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# Bioleaching of zinc from gold ores using *Acidithiobacillus ferrooxidans*

*Pakawadee Kaewkannetra*1), *Francisco Jose Garcia-Garcia*2), *and Tze Yen Chiu*3)

1) Department of Biotechnology, Faculty of Technology, Khon Kaen University, Khon Kaen 40002, Thailand
2) Corrosion and Protection Centre, School of Materials, The University of Manchester, Manchester M60 1QD, UK
3) Centre of Water Science, Cranfield University, Bedfordshire MK43 0AL, UK

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**Abstract:** The present work investigated microbial leaching of zinc from ore using *Acidithiobacillus ferrooxidans* (ATCC 14859). The ore samples, consisted of 13wt% zinc, were obtained from a gold mine in north-eastern Thailand. A shake flask study was performed on the ore samples using a rotary shaker under the following fixed conditions (250 $r \cdot min^{-1}$, 30°C for 16 d). The influence of various conditions, namely medium type (with and without iron), particle ore size (<20, 20-40, 40-60, 60-100, and >100 mesh), ore density (20 $kg \cdot m^{-3}$, 50 $kg \cdot m^{-3}$, and 100 $kg \cdot m^{-3}$), and pH of the medium (2, 2.5, 2.8, and 3), were investigated. The microbial leaching was assessed by determining the concentration of zinc in the medium and compared with the initial sample concentration. The results show that *Acidithiobacillus ferrooxidans* can successfully leach zinc by as much as 6 times compared with the control experiment (without *Acidithiobacillus ferrooxidans* ferrooxidans). The maximum efficiency (92.3%) for microbial leaching is obtained in iron-containing medium, 20-40 mesh ore sizes, 20 $kg \cdot m^{-3}$ ore density at pH 2.8, and the zinc content is found in the medium at about 120 $mg \cdot L^{-1}$.

**Key words:** bioleaching; *Acidithiobacillus ferrooxidans*; gold ores; gold mine

## 1. Introduction

Approximately 90% of total zinc in the world, estimated to be over 7 million tons, is produced through the extraction of zinc from sphalerite by roast-leach-electrowinning and pressure hydrometallurgy [1]. These processes are often associated with environmental and health risks, and hence other less detrimental alternatives are being explored [2-3]. The application of biotechnology is one such technology, which involves the use of microorganisms to extract specific metals from their ores. Typical bacteria studied and employed in the microbial leaching process include mesophilic and other chemo-autotrophic bacteria, *i.e. Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans* [4-6]. Because the current world focus on global warming and carbon footprints, bioleaching becomes increasingly important as an alternative extraction method to increase zinc production without the consequent production of $SO_2$.

Bioleaching of zinc minerals is thought to occur *via* two mechanisms, *i.e.* direct and indirect mechanisms [7]. In the direct mechanism, physical contact between *Acidithiobacillus ferrooxidans* and the sulfide mineral surface is essential and the cells catalyze 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. In the indirect mechanism, ferric iron as a chemical leaching agent is supplied by regeneration from the reaction product (ferrous iron) through biological oxidation by *Acidithiobacillus ferrooxidans*. Regardless of the mechanisms, factors, such as mineralogical composition and operating conditions, affect bioleaching efficiencies and ultimately feasibility for clean extraction [5, 8].

Many investigations have been carried out to develop commercial bioleaching for base metals in various countries with differing leaching effects reported in each study [4, 7, 9-10]. This has been attributed to the distinct properties of the ore specimens used in the various experiments. It is well known that different metals may be selectively leached from one another because of electrochemical galvanic interactions [11-12]. The selective leaching of metals is also

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**Corresponding author:** Tze Yen Chiu, **E-mail:** tychiu_99@yahoo.com

observed with zinc bearing ores, which often occurs as fine intergrowths of other sulphide minerals. In the presence of certain metals such as galena, the bioleaching of sphalerite is made passive, whereas in the presence of other metals such as pyrite or pyrolusite, sphalerite is selectively oxidised during bioleaching [11, 13-14].

Most of the gold mines in Thailand contain complex ores, which contain zinc amongst other heavy metals. The manganese dioxide, iron and lead contents in the naturally occurring ore waste from gold mines are substantially different from those obtained around the world. To the knowledge of authors, no studies have been reported for the bioleaching from complex ores obtained in Thailand. This study aimed to assess the potential amenability of these ores to bioleaching using widely studied bacteria (*Acidithiobacillus ferrooxidans*) for the treatment of Thai mining waste. In addition, the results from this study would provide more information for the management of waste within the Thai mining community. The optimal conditions for biological extraction using *Acidithiobacillus ferrooxidans* were investigated. The evaluated parameters included pH, medium type, samples size, and composition of ores.

