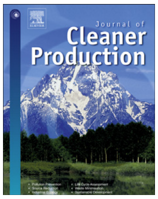
Journal of Cleaner Production 168 (2017) 814–822

Contents lists available at ScienceDirect

Journal of Cleaner Production

journal homepage: www.elsevier.com/locate/jclepro



Analysis of the effects of natural organic matter in zinc beneficiation

Rene A. Silva [a, b], Danilo Borja [a], Gukhwa Hwang [a], Gilsang Hong [a], Vishal Gupta [c], Scott A. Bradford [d], Yahui Zhang [b], Hyunjung Kim [a, *]

[a] Department of Mineral Resources and Energy Engineering, Chonbuk National University, 567, Baekje-daero, Deokjin-gu, Jeonju, Jeonbuk 54896, Republic of Korea
[b] Department of Process Engineering, Faculty of Engineering and Applied Science, Memorial University of Newfoundland, St. John's, NL A1B 3X5, Canada
[c] Research & Development, EP Minerals, 9785 Gateway Dr., Suite 1000, Reno, NV 89521, USA
[d] US Salinity Laboratory, USDA, ARS, Riverside, CA, USA

ARTICLE INFO

Article history:
Received 25 March 2017
Received in revised form
13 August 2017
Accepted 2 September 2017
Available online 4 September 2017

Keywords:
Natural organic matter
Mine tailings
Metal recuperation
Bioleaching
Zinc beneficiation

ABSTRACT

In this study, we present the analysis of the effects of Natural Organic Matter (NOM) in zinc beneficiation from abandoned mine tailings using bioleaching technologies. We used standardized Suwannee River Humic Acid (SRHA) as the NOM source to analyze the importance of the quality of the process waters and the possible side effects of NOM accumulated on mine tailings when exposed to environmental conditions. Our study proposes the use of process waters as an important variable to maintain high process efficiency. Concentrations of 20 ppm of SRHA suspended in the bioleaching medium reduced the Zn removal efficiency by 10% as compared with the process without SRHA. Similarly, at concentrations of 50 ppm of SRHA, the Zn removal efficiency decreased by 20% as compared with the process without SRHA. However, our study refutes the view that the accumulation of NOM on mine tailings could possibly reduce the process efficiency. We conclude that the suspended NOM was able to alter the process efficiency by reducing the bacterial attachment on the mine tailing's surface, which suggested the importance of the bacterial contact mechanism.

1. Introduction

Biohydrometallurgy is the use of microorganisms in mineral processing; this technique has been widely investigated as an alternative process for mineral enrichment and metal recuperation (Brierley and Brierley, 2013; Rawlings and Johnson, 2007; Rawlings and Silver, 1995). It is a time-tested process that operates either by dissolving the valuable metals for their recuperation in the bioleaching process or by dissolving the undesired metals from the valuable minerals as in the biooxidation process (Acevedo, 2000; Brierley and Brierley, 2013). In both the cases, the strong oxidizing agents produced by the microorganisms interact with the mineral, resulting in the breakage of the mineral matrix and the subsequent metal liberation (Brierley and Brierley, 2013; Rawlings and Johnson, 2007; Rawlings and Silver, 1995; Rohwerder et al., 2003). Because of the simplicity and feasibility of metal oxidation by microorganisms, the process has found several applications in the mining industry (Bosecker, 1997; Brierley and Brierley, 2001). Some examples of microbial applications include the increase in the ore grades in gold concentrates (Rawlings and Silver, 1995), extraction of copper from low grade/copper sulfide ores (Brierley and Brierley, 2013), detoxification of soils, treatment of sewage sludge contaminated with heavy metals (Mulligan et al., 2001; Pathak et al., 2009), and treatment of different types of wastes containing different metals (Hoque and Philip, 2011). Similarly, bioleaching is an alternative method for the detoxification of abandoned mine sites, providing a potential reduction of social and environmental problems allowing simultaneous economic benefits (Olson et al., 2003; Park et al., 2014).

Several operational parameters for biomining processes have been identified and studied during the development of this technology. Some parameters vary according to the type of microorganism, others on the mineral matrix involved in the process (Rawlings and Johnson, 2007; Rawlings and Silver, 1995). The selection of the process pH, temperature, and energy source is usually based on the type of microorganism used (Rawlings, 2007; Schippers, 2007). Parameters, such as aeration/agitation rate, reaction time, solid concentration/compaction, and particle size,

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[*] Corresponding author.
E-mail address: kshjkim@jbnu.ac.kr (H. Kim).

http://dx.doi.org/10.1016/j.jclepro.2017.09.011

depends more on the type of mineral matrix (Rawlings and Johnson, 2007; Rawlings and Silver, 1995). However, relatively new applications of biomining related to non-traditional metal sources like sewage sludge and electronic waste, have faced different operational challenges. As an example, a number of studies show that the metal extraction efficiency decreased as the concentration of organic compounds increased in a biotic media (Fang and Zhou, 2006; Mazuelos et al., 1999; Mulligan et al., 2001; Pathak et al., 2009). Also, Mazuelos et al. (1999) identified an inhibition of biooxidation processes by organic compounds derived from industrial operations. Similarly, Fang and Zhou (2006) and Pathak et al. (2009) suggested that organic matter dissolved from sewage sludge presents inhibitory effects over bioleaching processes as some microorganism are sensitive to various organic acids, sugars, and aminoacids. Because of the relatively recent development of these new bioleaching applications, the influence of organic compounds and natural organic matter (NOM) in traditional biomining applications has not yet been analyzed. NOM could be present in almost all industrial applications (Grigg, 2008); therefore, its analysis and monitoring could play a major role in increasing the process efficiency. More so, when cost-effective technologies are required to overcome the disadvantages associated with low-value metal concentrations in the substrate as in the metal recuperation from mine tailings.

