

Effect of pyrite, elemental sulfur and ferrous ions on EPS production by metal sulfide bioleaching microbes

Zhi-guo HE^{1,2}, Yan-ping YANG^{1,2}, Shan ZHOU^{1,2}, Yue-hua HU^{1,2}, Hui ZHONG³

1. School of Minerals Processing and Bioengineering, Central South University, Changsha 410083, China;

2. Key Laboratory of Biohydrometallurgy of Ministry of Education, Central South University, Changsha 410083, China;

3. School of Life Science, Central South University, Changsha 410083, China

Received 17 April 2013; accepted 3 March 2014

Abstract: Extracellular polymeric substances (EPS) produced by acidophilic bioleaching microorganisms play an important role in the production of acid mine drainage and metal sulfide bioleaching. EPS mediate the contact between microbial cells and growth substrates, having a pivotal role in organic film formation and bacterium-substratum interactions. The production and chemical composition of EPS produced by seven bioleaching strains grown with different substrates were studied. Analysis of the EPS extracted from these strains indicated that the EPS consisted of carbohydrates, proteins and galacturonic acid. The contents of EPS, carbohydrates, proteins and galacturonic acid of EPS were largely related to the kind of strain used and culture condition. The results show that EPS productions of microbes grown with pyrite were significantly higher than those of microbes grown with sulfur or $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$. The highest EPS production of the seven acidiphilic strains was (159.43 ± 3.93) mg/g, which was produced by *Leptospirillum ferriphilum* CBCSUCSU208015 when cultivated with pyrite.

Key words: acid mine drainage; carbohydrates; extracellular polymeric substances; galacturonic acid; metal sulfide; bioleaching

1 Introduction

Acidiphilic ferrous or sulphur oxidizing microorganisms are responsible for the phenomenon of acid mine drainage and have been adopted in metal sulfide bioleaching to recover trace metals [1]. It was reported that the attachment of these acidiphilic microorganisms onto the mineral surface is mediated by extracellular polymeric substances (EPS) which play a pivotal role in metal sulfide oxidation [2,3]. It is believed that the EPS could act with concentrate ferric ions and form a special layer in which the ferric ions oxidize mineral, releasing energy sources such as sulphur and ferrous ions for the acidiphilic microorganisms [4]. EPS are complex mixtures of polymers excreted by microorganisms, including products derived from lysis and hydrolysis, and organic matter adsorbed from the environment. They are mainly composed of carbohydrates, proteins and smaller quantities of humic substances, lipids, uronic and nucleic acids [5–9]. EPS characteristics and amounts can be influenced by several

factors such as the composition of the medium and incubation conditions [9–11], and such culture condition induced variation explains the diversity in EPS production noted in previous studies. HAMEIT et al [12] studied the composition of EPS of mesophilic bacteria (*A. ferrooxidans*, *A. thiooxidans* and *L. ferrooxidans*) grown with only single energy source; however, no previous work has systematically studied the effect of different energy sources on the EPS production by different kinds of bioleaching microorganisms which include the extreme thermophilic archaea, the mesophilic bacteria and the moderate thermophilic bacteria. The goal of this work was to study the production and composition of EPS produced by seven known acidiphilic metal sulfide bioleaching microbes, including four extreme thermophilic archaea, one moderate thermophilic bacteria and two mesophilic bacteria in order to unravel the effect of growth substrates on EPS production by these ferrous or sulphur oxidizers.

Sulfur, pyrite and ferrous ion were selected as the growth substrates because all of them have been found to exert important effects on the bioleaching of sulfide ores.

The main mechanism of bacterial catalysis in the dissolution of sulfide minerals was based on the bacterial oxidation of ferrous iron, with oxygen as the electron acceptor, while sulfur was the preferred energy source, but the formation of the sulfur layer also inhibited the dissolution of sulfide ores. The most abundant metal sulfide on earth, pyrite could offer iron ion and low valence state sulfur, which play an important role in the metal sulfide bioleaching [3,13,14].