## 2. Materials and methods

### 2.1. Bacteria and medium

Pure cultures of *Acidithiobacillus ferrooxidans* (ATCC 14859) which were received from King Mongkut's University of Technology Thonburi, Thailand, were cultivated aerobically at 30°C in a 9 K liquid medium (iron-containing medium) [5, 15]. Incubation was carried out in an Erlenmeyer flask on a shaker at 250 $r\cdot min^{-1}$. After bacterial growth reached the late logarithmic growth phase, the culture suspension was filtered through a 0.22-μm membrane (nucleopore) and washed several times with acidified water (pH=1.5) to eliminate iron(III). The bacteria was subsequently resuspended in medium without iron (0 K medium) and used as inocula.

### 2.2. Medium

The enriched salt solution, 9 K medium [15], used to cultivate the bacterial cultures contained 6 $g\cdot L^{-1}$ of $Fe_2(SO_4)_3$, 0.2 $g\cdot L^{-1}$ of $(NH_4)_2SO_4$, 0.1 $g\cdot L^{-1}$ of KCl, 0.1 $g\cdot L^{-1}$ of $K_2HPO_4$, and 0.4 $g\cdot L^{-1}$ of $MgSO_4\cdot 7H_2O$. In cases where iron was not added, the medium was termed 0 K medium. The media was sterilized by filtration through a 0.22 μm filter membrane.

### 2.3. Ore samples and size sieving

Ore samples, in the form of ZnS, used in this study, obtained from Thongkam mine Co., Ltd., Muang, Loie Province in north eastern area of Thailand, were ground and subsequently sieved using screen numbers 10, 20, 40, 60 and 100 mesh to obtain 5 different size fractions of 0.84-2.0, 0.42-0.84, 0.25-0.42, 0.15-0.25 and <0.15 mm, respectively. The initial zinc concentration was 130 g/kg as analyzed by Department of Mineral Resources, Ministry of Natural Resources and Environment of Thailand. The chemical composition of the ore was Zn 13.0wt%, S 30.0wt%, Fe 5.0wt%, Pb 3.6wt%, Cu 2.1wt%, $MnO_2$ 42.1wt%, Al and $Y_2O_3$ 4.2wt%. The ore samples were dried in a hot air oven at 105°C for 3 h and 15 g of each size group was autoclaved (Hiclave HV-85, Hirayama, Japan) at 103 kPa, 121°C for 15 min to sterilize the samples in order to prevent contamination from any microorganisms.

### 2.4. Microbial leaching experiments

Microbial leaching experiments were carried out in 250 mL Erlenmeyer flasks. Before use, the flasks were autoclaved at 103 kPa, 121°C for 15 min. After autoclaving, each flask was incubated under aseptic conditions and contained 3 mL of concentrated *Acidithiobacillus ferrooxidans* cells and 150 mL of 9 K liquid medium (unless stated otherwise) at the desired pH. pH adjustment was carried out on the enriched salt solution to the desired pH (2, 2.5, 2.8, and 3.0) using sulphuric acid ($H_2SO_4$) before it was transferred into each flask. To each flask, 3.0 g of ore corresponding to 20 $kg\cdot m^{-3}$ of ore density (unless stated otherwise) was added.

The effect of ore size fraction on zinc extraction was compared by adding various fractions to the Erlenmeyer flasks containing *Acidithiobacillus ferrooxidans* within the 9 K liquid medium. To investigate the effect of *Acidithiobacillus ferrooxidans*, control studies in the absence of *Acidithiobacillus ferrooxidans* were undertaken.

To facilitate the mixing of contents and the exchange of oxygen and carbon dioxide, the flasks were incubated on the orbital incubator shaker at 250 $r\cdot min^{-1}$ and 30°C. Table 1 presents the experimental conditions employed in the investigation of zinc leaching with *Acidithiobacillus ferrooxidans*.

### 2.5. Analytical techniques

2 mL aliquot of the leach solution were extracted from the flask (twice daily over 16 d) and centrifuged (Sorvall RC-26, Dupont, USA) at 12000 g for 5 min. The supernatant was decanted and the zinc concentrations were subsequently determined using an atomic absorption spectrophotometer (AAS 800, Perkin-Elmer, USA).