NOM enters the process when mine tailings are exposed over long periods to atmospheric conditions and anthropogenic activities such as surface waters, winds, flora and fauna, and reforestation activities (Grigg, 2008). Moreover, NOM can enter the bioleaching process through process waters that have not received proper treatment (Grigg, 2008). Previous studies have proposed that NOM at concentrations higher than 150 mg/L can directly affect the extraction of metals by inhibiting the bacterial metabolism and thereby reducing the production of oxidizing agents required for metal dissolution (Fang and Zhou, 2006; Mazuelos et al., 1999; Schwab et al., 2007). Moreover, the physical characteristics of NOM (Yang et al., 2012) and the high ionic strength of the bioleaching solution (Silva et al., 2015) provide the ideal conditions for NOM adsorption on the surface of the mineral matrix, which results in a reduction of the bacteria-mineral matrix interaction; this decreases the process efficiency.

In this study, for the first time, we present an analysis of the effects of NOM in zinc beneficiation from abandoned mine tailings using bioleaching technologies. Using this analysis, we highlight the importance of the quality of the process waters and the possible side effects generated by the accumulation of NOM on the surface of mine tailings when the substrates are exposed to environmental conditions. For this, we investigated the effects of the adsorption of NOM to the surface of the mine tailings and the effects of NOM suspended at different concentrations in the bioleaching solution. From the complex mixtures of organic compounds conforming to NOM, standardized humic acid was selected to systematically examine and determine the NOM effects (Yang et al., 2012). A pure culture of *Acidithiobacillus ferrooxidans*, which had been previously used in bioleaching literature as the main iron oxidizer microorganism was selected to perform the bioleaching experiment. In addition, the conditions selected to conduct this experiment (i.e., the initial pH, temperature, and rpm) were in accordance with the conditions used in previous literature (Hong et al., 2016; Lee et al., 2015; Park et al., 2014).

2. Materials and methods

2.1. Microorganism and culture media

To analyze the influence of organic matter in bioleaching experiments, we used a pure culture of the *A. ferrooxidans* strain KCTC 4515, which was provided by the Korean Research Institute of Bioscience and Biotechnology. Bacteria were cultured in the DSMZ medium 882 (Hong et al., 2016) with the pH values adjusted to 1.8 using H_2SO_4 (Acros Organics™) at 95% solution in water. The DSMZ medium 882 was formulated with 132.0 mg/L of $(NH_4)_2SO_4$, 53.0 mg/L of $MgCl_2 \times 6H_2O$, 27.0 mg/L of KH_2PO_4, 147.0 mg/L of $CaCl_2 \times 2H_2O$, 20.0 g/L of $FeSO4 \times 7H_2O$, 62.0 μg/L of $MnCl_2 \times 2H_2O$, 68.0 μg/L of $ZnCl_2$, 64.0 μg/L of $CoCl_2 \times 6H_2O$, 31.0 μg/L of H_3BO_3, 10.0 μg/L of Na_2MoO_4, 67.0 μg/L of $CuCl_2 \times 2H_2O$, and deionized (DI) water. As proposed by the standardized methodology for its formulation, DSMZ medium 882 was sterilized at 112 °C for 30 min. However, the Fe^{2+} ion concentration was monitored before and after the autoclaving process to dismiss any possibility of the oxidation of Fe^{2+} to Fe^{3+} ions (data not shown).

2.2. Preparation of mine tailings

Mine tailings were obtained from the Janggun Mine in the Bonghwa region, Gyeongsang, South Korea. Janggun is a closed mine dedicated to the extraction of Zn and Pb (Park et al., 2014). To systematically analyze the bioleaching experiment, mine tailings with a particle size fraction of 100–150 μm were selected. Preliminary experiments demonstrated that mine tailing increased organic matter in solution. Also, the amount of suspended organic matter decreased to almost nonexistent after several washing procedures with DI water (data not shown). For washing the tailings, a solid concentration was adjusted to 5% in 50 mL conical tubes (BD Falcon™, USA). The tubes were agitated for 1 h in a rotary agitator (Wisemix® RT-10, Witeg, Germany) at 70 rpm. After each hour of agitation, the tubes were rested for 10 min; the supernatant was then discharged and replaced with fresh DI water for subsequent washings. The washing of mine tailings continued until the absorbance of the discharged supernatant was constant and had a wavelength lower than 0.010 at 540 nm. Absorbance was used as an indirect method to confirm the reduction of NOM in suspension; therefore, we assume that a negligible amount of organic matter content was present on the mine tailings surface. Finally, the mine tailings were dried at 60 °C and used as zero NOM in the bioleaching experiments.

2.3. Preparation of SRHA

Suwannee River Humic Acid (SRHA; Cat #2S101H, Standard II, International Humic Substances Society) was selected to systematically examine and determine the NOM effects in bioleaching operations (Yang et al., 2012). An SRHA stock solution was prepared by dissolving 25.0 mg of a dry sample of standardized SRHA in 50 mL in DI water and stirring for 24 h in a dark flask. The solution was then vacuum filtered through a 0.22 μm cellulose acetate membrane. The pH of the solution was then adjusted to 8.0 using 0.1 M NaOH solution and was stored in the dark at 4 °C until it was used (Han et al., 2014a, 2014b; Hwang et al., 2015). The total organic carbon (TOC) content of the SRHA stock solution was determined to be 218.5 mg/L (Tekmer Fusion, Teledyme Instruments, USA). The desired SRHA concentrations were obtained by dilution of the stock solution.

