EXTRACELLULAR POLYMERIC SUBSTANCES (EPS) FROM BIOLEACHING SYSTEMS AND ITS APPLICATION IN BIOFLOTATION

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The use of EPS-producing heterotrophic and chemolithotrophic bacteria in bioflotation processes have been investigated for the last two decades. These studies have mostly been confined to laboratory microflotation tests using pure cultures. In this study the use of EPS, extracted from bioleaching consortia, to float chalcopyrite was evaluated and key process parameters such as pH, flotation time and the concentration of collector, bacterial cells and EPS were optimized. Analyses of the EPS extracted from various bioleach systems indicated that the EPS consisted mainly of carbohydrates, proteins and uronic acids. Microflotation tests using free EPS yielded a chalcopyrite recovery of 77 % when chalcopyrite was floated alone and 70 % during the separation of a mixture of pure chalcopyrite and pure pyrite compared to 32 % when using SIBX only. The results obtained suggested that the flotation of chalcopyrite could be significantly increased in the presence of EPS extracted from bioleaching populations.

Keywords: EPS, bioflotation**,** bacteria, bioleaching, biotechnology.

1. INTRODUCTION

The growing world demand for raw minerals has led to the increased exploitation of low grade-sulfide ores, necessitating processing of ores with complex mineralogy, particularly for base metals. This in combination with the more rigorous specifications for production of concentrates, stricter environmental legislation and a necessity to achieve lower operating costs has led to numerous investigations aimed at finding better processing techniques and more effective flotation reagents. Conventional flotation methods use highly selective inorganic modifiers such as cyanides, sulfides and ferro-cyanides, and their use has raised concern with regard to environmental issues (Bradshaw *et al*., 1998).

The use of microorganisms in mineral beneficiation has been elucidated with recent developments in biotechnology. Bioflotation is one such developing technology for processing ores. During bioflotation, microorganisms and associated extracellular metabolic products are used to selectively separate gangue ores (Deo and Natarajan, 1997) and have been reported as friendly modifiers, collectors and depressants (Santhiya *et al*., 2000, 2001; Patra and Natarajan, 2003, 2004, 2006, 2008; Chandraprabha *et al*., 2004, 2005; Chandraprabha and Natarajan, 2006; Deo and Natarajan, 1998). These microorganisms may act as bioreagents and induce hydrophobic properties once they have adhered to the mineral surfaces (Botero *et al*., 2008; Sharma and Rao, 2002). The precence of functional non- polar groups such as hydrocarbon chains and polar groups (carboxyl, hydroxyl, phosphates) at the microbial cellular surface or metabolic products can either directly or indirectly modify the mineral surfaces, lending the microbial culture

similar characteristics of surfactant molecules. The direct mechanism involves the adhesion of cells to mineral particles. The indirect mechanism refers to biological reagents such as secreted metabolites and EPS acting as surface-active reagents (Sharma and Rao, 1999) or to soluble fractions of the microorganisms derived from their rupture (Schneider *et al*., 1994; Raichur *et al*., 1997).

Numerous laboratory studies have demonstrated that it is possible to use microbial species as flotation reagents in selective separation. The selective flotation or depression of sulphides and oxides has been studied with a variety of microbes (e.g. *Mycobacterium phlei, Paenibacillus polymyxa, Rodococcus opacus*) and bioleaching bacteria (e.g. *Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans, Leptospirillium ferrooxidans*) (Raichur *et al* 1996, Zheng *et al* 1998, Deo and Natarjan, 1998, de Mesquita *et al* 2003, Patra and Natarajan, 2008, Botero *et al* 2008, Chandraprabha *et al* 2004, 2005, Yuce *et al* 2006, Hosseini *et al*., 2005, Kolahdoozan *et al* 2004, Chandraprabha and Natarajan 2006, Santhiya *et al*., 2001, Vilinska and Rao, 2008).

Of particular interest are bioleaching bacteria, acidophilic metal sulphide oxidizing species, which are already used in commercial biohydrometallurgical processes. These bacteria selectively attach to sulphide surfaces forming a biofilm or an EPS layer. Compared to conventional inorganic reagents, bacteria are non- toxic and environmentally benign, potentially providing an alternative to conventional flotation methods.

