

*Full Length Research Paper*

# **Bioleaching of dewatered metal plating sludge by *Acidithiobacillus ferrooxidans* using shake flask and completely mixed batch reactor**

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The objective of the present study was to evaluate the application of bioleaching technique to recover heavy metals (Zn, Cu, Ni, Pb, Cd and Cr) in dewatered metal plating sludge not containing sulphide or sulphate compounds. The effects of pH, pulp density of the sludge and agitation time were investigated in both shake flask and completely mixed batch reactor experiments. The leaching of heavy metals increased with decrease of pH and pulp density and increase of agitation time for both bioleaching experiments. The maximum metal leaching efficiencies of 93 and 97% Zn, 86 and 96% Cu, 85 and 93% Ni, 70 and 84% Pb, 65 and 67% Cd and 30 and 34% Cr were attained with the shake flask and the completely mixed batch reactor experiments, respectively, at a pH 2, a solid content of 2% (w/v) and a reaction temperature of  $25 \pm 2^\circ\text{C}$  during the bioleaching process (20 days). The rates of metal leaching for both bioleaching systems are well described by a kinetic equation related to time. Although the rates of metal leaching were in the decreasing order:  $\text{Zn} > \text{Cu} > \text{Ni} > \text{Pb} > \text{Cd} > \text{Cr}$  for both leaching experiments, bioleaching using the completely mixed batch reactor was slightly more efficient than the shake flask due to aeration of system. These results suggest that bioleaching may be an alternative or adjunct to conventional physicochemical treatment of dewatered metal plating sludge for the removal of hazardous heavy metals.

**Key words:** Bioleaching, waste sludge, heavy metals, *Acidithiobacillus ferrooxidans*

## **INTRODUCTION**

In the light of the expected shortage of non-renewable resources, increased efforts are absolutely necessary to recover metal from low grade materials and industrial wastes with the aid of new or improved technologies (Krebs et al., 1997). Generally, the metal removal from wastes has been carried out by chemical methods, prevalently acid treatments, at very low pH (1.5 - 2.0). However, some disadvantages of chemical treatments, for example, high cost, operational difficulty, high energy requirements and sometimes un-satisfactory yield of metal solubilization, made researchers and practitioners seek on alternative (Solisio et al., 2002; Kim et al., 2005;

Wu and Ting, 2006).

For several decades, the bioleaching techniques, as an alternative method, have been intensively applied at industrial scale in the fields of extractive metallurgy for low-grade ores (that is, containing gold, copper, manganese, nickel, or uranium at a concentration less than 0.5 wt.%) due to its environmentally friendly aspect and low cost, without strict requirements of raw material composition (Mazuelos et al., 1999; Bevilacqua et al., 2002; Chatain et al., 2005; Shi and Fang, 2005; Fang and Zhou, 2006).

Bioleaching is a natural process that involves interaction between several ionic species including iron, sulphate and nutrient transfer to microorganism. To do this, the acidophilic microorganisms such as *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans* are generally used (Das et

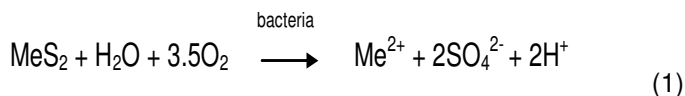
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**Table 1.** Physicochemical characteristics of the dewatered metal plating sludge samples.

Characteristic	Value
Solids (wt.%)	4.11 - 4.95
pH	9.01 - 9.45
Particle size (mm)	d <sub>90</sub> < 0.125
Concentration of heavy metals (mg/kg)	
Zn	22900 - 22980
Pb	828 - 850
Ni	601 - 729
Cu	625 - 691
Cd	325 - 341
Cr	210 - 212

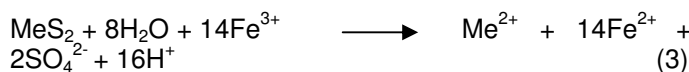
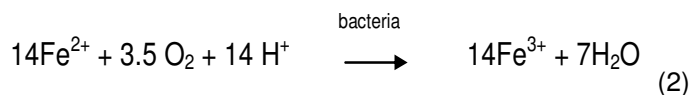
al., 1999).

The mechanism of bioleaching is usually discussed in terms of a direct bacterial attack on sulphide minerals and an indirect attack via ferric iron (Konishi et al., 1992). The direct dissolution of minerals is caused by the attack on sulphide by the enzymatic system of the microorganisms situated at the mineral surface and it is generally expressed by the following (Equation 1) (Silverman and Ehrlich, 1964; Free et al., 1991; Lizama and Suzuki, 1991):



Where MeS<sub>2</sub> is an insoluble metal sulfide and Me<sup>2+</sup> is a free metal ion.