2.4. Adsorbed and suspended natural organic matter conditions

Variations in the adsorbed and suspended NOM in the bioleaching systems were obtained by either exposing the washed mine tailings to solutions with different concentrations of SRHA before the bioleaching experiments or by varying the concentrations of SRHA in the bioleaching solution. For all the systems, washed mine tailings (Section 2.2) were used as the zero NOM concentration.

For the exposure of mine tailings to SRHA (adsorbed NOM), we first characterized the extent of adsorption of NOM on the surface of the mine tailings. For this, 0.25, 0.50, 1.00, and 2.00 g/L of mine tailings were each added to different flasks containing 50 mL SRHA solution at the different concentrations, i.e., 0, 5, 10, 20, and 50 ppm. The SRHA concentration was confirmed by analyzing the TOC of each solution. The conical tubes were then placed in a rotary agitator at 45 rpm until the adsorption equilibrium of SRHA on the mine tailings surface was confirmed after 12 h (data not shown). After the agitation time was concluded, the conical tubes were rested for 10 min before sampling the supernatant for determining the final TOC. The content in the conical tubes was then filtrated in vacuum using a cellulose acetate membrane (Advantec®, MFS Inc., Japan) of 0.45-μm pore size to separate the mine tailings and obtain the adsorbed NOM. The mine tailings rich in NOM were dried at room temperature. The amount of SRHA attached on the mine tailings was determined by subtracting the initial and final TOC concentrations, and the best configuration of solid concentration was selected for bioleaching experiments (Section 2.5).

To modify the concentration of NOM suspended in the bioleaching solution (suspended NOM), the bioleaching solution was adjusted to different concentrations of SRHA (0, 20, and 50 ppm) prior to the bacterial inoculation. This range of SRHA concentrations was selected to avoid inhibitory effects over bacterial culture as described by Fang and Zhou (2006) who observed a reduction of Fe^{2+} oxidation at organic matter concentrations higher than 150 ppm.

2.5. Bioleaching experiments

Bioleaching experiments were conducted using 500 mL flasks with a working volume of 300 mL of DSMZ culture medium 882. The solid concentration selected for the bioleaching experiments was fixed at 0.5% w/v (explained in Section 3.1) at a constant temperature of 30 °C and agitation speed of 150 rpm. The bacterial concentration was adjusted to 1×10^7 cells/mL with an initial pH corrected to 1.8 using 10 N H_2SO_4 (Sigma-Aldrich®). An additional abiotic experiment was conducted as the control experiment. All experiments were conducted for 720 h (30 days). The oxidation–reduction potential (ORP), pH, and metal concentration were measured periodically.

2.6. Metal concentration analysis

An aliquot of 2 mL was filtered through a 0.45-μm nylon syringe filter. The sample was then diluted (1/10) in 0.5 N nitric acid for storage. The metal concentration of Zn and Fe was obtained by inductively coupled plasma analysis. Moreover, the Fe^{2+} ion concentration was analyzed using the *o*-phenanthroline method (Koch et al., 1992). The Fe^{3+} ion concentration was calculated by mass balance between the total Fe and Fe^{2+} ion. The water losses were corrected on a daily basis by adding DI water with pH corrected to 1.8 with 10 N H_2SO_4 (Sigma-Aldrich®).

2.7. SRHA toxicity test on bacteria

To dismiss any possible negative effect of SRHA on bacterial growth, the growth of bacterial concentration was monitored in the presence of different SRHA concentrations until the beginning of the culture stationary phase (determined at 72 h). Flask experiments with 300 mL of DSMZ medium 882 with SRHA concentrations of 0, 20, and 50 ppm were inoculated to achieve initial bacterial concentrations of approximately 2.7×10^7 cells/mL. The bacterial concentration was then monitored every 24 h using a Burker–Turk counting chamber (Paul Marienfeld GmbH & Co., Germany) under phase-contrast microscopy (ODEO-2003 Triple, IPONACOLOGY, Japan) for cell counting.

2.8. Bacteria attachment on mine tailings

The bacterial attachment test determined the extent of bacterial attachments to the mine tailings. The attachment test was conducted under the same conditions as that of the bioleaching process ($pH = 1.8$, temperature = 30 °C, and initial cell concentration = 1.2×10^8 cells/mL). However, the solution ionic strength of 320 mM was achieved with NaCl instead of a culture medium to avoid cell growth. The attachment test was conducted in a 50 mL conical tube (BD Falcon™, USA) with a shaker (Wise-Shake SHR-1D, Witeg, Germany) at 70 rpm. The number of cells attached to the mine tailings was determined as the difference between the initial cell concentration and the cell concentration at specific time intervals (0, 40, and 80 min) using a Burker–Turk counting chamber. The maximum time of the interaction (80 min) was found to be the equilibrium time of cell adhesion (Kim et al., 2009, 2010).

2.9. Statistical analysis

All tests were conducted at least in triplicated to ensure reproducibility excepting the control experiment (i.e., abiotic condition), which was not replicated. In the figures, mean data is presented with error bars indicating one standard deviation. Differences between the mean values were analyzed using the Student's *t*-test and were considered statistically significant when $P < 0.05$.

3. Results and discussion

3.1. Characterization of experimental conditions

The mine tailings used for this research were obtained from a dormant mine that used to be one of the largest mineral ore producers in South Korea from the early 1940s–1980s. Its production significantly decreased by the end of the 1990s (Lee et al., 1998). Nevertheless, the old technologies for metal beneficiation did not allow the total extraction of valuable metals, which left behind metal concentrations in tailings. Presently, these metal concentrations can provide significant economic/environmental benefits (Dold, 2008; Hilson and Murck, 2000). As shown in the chemical characterization in Table 1, the mine tailings with particle size in the range of 100–150 μm still contain significant concentrations of Zn (approximately 5% w/w). X-ray Diffraction (XRD) analyses suggested sphalerite (ZnS) as the most common form of Zn available in

Table 1
Chemical characterization of mine tailings.