Studies showed that the use of xanthate in the flotation of pyrite could be greatly reduced by prior application of *A. ferrooxidans* whereas the effect on chalcopyrite was found to be marginal (Vilinska and Rao, 2008). *A. ferrooxidans* cells, which have a natural affinity for sulphide minerals, mostly pyrite, can therefore be used for selective separation of chalcopyrite from pyrite. The majority of studies using bioleaching bacteria and their derived products have concentrated on *A. ferrooxidans*, *A. thiooxidans* and *L. ferrooxidans*. To our current knowledge and understanding the EPS obtained from mixed bacterial consortia used in bioleaching operations have not been investigated for use in bioflotation.

The aim of this study was to investigate the application of mixed bioleaching consortia and their EPS as bioflotation reagents. The main objectives were (i) optimisation of operating parameters such as pH, flotation time, collector, EPS and bacterial cell concentration and (ii) evaluation of the efficiency of bioleaching bacteria and the EPS produced as bioflotation agents.

2. EXPERIMENTAL

2.1. Mineral samples

Pure mineral samples of chalcopyrite ($CuFeS_2$) were obtained from Gregory, Bottley & Lloyd, UK The chemical compositions of the chalcopyrite and pyrite used during the microflotation tests are presented in Table 1. Both samples were milled finer and a particle size of $d_{80} = 75\mu m$ was utilised for all experiments.

Table 1: Chemical characterisation of the pyrite and chalcopyrite samples used during the microflotation tests

Element (Mass %)	**Al**	**Si**	**Ca**	**Cr**	**Fe**	**Co**	**Cu**	**Pb**
Pyrite	0.23	0.51	0.058	<0.05	47.3	0.11	0.13	0.090
Chalcopyrite	<0.05	<0.05	0.080	0.48	31.1	<0.05	37.1	<0.05

2.2. Bacterial cell isolation and EPS extraction

Bioleach pulp samples used during cell isolation and EPS extraction were obtained from several continuously operated bioleaching systems. These reactors were maintained on various sulphide concentrates. The operating conditions and composition of the microbial consortia are summarised in Table 2.

Table 2: List of bioleaching reactors from which bacterial cells with bound EPS were isolated and free EPS extracted.

Operating Conditions (at steady state)			**Sulphide concentrate**	**Culture**	**Composition of the Microbial Population**
T (°C)	**pH**	**ORP (mV)**			
37	1.55	645	Sphalerite	Mesophiles	*Acidithiobacillus caldus* *Leptosprillium sp* *Sulfobacillus sp*
35	1.40	670	Pyrite	Mesophiles	
45	1.60	615	Sphalerite	Moderate thermophiles	*Acidithiobacillus caldus* *Leptosprillium sp* *Sulfobacillus sp* *Ferroplasma sp*
45	1.50	674	Chalcopyrite-pyrite	Moderate thermophiles	
70	1.64	635	Chalcopyrite-pyrite	Extreme thermophiles	*Acidianus sp* *Metallosphaera sp* *Sulfolobus sp*

The samples were allowed to stand for an hour to allow the solids to settle. The solid free liquor was centrifuged (6000 rpm, 4°C, 20 min) and the cell pellet re-suspended in 50 ml of nutrient solution (1g/l $(NH4)_2SO_4$, 0.1g/l KCl, 0.5g/l $MgSO_4.7H_2O$ and 0.5g/l K_2HPO_4) before a cell count was performed using a Neubauer cell counting chamber under a microscope. All analytical grade chemicals reagents used were obtained from Merck, South Africa. It should be noted that unless otherwise stated all microbial isolations were conducted in duplicate.

The supernatant obtained during the isolation of bacterial cells with the bound EPS was used for the extraction of only the free EPS found in the bioleach pulp. Cold ethanol (2.2 x volume of supernatant) was added to precipitate the EPS (4°C, 2 hours). The precipitated EPS was collected by centrifugation (6000 rpm, 4°C, 20 min) and the EPS pellet was dried overnight at 40°C. The dry weight of the EPS was determined before it was biochemically characterised for the presence of carbohydrates, proteins, uronic and humic acids. All EPS extractions were conducted in duplicate.

2.3. Biochemical characterisation of the EPS

The biochemical composition of the free EPS only was determined using the following described methods. The Bradford assay was used to determine the protein content, using bovine serum albumin as standard (Bradford, 1976) and the total carbohydrate content was measured using the phenol-sulphuric assay with glucose (Merck, South Africa) as the standard (Fox and Robyt, 1990). The uronic acid assay was used to determine the uronic acid content with glucuronic acid (Sigma Aldrich, South Africa) as a standard.