In the indirect mechanism, microorganisms act indirectly since they regenerate ferric iron through the oxidation of ferrous iron in solution (Equations 2 - 3) (Boon et al., 1998; Fowler and Crundwell, 1998):



The contributions of the leaching mechanisms depend on the types of sulphide mineral and on the operating conditions (Konishi et al., 1992).

The objective of the present study was to evaluate the application of this bioleaching technique to recover heavy metals (Zn, Cu, Ni, Pb, Cd and Cr) in dewatered metal plating sludge not containing sulphide or sulphate compounds. The effects of pH, pulp density of the sludge and agitation time were investigated in both shake flask and completely mixed batch reactor experiments.

## MATERIALS AND METHODS

### Dewatered metal plating sludge

Dewatered metal plating sludge samples were collected in polyethylene bags from the Karame Metal Plating Industries in Fatsa-Ordu, Turkey. To avoid any changes in physicochemical properties, the sludge was stored at 4°C and used throughout the study.

The pH of the sludge samples and supernatant was determined according to Alloway's procedures (Alloway, 1995). Wet sieving technique was used to determine the particle size of sludge samples and found that to have a small particle size with greater than about 90 wt.% passing a number 120 sieve (0.125 mm). Solid contents were measured according to the method of the American Public Health Association (APHA) (2005). Total concentrations of heavy metals were determined using an atomic absorption spectrophotometer (AAS, model Perkin Elmer 3100) after acid digestion following US EPA Method 3050B (1996) by averaging the results of six determinations. The physicochemical characteristics of the dewatered sludge samples are presented in Table 1. All analysis was carried out in duplicate (Bayat and Sari, 2010).

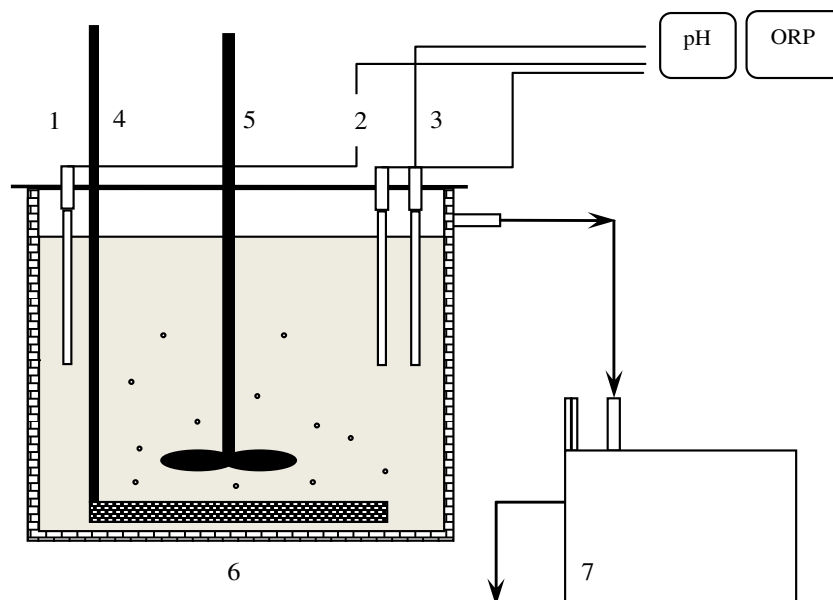
### Microorganism and culture medium

A culture of iron-oxidizing microorganisms, whose origin was an acid mine drainage water of Galena (PbS) and Sphalerite (ZnS) processing plant of Pinar Mining Company (Adana, Turkey), was used. Characterization of the culture (Department of Biology, Cukurova University, Adana, Turkey) showed that the main bacteria strains were *A. ferrooxidans*. This gram negative bacteria is characterized by nonsporulating rods, 0.5 – 0.6 µm wide and 1.0 – 2.0 µm long with rounded ends, and occurring single or in pairs, or rarely in short chains. The bacteria are also known to be motile by means of a single polar flagellum (Jensen and Webb, 1995). All these characteristics were observed during the cultivation of the strain used. The composition of the nutrient growth medium (9 K) and maintenance of cells was 3 g (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.5 g K<sub>2</sub>HPO<sub>4</sub>, 0.5 g MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.1 g KCl, 0.014 g Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O and 44.2 g FeSO<sub>4</sub>·7H<sub>2</sub>O (as a substrate) per liter of deionized water (Bojinova and Velkova, 2001).

