Microbial Leaching of Zinc from Low Grade Ore
ไมโครเบียลลิชชิ่งสังกะสีจากกากแร่เกรดต่ำ

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Abstract

The present work investigates microbial leaching of zinc from low grade ore using *Acidithiobacillus ferrooxidans* (ATCC 14859). The initial low grade ore samples were collected from north-eastern Thailand, (Thongkum Mine, Amphoe Muang, Loei Province), and consisted of 13 % of initial zinc or 130 g kg^{-1}. The experiments were undertaken at the shaken flask scale under controlled conditions of shaking speed 250 rpm, temperature 30 °C, time 16 days and 4 factors were varied; type of medium (0 k and 9 k medium), particle ore size (<20, 20-40, 40-60, 60-100 mesh and >100 mesh), percentage of ore density (2%, 5% and 10%) and the initial pH of the medium (2, 2.5, 2.8 and 3). The microbial leaching was assessed by determining the concentration of zinc in the medium and comparing it with the initial sample concentration. The results show that *A. ferrooxidans* can successfully leach zinc by as much as 6 times compared with the control experiment (without addition of *A. ferrooxidans*). The maximum efficiency (92.3%) for microbial leaching was obtained in 9 K medium, 20-40 mesh ore sizes, 2% ore density at pH 2.8 and the zinc content was found in the medium to be about 120 mg l^{-1}.

Keywords: Microbial Leaching, *Acidithiobacillus ferrooxidans*, Low grade ore
คำสำคัญ: ไมโครเบียลลิชชิ่ง *Acidithiobacillus ferrooxidans* สินแร่เกรดต่ำ

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บทคัดย่อ

งานวิจัยนี้เป็นการศึกษาการสกัดสังกะสีออกจากกากแร่เกรดต่ำด้วยจุลินทรีย์ที่เรียกว่า “ไมโครเบียลลิชชิ่ง” โดยใช้แบคทีเรียสายพันธุ์บริสุทธิ์ *Acidithiobacillus ferrooxidans* (ATCC 14859) และใช้ตัวอย่างกากแร่จากเหมืองแร่ทุ่งคำ (อำเภอเมือง จังหวัดเลย) ที่ผ่านการถลุงเพื่อแยกสินแร่ที่มีค่าออกแล้ว พบว่ากากแร่ประกอบด้วยสังกะสีคิดเป็นปริมาณร้อยละ 13 หรือมีความเข้มข้น 130 กรัมต่อกิโลกรัม จากนั้นนำมาศึกษาการเกิดไมโครเบียลลิชชิ่งในฟลาสก์ ที่ควบคุมความเร็วในการเขย่า 250 รอบต่อนาที อุณหภูมิ 30 องศาเซลเซียส และใช้เวลาเลี้ยงเชื้อทั้งหมด 16 วัน โดยมีการแปรผันปัจจัยที่มีอิทธิพลต่อการเกิดไมโครเบียลลิชชิ่ง 4 ปัจจัย ได้แก่ อาหารเลี้ยงเชื้อจุลินทรีย์ (สูตร 0 k และ 9 k) ขนาดของกากแร่ (<20, 20-40, 40-60, 60-100, >100 เมช) ความหนาแน่นของกากแร่ (2%, 5%, 10%) และค่าพีเอชเริ่มต้นของอาหารเลี้ยงเชื้อ (2, 2.5, 2.8 และ 3) การเกิดไมโครเบียลลิชชิ่งจะประเมินจากการเปรียบเทียบความเข้มข้นของสังกะสีที่พบในอาหารเลี้ยงเชื้อกับความเข้มข้นเริ่มต้นของสังกะสีในกากแร่ ผลการทดลองพบว่าเชื้อ *A. ferrooxidans* สามารถสกัดสังกะสีออกมาจากกากแร่ได้มากกว่าชุดควบคุม (ชุดที่ไม่มีการเติมเชื้อ) ถึง 6 เท่า ประสิทธิภาพสูงสุดในการเกิดไมโครเบียลลิชชิ่งเท่ากับ 92.3% ภายใต้สภาวะที่มีอาหารเลี้ยงเชื้อสูตร 9 k โดยใช้ขนาดของกากแร่ 20-40 เมช และมีความหนาแน่นของกากแร่ 2% ที่พีเอช 2.8 โดยปริมาณสังกะสีที่พบในอาหารเลี้ยงเชื้อมีความเข้มข้นทั้งหมดประมาณ 120 มิลลิกรัมต่อลิตร

Introduction

Mining is one of humankind's oldest activities, and the techniques used to extract minerals have not changed for centuries. Normally, ores are dug from the earth, crushed and then minerals are extracted by extreme heat or using highly toxic chemicals. Therefore, the environmental and health effects of traditional mining technologies have been deleterious (Tyagi and Cavillard, 1987; Veglio and Beolchini, 1997; Leduc et al., 1997). To confront this problem and because of the potential for industrial application, biotechnology may be one answer to this problem. It may permit taking different minerals and valuable metals from low grade ores such as copper, zinc, silver and uranium etc. and economically converting them into useful products (Tyagi and Cavillard, 1987; Veglio and Beolchini, 1997; Leduc et al., 1997).