2 Experimental

2.1 Culture of microorganisms

The microbial strains used in this study included *Acidianus manzaensis* CBCBSUCSU208050, *Acidianus brierleyi* CBCBSUCSU208128, *Metallosphaera sedula* CBCBSUCSU208044, *Sulfolobus metallicus* CBCBSUCSU208047, *Acidithiobacillus ferrooxidans* CBCBSUCSU206060, *Leptospirillum ferriphilum* CBCBSUCSU208015 and *Sulfobacillus thermosulfidooxidans* CBCBSUCSU208125. All of these strains were isolated and kept by CCTCC. The four strains: *A. manzaensis* CBCBSUCSU208050, *A. brierleyi* CBCBSUCSU208128, *M. sedula* CBCBSUCSU208044, *S. metallicus* CBCBSUCSU208047 belong to the extreme thermophilic archaea which can oxidize both pyrite and sulfur; *A. ferrooxidans* CBCBSUCSU206060 and *L. ferriphilum* CBCBSUCSU208015 belong to the mesophilic bacteria, in which *A. ferrooxidans* CBCBSUCSU206060 can oxidize pyrite, ferrous ion and sulfur while *L. ferriphilum* CBCBSUCSU208015 can oxidize both pyrite and ferrous ion; *S. thermosulfidooxidans* CBCBSUCSU208125 is a moderate thermophile capable of oxidizing both pyrite and ferrous iron. The medium used for culturing consisted of the following compounds: 30 g/L $(\text{NH}_4)_2\text{SO}_4$, 0.5 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g/L K_2HPO_4 , 0.1 g/L KCl, 0.01 g/L $\text{Ca}(\text{NO}_3)_2$, 10 g/L pyrite, 10 g/L sulfur or 44.7 g/L $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ was added as the energy source. The archaea strains were cultured at 65 °C and pH 2.0 supplemented with 0.02% yeast extract and the moderate thermophiles were cultured at 40 °C and pH 2.0 supplemented with 0.02% yeast. The mesophilic bacteria, *A. ferrooxidans* CBCBSUCSU206060, grew at 30 °C and pH 2.0; *L. ferriphilum* CBCBSUCSU208015 were cultured at 30 °C and pH 1.6.

2.2 Preparation of cell samples

Cells were grown to later exponential phase and the samples were allowed to stand for 1 h to allow the solids to settle down, then the cultures were filtered through filter paper (30 μm), and the filtrate was centrifuged followed by a repeated washing in sterile deionized water (pH 2.0) in order to remove anytrapped ions.

2.3 Mineral components

The main contents of pyrite were as follows (mass fraction): 52.92% S, and 44.7% Fe. Mineral powders used in the experiments had a size distribution of less than 74 μm (95% of grains).

2.4 Extraction of EPS

Three of the bioleaching microorganisms were used to optimize the EPS extraction protocol that was used for this study. The optimized procedure was as follows. In order to harvest the EPS from the cells, 0.1 g wet mass of cell pellets were resuspended in 10 mmol/L Tris-HCl [Ph 7], 1 mmol/L EGTA and 10^{-3} mmol/L N-dodecyl-N, N-dimethyl-3-ammonio-1-propane-sulfonate, then the solution was shaken using a vortex (0–1400 r/min) for 30 s at 4 °C to ensure equal distribution of cells. Then, the solution was shaken again (170 r/min, 4 °C, 12 h) and filtered through 0.2 μm filters in order to separate out the soluble fractions containing the EPS. The soluble fractions were stored in aliquots at –20 °C until further analysis, while the dry filters were used to obtain the mass of the cell pellets [15,16]. Contamination by membrane fragments of damaged cells, possibly caused by repeated shaking, was assessed via the detection of 2-keto-3-deoxyoctonate (KDO) and DNA. KDO is a part of the cell membrane in gram negative bacteria, and can be used as a marker for contamination by membrane components. Likewise, the detection of DNA in the EPS fraction may also indicate cell breakage and potential contamination. Therefore, below detection or low levels of both DNA (1%–1.4% w/w) and KDO (0.04–0.08 mg/g) would indicate that the extracted EPS was not contaminated by significant amounts of intracellular materials in this experiment [16,17].

2.5 Chemical analysis of EPS

The total quantity of cells and extracted EPS was measured by the mass of solids after lyophilization. Quantitative estimation of protein content for each aliquot was obtained by employing the principle of protein-dye binding method using bovine serum albumin as standards [18] and the saccharide content in EPS was measured by the anthrone method with glucose as the standard [19]. Galacturonic acid was analyzed by fosulfuric acid-carbazole colorimetry methods [20]. The spectrophotometer NanoDrop ND-100 was used to quantify DNA in the extracted EPS samples. The procedure of quantitative analysis KDO referenced that by SEGURA et al [17]. Experimental procedures for these assays have been described previously [15]. Finally, the concentration of iron in the EPS solution was analyzed by ICP-AES. All experiments were conducted in triplicate. All the data were the average number and the error bar represented standard deviation.

3 Results

3.1 Comparison of EPS production by seven bioleaching microbes

The amount of DNA detected in the extracted EPS was in the range of 1%–1.4% of the total cell mass, and the KDO contents in the extracted EPS were in the range of 0.04–0.08 mg/g. The low amounts of DNA and KDO in the extracted EPS and the content range usually indicated negligible contamination by intracellular substances in the collected EPS [15,17].

Figure 1 shows the amount of total extracted EPS for each cultured strain. The amount of EPS varied according to the culture condition and the exact strain. Among the seven strains, *L. ferriphilum* CBCBSUCSU208015 cultivated with pyrite had the highest overall EPS production with a total amount of up to (159.43 ± 3.93) mg/g. The EPS production of *A. ferrooxidans* CBCBSUCSU206060 cultivated with sulfur was the lowest at only (38.27 ± 5.1) mg/g. We also found that the EPS yields of the microbes cultured with pyrite were significantly higher than those of the microbes grown with sulfur or $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ for the same strain. EPS productions of the extreme thermophile archaea were generally much less than those of the moderate thermophilic or mesophilic bacteria when cultured with the same substrate. For extreme, in thermophilic archaea cultivated with the same energy source (pyrite or sulfur), the EPS production of *A. manzaensis* CBCBSUCSU208050 reached (121.98 ± 3.55) mg/g and (60.57 ± 2.87) mg/g when cultivated with pyrite