Prior to inoculation in a shake flask and a completely mixed batch (CMB) reactor, initial conditioning of the culture (10% (v/v)) was performed in 100 ml of 9 K medium in a 250 ml Erlenmeyer flask at 25 ± 2°C on a shaking incubator (Elektro-mag M-200) at 120 rpm. The initial pH was adjusted to be in the range 2.5 – 3.0 using 10 N H<sub>2</sub>SO<sub>4</sub>. In order to enhance to cell activity, serial transfer to fresh 9K medium was done several times. The bacterial cultures were tested by microscopic cell counting in a Petroff-Hausser counting chamber. When the culture was in the exponential growth phase (around 10<sup>6</sup> cells/ml), a sample was taken to inoculate a bioleaching experiment (Bayat and Sari, 2010).

### Leaching techniques

The shake flask bioleaching experiments were performed in 250 ml erlenmeyer flasks containing 100 ml of 9K liquid medium (3g (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.5g K<sub>2</sub>HPO<sub>4</sub>, 0.5g MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.1g KCl, 0.014g Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O and 44.2g FeSO<sub>4</sub>·7H<sub>2</sub>O (as a substrate) per liter of deionized water) supplemented with 2 - 24% dewatered metal plating sludge were inoculated with 10% (w/v) solution of *A. ferrooxidans*. To facilitate mixing of the contents and transfer of O<sub>2</sub> and CO<sub>2</sub>, the flasks were agitated at 120 rpm in an incubating shaker (Elektro-mag M-200) at 25 ± 2°C. The initial pH was adjusted to be in the range of 2.5 – 3.0 using 10 N H<sub>2</sub>SO<sub>4</sub>. Sample aliquots of 10 ml were withdrawn from the flasks at 2 day intervals during the



**Figure 1.** Schematic diagram of completely mixed batch (CMB) reactor. 1, pH probe; 2, ORP probe; 3, temperature probe; 4, air diffuser; 5, stirrer; 6, completely mixed batch reactor; 7, water bath circulator.

leaching period of 20 days for determination of Zn, Cu, Ni, Pb, Cd and Cr.

The biological leaching experiments were carried out in a 3 L completely mixed batch (CMB) reactor with 15 cm ID and 17 cm in height equipped by pH controller, temperature controller, stirrer and an aerator system (Figure 1). The pH of the slurry was initially basic and 10 N H<sub>2</sub>SO<sub>4</sub> was necessary to decrease the pH (2.5 – 3) in order to create a favourable environment for the employed microbial strain. The agitation was kept to 120 rpm, the air flow-rate was fixed at 0.5 l/min and the temperature was maintained at 25 ± 2°C using a water bath. A 10% (v/v) (200 ml) growing *A. ferrooxidans* culture was added into the reactor containing 2 l of a 9K medium and different solids contents (Total Solids (TS); 2 - 24% (w/v)). The treatment was carried out until the solubilized metal content reached stationary values. Aliquot samples of 10 ml were drawn at every 2 days from the reactor over the leaching period (20 days) (Bayat and Sari, 2010).

Both bioleaching samples were filtered through 0.45 µm pore size filters (Sartorius cellulose acetate filter). After filtration, the concentrations of Zn<sup>2+</sup>, Cu<sup>2+</sup>, Ni<sup>2+</sup>, Pb<sup>2+</sup>, Cd<sup>2+</sup> and Cr<sup>6+</sup> in the supernatant were analyzed using a Perkin Elmer Model 3100 Atomic Absorption Spectrophotometer (AAS) at 213.9, 324.8, 232, 217, 228.8 and 357.9 nm for Zn<sup>2+</sup>, Cu<sup>2+</sup>, Ni<sup>2+</sup>, Pb<sup>2+</sup>, Cd<sup>2+</sup> and Cr<sup>6+</sup>, respectively, and if necessary, samples were diluted with distilled water. The loss of water content in the bioleaching systems due to evaporation and sampling was replenished with doubled distilled water by weight difference method. The control tests without the inoculation of bacteria and addition of nutrient medium (9K) were performed at 2% (w/v) solids contents of suspension during the same bioleaching period for comparison. All the experiments were carried out in duplicate. The average deviation between the replicates was found to be within ±5%.