	Particle Size [μm]	Fe [ppm]	Zn [ppm]	As [ppm]	Pb [ppm]	Cu [ppm]
Mine tailing	100–150	172700	50600	34600	4200	2600

the sample (data not shown). Other concentrations of metals are also presented representing further opportunities for metal beneficiation.

Fig. 1 shows the current disposition of mine tailings at the Janggun Mine. There is a clear exposition of mine tailings to the environmental conditions (e.g., rain, snow, animals, and plants) allowing the NOM to interact with mine tailings and thereby begin the bioleaching process (Grigg, 2008). To understand the effect of NOM on the bioleaching process, we characterized the extent of NOM attachment on the surface of mine tailings, as described in Section 2.4. From Fig. 2, it can be observed that adsorbed NOM amount increased with increasing humic acid concentration at particular solid concentration, as expected. NOM adsorption decreased with increasing solid concentration likely due to insufficient amount of humic acid present in the solution to provide complete coverage to the particles. In addition, from Fig. 2, we can observe that there are no major differences between the extent of adsorption using solutions of 10 and 20 ppm of SRHA. Also, the adsorption observed in the SRHA solutions of 1 ppm was very low and unstable to produce reliable data during bioleaching experiments. Therefore, for this research, we selected only a solid concentration of 0.5% w/v and mine tailings exposed to SRHA concentrations of 20 ppm and 50 ppm to conduct the bioleaching experiments. The adsorption of NOM on mine tailings was conducted in solutions with neutral pH in order to resemble the adsorption of NOM in natural conditions.

3.2. *Effect of adsorbed NOM on the mine tailings surface*

The effects of NOM on bioleaching were analyzed by comparing the removal efficiencies of Zn from mine tailings containing different amounts of NOM adsorbed onto their surfaces. After the characterization of the adsorption of NOM on mine tailings (Section 3.1), we conducted bioleaching experiments using mine tailings exposed to solutions of 0, 20, and 50 ppm of SRHA, which acted as the source of NOM. Moreover, an abiotic condition was included to compare our bioleaching results with a system lacking bacteria. Fig. 3 presents the obtained Zn extraction trend.

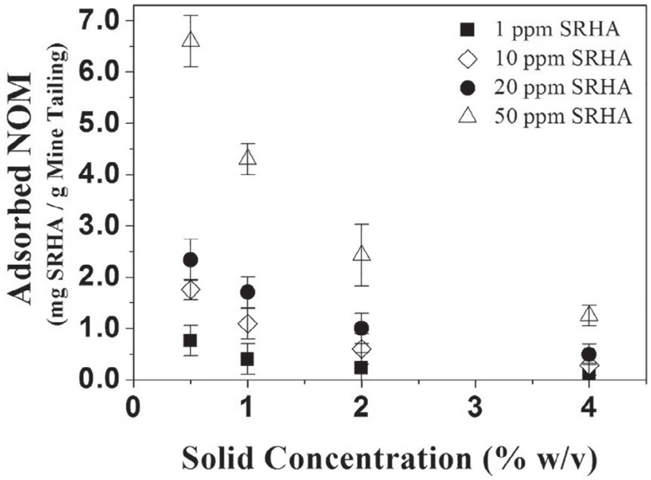

Fig. 2. Amount of NOM in the form of SRHA attached per gram of mine tailings when exposed to different concentrations of SRHA. The experiments were conducted at room temperature and neutral solutions.

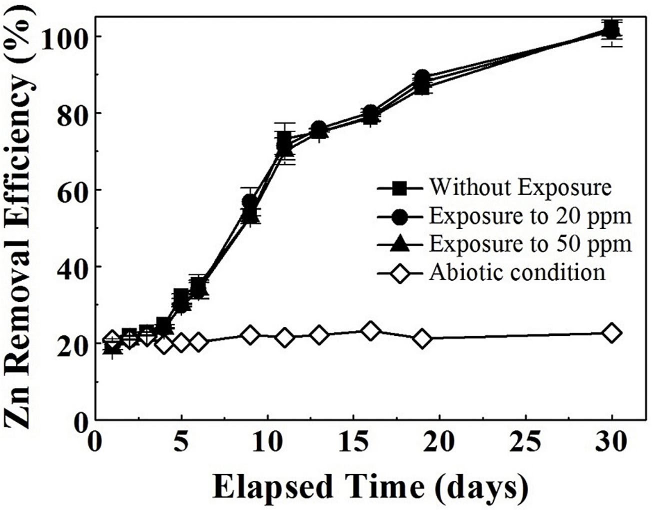

Fig. 3. Zn removal efficiency for mine tailings exposed to solutions containing different amounts of NOM in the form of SRHA.

It can be observed that regardless of the amount of NOM adsorbed to the surface of mine tailings, the same Zn bioleaching trend was obtained. The absence of bacteria in the bioleaching system only produced a Zn removal efficiency of 23%, whereas the rest of the biotic conditions showed extraction efficiencies above 70% by the 11th day of the reaction. At the end of the experiment, all the biotic conditions showed extractions close to 100%, proving that despite the amounts of NOM on the surface of the mine tailings, the presence of bacteria in the system was a major factor influencing the Zn bioleaching. This implies that the interaction of NOM with mine tailings due to natural surface conditions does not negatively

Fig. 1. Tailings pile at Janngun Mine, South Korea. (a) Perennial vegetation growing in the surroundings of the pile. The observed slope allows surface streams to form during the rainy season. (b) The pile exposed to natural surface conditions such as rain, snow, wind, and wild life.

affect the bioleaching processes.