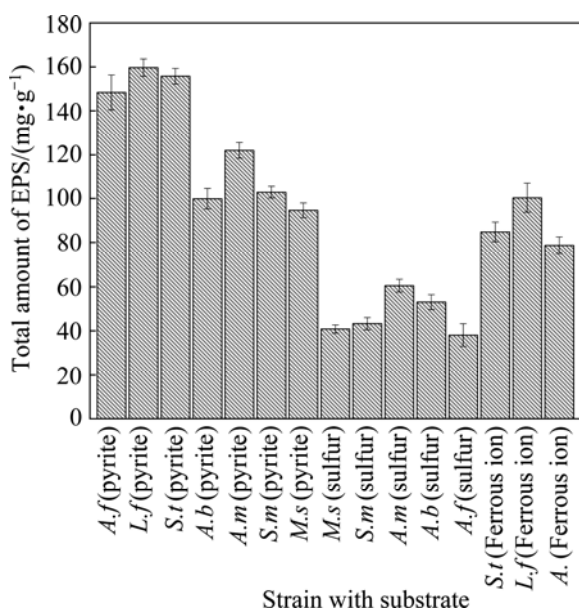


Fig. 1 Comparison of EPS production by seven bioleaching strains cultured with different substrates

or sulfur respectively, significantly higher than those of the other three extreme thermophilic archaea (*A. brierleyi* CBCBSUCSU208128, *M. sedula* CBCBSUCSU208044 and *S. metallicus* JCM9184).

In Fig. 1, the total amount of EPS displayed was the ratio of the chemical content of EPS and the dry mass of cell. The error bars represent SD, $n=3$. The strains cultivated with pyrite were *A. manzaensis* CBCBSUCSU208050, *A. brierleyi* CBCBSUCSU208128, *M. sedula* CBCBSUCSU208044, *S. metallicus* JCM9184, *A. ferrooxidans* CBCBSUCSU206060, *L. ferriphilum* CBCBSUCSU208015 and *S. thermosulfidooxidans* CBCBSUCSU208125. The strains cultivated with sulfur were *A. manzaensis* CBCBSUCSU208050, *A. brierleyi* CBCBSUCSU208128, *M. sedula* CBCBSUCSU208044, *S. metallicus* JCM9184, *A. ferrooxidans* CBCBSUCSU206060. The strains cultivated with ferrous ion were *A. ferrooxidans* CBCBSUCSU206060, *L. ferriphilum* CBCBSUCSU208015 and *S. thermosulfidooxidans* CBCBSUCSU208125.

3.2 Protein contents in EPS

The protein contents of the extracted EPS fractions are displayed in Fig. 2. We found that among those microbes cultivated with pyrite, the extreme thermophilic archaea *A. manzaensis* CBCBSUCSU208050 had the highest EPS protein content, up to (4.04 ± 1.34) mg/g, and *L. ferriphilum* CBCBSUCSU208015 had the second highest EPS protein content, up to (3.08 ± 1.14) mg/g.

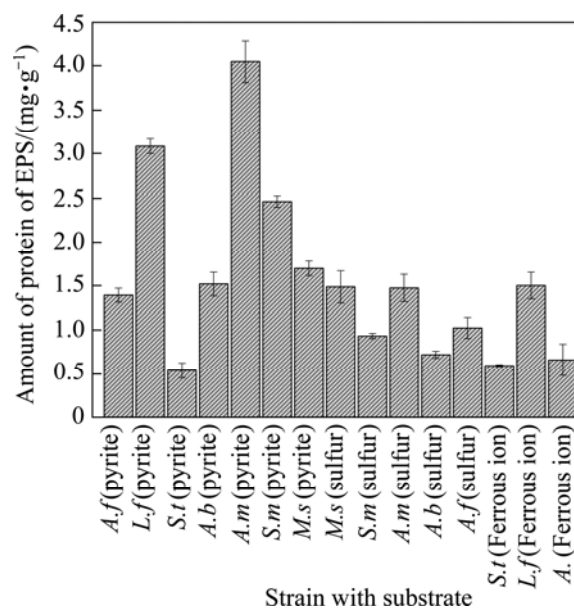


Fig. 2 Protein contents of EPS from seven acidiphilic strains cultured with different substrates (The amount of protein in EPS displayed was the ratio of the chemical content of protein of EPS and the dry mass of extracted EPS; the error bars represent SD, $n=3$)

When grown with sulfur, *A. manzaensis* CBCBSUCSU-208050 and *M. sedula* CBCBSUCSU208044 had the same EPS protein content, with a value higher than bacterial strains cultured with sulfur. For microbes grown with ferrous ion, *L. ferriphilum* CBCBSUCSU208015 had the most abundant protein in EPS, which was (1.50 ± 0.91) mg/g. Furthermore, the protein content in EPS was insensitive to the growth substrates (pyrite or ferrous ion) for *S. thermosulfidooxidans* CBCBSUCSU-208125 with a content of only (0.54 ± 0.16) mg/g. The results for the other six strains showed that the protein contents in EPS grown with pyrite were higher than those grown with sulfur or ferrous ion. As an example, the protein content in the EPS from *A. ferrooxidans* CBCBSUCSU206060 was (1.39 ± 1.08) mg/g when grown with pyrite, while it was only (1.03 ± 0.25) mg/g or (0.66 ± 0.31) mg/g, respectively, when grown with sulfur or ferrous ion.