**Table 1. Experimental conditions employed to leach zinc using *Acidithiobacillus ferrooxidans* (150 mL of 9 K liquid medium)**

| pH | Ore density / ($kg \cdot m^{-3}$) | Mass of ore sample / g |
|---|---|---|
| 2 | 20 | 3 |
|  | 50 | 7.5 |
|  | 100 | 15 |
| 2.5 | 20 | 3 |
|  | 50 | 7.5 |
|  | 100 | 15 |
| 2.8 | 20 | 3 |
|  | 50 | 7.5 |
|  | 100 | 15 |
| 3.0 | 20 | 3 |
|  | 50 | 7.5 |
|  | 100 | 15 |

Note: the number of *Acidithiobacillus ferrooxidans* is $15 \times 10^6$.

## 3. Results and discussion

### 3.1. Microbial growth

After the medium was inoculated with the cell population count of approximately $5 \times 10^6$ $mL^{-1}$ of *Acidithiobacillus ferrooxidans* and subsequently cultivated aerobically (see Section 2.1.). Its growth was monitored by counting the cells using a haemacytometer. Fig. 1 depicts the growth curve of *Acidithiobacillus ferrooxidans* in terms of colony forming units (CFU) as a function of cultivation time. The exponential phase of bacterial growth started after 10 h and continued until 16 h when it reached the stationary phase. This suggested that the harvesting cell would be undertaken to get the quantitative cell for use in the further step.

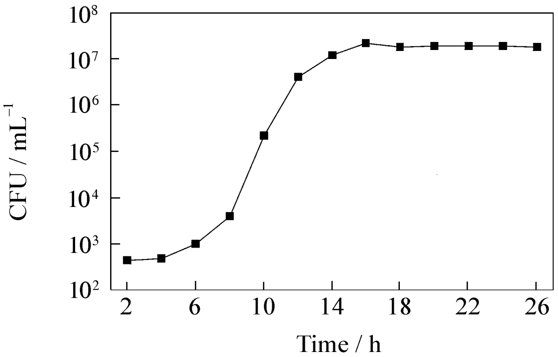

**Fig. 1. Growth curve of *Acidithiobacillus ferrooxidans*.**

### 3.2. Effect of the presence and absence of bacteria strain

Fig. 2(a) depicts the zinc concentrations observed as a function of leaching time in the presence and absence of *Acidithiobacillus ferrooxidans*. In the absence of bacteria, the leaching rate of zinc was almost constant at less than 4 $mg \cdot L^{-1}$ after 16 d of leaching. In the presence of bacteria, the zinc dissolution rate increased and the zinc dissolution curve reflected the typical characteristic lag period of the bioleaching process. Similar zinc concentrations in the leachate were obtained at day 10 and day 4 in the absence and presence of *Acidithiobacillus ferrooxidans*, respectively. These observations can be explained by the following reactions involved in the biological oxidation of zinc sulphide [16]:

$$ZnS+2Fe^{3+} \rightarrow Zn^{2+}+2Fe^{2+}+S \quad (1)$$

$$ZnS+0.5O_2+H_2SO_4 \rightarrow ZnSO_4+H_2O+S \quad (2)$$

$$4Fe^{2+}+O_2+4H^+ \xrightarrow{\text{bacteria}} 4Fe^{3+}+2H_2O \quad (3)$$

$$S+1.5O_2+H_2O \xrightarrow{\text{bacteria}} H_2SO_4 \quad (4)$$

$$ZnS+MnO_2+2H_2SO_4 \rightarrow ZnSO_4+MnSO_4+2H_2O+S \quad (5)$$

In the absence of bacteria, zinc sulphide dissolution was principally chemical and was mediated by the protons present in the solution. This corresponded with the changes in pH *vs.* time (Fig. 2(b)).