The results obtained for Zn bioleaching are supported by similar trends obtained by the analysis of pH values, the redox potentials of the solution, and the concentrations of the redox pair of Fe^{2+} and Fe^{3+}. As shown in Fig. 4a, the pH value for the abiotic condition remained almost stable at approximately 2.1; however, the pH variations for the biotic conditions were similar regardless of the amount of NOM adsorbed to the surface of the mine tailings. Nevertheless, the general trend for the pH variations was observed to be the opposite to the standard bioleaching reaction (Eq. (1)), wherein an increase in the pH is expected to occur by the consumption of H^+ ions in the bacterial oxidation of Fe^{2+} to Fe^{3+}. The initial increase in pH observed in Fig. 4a is considered to be only because of the addition of mine tailings into the bioleaching reactor.

$$2Fe^{2+} + \frac{1}{2}O_2 + 2H^+ \xrightarrow{A\cdot f} 2Fe^{3+} + H_2O. \tag{1}$$

In contrast to the pH reduction, an increase in the solution redox potential was observed (Fig. 4b), as suggested by Eq. (1) and the following Nernst equation (Nicol and Lázaro, 2002) related to the electrochemical potential:

$$E = E^0 + [RT/ZF]\ln\left[Fe^{3+}/Fe^{2+}\right] \tag{2}$$

Where E represents the electrochemical potential; E^0 the standard electrochemical potential; R the gas constant; T the absolute temperature; Z the transferred electrons; and F the Faraday constant. The increase in the solution redox potential represents a higher production of the oxidizing agent (i.e., Fe^{3+}) and a reduction in the Fe^{2+} concentration representing a healthy bacterial system (Ngoma et al., 2015). However, this increase is not observed in the abiotic condition, confirming that the bacteria alone were responsible for the oxidation of Fe^{2+} to Fe^{3+}. Therefore, we can conclude that bacteria were responsible for the bioleaching of Zn. The variation in the concentration of the Fe species, Fe^{2+} and Fe^{3+}, can also be observed in Fig. 4c and d, respectively. As observed in Fig. 4c and d, the abiotic condition did not show a reduction in Fe^{2+}; consequently, they did not show an increase in Fe^{3+} ions either, as suggested in Eq. (1). In the case of biotic conditions, a similar reduction of Fe^{2+} and Fe^{3+} was observed for all conditions. Nevertheless, similar to the pH values, Fe^{3+} showed a behavior opposite to what is described by Eq. (1) and Eq. (2). Previous literature has shown the reduction of Fe^{3+} in accordance with the reduction of the total Fe species (Fig. 4d) because of the formation of Fe precipitates (Hong et al., 2016; Silva et al., 2015). Jarosites and iron hydroxides are among the most common Fe precipitates formed during the bioleaching processes, following the reactions described in Eq. (3) and Eq. (4); in these two equations, M could be K^+, Na^+, NH_4^+, Ag^+, or H_3O^+ (Daoud and Karamanev, 2006; Park et al., 2014; Silva et al., 2015). Hence, the reduction of pH can also be described by the production of H^+ during Fe^{3+} precipitations.

$$\text{Iron hydroxides:}\ Fe^{3+} + nH_2O \rightarrow Fe(OH)_n^{3+(-n)} + nH^+ \tag{3}$$

$$\text{Jarosites:}\ 3Fe^{3+} + M^+ + 2HSO_4^- + 6H_2O \rightarrow MFe_3(SO_4)_2(OH)_6 + 8H^+ \tag{4}$$

3.3. Effect of NOM suspended in a bioleaching solution

Unlike the experiments using adsorbed NOM (Fig. 3), the NOM available in the bioleaching solution showed differences in efficiency for Zn bioleaching (Fig. 5). In Fig. 5, the Zn bioleaching trend is presented for experiments with NOM concentrations of 0, 20, and 50 ppm of SRHA. The suspended SRHA represents the NOM entering the bioleaching process because of the low quality of the process waters. An abiotic experiment was also conducted as a control experiment to compare a possible annihilation of bacteria