(Mojica *et al*., 2007) and the humic acid content was measured using humic acid (Sigma Aldrich, South Africa) as the standard (Frolund *et al*., 1995). All samples were analyzed using the NanoDrop 1000 Spectrophotometer.

2.4. Optimisation of process parameters for microflotation tests

During the process optimisation tests all experiments were carried out using a modified Hallimond tube (Fuerstenau *et al*., 1957) by passing nitrogen gas at a flow rate of 200 ml/min for 2 min, at pH 4 unless otherwise stated. For each flotation test 1g of chalcopyrite was used and the floated chalcopyrite was collected, dried and weighed to determine the percentage chalcopyrite floated.

2.4.1. Collector concentration

Industrial grade sodium isobutyl xanthate (SIBX) was used as a collector for chalcopyrite flotation. The effect of various SIBX concentrations, ranging from 0.5×10^{-5}M to 5×10^{-4} M, on the flotation of chalcopyrite was investigated in the absence of bacterial cells and EPS.

2.4.2. Bacterial cell concentration

A series of tests were conducted on pure chalcopyrite to determine the optimal cell concentration to be used during microflotation tests. The final cell concentrations evaluated ranged from 1×10^6 to 1×10^8 cells/g chalcopyrite.

2.4.3. EPS concentration

During the optimisation of the free EPS concentration required for optimal flotation of chalcopyrite, dried EPS was re-dissolved in dH_2O and the final EPS concentrations evaluated were 1.7×10^{-3}, 8.6×10^{-3}, 1.7×10^{-2}, 3.5×10^{-2}, 6.9×10^{-2}, 1.7×10^{-1} and 3.1×10^{-1} mg/g chalcopyrite.

2.4.4. Flotation time

A series of microflotation tests were conducted using 1 g of pure chalcopyrite and the optimised SIBX concentration to determine the optimal flotation time of chalcopyrite. It should be noted that no EPS or bacterial cells were added and the flotation times evaluated varied from 1 to 60 min.

2.4.5. pH

To determine the effect of pH on chalcopyrite recovery, a series of tests were conducted using bacterial cells (1×10^6 cells/g chalcopyrite), EPS (3.45×10^{-2} mg/g chalcopyrite) and SIBX (1×10^{-5} M) at a fixed flotation time of 20 min with the pH maintained at either pH 4 or pH 9.

2.5. Microflotation tests at optimised conditions using bioleaching cultures and EPS extracted from various reactor systems

All microflotation tests were carried out using a modified Hallimond tube as described for the previous tests. The optimised process parameters determined in section 2.4 were used for all tests. Prior to flotation, 1 g of the mineral sample (d_{80} = 75µm) was conditioned for 20 min in the presence of EPS (3.45 x 10^{-2} mg/g chalcopyrite) or bacterial cells (1 x 10^{6} cells/g chalcopyrite). Prior to collector addition the pH was adjusted to the required pH of 9 by the addition of NaOH. The mineral sample together with the EPS or bacterial cells was conditioned in the presence of the collector (1 x 10^{-5} M SIBX) for 5 min. The floated and tailings samples were collected separately, filtered, dried, weighed and the recovery calculated. The floated residues were subsequently analysed using ICP analysis for further confirmation of the recovery of chalcopyrite. All flotation experiments were conducted in duplicate unless otherwise stated.

3. RESULTS AND DISCUSSION

3.1. Process optimisation

During process optimisation free EPS and cells containing bound EPS were extracted from a continuously operated pyrite reactor (45 °C). Laboratory microflotation tests were conducted in a Hallimond tube and the various process parameters such as the flotation time, pH, collector, bacterial cell and EPS concentration were optimised. These optimised conditions were used in subsequent studies to evaluate the use of free EPS and bacterial cells containing bound EPS, from various bioleaching systems, as potential bioflotation agents.

3.1.1. Biochemical characterisation of the free EPS

The free EPS obtained after ethanol precipitation was biochemically characterised and found to consist of carbohydrates (79 %), proteins (19 %), uronic acids (0.68 %) and humic acids (0.23 %). Of the total dry mass obtained during ethanol precipitation, 70 % of the mass obtained could be accounted for by the above mentioned constituents. The unaccounted constituents (30 %) could be attributed to lipids, DNA and even metal ions, such as Fe which are known to be present in EPS.