The microbial leaching process, typically using mesophilic bacteria such as *Acidithiobacillus ferrooxidans* (previously known as *Thiobacillus ferrooxidans*) and other chemo-autotrophic bacteria such as *Acidithiobacillus thiooxidans* (formerly known as *Thiobacillus thiooxidans*) and *Leptospirillum ferrooxidans* (Deveci et al., 2004), has become increasingly important and has been proven to be an attractive alternative for the extraction of valuable metals or different minerals from refractory ores (Tyagi and Cavillard, 1987; Konishi et al., 1992; Veglio and Beolchini, 1997; Leduc et al., 1997 and recently Shi et al., 2006). The mechanism of microbial leaching is usually discussed in terms of a direct mechanism that requires physical contact between *A. ferrooxidans* and the sulfide mineral surface, the cells catalyzing the oxidation of Fe(II) sulphide to Fe(III) sulphate through a number of enzymes such as rusticyanin, Fe(II)-cytochrome c-552 oxidoreductase and cytochrome c-552 while in an indirect mechanism, the ferric iron as a chemical leaching agent is supplied by regeneration from the reaction product (ferrous iron) through biological oxidation by *A. ferrooxidans*. Both of the mechanisms depend on type of sulphide mineral and operating conditions (Konishi et al., 1992; De et al., 1997). In this research work, the ability of *A. ferrooxidans* under variation of pH, type of medium, sample size fraction and percentage of ore density was evaluated to obtain the optimal condition for extraction of zinc from low grade ores using the microbial leaching process (at the shaken flask scale).

Material and Methods

1. *Bacteria*

Pure culture strains of *A. ferrooxidans* ATCC 14859 received from King Mongkut's University of Technology Thonburi, were cultivated aerobically in an Erlenmeyer flask using an incubating shaker with speed 250 rpm, at 30 ^{o}C and grown in 9 K medium (Konishi et al., 1992; Donati et al., 1997). They were then harvested at the late logarithmic phase of growth. The culture suspension was filtered through a 0.22 μm filter (Nucleopore) and washed several times with acidified water (pH=1.5) to eliminate ferric ion. Finally, the bacteria pellet was suspended in medium without iron (0 K medium) and used as inocula.

2. *Medium*

The growth of *A. ferrooxidans* was conducted in an enriched salt solution called a 9 K medium (Donati et al., 1997), containing 6 g l^{-1} ferric ion [as $Fe_2(SO_4)_3$] instead of 9 g l^{-1} ferrous ion, $(NH_4)_2SO_4$ (0.2 g l^{-1}), KCl (0.1 g l^{-1}), K_2HPO_4 (0.1 g l^{-1}) and $MgSO_4.7H_2O$, (0.4 g l^{-1}). In some cases, iron was not added (called a 0 K medium). Then, media was sterilised by filtration through a 0.22 μm filter.

3. *Ore samples and size sieving*

Low grade ore samples used in this study, obtained from Thongkam mine Co., Ltd., Amphoe Muang, Loei Province in the north eastern area of Thailand, were ground and were then sieved (using screens no. 10, 20, 40, 60 and 100 mesh) to obtain 5 groups of size fraction (< 20, 20-40, 40-60, 60-100 and >100 mesh). The initial composition of zinc was 130 g kg^{-1} (analyzed by the Dept. of Mineral Resources, Ministry of Natural Resources and Environment of Thailand). The low grade ore samples were then dried in a hot air oven at 105^{o}C for 3 hrs and 15 g of each group of size fraction was sterilised using an autoclave at 15 psi and 121^{o}C for 15 min to prevent the contamination of other micro-organisms.