3.3 Saccharide contents in EPS

Figure 3 shows the saccharide contents in EPS. Growth substrates (pyrite, element sulfur and ferrous) were found to have the greatest influence on the proportion of saccharide in EPS. For the microbes cultivated with pyrite, *A. ferrooxidans* CBCBSUCSU-206060 had the highest EPS saccharide content, which was (4.51 ± 2.24) mg/g. *L. ferriphilum* CBCBSUCSU-208015 had the second highest EPS saccharide content, which was (3.74 ± 0.73) mg/g. For microbes grown with sulfur, all seven strains used in this study had a small

amount of saccharide in the EPS. For microbes grown with ferrous ion, *L. ferriphilum* CBCBSUCSU208015 had the highest saccharide content, up to (2.95 ± 1.43) mg/g. Generally, mesophilic bacteria had higher saccharide contents in EPS compared with both moderate thermophilic bacteria and the extreme thermophilic archaea grown with the same substrate. Within the same strain, the saccharide content of the EPS was higher when grown on pyrite in comparison to growth with sulfur or ferrous iron. For example, *A. ferrooxidans* CBCBSUCSU206060 had (4.51 ± 2.24) mg/g of saccharide in EPS when grown with pyrite, while only (2.63 ± 1.09) or (0.87 ± 0.35) mg/g of saccharide was present in the EPS when grown with sulfur or ferrous, respectively.

3.4 Iron contents

Figure 4 shows the iron content in EPS for the strains studied. It was found that iron was below detection in the EPS of microbes grown with sulfur. For microbes grown with pyrite, both *L. ferriphilum* CBCBSUCSU208015 and *S. thermosulfidooxidans* CBCBSUCSU208125 had high contents of iron in their EPS, (3.63 ± 0.25) mg/g and (3.66 ± 0.38) mg/g, respectively. For microbes cultivated with ferrous iron, *L. ferriphilum* CBCBSUCSU208015 had the highest EPS iron content, (2.33 ± 0.09) mg/g. The iron contents of the EPS of pyrite-grown bacteria were twice as much as those of ferrous-grown bacteria. *A. ferrooxidans* CBCBSUCSU206060 cultured with pyrite had

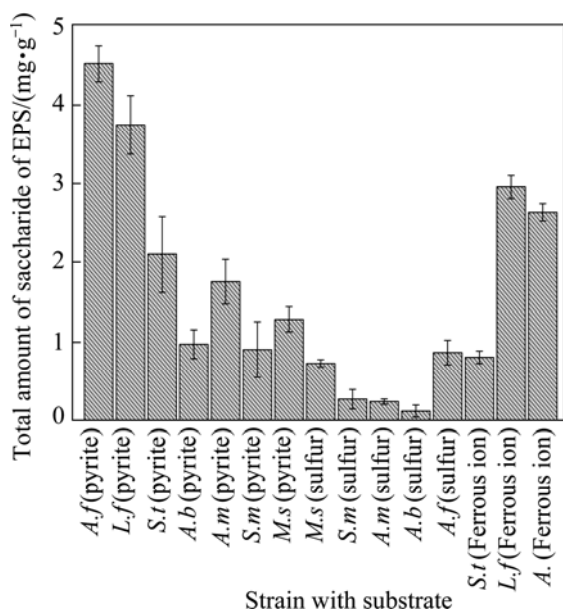


Fig. 3 Saccharide contents of EPS from seven bioleaching strains cultured with different substrates (The amount of saccharide in EPS displayed was the ratio of the chemical content of saccharide of EPS and the dry mass of extracted EPS. The error bars represent SD, $n=3$)

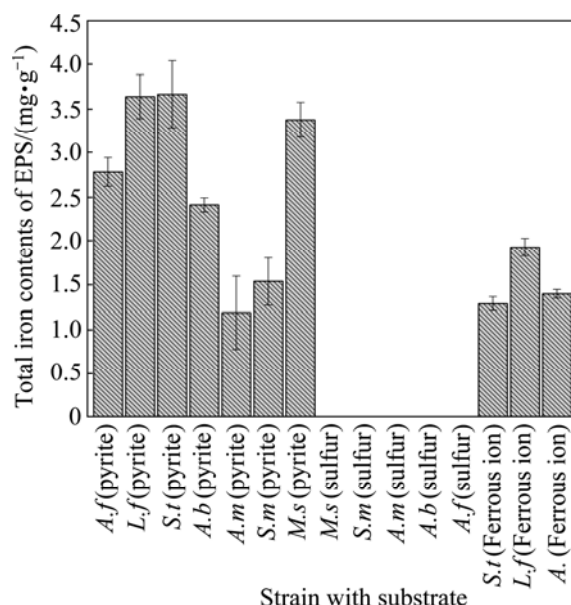


Fig. 4 Iron contents of EPS from seven bioleaching strains cultured with different substrates (The amount of iron in EPS displayed was the ratio of the chemical content of iron of EPS and the dry mass of extracted EPS. The error bars represent SD, $n=3$)

(2.78 ± 1.64) mg/g iron in EPS, in contrast to only (1.40 ± 0.48) mg/g when cultured with ferrous ion. Among the four archaea strains, pyrite-grown *M. sedula* CBCBSUCSU208044 had the highest iron content in EPS, (3.37 ± 0.02) mg/g.