3.2. Optimisation of the microflotation tests

3.2.1. Collector concentration

The results in Figure 1A indicated that by increasing the SIBX concentration from 0.5 x 10^{-5} M to 1 x 10^{-5} M, the chalcopyrite recovery could be increased from 7.5 to 10 %. However at collector dosages above 1 x 10^{-5} M SIBX, the recovery decreased reaching 5.5 % at the highest SIBX concentration of 5 x 10^{-4} M. This decrease could occur as a result of hemi-micelle formation. Collectors of the type $CH_3(CH_2)n\text{-}2CH_2\text{-}P$, where *P* is a polar group and *n* is the number of carbon atoms in the straight alkyl chain, will induce flotation of an appropriate mineral at a lower concentration as *n* increases and has been reported for such systems as xanthates on sulfides (Somasundaran and Fuerstenau, 2008). The results from this study indicated that the optimum SIBX concentration to be used to obtain maximum flotation and to minimise micelle formation was 1 x 10^{-5} M SIBX.

3.2.2. Bacterial cell concentration

Figure 1B indicated that addition of lower bacterial concentrations i.e. 1 x 10^6 and 1 x 10^4 cells/g chalcopyrite resulted in the best chalcopyrite recovery with further increases in cell concentration resulting in a decrease in recovery from ~16 to ~9 %. It was therefore decided to use 1 x 10^6 cells/g chalcopyrite in further experiments.

3.2.3. EPS concentration

The results indicated that by increasing the EPS concentration from 1.7 x 10^{-3} to 3.5 x 10^{-2} mg/g chalcopyrite, recovery could be increased from 27 to 39 % (Figure 1C). However, further increases in EPS concentrations had a negative effect on chalcopyrite floatability. This decreased recovery could be as a result of the $CuFeS_2$ particles being affected by the high viscosity from the excessive levels of EPS in the solution (Li *et al.*, 2008). The presence of excessive biopolymers can result in decreased flotation capability as there would be insufficient free space for bio-polymers to attach to the particle surface. The optimal EPS concentration to be used for chalcopyrite flotation under these operating conditions was 3.5 x 10^{-2} mg/g.

3.2.4. Flotation time

Previous studies utilised pH 4 during microflotation tests using bacteria (Vilinska and Rao, 1998), therefore during this study pH 4 was used during process optimisation. Figure 1D indicated that at pH 4, with an increase in flotation time, more chalcopyrite could be recovered. At 20 min a maximum recovery of 27 % could be achieved compared to the 9 % obtained at 2 min, however no significant increase was noted thereafter.

3.2.5. pH

Optimisation of the collector, bacterial cell and EPS concentrations as well as the flotation time did not result in the high chalcopyrite flotation recoveries as obtained in literature (Vilinska and Rao, 2008: Patra and Natarajan, 2008). In order to obtain chalcopyrite recoveries higher than 27 %, the pH was increased to pH 9, which is the pH routinely used in larger flotation circuits when using SIBX as collector, since xanthate based collectors are more functional at higher pH (Bradshaw *et al*., 1998; Bradford *et al*., 19980.

The results indicated (Figure 1E) that at pH 9, with a 20 min flotation time and using the optimised bacterial cell, EPS and SIBX concentrations, higher recoveries of chalcopyrite (35-58 %) could be obtained when compared to pH 4 (18-32 %). The data also indicated that when operating at optimal conditions, EPS is more efficient at chalcopyrite flotation than either the bacterial cells or the collector. At the optimal pH of 9, 58 % chalcopyrite recovery could be obtained using free EPS (extracted from a pyrite system) when compared to the bacterial cells (41%) and SIBX only (32 %).

