumulate higher concentrations of iron(II), the order of the iron(II) accumulation is opposite of the ilmenite concentration order in which the incubation with the lowest ilmenite concentration (0.05 gml) showed the highest iron(II) accumulation while the incubation with highest ilmenite concentration (0.30 g/ml) showed the lowest iron(II) accumulation. As for the extend of reaction, incubation with 0.10 g/ml ilmenite showed the highest iron(II) accumulation as the concentration reached up to 1.4 mM which was achieved after 14 days of incubation. The extend of reaction for other concentrations of ilmenite, however, reach the stationary phase after 7 days of incubations. This result indicated ilmenite concentrations higher than 0.10 g/ml (0.2 and 0.30 g/ml) decelerate the iron(III) oxides reductive dissolution activity by *S. oneidensis* MR-1, possibly because of toxicity effects of ilmenite ore at higher concentrations [16]. As for 0.05 g/ml of ilmenite, although the initial reductive dissolution activity was higher than 0.10 g/ml of ilmenite, lower initial concentration of substrate lowers the extend of reductive dissolution reaction. For the abiotic control supplemented with 0.10g/ml of ilmenite, no noticeable increment of iron(II) accumulation can be observed, this indicated that accumulations of iron(II) concentrations are the result of *S. oneidensis* MR-1 respiratory activities.

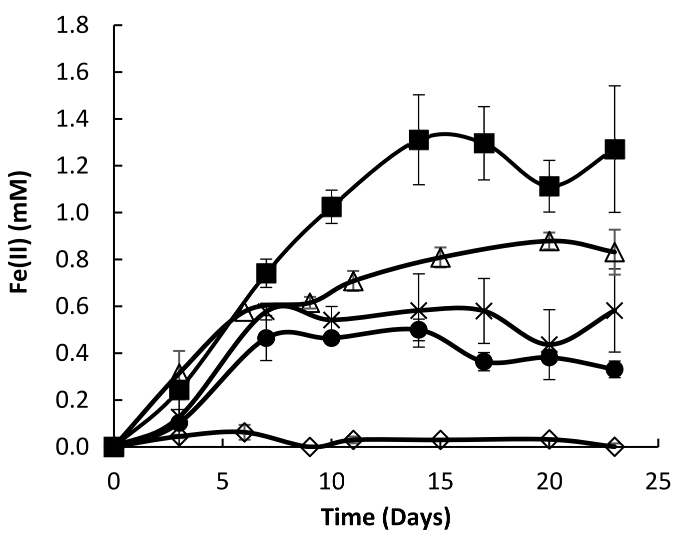

FIGURE 1. Iron(II) accumulation in growth media. concentration of *S. oneidensis* MR-1 with five different concentrations of ilmenite ore in LB broth at 562 nm for day 0 to day 23. Key: (◊) Abiotic control; (△) 0.05 g/ml; (■) 0.10 g/ml; (×) 0.20 g/ml; (●) 0.30 g/ml. The error bars represent the standard deviations.

Mineral Phase Constitution of Ilmenite

X-ray diffraction (XRD) analysis was performed to observe crystallographic structure changes in ilmenite grains due to reductive dissolution of iron(III) by *S. oneidensis* MR-1. The corresponding XRD patterns of raw ilmenite, abiotic control 0.1 g/mL ilmenite and biotic (incubated with *S. oneidensis* MR-1) 0.1 g/mL ilmenite are demonstrated in Fig. 2. For raw ilmenite, the peaks found on the diffractogram showed ilmenite ($FeTiO_3$) and hematite (Fe_2O_3) as the main mineral phases. However, the mineral phases in the bioleached residue of biotic 0.1 g/mL ilmenite sample changed to rutile (TiO_2), and hematite. New diffraction peaks appeared after bioleaching indicating rutile peaks might be due to the occurrence of ilmenite dissolution [17]. Extra new peak for hematite appeared after the bioleaching suggested that the iron (II) oxides resulted from the reductive dissolution process had reacted with the oxygen gas from the environmental and formed into hematite. Yet, unreacted ilmenite peak is still visible in the bioleached sample. These results indicate that *S. oneidensis* MR-1 activity changed the mineral phase constitution of the ilmenite ore.

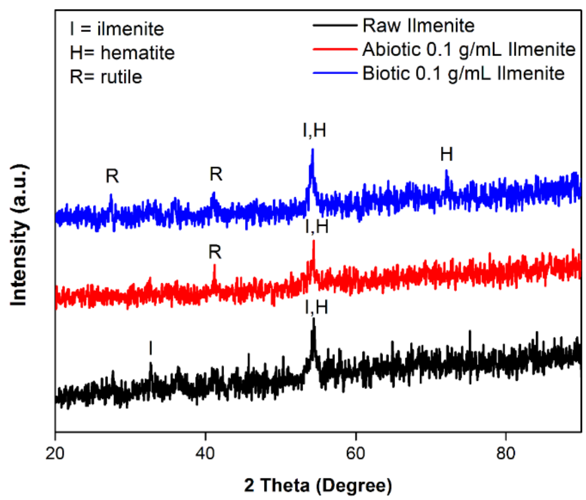

FIGURE 2. XRD patterns of raw ilmenite, abiotic 0.1 g/mL ilmenite and biotic 0.1 g/mL ilmenite.

CONCLUSION

In this study, we demonstrated that iron(III) impurities contained in ilmenite ore can dissolved by reduction activity of IRB, *S. oneidensis* MR-1. Reductive dissolution of ilmenite by *S. oneidensis* MR-1, leached out iron(III) impurities and transformed the mineral phase of the ilmenite ore as indicated in XRD analysis. Incubation of *S. oneidensis* MR-1 with different concentrations of ilmenite indicated that 0.10g/ml of ilmenite is the optimum ilmenite concentration for current experimental set-ups. Although industrial application of IRB in bioleaching of ilmenite requires further optimization studies, results from this study suggested that IRB could be an intriguing microorganism for application in bioleaching process of ilmenite and other iron-containing mineral ores.

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