3.5 Galacturonic acid contents in EPS

Figure 5 shows the galacturonic acid content in EPS for all strains studied. *M. sedula* CBCBSUCSU208044 had the highest overall galacturonic acid content of (3.56 ± 0.72) mg/g when cultured with pyrite. *A. manzaensis* CBCBSUCSU208050 and *L. ferriphilum* CBCBSUCSU208015 had the next highest contents of galacturonic acids in EPS, (3.25 ± 0.09) mg/g and (3.39 ± 0.27) mg/g, respectively. Within the same strain, cells cultured with pyrite had higher galacturonic acid contents than those cells grown with ferrous iron or elemental sulfur. Pyrite-grown *A. ferrooxidans* CBCBSUCSU206060 had (2.43 ± 1.13) mg/g of galacturonic acids in EPS, but only (1.20 ± 0.07) mg/g or (0.25 ± 0.39) mg/g, respectively, when grown with ferrous iron or sulfur. For microbes cultured with the same substrate, the extreme thermophilic archaea strains had higher galacturonic acid contents within their EPS compared with bacterial strains.

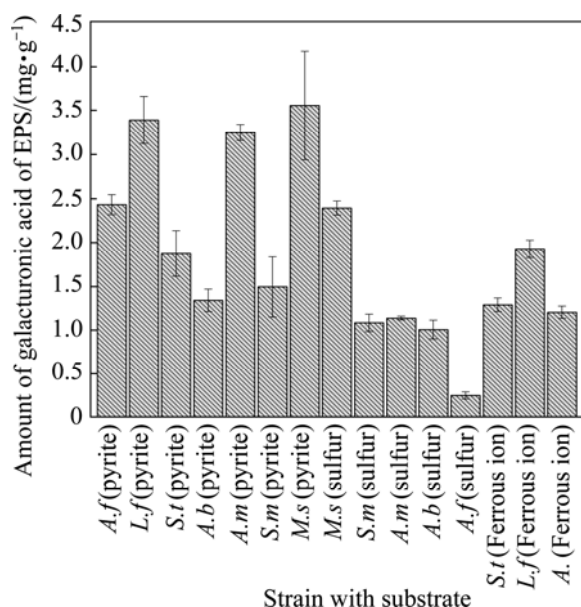


Fig. 5 Galacturonic acid contents of EPS from seven bioleaching strains cultured with different substrates (The amount of galacturonic acid in EPS displayed was the ratio of the chemical content of galacturonic acid of EPS and the dry mass of extracted EPS. The error bars represent SD, $n=3$)

4 Discussion

Several EPS extraction methods have been

developed previously; however, no universal method has yet been adopted due to the difficulty in establishing a balance between high yields and the minimum cell lysis and contamination [21]. In fact, most of the common extraction methods such as sonication, formaldehyde, sulfuric acid, and formaldehyde plus NaOH promote leakage of intracellular material [21,22]. In a comparison of these common extraction methods, it was found that the suitable method for the extraction of EPS from bioleaching microorganisms was the method described in this study and was subsequently used for all of the strains, which also guaranteed that we can compare the data among the seven bioleaching microbes cultured with different energy sources.

Some studies have found that the total yield of EPS depends on the composition of the medium (carbon and nitrogen sources, growth factors etc), the culture conditions such as temperature, pH value, oxygen tension [23], and the particular strain used [3]. In this study, it was also found that the amount of EPS extracted from the selected bioleaching microbes was severely affected by growth substrates and correlated with strain types. ZENG et al [16] found that the EPS from bioleaching microorganisms was primarily composed of sugars and fatty acids and while the protein content was relatively low. The amount of extracted EPS varied according to different growth stages, which was mainly selected according to the growth trend of bioleaching microorganisms, and that furthermore once the EPS were produced by the microbes, it was difficult to remove them from the bioleaching system [16]. However, several studies have also reported that microorganisms produced less EPS when consuming substrate and grew rapidly. Rather, cells were observed to produce more EPS when growing slowly, and the EPS production rate is inversely proportional to the substrate consumption rate [24,25]. In contrast, some other studies found that the EPS production rate was independent of growth rate [26,27] and that the amount of EPS produced by the different strains did not correlate with their oxidation rates [3]. Up to now, the general correlations of the EPS production and their chemical composition with the microbial leaching activity are still unclear among bioleaching microbes.