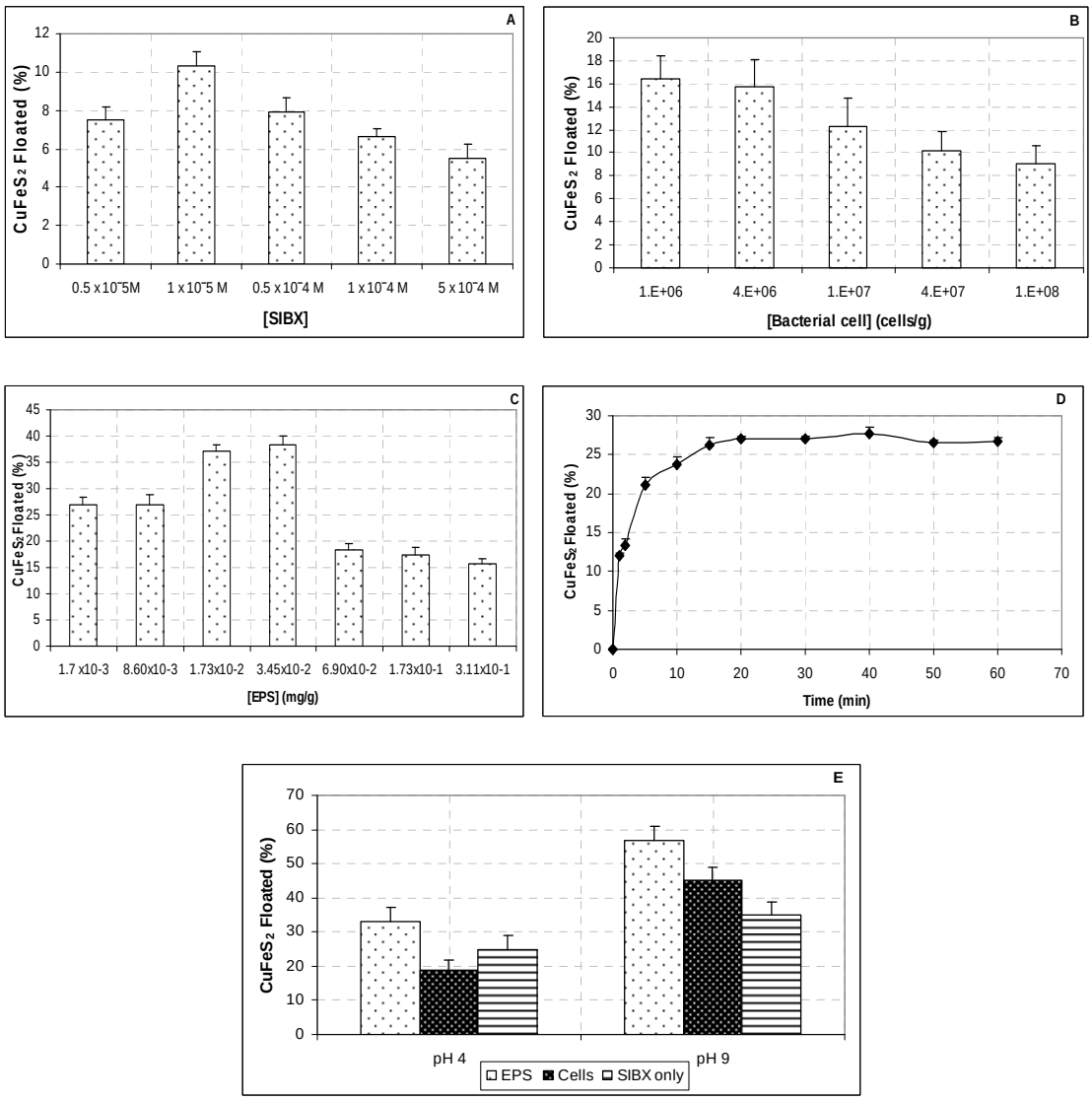


Figure 1:Process optimisation of microflotation operating parameters and the effect of (A) SIBX collector concentration (B) bacterial cell concentration (C) EPS concentration (D) flotation time and (E) pH on the flotation of chalcopyrite. (The error bars represent the standard deviation).

