学　位　論　文　内　容　の　要　旨

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学　位　論　文　題　名

Study on interaction between bacteria and hematite under seawater condition for the elution of iron
(海水条件下での鉄溶出を目的としたバクテリアとヘマタイトの相互作用に関する研究)

The development of rural area and the rapid growth of city leads to varieties of environmental issues such as deforestation and mineral supply limitation. One of the serious problems is the limitation of iron supply from the forest, which lead to the depletion of seaweed in coastal area. Since eluted-iron is essential for reproduction and growth of seaweed, the trace amount of iron supply is required. However, it is difficult for eluted-iron to maintain in the dissolved form in seawater condition. For this reason, the organic matter in the fertilizer has been introduced to the iron oxide which is the component of the steel-making slag. This kind of fertilizer is called as Fe-fertilizer to supply the iron into seawater. The trace amount eluted-iron using Fe-fertilizer technique has been studied and barren seaweed was recovered within a few years. There is an evidence showing the bacteria might play an important part for this iron dissolution.

This thesis is mainly focusing on the iron oxide application to various environmental problem using bacterial activities. The main challenging point is to clarify the mechanism of bacteria to the iron dissolution along with the absorption properties of the bacteria related to future application in the future study.

In Chapter 1, the research background, the literature review, the research objectives and the originality of the thesis are presented.

In Chapter 2, iron elution mechanism associated with bacteria cells are discussed. The property of bacteria cells and their activities has been examined. The microorganism was isolated from Fe-fertilizer incubated in coastal seawater for 6 months, and was identified as *Exiguobacterium oxidotolerans* by 16S rDNA analysis. The iron elution of the bacteria was demonstrated based on the increase of dissolved iron by incubation with $Fe_2O_3$(hematite) under a seawater-like condition. The value of ORP was changed from ca. 0 mV to ca. –400 mV by inoculation of the bacteria, which causes reductive elution of Fe. The concentration of eluted iron was significantly affected by Anthraquinone-2,7-disulfonate (AQDS), which is used as a model for organic matter in the system. The observation of eluted iron concentration has shown higher concentration in AQDS containing sample at 38 mg $L^{-1}$ compared to the sample containing only *E. oxidotolerans* with hematite which detected at 22.3 mg $L^{-1}$. The relatively high concentration of organic acids (Oxalic acid, 11,300 mg $L^{-1}$) detected in samples containing *E. oxidotolerans* and hematite. The hematite surface was analyzed by using X-ray photoelectron spectroscopy (XPS), field emission scanning electron microscope (FE-SEM) and attenuated total reflectance Fourier transform infrared (ATR-FTIR) after 30-days exposure to bacterial culture. The surface alteration has been observed in the treated hematite, indicating the evidence of iron elution into the culture medium. Based on the results, it was proved that *E. oxidotolerans* is capable of

Fe reductive elution of iron from $Fe_2O_3$into seawater. Anthraquinone-2,7-disulfonate (AQDS), which can play as an electron acceptor/donor between microbe and insoluble $Fe_2O_3$ particles, enhanced the effect of iron bio-leaching.

In Chapter 3, the interaction between bacteria cell and hematite surface has been investigated to clarify the major reaction in the iron elution. We proposed two possible mechanisms of iron elution focusing on the contact condition between bacteria and hematite surface. Two system of iron elution has been examined. One is direct elution system and another is indirect elution, where bacteria and hematite are separated by dialysis tube. The elution test was conducted for 10 days, and iron elution, metabolite production (oxalic acids), ORP and pH were monitored. The effect of organic acids which are usually produced by *E. oxidotolerans* was also examined in indirect elution system by adding external organic acids (i.e. chemical reagents). Adequate iron elution was detected only in direct elution system (30.57 mg $L^{-1}$). Hematite surface observation using FE-SEM, ATR-FTIR and SEM-EDS also revealed the surface alteration and particles dispersion in direct elution system. Our study could conclude direct interaction of bacteria cell with hematite would mainly facilitate iron elution, probably due to the electron transfer via cell membrane to hematite, resulting in reductive elution of hematite.

In Chapter 4, the adsorption ability of bacteria cell to hematite surface has been examined using genetically-modified bacteria. Based on the result in chapter 3, direct interaction between bacteria and hematite surface played an important part in electron transfer to reduce insoluble-Fe(III) to eluted-Fe(II). Efficient and active adsorption of bacteria cells to hematite surface might lead to improvement in iron dissolution. *Escherichia coli* displaying iron-binding peptide on its cell surface has been prepared to enhance the adsorption ability. The two types of iron-binding peptide (IBP1 and IBP2) has been selected to evaluate the adsorption efficiency to the hematite. We introduce IBPs into outer membrane protein (OmpA) to construct fusion genes (IBPs-OmpA) in this work. Construction of expression vector, transformation of the cell, and protein expression in *E. coli* have been successful. The adsorption of *E. coli* expressing IBP1 or IBP2 has been conducted using fluorescent microscope after staining the cells by LIVE/DEAD staining kit. IBP2-displaying cell was revealed to show higher adsorption efficiency at pH 7-7.4 while the cells could not bind to hematite surface at pH 8. The IBP2-displaying cell showed specific adsorption to hematite among other solid materials such as titanium, copper and glass.

Finally, Chapter 5 is a summary of all the results obtained in each chapter and some guide for future research works in the above areas.