With respect to variation in the amount of protein in the EPS, we found that it was largely affected by energy sources and the exact kind of bioleaching strain, furthermore. It was also found that protein accounted for only a small fraction of the EPS (0.32%–3.3% in mass fraction). GOVENDER and GERICHKE [28] found that the EPS consisted predominantly of carbohydrates and smaller amount of protein with trace levels of humic and

uronic acids. ROHWERDER and SAND [29] and ZHANG et al [30] found that the extracellular proteins and outer membrane proteins for *Acidithiobacillus* and *Acidiphilium* played a key role in the sulfur mobilization and sulfur transportation, respectively. For *A. ferrooxidans*, the general pattern of the type of extracellular proteins was affected by the kind of energy source (e.g. sulfur or ferrous iron) and the extracellular proteins played a role in various functions such as conjugal transfer, sulfur activation, polysaccharide deacetylation [30].

The results of this study show that the EPS consists of saccharides (0.23%–3.1% in mass fraction). Most saccharides in EPS were found to be composed of the neutral sugars glucose, galactose and/or rhamnose [31–35]. Some saccharides also consisted of acetylated amino sugars [36,37]. The overall amount and saccharide composition in EPS depend on the strain type, the culture condition, and the type of carbon source [11]. Some studies have also found that saccharide was utilized faster than protein in the EPS when EPS was used as a substrate [38].

We observed that the iron (III) ion content in EPS was significantly affected by energy sources and the kind of strains. KINZLER et al [3] found that the total iron (III) ion content in EPS was significantly higher for the more active *A. ferrooxidans* strains R1 and C-52 than that for the less active strains R7 and SPIII/3. GEHRKE et al [15] and ZENG et al [16] found that with the amount of EPS increasing compared to the mass of cell pellet, enough uronic acid was produced to combine with ferric ions to form complexed ferric ions. It was also found that ferric ions could occur stoichiometrically with the complexing glucuronic acid [39]. The ferric ions complexed within the EPS were found to play a pivotal role in attachment to mineral surfaces [40]. It has been reported that the primary attachment of microbes to pyrite is mediated by positively charged exopolymer-complexed iron (III) ions, allowing an electrochemical interaction with the negatively charged surface [15]. The ferric ions in the EPS are also thought to be the primary means by which microbes dissolve the mineral sulfide surface via oxidative attack [3]. These various roles of iron (III) in the EPS make it a critical aspect of the function and physiology of bioleaching microbes.

5 Conclusions

1) The amount and chemical composition of EPS produced by acidophilic bioleaching microbes depend on the strain type and growth substrates. The extreme thermophilic archaea had the lowest saccharides content in EPS, while mesophilic bacteria had the highest one.

2) Bacteria produced much greater amount of EPS than archaea when grown with pyrite. The highest total amount of EPS among the seven acidiphilic bioleaching microbes was (159.43±3.93) mg/g by *L. ferriphilum* CBCBSUCSU208015 cultivated with pyrite.

3) The amount of EPS yielded by bioleaching microbes cultured with pyrite was much higher than that of microbes cultivated with sulfur or ferrous ion.

References

- [1] JOHNSON D B. Biodiversity and ecology of acidophilic microorganisms [J]. FEMS Microbiology Ecology, 1998, 27: 307–317.
- [2] BEECH W B, SUNNER J. Biocorrosion: Towards understanding interactions between biofilms and metals [J]. Current Opinion Biotechnology, 2004, 15(3): 181–186.
- [3] KINZLER K, GEHRKE T, TELEGDI J, SAND W. Bioleaching—A result of interfacial processes caused by extracellular polymeric substances (EPS) [J]. Hydrometallurgy, 2003, 71(1–2): 83–88.
- [4] SAND W, GEHRKE T. Extracellular polymeric substances mediate bioleaching/biocorrosion via interfacial processes involving iron(III) ions and acidophilic bacteria [J]. Research Microbiology, 2006, 157: 49–56.
- [5] SPONZA D. Extracellular polymeric substances and physicochemical properties of flocus in steady and unsteady-state activated sludge systems [J]. Process Biochemistry, 2002, 37: 983–998.
- [6] DOMINGUEZ L, RODRIGUEZ M, PRATS D. Effect of different extraction methods on bound EPS from MBR sludges. Part I: Influence of extraction methods over three dimensional EEM fluorescence spectroscopy fingerprint [J]. Desalination, 2010, 261: 19–26.
- [7] MCSWAIN B S, IRVINE R L, HAUSNER M, WILDERER P A. Composition and distribution of extracellular polymeric substances in aerobic flocs and granular sludge [J]. Applied Environment Microbiology, 2005, 71: 1051–1057.
- [8] HORN H, NEU T R, WULKOW M. Modelling the structure and function of extracellular polymeric substances in biofilms with new numerical techniques [J]. Water Science Technology, 2001, 43: 121–127.
- [9] LASPIDOU C S, RITTMANN B E. A unified theory for extracellular polymeric substances, soluble microbial products, and active and inert biomass [J]. Water Resource, 2002, 36: 2711–2720.
- [10] YU R L, OU Y, TAN J X, WU F D, SUN J, MIAO L, ZHONG D L. Effect of EPS on adhesion of *Acidithiobacillus ferrooxidans* on chalcopyrite and pyrite mineral surfaces [J]. Transactions of Nonferrous Metals Society of China, 2011, 21: 407–412.
- [11] LOOISJESTEIJN P J, BOELS I C, KLEEREZEM M, HUGENHOLTZ J. Regulation of exopolysaccharide production by *Lactococcus lactis subsp. cremoris* by the sugar source [J]. Applied Environment Microbiology, 1999, 65: 5003–5008.
- [12] HAMEIT K, GOKSEL A, KOCK D, KLOCK J H, GEHRKE T, SAND W. Adhesion to metal sulfide surfaces by cells of *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans* [J]. Hydrometallurgy, 2006, 83: 245–254.

