Pyritic Sulfur Removal from Lignite by *Thiobacillus ferrooxidans*: Optimization of a Bioleaching Process

Nattaya Ruamsap and Ancharida Akaracharanya

The optimal conditions for bioleaching process of pyritic sulfur from lignite by *Thiobacillus ferrooxidans* Y4-3 were as follows: inoculum grown in 9K medium at 30°C with shaking at 200 rpm for 7 days, 10% (v/v) of the inoculum was inoculated in 10% (g/100 ml) of 45 μm-lignite particles suspended in distilled water at the initial pH of 2.0, and incubated at the above conditions for 8 days. Under the optimal conditions, *T. ferrooxidans* Y4-3 desulfurized 11.52% of the total pyritic sulfur from lignite.

**Key words:** *T. ferrooxidans*, bioleaching, desulfurization and lignite.

*Department of Microbiology, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand.*
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ภาวะที่เหมาะสมของกระบวนการไบโอลิชชิงเพื่อการขจัดกํามะถันไพไรต์ออกจากลิกไนท์โดย *Thiobacillus ferrooxidans* Y4-3 เป็นดังนี้ เลี้ยงเชื้อเริ่มต้นในอาหารเลี้ยงเชื้อเหลวสูตร 9K ที่อุณหภูมิ 30°C ให้อากาศโดยการเขย่าที่ความเร็ว 200 รอบต่อนาที เป็นเวลา 7 วัน ปลูกเชื้อเริ่มต้นที่ได้ 10% (ปริมาตร/ปริมาตร) ลงในลิกไนท์ขนาดอนุภาค 45 ไมครอน แขวนลอยในน้ำกลั่น 10% (กรัม/100 มล.) ปรับค่าความเป็นกรด-ด่างเริ่มต้นเป็น 2.0 แบ่งที่ภาวะเดิมเป็นเวลา 8 วัน ที่ภาวะที่เหมาะสม *T. ferrooxidans* Y4-3 สามารถขจัดกํามะถันไพไรต์ออกจากลิกไนท์ได้ 11.52%

กําหนด *T. ferrooxidans* ไบโอลิชชิง การขจัดกํามะถัน ลิกไนท์
INTRODUCTION

There is a huge quantity of lignite in the Northern part of Thailand. Mae Moh district, Lamphang province is considered to have the largest lignite coal mine in South East Asia. There have been efforts to utilize lignite without causing environmental pollution. Lignite is a kind of coal which is formed from accumulated vegetable matter that has been altered by decay and by various amounts of heat and pressure. Coal is divided into 4 ranks from lignite (brown coal), sub-bituminous coal, bituminous coal, to anthracite. Different amounts of heat and pressure during the geochemical stage of coal development cause these differences in rank. Coal is composed mainly of carbon, hydrogen, and oxygen, with lesser amounts of nitrogen, sulfur and other elements. Sulfur in coal is present in two major forms; 1) Organic sulfur which is chemically bound to carbon and is part of the structure of coal and cannot be removed without destroying some of the integrity of the coal. 2) Inorganic sulfur: 2.1) pyritic sulfur such as iron pyrite (FeS₂). Iron pyrite is a cubic crystal dispersed throughout the coal matrix. Pyrite crystals can be removed by various techniques without destroying the chemical structure of coal. 2.2 sulfate which usually originates from oxidation of pyrite.

As lignite is sulfur-contaminated, burning tens of thousand tons of lignite per day as fuel for electricity generation will inevitably cause sulfur dioxide in the air, entailing toxicity to human beings, animals, plants, and causing acid rain resulting in damage to premises and aquatic ecology. Presently, the Electricity Generation Authority of Thailand entraps all sulfur dioxide emitting from lignite burning as calcium sulfate by fuel gas desulfurization. The fuel gas desulfurization equipment has been found to be too costly and its operating life cycle is much shorter than it should be, due to corrosion damage by sulfuric acid. Consequently, reducing sulfur contamination in lignite prior to burning will help lower sulfur dioxide and sulfuric acid emission, thus prolonging the functioning capability of the fuel gas desulfurization.