#### Kinetic study

For bioleaching, a kinetic model (Froment and Bischoff, 1979;

Blancarte-Zurita et al., 1987; Chen and Lin, 2000; Bayat et al., 2009) was employed, the equations of which are:

$$-\frac{dM}{dt} = k(M_s - M) \quad (4)$$

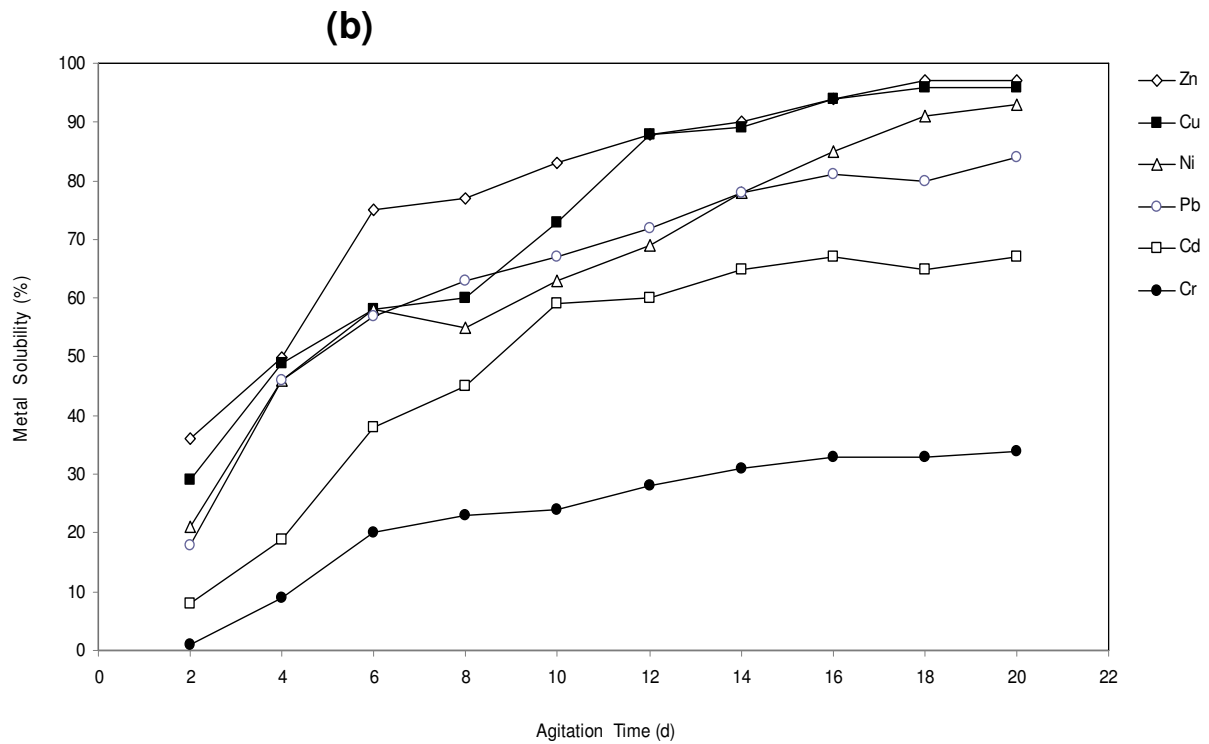
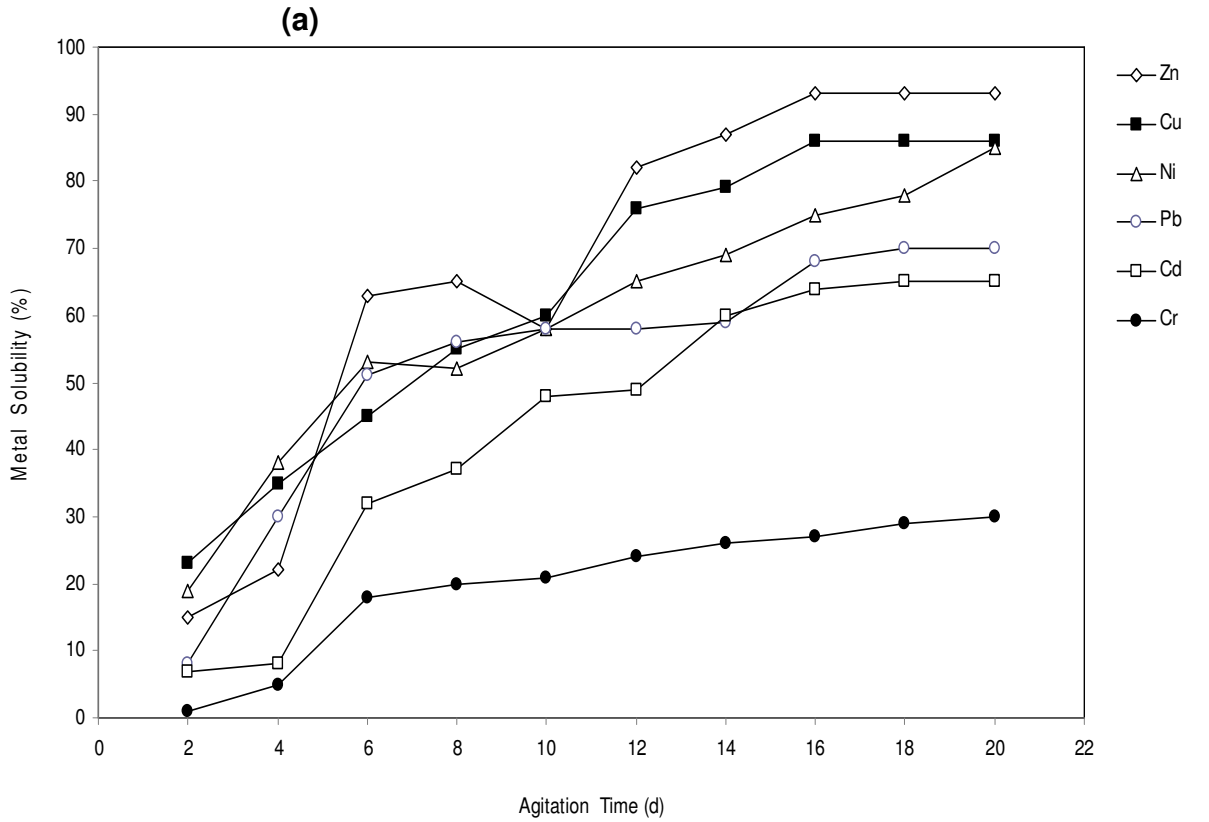
where  $k$  is the rate constant,  $M_s$  and  $M$  are the initial weight of metal in the sludge (mg) and weight of metal in the aqueous phase (mg), respectively, and  $t$  is the leaching time (day). By integrating Equation 4 between the initial moment and the conditions corresponding to a time  $t$ , Equation 5 was obtained, from which the value of the rate constant,  $k$ , can be deduced:

$$\ln \left[ \frac{M_s}{M_s - M} \right] = kt \quad (5)$$

## RESULTS AND DISCUSSION

### Effect of agitation time on metal solubilization

Figures 2a and b shows the solubilization of heavy metals (Zn, Cu, Ni, Pb, Cd and Cr) from the dewatered metal plating sludge with constant solid content (2% (w/v)) at initial pH 2 and at 25 ± 2°C during the 20 days of both bioleaching. The results indicated that the percent solubilization of heavy metals increased with an increase in agitation time for both bioleaching experiments. It can also be inferred from Figures 2a and b that except Cr, more than 55% of heavy metals (Zn, Cu, Ni, Pb and Cd) in the solid phase was solubilized in 10 days and



**Figures 2.** Metal solubilization with time in bioleaching process (pulp density: 2% (w/v), agitation: 120 rpm, temperature: 25 ± 2°C). Shaked flask and (b) completely mixed batch reactor.

thereafter, rose slowly before attaining stationary values.

The profiles of heavy metals solubilization with time were similar to the earlier reports on removal of metals from metal plating sludge and other wastes by species of *Acidithiobacillus* (Blais et al., 1992; Shanableh and Ginige, 2000; Bojinova and Velkova, 2001; Solisio et al., 2002; Chan et al., 2002; Wong et al., 2004; Kim et al., 2005; Zhang et al., 2008).

### Effect of pH on metal solubilization

Figures 3a and b present the solubilization obtained for zinc, copper, nickel, lead, cadmium and chromium ions as a function of pH (sludge acidification) for a constant solid content (2% (w/v)) at  $25 \pm 2^\circ\text{C}$  during the 20 days of both bioleaching experiments.

The data in Figures 3a and b indicate the rates of metal solubilization with increased bioacidification. By day 20 (pH 2 for shake flask experiments and pH 1.95 for completely mixed batch reactor experiments), the heavy metal solubilization results reached 93% for Zn, 86% for Cu, 85% for Ni, 70% for Pb, 65% for Cd, 30% for Cr from shake flask experiments and 97% for Zn, 96% for Cu, 93% for Ni, 84% for Pb, 67% for Cd, 34% for Cr from completely mixed batch reactor experiments. The control run showed low metal solubilization (16% for Zn, 11% for Cu, 6% for Ni, 3% for Pb, 1% for Cd, 0% for Cr from shake flask experiments and 20% for Zn, 15% for Cu, 10% for Ni, 5% for Pb, 2% for Cd, 0% for Cr from completely mixed batch reactor experiments) principally because of the high pH values (pH 6.25 for shake flask experiments and pH 5.90 for completely mixed batch reactor experiments) at the end of both experiments.