- [13] MERUANE G, VARGAS T. Bacterial oxidation of ferrous iron by *Acidithiobacillus ferrooxidans* in the pH range 2.5–7.0 [J]. Hydrometallurgy, 2003, 71: 149–158.
- [14] CHEN P, YAN L, LENG F F, NAN W B, YUE X X, ZHENG Y N, FENG N, LI H Y. Bioleaching of realgar by *Acidithiobacillus ferrooxidans* using ferrous iron and elemental sulfur as the sole and mixed energy sources [J]. Bioresource Technology, 2011, 102: 3260–3267.
- [15] GEHRKE T, TELEGDI J, THIERRY D, SAND W. Importance of extracellular polymeric substances from *Thiobacillus ferrooxidans* for bioleaching [J]. Applied Environment Microbiology, 1998, 64(7): 2743–2747.
- [16] ZENG W M, QIU G Z, ZHOU H B, LIU X D, CHEN M, CHAO W L, ZHANG C G, PENG J H. Characterization of extracellular polymeric substances extracted during the bioleaching of chalcopyrite concentrate [J]. Hydrometallurgy, 2010, 100: 177–180.
- [17] SEGURA R L, AGUILA-ARCOS S, UGARTE-URIBE B, VECINO A J, de la CRUZ F, GOÑI F M, ALKORTA I. The transmembrane domain of the T4SS coupling protein TrwB and its role in protein–protein interactions [J]. Biochimica Biophysica Acta, 2013, 1828(9): 2015–2025.
- [18] HE Z, ZHONG H, HU Y, XIAO S, LIU J, XU J, LI G. Analysis of differential-expressed proteins of *Acidithiobacillus ferrooxidans* grown under phosphate starvation [J]. Journal of Biochemistry and Molecular Biology, 2005, 38(5): 545–549.
- [19] LIU X Y, LI L, SHI H W, CAI Y P. Influence of light intensity on soluble sugar, organic acid and Vc content of *Pyrus bretschneideri* cv. Dangshan Su pear fruit in its growth phase [J]. Pratacultural Science, 2007, 43(7): 34–37.
- [20] LIN Y, HUANG L J, TIAN G Y. An improved method for the quantitative determination of uronic acid [J]. Chinese Traditional and Herbal Drugs, 1999, 30(11): 347–351.
- [21] AZEREDO J, HENRIQUES M, SILLANKORVA S, OLIVEIRA R. Extraction of exopolymers from biofilms: the protective effect of glutaraldehyde [J]. Water Science Technology, 2003, 47(5): 175–179.
- [22] LIANG Z W, LI W H, YANG S Y, DU P. Extraction and structural characteristics of extracellular polymeric substances (EPS), pellets in autotrophic nitrifying biofilm and activated sludge [J]. Chemosphere, 2010, 81: 626–632.
- [23] DEGEEST B, VANINGELGEM F, DEVUYST L. Microbial physiology, fermentation kinetics, and process engineering of heteropolysaccharide production by lactic acid bacteria [J]. International Journal of Dairy, 2001, 11: 747–757.
- [24] EVANS E, BROWAN M W, GILBERT P. Iron chelator, exopolysaccharide and protease production in *Staphylococcus epidermidis*: A comparative study of the effects of specific growth rate in biofilm and planktonic culture [J]. Microbiology, 1994, 140(15): 3–7.
- [25] ROBINSON J A, TRULEAR M G, CHARACKLIS W G. Cellular reproduction and extracellular polymer formation by *Pseudomonas aeruginosa* in continuous culture [J]. Biotechnology Bioengineer, 1984, 26(14): 9–17.
- [26] HSIEH K M, MURGEL G A, LION L W, SHULER M L. Interactions of microbial biofilms with toxic trace metals observation and modeling of cell growth, attachment, and production of extracellular polymer [J]. Biotechnology Bioengineer, 2007, 44(2): 19–31.
- [27] TURAKHIA M H, CHARACKLIS W G. Activity of *Pseudomonas aeruginosa* in biofilms: Effect of calcium [J]. Biotechnology Bioengineer, 1988, 33(4): 6–14.
- [28] GOVENDER Y, GERICKE M. Extracellular polymeric substances (EPS) from bioleaching systems and its application in bioflotation [J]. Minerals Engineer, 2011, 24: 1122–1127.
- [29] ROHWERDER T, SAND W. The sulfane sulfur of persulfides is the actual substrate of the sulfur-oxidizing enzymes from *Acidithiobacillus* and *acidiphilium* [J]. Microbiology, 2003, 149(7): 1699–1710.
- [30] ZHANG C G, XIA J L, DING J N, YANG X D, NIE Z Y, QIU G Z. Cellular acclimation of *Acidithiobacillus ferrooxidans* to sulfur biooxidation [J]. Journal of Miner Metallur Process, 2009, 26(1): 30–34.
- [31] FABER E J, ZOON P, KAMERLING J P, Vliegenthart J F G. The exopolysaccharides produced by *Streptococcus thermophilus* Rs and Sts have the same repeating unit but differ in viscosity of their milk cultures [J]. Carbohydrate Research, 1998, 310: 269–276.
- [32] IBARBURU I, SORI-DIAZ M E, RODRIHUEZ-CARVAJALMA, VELASCOL S E, TEJEROMATEO P, GIL-SERRANO A M, IRASTORZAL A, DUENAS M T. Growth and exopolysaccharide (EPS) production by *Oenococcus oeni* I4 and structural characterization of their EPS [J]. Journal of Applied Microbiology, 2007, 103(2): 477–486.
- [33] GOKCEN Y C, BELMA A, YAVUZ B. Characterization and production of the exopolysaccharide (EPS) from *Pseudomonas aeruginosa* G1 and *Pseudomonas putida* G12 strains [J]. Carbohydrate Polymers, 2008, 73: 178–182.
- [34] LEMOINE J, CHIRAT F, WIERUSZESKI J M, STRECKER G, FAVRE N, NEESER J N. Structural characterization of the extracellular polysaccharides produced by *Streptococcus thermophilus* Sfi39 and Sfi12 [J]. Applied Environment Microbiology, 1997, 63: 3512–3518.
- [35] YAMAMOTO Y, NUNOME T, YAMAUCHI R, KATO K, SONE Y. Structure of an exocellular polysaccharide of *Lactobacillus helveticus* TN-4, a spontaneous mutant strain of *Lactobacillus helveticus* TY1-2 [J]. Carbohydrate Research, 1995, 275: 319–332.
- [36] ROBIJIN G W, WIENK H L J, VANDENBERG D J C, HAAS H, KAMERLING J P, Vliegenthart J F G. Structural studies of the exopolysaccharide produced by *Lactobacillus paracasei* 34-1 [J]. Carbohydrate Research, 1996, 285: 129–139.
- [37] STINGELE F J R, NEESER J R, MOLLET B. Identification and Characterization of the EPS (exopolysaccharide) gene cluster from *Streptococcus thermophilus* Sfi6 [J]. Journal of Bacteriol, 1996 178: 1680–1690.
- [38] ZHANG X Q, PAUL L, BISHOP P L. Biodegradability of biofilm extracellular polymeric substances [J]. Chemosphere, 2003, 50: 63–69.
- [39] GEESEY G G, JANG L. Interactions between metal ions and capsular polymers [C]//BEVERIDGE T J, DOYLE R J. Metal Ions and Bacteria. New York: John Wiley & Sons, 1989: 325–357.
- [40] SAND W, GEHRKE T, JOZSA P G, SCHIPPERS A. Direct versus indirect bioleaching [C]//AMILS R, BALLESTER A. Biohydrometallurgy and the Environment Toward the Mining of the 21st Century, Part A. Amsterdam: Elsevier, 1999: 27–49.