4. *Microbial leaching experiments*

Typically, microbial leaching experiments were carried out in 250 ml Erlenmeyer flasks. Enriched salt solution (150 ml) adjusted to the desired pH (2, 2.5, 2.8 and 3.0) was transferred into each flask to which 3, 7.5 and 15 g of the ore/concentrate sample (corresponding to 2, 5 and 10 % w/v ore density) was added. Moreover, the ore size fraction was varied (<20, 20-40, 40-60, 60-100 and >100 mesh) and the addition of *A. ferrooxidans* compared to the experiment with no added *A. ferrooxidans*. The flasks were then autoclaved at 15 psi and 12 ^{o}C for 15 min. Following autoclaving, each flask was incubated under aseptic conditions with 3 ml of concentrated *A. ferrooxidans* cells to medium used (0 K medium and 9 K medium) producing a final volume of slurry of approximately 150 ml. To facilitate mixing of the contents and exchange of oxygen and carbon dioxide, the flasks from each condition were incubated on the orbital incubator shaker at 250 rpm and optimal growth temperature of 30 ^{o}C for mesophilic bacteria such as

A. ferrooxidans (Deveci et al., 2004). Table 1 shows the experimental design conditions used to investigate zinc leaching using *A. ferrooxidans*.

5. *Analytical techniques*

Samples of 2 ml aliquot of the leach solution were taken periodically from each flask (twice a day for 16 days), centrifuged at 12000 g for 5 min and the supernatant of the leaching system was then analysed for zinc concentration using Atomic Absorption Spectrophotometer (AAS). For monitoring pH, the pH was adjusted using 18 M sulphuric acid (H_2SO_4) when it deviated toward neutrality from the initially present values.

Results and Discussion

After the medium was inoculated with strains of *A. ferrooxidans* (approximately 5 x 10^6 cell/ml) and cultivated aerobically (as mentioned in *1.*), growth was monitored by cell counting using a haemacytometer. Figure 1 shows the growth curve of *A. ferrooxidans* as a function of cultivation time and the log phase was obtained from 10-16 hrs. This suggests when cell harvesting should be undertaken to get the cell number for use in the further steps.

Figure 2 shows the zinc concentrations found as functions of cultivation time in the experiments in which *A. ferrooxidans* was added to the flask during the leaching experiment, compared with the experiment with no added microbes. The results show that *A. ferrooxidans* can successfully leach zinc by as much as 6 times at 16 days compared with the control experiment (without addition of *A. Ferrooxidans*).

Consequently, Figure 3 shows the percentage of zinc leaching due to 5 different groups of ore size fraction as a function of time. As time increases, the percentage of leaching increases and the highest percentages in leaching for all size fractions are obtained after 16 days of incubation. This means that *A. ferrooxidans* can grow and leach zinc into the liquid medium especially in the case of size fraction of 20-40 mesh. The efficiency of zinc leaching reached 93%, which is in good agreement with previous studies, in which it is stated that the highest zinc leaching by *A. ferrooxidans*, *A. thiooxidans* and *L. ferrooxidans* have been reported in a range of 84% - 98 % (Deveci et al., 2004; Tipre and Dave, 2004). This depends on the different size fraction of ore and the percentage of ore density used. In addition, our results have similar trends to other studies where the highest leaching occurred with the smallest ore size fraction and ore density used.

The results of zinc concentration found in the presence of 9 K medium comparing different percentages of ore density (2%, 5% and 10%) as functions of time at the optimal pH of 2.8 are shown in Figure 4. It was found that all experiments had increasing zinc concentration as the cultivation time increased. The highest zinc concentration for the 2% ore density was around 120 mg l^{-1} after 16 days and obtained a high efficiency of zinc leaching at around 92%. Meanwhile the experiments with 5% and 10% ore density obtained final concentrations of zinc at about 100 mg l^{-1} and 75 mg l^{-1}, equivalent to 73% and 57%, respectively. In a previous study, it is stated that in the stirred tank reactor (STR) batch leaching results in a decline in the percentage of zinc concentrate with increasing density (5%, 10%, 20% and 25%), unlike in the shake flask study. This could be because of the different oxygen reactions and oxygen mass transfer prevailing in the shaken flask and the STR (Tipre and Dave, 2004).

Conclusions

It is clear that good results can be obtained under various combinations of parameters. It was expected that the ferrous iron oxidizing ability of the *A. ferrooxidans* is significantly affected by the presence of zinc in the low grade ores and the best results would be achieved under optimal conditions, where the initial pH of the suspensions containing 9 K medium was about 2.8 with fraction size of ores in the range of 20-40 mesh. The 2 % ore density gave the highest efficiency (up to 92.3 %) for *A. ferrooxidans* in the microbial leaching process after 16 days cultivation, showing that the ability of *A. ferrooxidans* to extract zinc from low grade ore is as much as six times greater than in the control experiment (without addition of *A. ferrooxidans*). Changes in the efficiency of *A. ferrooxidans* confirm that particle size fraction, medium, pH and percentage of ore density are important in microbial leaching and that the choice of these factors can influence the optimal conditions for use.