3.3. Biochemical characterization of extracted free EPS from various bioleaching systems

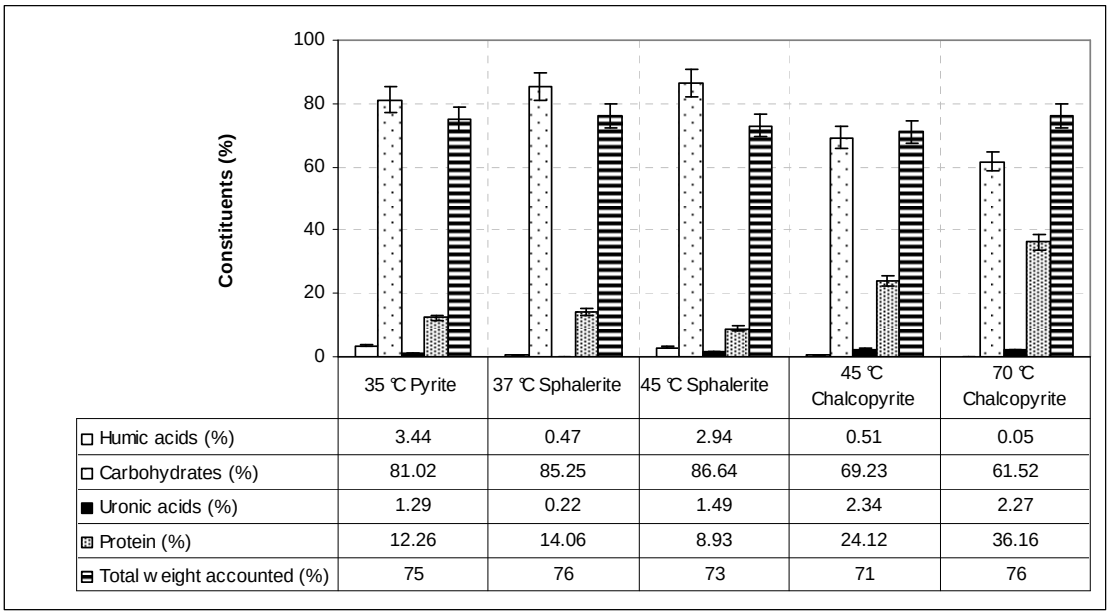
In section 3.1 the process parameters were optimised using free EPS extracted from a continuously operated (45 °C) pyrite bioleaching system. The optimised conditions as described in the previous section i.e. the flotation time (20 min), pH (pH 9), collector concentration (1x 10^{-5} M SIBX), bacterial cell concentration (1 x 10^{6} cells/ g chalcopyrite) and EPS concentration (3.5 x 10^{-2} mg/g chalcopyrite) were subsequently used in further studies to evaluate the use of free EPS and cells with bound EPS, from bioleaching systems at various operating conditions (Table 2), as potential bioflotation agents.

Biochemical characterisation of the EPS indicated that in all cases over 70 % of the dry weight obtained could be attributed to EPS with a ~ 30 % of the mass unaccounted for. The unknown constituents might be attributed to lipids, DNA and even metal ions which are known to be present in EPS. The EPS was found to consist mainly of carbohydrates and smaller amounts of protein with trace levels of humic and uronic acids (Figure 2).

Figure 2 indicated that the free EPS extracted from the sphalerite and pyrite systems at 35, 37 and 45 °C consisted of mainly carbohydrates (over 80 %) with lower levels of protein detected varying between 9 and 14 %. Although the free EPS extracted from the chalcopyrite systems at 45 and 70 °C consisted mainly of carbohydrates (over 60 %), significantly higher amounts of protein were recorded, with the highest protein level of 36 % detected in the extreme thermophile system.

The carbohydrate : protein ratio (C:N) of the EPS varied significantly between the chalcopyrite and the pyrite and sphalerite systems. The free EPS extracted from pyrite and sphalerite systems showed C:N ratios of 6.6 (35° C pyrite), 6.1 (37 °C sphalerite) and 9.7 (45°C sphalerite) whilst the chalcopyrite systems had lower C : N ratios of 2 (45 °C) and 1.7 (70 °C).

The results obtained were contrary to published literature that indicated the EPS from bioleaching bacteria (*A. ferrooxidans* and *L. ferrooxidans*) consisted mainly of neutral sugars and lipids (Gehrke *et al*., 1998; 2001, Rohwerder *et al*., 2003, Harneit *et al*., 2006). This difference could be a result of the previous studies isolating EPS from pure mesophile cultures grown in the presence of pyrite, sulphur or ferrous sulphate, while the EPS in this study was extracted from mixed cultures (mesophile, moderate thermophiles and extreme thermophiles) growing on various mineral resources such as pyrite, sphalerite and chalcopyrite. From the results obtained in this study it could be suggested that the free EPS obtained from microbes growing on chalcopyrite have a higher affinity for chalcopyrite during microflotation compared to the EPS extracted from other systems.