Some microorganisms can oxidize pyritic sulfur in coal to sulfate. Since the oxidation reaction occurs at normal temperature and pressure, the cost is low and the generated energy of the lignite remains consistent. The absence of dangerous chemical treatment will be environment friendly. Currently, because microbial desulfurization treated coal can be directly burnt as fuel by fluidization, a high potential exists for using this technique. The process of microbial desulfurization of coal is also known as coal bioleaching. *Thiobacillus ferrooxidans* is an acidophilic chemolithotrophic bacteria which has an important role in coal bioleaching. Its optimal pH for growth is 2.0-2.5 and it does not require organic carbon for growth because it uses carbon dioxide in the air as a carbon source. *T. ferrooxidans* oxidizes pyritic sulfur to sulfate by 2 mechanisms.

1) Under acidic conditions, *T. ferrooxidans* oxidizes soluble ferrous ions to ferric ions and the ferric ions react with pyrite. (4)

\[
4\text{FeSO}_4 + \text{O}_2 + 2\text{H}_2\text{SO}_4 \rightarrow 2\text{Fe}_2\text{(SO}_4)_3 + 2\text{H}_2\text{O} \\
\text{FeS}_2 + 2\text{Fe}_2\text{(SO}_4)_3 \rightarrow 3\text{Fe}_2\text{(SO}_4)_3 + 2\text{S}_3^{2-}
\]

2) *T. ferrooxidans* oxidizes sulfur and sulfide to sulfate by sulfur (sulfide) : ferric ion oxidoreductase (5) and oxidizes sulfite to sulfate by sulfite : ferric ion oxidoreductase (6) or sulfite oxidase.
The objective of this research is to optimize the use of *T. ferrooxidans* for lignite bioleaching.

**MATERIALS AND METHODS**

**Lignite sample**

Lignite samples (0.45 µm particle) were obtained from Mae Moh lignite mine, Mae Moh district, Lamphang province, Thailand. They contained 10 mg/g pyritic sulfur and the pH was 5.1. Pyritic sulfur in lignite was analyzed by ASTM Standards, D2492. The pH of lignite particles suspended in distilled water was assumed to be the pH of lignite.

**Bacterial strains and culture conditions**

*T. ferrooxidans* ATCC19859, a high efficient pyritic sulfur removal strain, was obtained from American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia, USA. *T. ferrooxidans* Y4-3, a high transformation efficiency strain, was obtained from Dr. Chihiro Inoue, Central Research Laboratory, Dowa Mining Co. Ltd., Hachioji, Tokyo, Japan and Dr. Tomonobu Kusano, Nara Institute of Science and Technology, Nara, Japan. *T. ferrooxidans* cells were grown in 9K medium (200 ml of 25% (w/v) FeSO₄·7H₂O solution (pH adjusted to 2.2 with 10 N H₂SO₄) was mixed with 800 ml of basal salts solution at 30°C with shaking (200 rpm): basal salts solution was as follows: (NH₄)₂SO₄, 3 g; K₂HPO₄, 0.1 g; MgSO₄·7H₂O, 0.5 g; KCl, 0.1 g; Ca(NO₃)₂·2H₂O, 0.02 g; in 800 ml distilled water adjusted pH to 2.3 with 10 N H₂SO₄) or on TSM solid medium.

**Optimization of bioleaching process**

**Inoculum**

A single colony of *T. ferrooxidans* was inoculated into 9K medium, incubated at 30°C with shaking at 200 rpm for 10 days, transferred at 10% (v/v) to fresh 9K medium and incubated under the same conditions was used as inoculum.

**Bioleaching process**

Inoculum at 1% (v/v) was inoculated into 10% pulp density (g/100 ml) lignite particles suspended in 9K medium without ferrous sulfate and incubated at 30°C with shaking at 200 rpm for 10 days. The 9K medium was used in place of inoculum in the control experiment.

**Optimization conditions**

The following bioleaching conditions were varied: suspension medium, initial pH, incubation temperature, % pulp density (lignite particle (g) suspended in 100 ml of suspension medium), inoculum size, inoculum age and incubation time.