Acknowledgments

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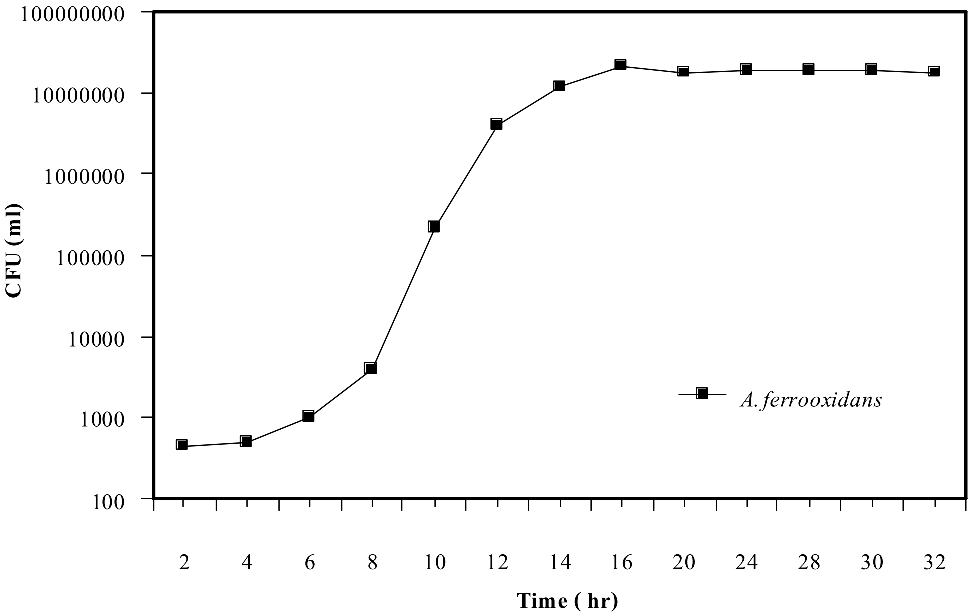

Figure 1. Growth curve of *A. ferrooxidans*.

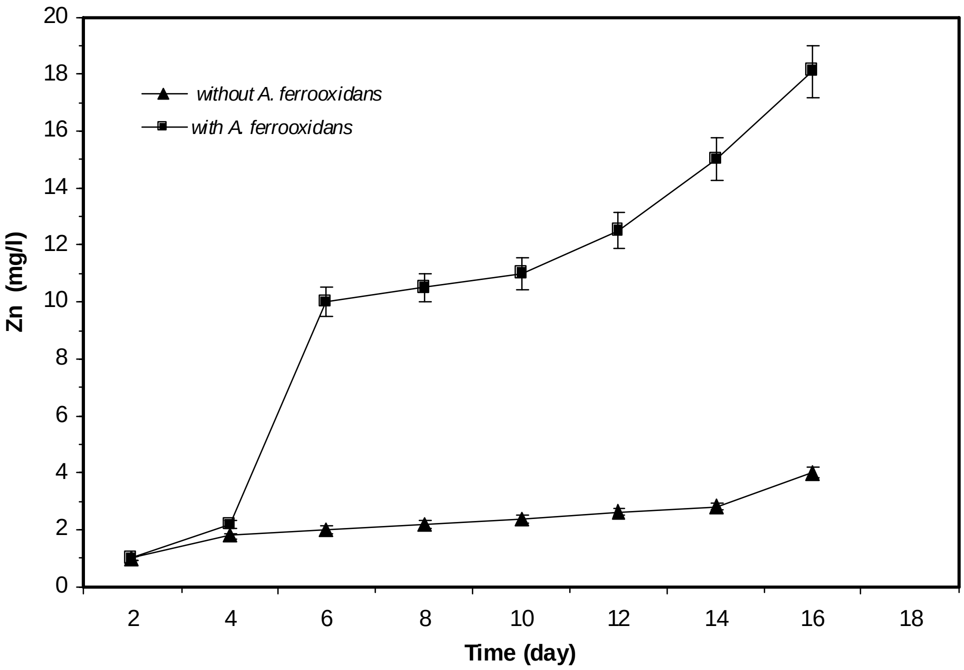

Figure 2. Comparison of zinc leaching concentration found in the experiments with and without addition of *A. ferrooxidans*.