黄铁矿、单质硫、亚铁离子对 浸矿微生物产生胞外多聚物的影响

贺治国^{1,2}, 杨彦平^{1,2}, 周 珊^{1,2}, 胡岳华^{1,2}, 钟 慧³

1. 中南大学 资源加工与生物工程学院, 长沙 410083;
2. 中南大学 生物冶金教育部重点实验室, 长沙 410083;
3. 中南大学 生命科学学院, 长沙 410083

摘 要: 嗜酸浸矿微生物产生的胞外多聚物(EPS)在酸性矿坑水的产生和硫化矿的浸出过程中, 有着非常重要的影响, 胞外多聚物(EPS)介导细胞与能源物质的接触, 对有机薄膜的形成和细菌与基础物之间的相互作用起着重要的作用。对 7 株浸矿菌在不同能源培养物下产生的 EPS 的量以及 EPS 的化学成分进行研究, 发现 EPS 含有化学成分糖、蛋白质、糖醛酸等, 细菌的种类和能源物质对 EPS 的量和成分有很大影响。结果表明, 以黄铜矿为能源物质的细菌产生的 EPS 要比以单质硫和亚铁为能源物质产生的 EPS 量多, EPS 含量最高为 (159.43 ± 3.93) mg/g, 是由 *Leptospirillum ferriphilum* CBCBSUCSU208015 在黄铁矿为能源物质下产生的。

关键词: 酸性矿坑水; 糖类; 胞外多聚物; 半乳糖醛酸; 金属硫化矿; 生物; 浸出

(Edited by Hua YANG)