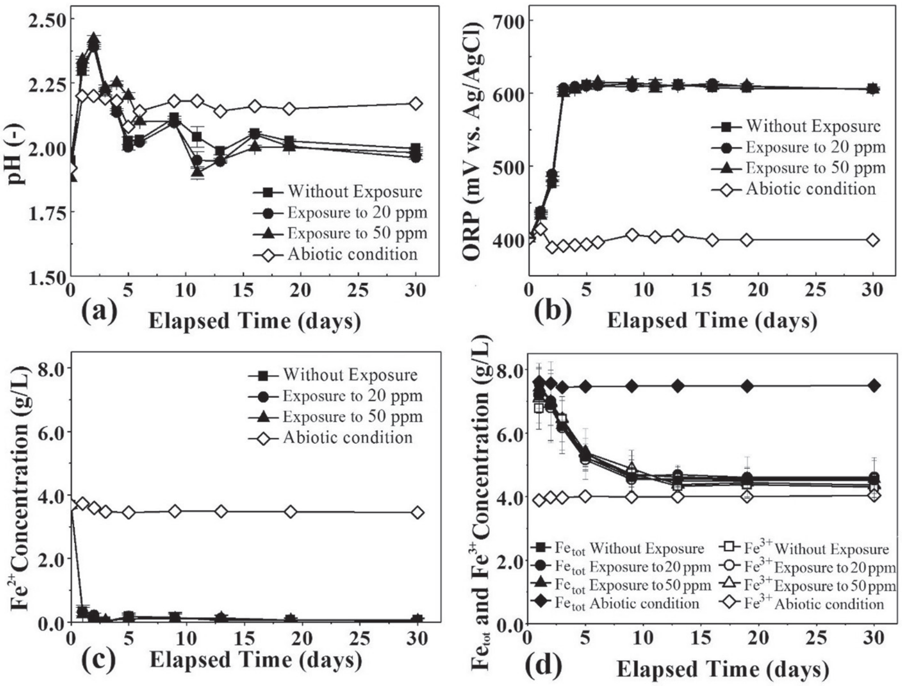

Fig. 4. Trends for (a) pH, (b) solution oxidation-reduction potential, (c) Fe^{2+} concentration, and (d) total Fe (Fe_{tot}) and Fe^{3+} concentrations for mine tailings exposed to solutions containing different amounts of NOM in the form of SRHA.

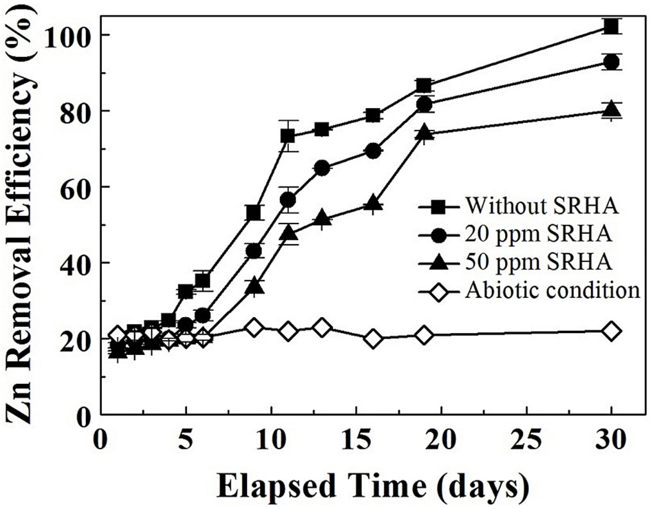

Fig. 5. Zn removal extraction efficiency for different concentrations of NOM in the bioleaching solution.

arising from the addition of NOM into the system. The difference in the Zn bioleaching trends in the presence of NOM was evident soon after starting the bioleaching experiments. This difference was maintained until the end of the experiment with a final difference in Zn removal of 10% for the system containing 20 ppm of NOM and a reduction of approximately 20% for the system with 50 ppm.

However, the trends obtained for the analyses of pH values, redox potentials, and Fe^{2+} concentrations observed when NOM was suspended in bioleaching media did not resemble the Zn removal trend shown in Fig. 5. Instead, all the parameters showed similar trends regardless of the variations in the concentration of NOM in the solution. For example, the pH trend was observed to show the same behavior for all conditions regardless of the concentration of NOM (Fig. 6a). Similarly, the solution redox potential increased for all conditions above 600 mV during the first three days of the reaction (Fig. 6b), suggesting a high bacterial oxidation of Fe^{2+} to Fe^{3+} (Fig. 6c).

From the data collected, the concentration of Fe^{3+} and total Fe appeared to decrease at a faster rate as the concentration of NOM increased in the solution (Fig. 6d), forming Fe^{3+} precipitates (Section 3.2). Similar to the faster increase in the formation of precipitates, we observed a decrease in the bioleaching of Zn in accordance with the concentration of NOM (Fig. 5). However, such Zn reduction was not observed in the experiments with the adsorbed SRHA (Fig. 3) even at similar rates of total Fe reduction (Fig. 4d). Some authors have pointed out the possibilities of co-precipitation of the target metal with Fe precipitates, reducing the bioleaching metal removal efficiency (Jensen and Webb, 1995; Park et al., 2014). However, one of the widely used hydrometallurgical processes for Zn purification from Fe rich solutions is the removal of Fe by induced precipitation wherein jarosites and Fe-hydroxides are formed (Dutrizac and Jambor, 2000; Gordon and Pickering, 1975; Zhang et al., 2016). The co-precipitation of Zn with species formed during Fe/Zn-humic acid systems can also be