These metal solubilization trends indicate that the degree of metal leaching was dependent on the type of metal and sludge (Shanableh and Ginige, 2000; Chen and Lin, 2001).

### Effect of pulp density of sludges on metal solubilization

The leaching rate of heavy metals by *A. ferrooxidans* with 10% of the inoculation under different pulp densities (2 - 24% (w/v)) are shown in Figures 4a and b. It is found in Figures 4a and b that the increasing pulp density of the sludge results in leaching rate of heavy metals decreasing for both systems. This is because the high solid content of pulp hinders oxygen and carbon dioxide to transfer to strains and shear stress of mineral is harmful to bacteria which lead to lag phase of strains longer (Zhang et al., 2008).

Such decrease in the extent of metal solubilization due to increase in the sludge pulp density was likely due to the buffering capacity of dewatered metal sludges (Jain and Tyagi, 1992; Kim et al., 2005). Since pH plays a

critical role in metal solubilization, buffering effects of the sludge on inhibition of low pH maintenance might retard the rate of metal leaching. The initial acidity of the solution became neutralized by the high pH of the sludge itself and bacterially produced protons might be consumed by natural buffering agents of the sludge (Kim et al., 2005).

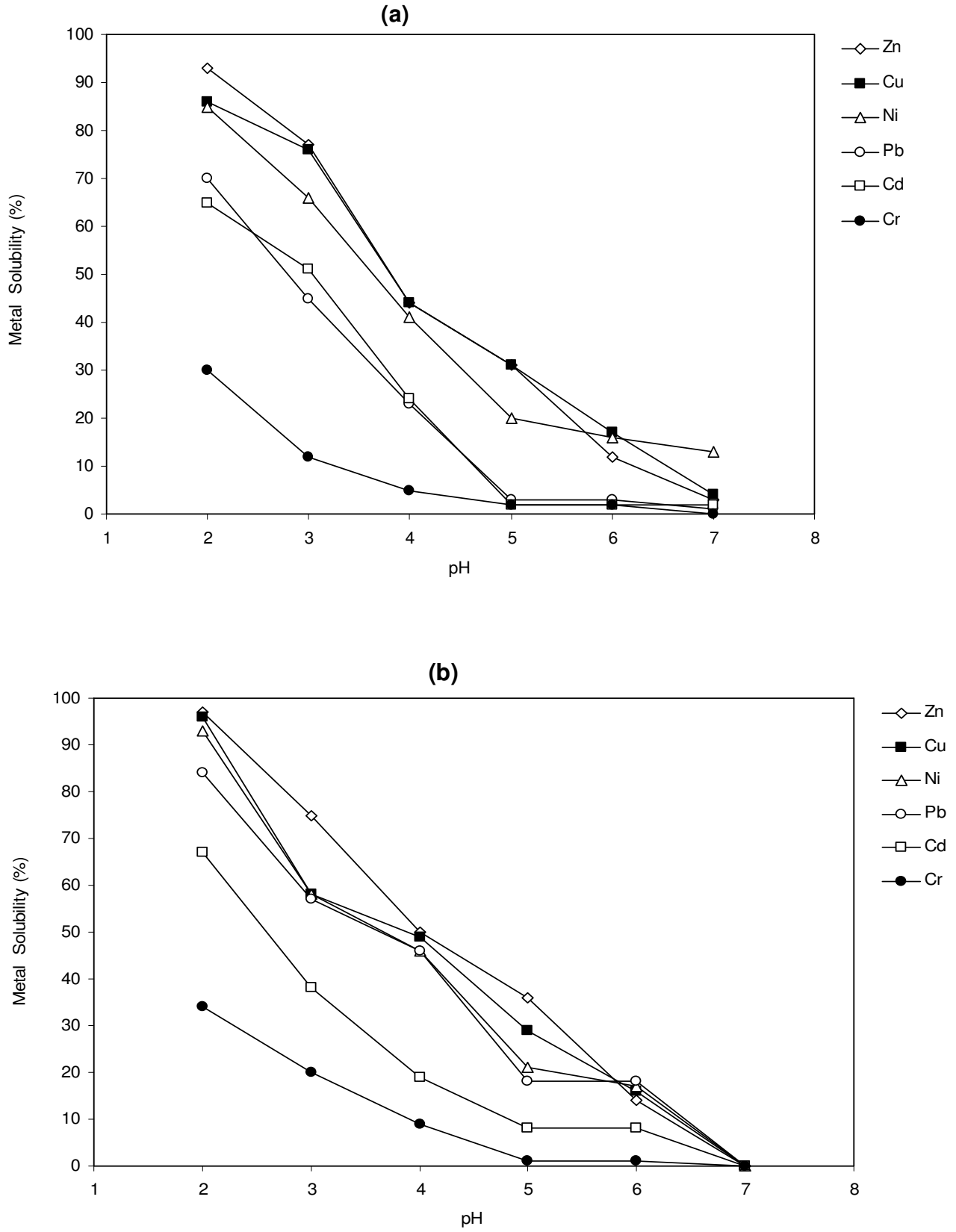