**Analysis of pyritic sulfur removal**

The filtrate obtained from filtering out the lignite particles from the culture using Whatmann No.1 filter paper was examined for the amount of sulfate resulting from bacterial pyritic sulfur oxidation by a standard turbidimetric method. Mix 0.2-1.0 ml of sample in distilled water and made up to 100 ml. Add 20 ml of buffer solution (3% MgCl₂·6H₂O, 0.5% sodium acetate, 0.1% potassium nitrate, 2% acetic acid) and mixed in stirring apparatus. While stirring, added a spoonful (~0.75 g) of BaCl₂ (20-30 mesh) crystals and begin timing immediately. Stirred for 60±2 sec at constant speed. Measure of barium sulfate turbidity at OD₄₂₀. Estimated sulfate concentration in sample from correlation curve between sulfate concentration and OD₄₂₀ as shown in Figure 1.
Pyritic Sulfur Removal from Lignite by Thiobacillus ferrooxidans : Optimization of a Bioleaching Process

RESULT AND DISCUSSION

Comparison of pyritic sulfur removal efficiency between *T. ferrooxidans* Y4-3 and *T. ferrooxidans* ATCC19859

Hoffmann et al.\(^8\) reported that the *T. ferrooxidans* ATCC19859 removed 90-98% pyritic sulfur from coal within 8-12 days. Therefore, the pyritic sulfur removal efficiency of *T. ferrooxidans* Y4-3 and *T. ferrooxidans* ATCC19859 was compared. After bioleaching proceeded, culture filtrate of *T. ferrooxidans* Y4-3 and *T. ferrooxidans* ATCC19859 contained sulfate 0.211 and 0.122 mg/g lignite, respectively. The results indicated that *T. ferrooxidans* Y4-3 removed 1.32% pyritic sulfur and *T. ferrooxidans* ATCC19859 removed 0.76% pyritic sulfur from lignite under test conditions (Figure 2). *T. ferrooxidans* Y4-3 was used in further experiments on optimization of the bioleaching process.

The factors led to the higher rate of pyritic sulfur removal by *T. ferrooxidans* were:\(^8\) 1) lignite sample; higher concentration of pyritic sulfur contaminated and lower concentration of bioleaching inhibitors. 2) higher ratio of ammonium to phosphate in culture medium. 3) higher surface area of pyrite which obtained from smaller particle size or higher percentage of pulp density. The experiment of pyritic sulfur removal by *T. ferrooxidans* ATCC19859\(^8\) was done by suspending lignite particle (30-40 mg/g pyritic sulfur contaminated) at 20% pulp density in a very high ratio of ammonium to phosphate (90:1) culture medium. In our experiment both *T. ferrooxidans* Y4-3 and *T. ferrooxidans* ATCC19859 can grow equally well in the 9K medium (data not shown).

Optimization of bioleaching conditions

Various bioleaching conditions for *T. ferrooxidans* Y4-3 were examined.

Suspension medium

Lignite particles were suspended in 9K medium without ferrous sulfate and in distilled water. After bioleaching had proceeded, the culture filtrate contained sulfate at 0.211 and 0.220 mg/g lignite, indicating 1.32 and 1.37% pyritic sulfur was removed, respectively (Figure 3). Distilled water was used as the suspension medium in further experiments. The ratio of ammonium to phosphate in the 9K medium without ferrous sulfate used was 10:1 which did not differentiate the bioleaching capability of *T. ferrooxidans* Y4-3.
Figure 3. Effect of suspension medium (a), initial pH (b), incubation temperature (c), lignite pulp density percentage (d), inoculum size (e), inoculum age (f) and incubation time (g) on pyritic sulfur removal by *T. ferrooxidans* Y4-3.

**Initial pH**

The initial pH of lignite particles suspended in distilled water was 5.1, whereas the optimal pH for growth of *T. ferrooxidans* is 2-2.3\(^{15}\) and the optimal pH for sulfur bioleaching by *T. ferrooxidans* is 1-2.5.\(^{16}\) The initial pH of lignite particles suspended in distilled water was varied: 1.5, 2.0, 2.5 and 3.0. After bioleaching proceeded, the culture filtrate contained sulfate 0, 0.873, 0.209 and...
0.192 mg/g lignite, respectively. This indicated that 0, 5.45, 1.3 and 1.2% pyritic sulfur was removed, respectively (Figure 3). The initial pH of 2.0 was used in further experiments.