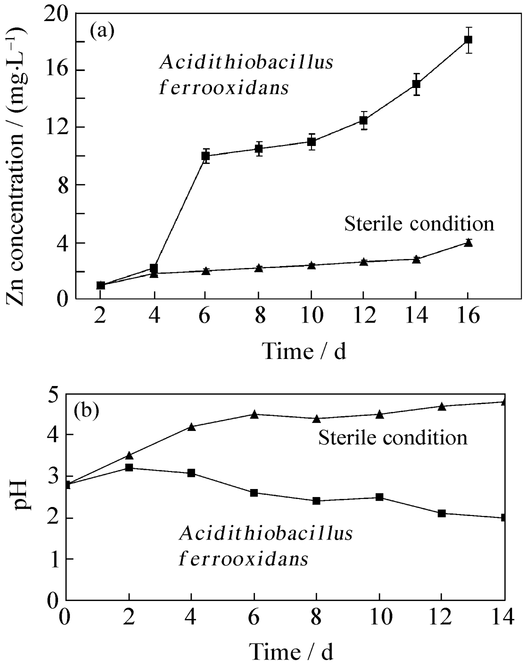

**Fig. 2. Effect of *Acidithiobacillus ferrooxidans*: (a) concentrations of zinc found in the leachate at pH 2.8; (b) evolution of pH during leaching as a function of time.**

The pH values during leaching experiments were monitored because pH was an important parameter in bioleaching and could serve as an indication of the biological activity. Before inoculation, the ores were pre-leached with sulphuric acid in order to neutralize the alkaline components within the ore. In the abiotic system, the pH increased to approximately 5.0. This arose from the consumption of acid during the protonic attack of the sphalerite and possible galvanic interaction between ZnS and $MnO_2$ [14, 16-17] according to Eqs. (2) and (5), respectively. In the inoculated test, an initial increase of pH was observed and was probably due to the same reasons as in the sterile control. After this period, the acidity increased because of the oxidation of elemental sulphur by *Acidithiobacillus ferrooxidans* (Eq. (4)). The pH decreased progressively probably as a result of iron hydrolysis and the consequent precipitation of ferric compounds through the indirect mechanism.

The difference between the observed zinc concentrations found in the leachate obtained with and without *Acidithiobacillus ferrooxidans* became more distinct after day 4. At day 4, the difference was approximately 0.5 $mg\cdot L^{-1}$, whereas the difference was 12 $mg\cdot L^{-1}$ at day 16. This was suggestive of the bacteria adapting to the system, which was in agreement with the results obtained by Liao and Deng [19]. They reported an enhanced zinc extraction percent in the presence of adapted bacteria as compared to non-adapted bacteria.

### 3.3. Effect of initial pH and medium

The influence of pH on zinc extraction in the presence of *Acidithiobacillus ferrooxidans* is shown in Fig. 3(a). The highest zinc concentration present in the leachate over the experimental period was observed when the initial pH was at pH 2.8. When the initial pH of the suspension was higher or lower than pH 2.8, the zinc extraction decreased notably. Above pH 2.8, according to Eq. (2) because of decreasing $H^+$ concentration, the zinc extraction decreased. The optimal pH found in this study was slightly higher than those reported in Refs. [4, 19-20]. Note that this optimal initial pH was unusual because most of the Fe(III) might slowly precipitate as jarosite, which was found to hinder the bacterial activity. However, it was important to note that the ores used in this study contained very small amounts of iron (6wt%), which could dramatically reduce the amount of precipitate formed. As a consequence, this could lead to a significant increase of the bacterial oxidizing activity of *Acidithiobacillus ferrooxidans* at pH above 2.5 [21]. This was in agreement with the findings of Giaveno *et al.* [22], who found a higher percentage of zinc leached from an ore that possessed a higher diffusion resistance and attributed this partially to the presence of a lower iron content. A recent study concluded that jarosite had little influence on zinc leaching as a consequence of the incomplete coverage of mineral particles [23]. Furthermore, Hsu and Harrison [24] undertook bioleaching experiments at pH 2.8 and found that the rate of zinc leaching was typical to those found by others. Another factor that might play a role was the possible galvanic interactions between ZnS and $MnO_2$ because $MnO_2$ was present at a relatively high content. Hydrogen ions were found to have a less prominent effect on the galvanic current observed for $MnO_2$-ZnS couple [14]. These interactions were not well understood but the increased galvanic reaction rate was postulated to arise from the removal of sulphur in the simultaneous bioleaching system containing zinc sulphide and manganese dioxide [25]. The high zinc extraction efficiency seemed to suggest that the passivation of sphalerite by galena might not be significant. This could arise from the low amount of lead present in the ores (<4wt%) and the high presence of $MnO_2$. The decreased degree passivation of sphalerite by galena was reported elsewhere under two different conditions: (1) as a result of decreased lead concentrations in the ores [26]; (2) from the addition of chalcopyrite (a mineral that encouraged zinc leaching from sphalerite) to a mixture of minerals containing galena and sphalerite [27]. Further work needs to be undertaken to ascertain if galvanic interactions play a vital role. This highlights need to carry out leaching optimisation studies on different ore sources.