### Kinetic studies

The kinetic model (Equations 4 and 5) was used to describe the leaching of heavy metals (Zn, Cu, Ni, Pb, Cd and Cr) for both bioleaching systems. Table 2 shows that the fitting of the results (the rate constants of the kinetic equations ( $k$ ) and the coefficient of determination of linear regression ( $R^2$ )) from experiments at the optimum bioleaching conditions (pH 2, 2% (w/v) solids, 120 rpm, reaction temperature:  $25 \pm 2^\circ\text{C}$ , time: 20 days).

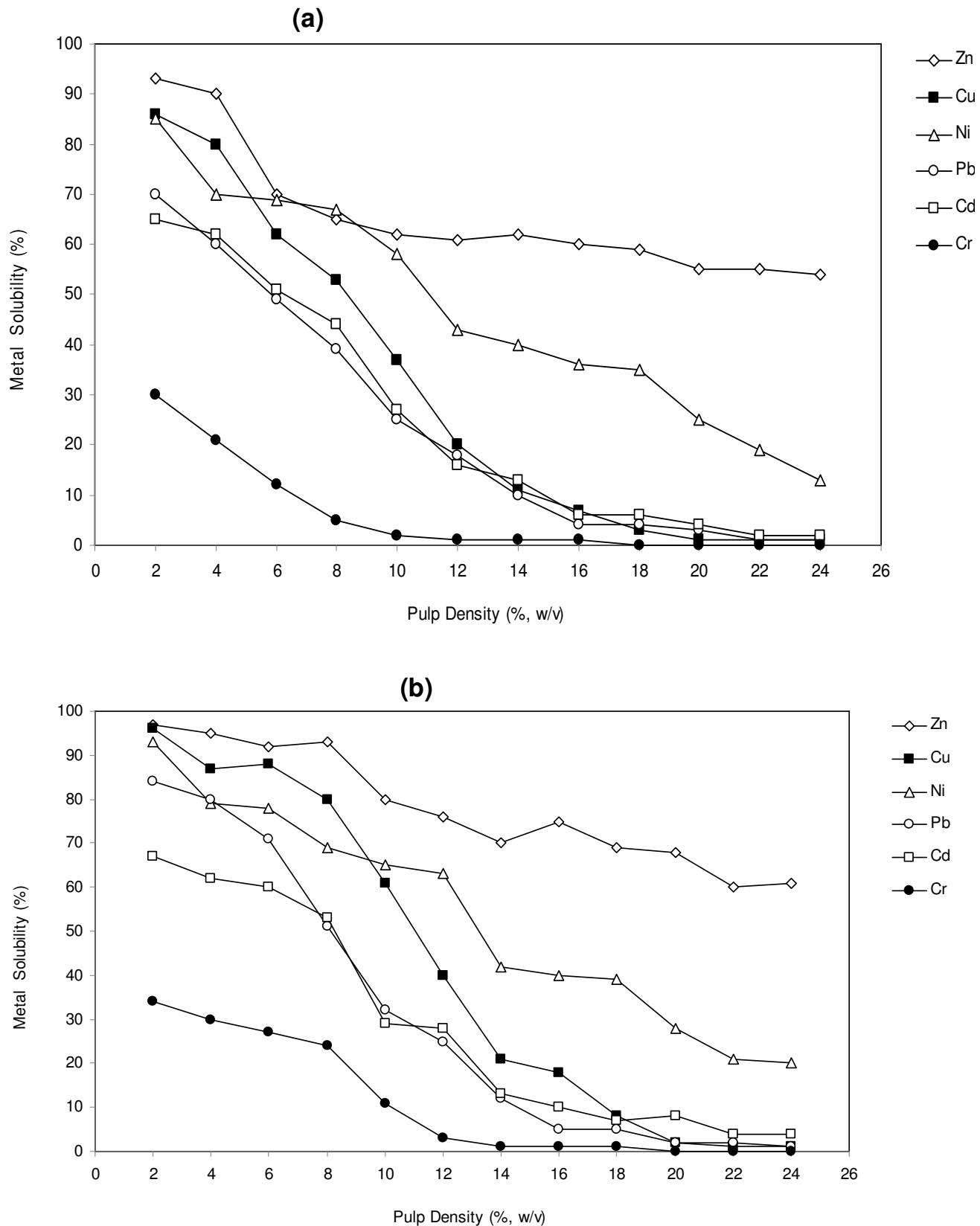
According to the rates of metal leaching results, the efficiency of metal solubilization is in the decreasing order: Zn > Cu > Ni > Pb > Cd > Cr for both bioleaching experiments. The maximum leaching results from the shake flask experiments and the completely mixed batch reactor experiments were 93 - 97% for Zn, 86 - 96% for Cu, 85 - 93% for Ni, 70 - 84% for Pb, 65 - 67% for Cd and 30 - 34% for Cr, respectively. It appears that bioleaching using the completely mixed batch (CMB) reactor was slightly more efficient than the shake flask due to the aeration system as well as the agitation system. The purpose of aeration and agitation in the microbial leaching process is to achieve oxygen transfer and to mix the solid substrate and nutrient solution thoroughly so that a uniform suspension of sludge particles and bacteria can be maintained (Couillard and Zhu, 1992; Chen and Lin, 2000).