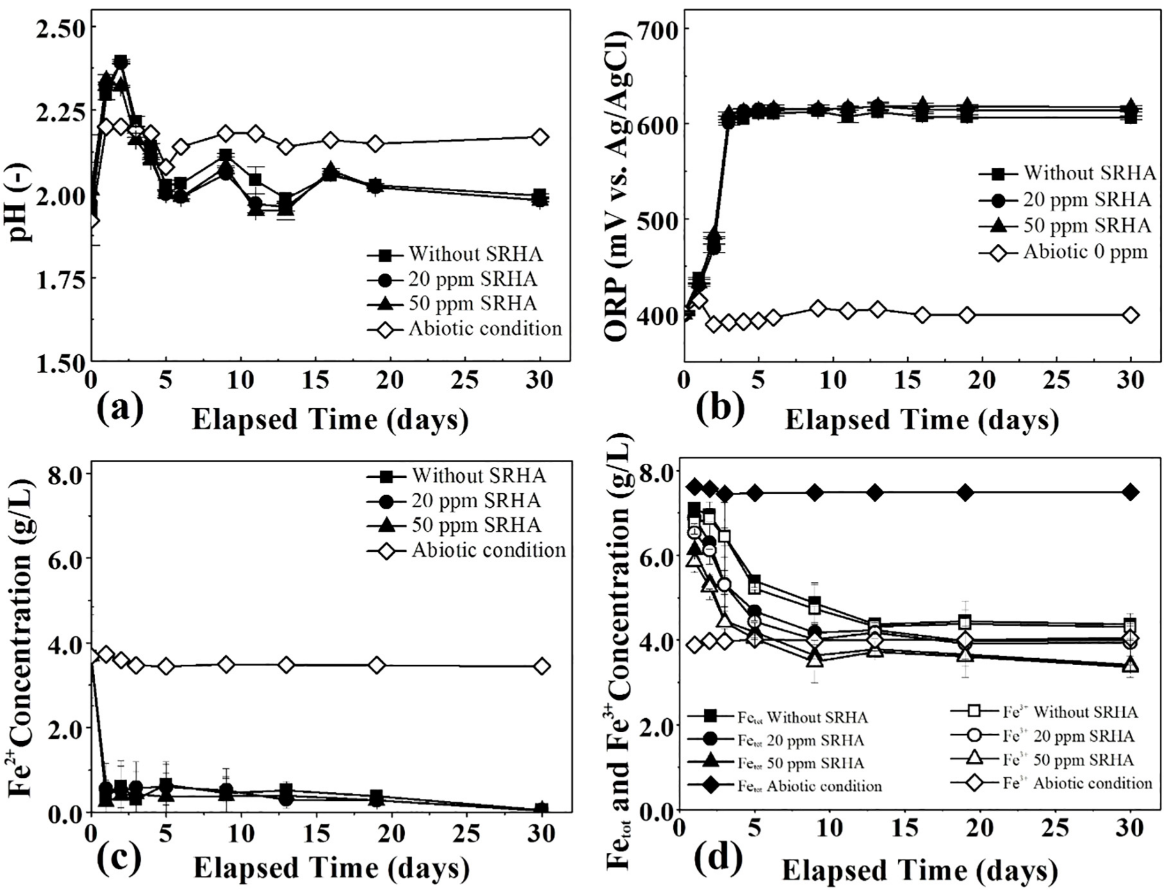

Fig. 6. Trends for (a) pH, (b) solution oxidation-reduction potential, (c) Fe^{2+} concentration, and (d) total Fe (Fe_{tot}) and Fe^{3+} concentrations in time when different concentrations of NOM were adjusted in the bioleaching solution.

discharged because the ligand sites provided by humic acid favor the sorption of Fe (III) ions over Zn (II) ions given the experimental conditions (Kerndorff and Schnitzer, 1980). Therefore, it is not possible to attribute the reduction in the Zn removal to the co-precipitation of Zn-Fe compounds. Nevertheless, the precipitation of Fe eventually depletes Fe^{2+} used by the bacteria as an energy source, which lowers the bacterial concentration and the production of the oxidizing agent (i.e., Fe^{3+}) required for metal leaching.

In addition, we analyzed the possible effects of NOM on the bacterial growth by obtaining a series of bacterial cultures at different concentrations of NOM (Fig. 7a). As observed, the bacterial concentrations seem to increase at a similar rate regardless of the concentration of NOM in the solution. Therefore, we concluded that NOM does not have a negative effect on bacterial growth; hence, the differences in Zn removal observed in Fig. 5 were not caused by the possible toxicity of SRHA. In contrast, it seemed most probable that the lower bioleaching of Zn was caused by lower bacterial mine tailing interactions (i.e., bacterial attachments) caused by higher NOM concentrations in the solution. To prove this, we conducted bacterial attachment experiments in the presence of different NOM concentrations. As seen in Fig. 7b, when the concentration of NOM increased, the bacterial attachment decreased, which suggested that NOM plays a key role in bacterial attachment, as reported by previous studies (Bradford et al., 2006; Tong et al., 2011). Fig. 7b also shows the attachment of bacteria on mine tailings that were previously exposed to SRHA (adsorbed NOM). There is no significant difference between the bacterial attachments on systems with mine tailings with adsorbed SRHA and systems without SRHA; this confirmed that the reduction of bacterial attachment was because of the SRHA in the solution. Further discussion about the bacterial mine tailings interaction is presented in Section 3.4.

3.4. *Effect of NOM on the bacteria-mine tailings interaction*

After dismissing the various possible causes for the differences in Zn removal efficiency (Section 3.2 and 3.3), we have suggested the reduction in the interaction between bacteria and mine tailings as the major reason for the reduction of process efficiency. As shown in Fig. 7b, a considerable reduction in the bacterial attachment is observed in the presence of suspended NOM. When the concentration of NOM was high, a high reduction in the attachment was observed. After 80 min, the attachment experiments containing 50 ppm of NOM showed that 7.81×10^9 bacterial cells were attached per gram of mine tailings. In the absence of NOM, however, a total of 2.44×10^{10} cells remained attached in 1 g of mine tailing. The reduction of bacterial attachment was found to be approximately 68% for the system containing 50 ppm of NOM and 19% for the system with 20 ppm of NOM. The differences in bacterial attachment were obtained as follows:

$$\text{Attachment Reduction}(\%) = \left[\left(B_{att}^{0} - B_{att}^{50}\right) \big/ B_{att}^{0}\right] \times 100 \qquad (5)$$

where B_{att}^{0} represents the attached bacteria in the absence of NOM, and B_{att}^{50} represents the attached bacteria in the presence of 50 ppm of SRHA.