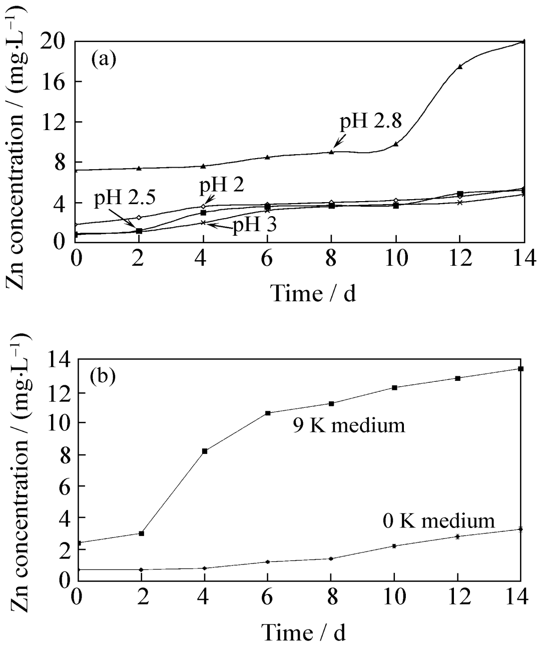

**Fig. 3. Plots of zinc concentration as a function of (a) pH and (b) medium.**

When 0 K medium was used, less than 4 $mg\cdot L^{-1}$ of zinc was obtained in the leachate, whereas in the presence of ferric ions, the zinc concentration increased by 3 to 4 fold (see Fig. 3(b)). This was expected since ferric ions provided the substrate for bacterial growth. This observation was in agreement with the results of previous workers who illustrated the dependence of zinc dissolution on ferric ions [28]. Once more, in 9 K medium, zinc dissolution increased after day 4. This was also another evidence of the importance of the bacterial acclimatisation in the bioleaching of ZnS.

### 3.4. Effect of ores size and ore density

Fig. 4 depicts the percentage of zinc leaching from five different groups of ores sizes as a function of leaching time. The decrease in ore size ranging from 0.84-2.0 mm to 0.42-0.84 mm was accompanied by an increase in zinc leaching. By decreasing the particle size, the surface area per unit mass of the mineral was increased which improved mass transfer, and enhanced bioleaching rates were achieved [29]. Decreasing the particle size to less than 0.42 mm, however, did not improve bioleaching. The optimum size fraction was 0.42-0.84 mm where the efficiency of zinc leaching reached 93wt%. The presence of a threshold particle size value was in agreement with the results of Nemeti *et al.* [29] who reported that although decreasing the particle size enhanced the bioleaching rate of pyrite, there was, however, a threshold particle size value below which a further decrease in the size led to a decrease in the dissolution rate. Below the threshold particle size, the particle mass and terminal settling velocity would decrease with an increasing tendency of the finer particles to completely follow the fluid motion in eddy streamlines [30]. This resulted in a decrease in the probability/frequency of direct and physical contact between *Acidithiobacillus ferrooxidans* and the sulfide mineral.

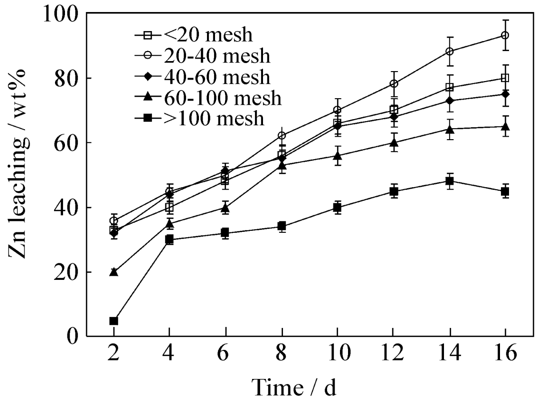

**Fig 4. Efficiency of zinc leaching from various ore sizes using *Acidithiobacillus ferrooxidans* as a function of time, pH 2.8.**