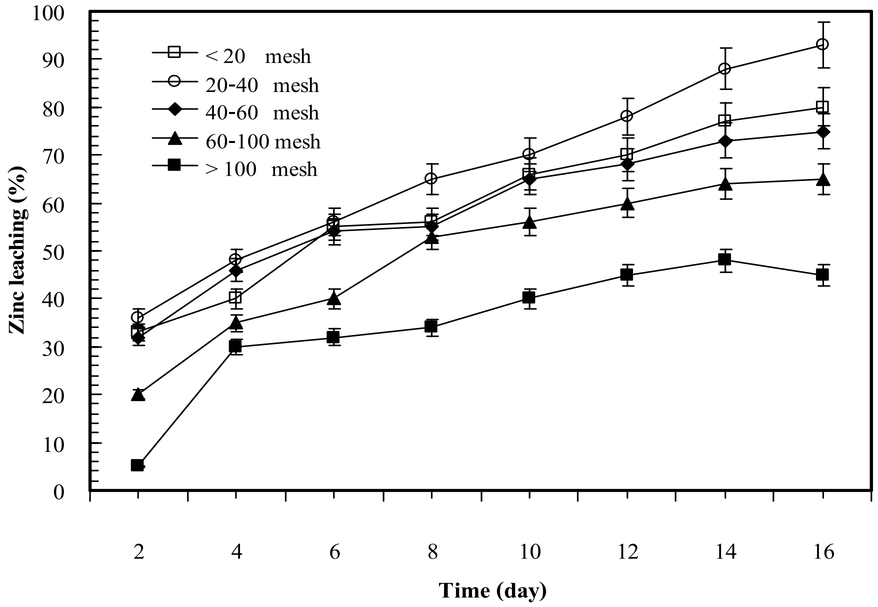

Figure 3. Efficiency of zinc leaching from various ore size fractions using *A. ferrooxidans* as a function of time.

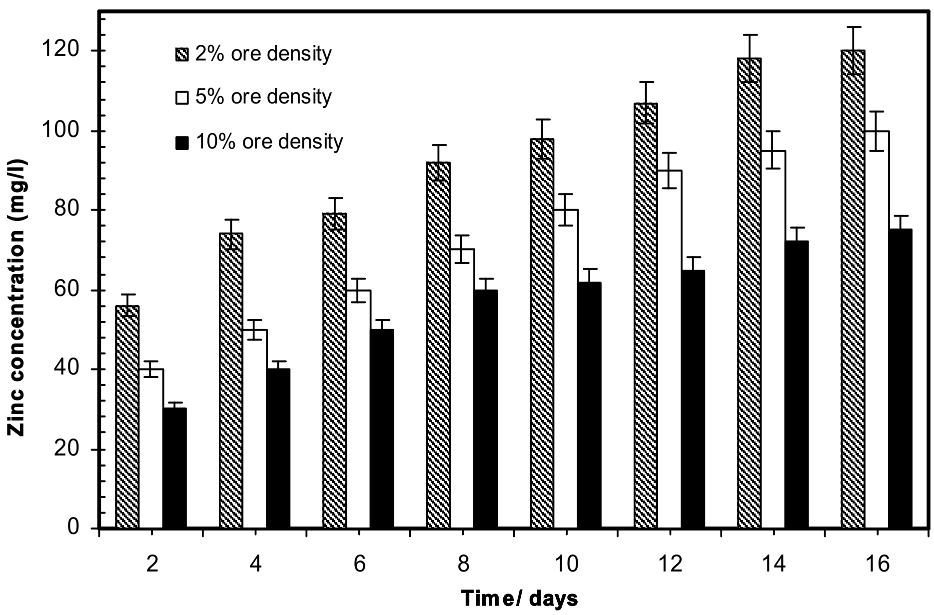

Figure 4. Concentration of zinc leaching using *A. ferrooxidans* as a function of time with different percentage ore densities.

Table 1. Experimental design conditions used to investigate zinc leaching using *A. ferrooxidans*.

pH	% ore density (w/v)	ore sample (g)	9 K medium (ml)	Concentrated *A. ferrooxidans* cells (ml)
2	2	3	150	3
	5	7.5	150	3
	10	15	150	3
2.5	2	3	150	3
	5	7.5	150	3
	10	15	150	3
2.8	2	3	150	3
	5	7.5	150	3
	10	15	150	3
3.0	2	3	150	3
	5	7.5	150	3
	10	15	150	3