### Conclusion

Bioleaching of dewatered metal plating sludges by *A. ferrooxidans* was studied in shake flask experiments and completely mixed batch (CMB) reactor experiments in which the following factors were examined to obtain the highest efficiency of metal solubilization; agitation time, pH and pulp density of the sludge. The degree of metals leaching increased with decreasing of pH and pulp density and increasing of agitation time and was dependent on the type of metal and sludge. The maximum metal leaching efficiencies of 93 and 97% Zn, 86 and 96% Cu, 85 and 93% Ni, 70 and 84% Pb, 65 and 67% Cd and 30 and 34% Cr were attained with the shake flask and the completely mixed batch reactor experiments, respectively, at a pH 2, a solid content of 2% (w/v), and a reaction temperature of  $25 \pm 2^\circ\text{C}$  during the bioleaching process (20 days). According to the kinetic study results, the rates of metal leaching are in the decreasing order: Zn > Cu > Ni > Pb > Cd > Cr for both



**Figure 3.** Effect of pH on metal solubilization in bioleaching process (pulp density: 2% (w/v), agitation time: 20 days, agitation: 120 rpm, temperature:  $25 \pm 2$  °C). Shaked flask and (b) completely mixed batch reactor.



**Figure 4.** Effect of pulp density of sludge on metal solubilization in bioleaching process (agitation time: 20 days, agitation: 120 rpm, temperature:  $25 \pm 2^\circ\text{C}$ ). (a) Shaked flask and (b) completely mixed batch reactor.

**Table 2.** The rate constants of the kinetic equations ( $k$ ) and the coefficient of determination of linear regression ( $R^2$ ) from experiments at the optimum bioleaching conditions\*.

Heavy metals	Bioleaching			
	Shake Flask		CMB Reactor	
	K (d <sup>-1</sup> )	R <sup>2</sup>	k (d <sup>-1</sup> )	R <sup>2</sup>
Zn	0.1469	0.9665	0.1734	0.9785
Cu	0.0498	0.9226	0.1762	0.9659
Ni	0.0533	0.8407	0.1267	0.9426
Pb	0.0188	0.8715	0.0832	0.9553
Cd	0.0872	0.9532	0.0595	0.8868
Cr	0.0948	0.9694	0.0214	0.8944

\*Optimum conditions: pH 2, 2% (w/v) solids, 120 rpm, reaction temperature: 25 ± 2°C, time: 20 days.

bioleaching experiments and bioleaching using the completely mixed batch (CMB) reactor was slightly more efficient than the shake flask due to the aeration system which achieved oxygen transfer very well. These results suggest that bioleaching using a reactor with aeration and agitation systems may be an alternative or adjunct to conventional physicochemical treatment of dewatered metal plating sludge for the removal of hazardous heavy metals.

## ACKNOWLEDGEMENTS

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