Fig. 5 depicts the zinc concentration in the leachate as a function of leaching time under various ore densities *i.e.* 20, 50, and 100 kg·m$^{-3}$. Decreasing the ore density led to increase in percentage zinc leached. The highest zinc concentrations of 120, 100, and 75 mg·L$^{-1}$ were found for the ore densities of 20, 50, and 100 kg·m$^{-3}$, respectively. This result was in agreement with results reported by other researchers working with complex concentrates of copper and zinc [31-32], complex sulphides of copper, lead and zinc [33], and copper concentrates [34-35]. The decrease in the percentage of metal dissolution when the ore density increased might arise from a lower availability of oxygen and CO$_2$, which were essential nutrients for *Acidithiobacillus ferrooxidans* growth.

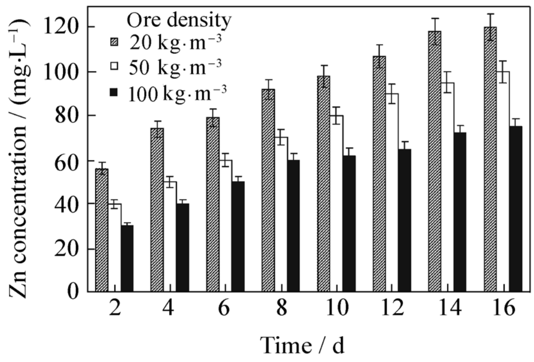

**Fig. 5. Concentration of zinc leaching using *Acidithiobacillus ferrooxidans* as a function of time under various ore densities, pH 2.8.**

### 3.5. Kinetics of zinc bioleaching

During bioleaching, elemental sulphur and other insoluble products formed a layer over the reactant surface and increased the path length for the diffusion of ions [36]. If the bioleaching kinetic was controlled by diffusion through these products, the kinetics might be correlated graphically as the following equation [37]:

$$K_{\mathrm{p}}t = 1-2/3x-(1-x)^{2/3} \tag{6}$$

where $K_{\mathrm{p}}$ is the parabolic rate constant (d$^{-1}$); $t$ is the time (d); and $x$ is the fraction of reacted zinc.

Based on experimental data in Fig. 4, a plot of parabolic rate constant ($K_{\mathrm{p}}$) *vs.* time (Fig. 6) became linear after about 6-8 d of bioleaching.

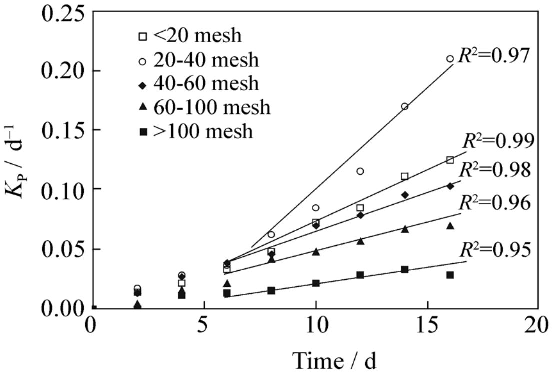

**Fig. 6. Parabolic rate constant versus time from zinc bioleaching data in Fig 4.**

These results clearly indicated that zinc leaching obeyed the model of shrinking core-product layer diffusion in a period of steady bioleaching after 6-8 d, in accordance with Eq. (6). Lizama *et al.* [38] revealed that the bioleaching kinetics of zinc sulphide could be divided into two stages: (1) a period of colonization of the ore by bacteria; (2) a steady rate period where the reaction could be described by classical shrinking-core kinetics. It was observed those phenomena in this work and agreed well with the former study.

## 4. Conclusion

The amenability of Thai ores for zinc bioleaching using *Acidithiobacillus ferrooxidans* is studied under various operating conditions (medium type, particle size, pH, and ore density). The zinc bioleaching efficiency of up to 92.3% is achieved under the following optimal conditions: 9 K medium, particle ore sizes of 20-40 mesh, ore density of $20 \cdot \mathrm{kg\ m}^{-3}$ and pH 2.8. The slightly higher optimal pH found in this study than those reported in literatures is attributed to the composition of the ores. These ores contain small amounts of iron, which can reduce jarosite formation, and high percentage of manganese dioxide, which is believed to encourage selective zinc leaching through possible galvanic interactions. The results from this study confirm that medium type, particle size, pH, and ore density are important parameters to consider in microbial leaching and highlights the need to carry out bioleaching optimisation studies on different ore sources.

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