**Incubation temperature**

*T. ferrooxidans* Y4-3 was inoculated into lignite particles suspended in distilled water at initial pH 2.0. The bioleaching mixtures were incubated at 30°C, 30+2°C, 35°C and 37°C. After bioleaching proceeded, the culture filtrate contained sulfate 0.873, 0.749, 0.634 and 0.523 mg/g lignite, indicating that 5.45, 4.68, 3.96 and 3.26% pyritic sulfur was removed, respectively (Figure 3). The bioleaching mixtures were incubated at 30°C in further experiments. Optimal bioleaching temperature of *T. ferrooxidans* is 28-30°C. The room temperature was 30°C; therefore, temperatures lower than 30°C were not examined because they would not appropriate for the application.

**Pulp density**

*T. ferrooxidans* Y4-3 was inoculated into lignite particles suspended in distilled water at initial pH 2.0 and the bioleaching mixture was incubated at 30°C. The pulp density percentage of lignite particles was varied: 5, 10, 20 and 30% (g/100 ml). After bioleaching proceeded, the culture filtrate contained sulfate 0.608, 0.873, 0.286 and 0.089 mg/g lignite, indicating that 3.8, 5.45, 1.79 and 0.56% pyritic sulfur was removed, respectively (Figure 3). The lignite pulp density at 10% (g/100 ml) was used in further experiments. Lower lignite pulp density percentage resulted in lower available of pyrite surface area, while higher lignite pulp density percentage resulted in lower dissolved oxygen and higher concentration of growth and bioleaching inhibitors.

**Inoculum size**

*T. ferrooxidans* Y4-3 was inoculated at 1, 5, 10, 15 and 20% (v/v) into 10% (g/100 ml) lignite particles suspended in distilled water at initial pH 2.0 and the bioleaching mixture was incubated at 30°C. After bioleaching proceeded, the culture filtrate contained sulfate 0.873, 1.084, 1.12, 1.104 and 1.076 mg/g lignite, indicating that 5.45, 6.77, 6.99, 6.89 and 6.72% pyritic sulfur was removed, respectively (Figure 3). The inoculum size of 10% (v/v) was used in further experiments. Pyritic sulfur removal was not increased with an inoculum size increment of 10 to 20% (v/v). The pyrite surface area might available for only 10% (v/v) inoculum.

**Inoculum age**

*T. ferrooxidans* Y4-3 grown in 9K medium for 5, 7 and 10 days was inoculated at 10% (v/v) into 10% (g/100 ml) lignite particles suspended in distilled water at initial pH 2.0 and the bioleaching mixture was incubated at 30°C. After bioleaching proceeded, the culture filtrate contained sulfate 0.840, 1.623 and 1.120 mg/g lignite, indicating that 5.24, 10.13 and 6.99% pyritic sulfur was removed, respectively (Figure 3). *T. ferrooxidans* Y4-3 grown in 9K medium for 7 days was used as inoculum in further experiments. The culture of *T. ferrooxidans* grown in 9K medium for 6 days contains the highest active cell number.

**Incubation time**

*T. ferrooxidans* Y4-3 grown in 9K medium for 7 days was inoculated at 10% (v/v) into 10% (g/100 ml) lignite particles suspended in distilled water at initial pH 2.0 and the bioleaching mixture was incubated at 30°C for 2, 4, 6, 8, 10 and 12 days. After bioleaching proceeded, the culture filtrate contained sulfate 0.970, 1.607 and 1.662, 1.846, 1.623 and 1.109 mg/g lignite.
mg/g lignite, indicating that 6.05, 10.13, 10.37, 11.52, 10.13 and 6.92% pyritic sulfur was removed, respectively (Figure 3). Ferric ions obtained from ferrous oxidation was used as electron donor for sulfur bioleaching. However ferric ions could form Fe(OH)₃ and MFe₂(SO₄)₃ precipitate covered the available pyrite surface area and pyritic sulfur removal was reduced. The bioleaching mixtures incubated for 8 days gave maximum pyritic sulfur removal at 11.52%. After optimization of bioleaching conditions, pyritic sulfur removal was increased from 1.32 to 11.52% or 8.72 times.

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