The bacterial attachment (i.e., the contact mechanism) and the bacteria suspended in the solution (i.e., non-contact mechanism) have been established as the main interactions between the bacteria and mine tailings during the bioleaching process (Rohwerder et al., 2003; Sand et al., 2001). In addition, a third interaction has been established as the combination of the bacterial attachment and the suspended bacterial mechanisms, which is a cooperative mechanism (Rohwerder et al., 2003; Sand et al., 2001). The contact mechanism plays an important role in bioleaching operations because it enhances the metal extraction (Sand et al., 2001; Silva et al., 2015). The enhancement in the metal removal is most likely because of a higher presence of Fe^{3+} ions in the bacterial extracellular polymeric substance that is directly available on the surface of the mine tailings when the bacteria are attached (Silva et al., 2015; Tributsch, 2001). This attachment resulting from the interaction between the bacteria and the mine tailings was identified as mainly governed by the Derjaguin–Landau–Verwey–Overbeek (DLVO) interaction that is determined by the sum of the attractive van der Waals force and the electrostatic force (Han et al., 2016; Jucker et al., 1998; van Loosdrecht et al., 1989; van Loosdrecht et al., 1990). Depending on the type (i.e., repulsive or attractive) and the magnitude of the electrostatic force, the total interaction between the bacteria and the mine tailings could be favorable (i.e., without any energy barrier) or unfavorable (i.e., with an energy barrier; Han et al., 2016; Kim et al., 2009; Tong et al., 2005). Previous studies (Tong et al., 2010, 2011) show that electrostatic forces were significantly reduced in the solutions with high ionic strengths (>100 mM), and the DLVO theory likely predicts favorable interactions between two substrates. In this study, we have calculated a high ionic strength (>300 mM) in the bioleaching solution; therefore, we suggest that the interaction between the bacteria and the mine tailings is likely favorable regardless of the presence of NOM. However, the cell adhesion test results are not consistent with this prediction because low cell adhesion was obtained at high NOM conditions. This indicated that the cell–mine tailing interaction is influenced by non-DLVO forces.

After observing similar Zn extraction trends regardless of the amount of NOM adsorbed onto mine tailings (Fig. 3), the introduction of suspended NOM was identified as the only factor responsible for the decrease in the Zn removal efficiency (Fig. 5).

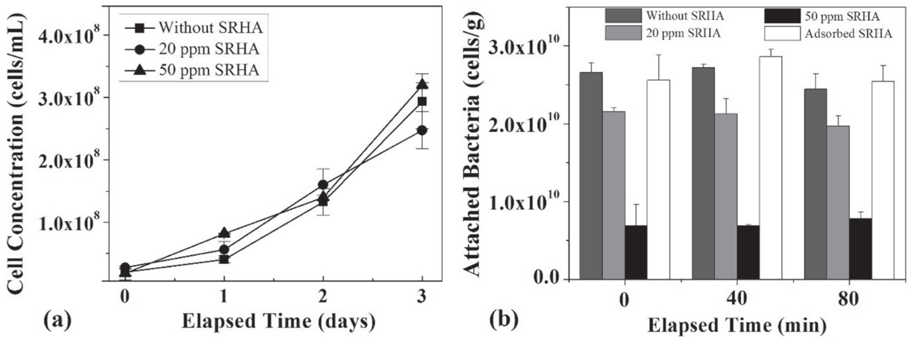

Fig. 7. (a) Effects of bacterial growth for different concentrations of NOM in the SRHA solution (b) Bacterial attachment per gram of mine tailing at different concentrations of SRHA solutions and of mine tailings with adsorbed SRHA. (The adsorption was obtained when mine tailings were exposed to solutions of 50 ppm of SRHA.)

One plausible explanation for the observed bioleaching and cell adhesion trend is the formation of steric repulsion between bacteria and mine tailings in systems with NOM. It is believed that when NOM is introduced into the system (i.e., as a suspension), it is adsorbed on the surface of the mine tailings and likewise on the bacterial membrane (Bradford et al., 2006; Tong et al., 2011; Yang et al., 2012). This attachment, therefore, renders both surfaces as a brush-like surface layer (Abu-Lail and Camesano, 2003; Yang et al., 2012). In a system with brush-layered colloids, the classic DLVO model often fails to fully explain the interactions detected experimentally because of the presence of steric forces caused by brush layers on two interacting colloids or at the colloid–solid substrate interface (Abu-Lail and Camesano, 2003; Jiang et al., 2012; Jucker et al., 1998). Therefore, it is believed that the introduction of NOM in the bioleaching process negatively affects the efficiency of bioleaching operations. However, this negative effect can only be observed when NOM is suspended in the bioleaching solution, allowing NOM to interact with both substrates (i.e., mine tailings and bacteria); the negative effect is not observable when NOM is only adsorbed on the mine tailings surface, as shown in Fig. 7b and in Section 3.2.

4. Conclusions

The NOM entering the bioleaching processes by attaching to the surface of the mine tailings does not produce negative impacts on the process efficiency (Fig. 3). NOM entering the bioleaching process because of the low quality of the process waters has the potential to decrease the process efficiency (Fig. 5). Different SRHA concentrations in the bioleaching medium, representing suspended NOM, were able to reduce the Zn removal efficiency by 10% at concentrations of 20 ppm and by 20% at concentrations of 50 ppm. In the system containing attached NOM, the increase in the attached amount did not affect the Zn removal efficiency. The suspended NOM was able to affect the process by reducing the bacterial attachment on the mine tailings surface. This reduction of bacterial attachment is most likely because of unfavorable interactions between the bacteria and mine tailings, both of which had brush layers and exhibited steric repulsion. Therefore, special attention to NOM in process waters is necessary to reduce the chances of lowering the Zn removal efficiency.

Acknowledgements

This work was supported by the Korea Energy and Mineral Resources Engineering Program (KEMREP) and the National Research Foundation of Korea through the Human Resource Training Project for Regional Innovation (2015H1C1A1035930). The authors would like to extend their acknowledgements to Heejae Kim for his help during the research process.

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