	35 ℃ Pyrite	37 ℃ Sphalerite	45 ℃ Sphalerite	45 ℃ Chalcopyrite	70 ℃ Chalcopyrite
Humic acids (%)	3.44	0.47	2.94	0.51	0.05
Carbohydrates (%)	81.02	85.25	86.64	69.23	61.52
Uronic acids (%)	1.29	0.22	1.49	2.34	2.27
Protein (%)	12.26	14.06	8.93	24.12	36.16
Total weight accounted (%)	75	76	73	71	76

Figure 2: Biochemical characterisation of the EPS constituents obtained from the various bioleaching reactors. (The error bars represent the standard deviation).

3.4. Microflotation tests comparing bacterial cells with bound EPS and free extracted EPS as bioflotation agents

The results indicated that bacterial cells containing bound EPS was not efficient at chalcopyrite flotation with chalcopyrite recoveries of between 32 – 43 % obtained (Figure 3). These results are unexpected when compared to other studies which indicated that bacteria growing on a chalcopyrite rich concentrate would be better adapted and suited for the flotation of $CuFeS_2$ (Patra and Natarajan, 2008).

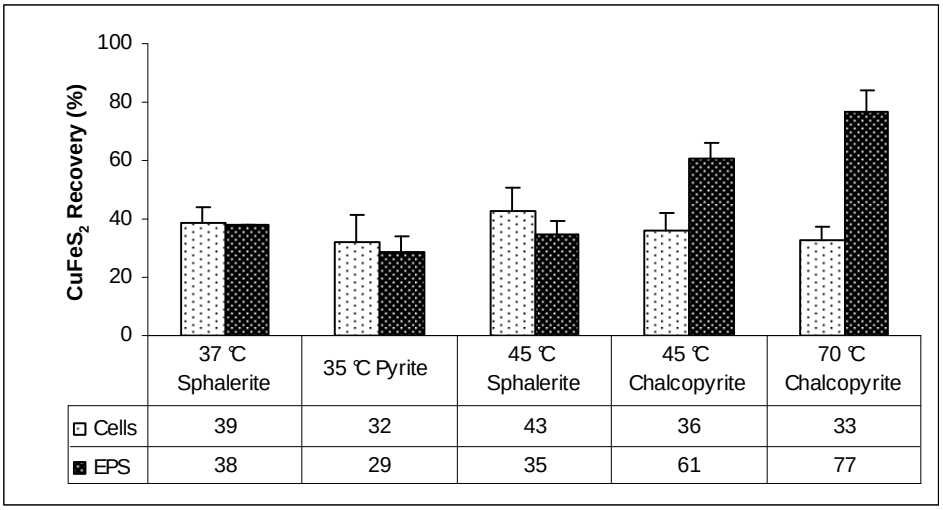


Figure 3: Comparison of the efficiency of using bacterial cells with bound EPS or free EPS as a bioflotation reagent. (The error bars represent the standard deviation).

Figure 3 indicated that using the free EPS extracted from the chalcopyrite systems resulted in higher chalcopyrite recoveries of 66 % (45 °C) and 77 % (70 °C) when compared to the EPS extracted from the systems operated at lower temperature with pyrite and sphalerite. The $CuFeS_2$ recoveries of 66 % and 77 % obtained during this study were within the range of 60- 80 % obtained by other researchers (Chandraprabha *et al*., 2004; 2005, Kolahdoozan *et al*., 2004, Hosseini *et al*., 2005, Chandraprabha and Natarajan, 2006, Yuce *et al*., 2006). However during this study we used a consortium of bacteria operating at temperatures ranging between 35 - 70 °C whereas those published reports utilised pure mesophilic cultures.

Figure 2 indicated that the EPS isolated from 45 and 70 °C chalcopyrite systems consisted mainly of carbohydrates, but contained higher amounts of proteins, uronic acids and carbohydrate: protein ratios when compared to the free EPS extracted from other

systems evaluated. The carbohydrate to protein ratio has been shown to have important implications in bioflotation. The metabolically secreted proteins in EPS have been known to induce hydrophobocity on various minerals whilst polysaccharides confer hydrophilicity on others (Vilinska and Rao, 2008). Proteins essentially serve as a hydrophobic agent with higher surface hydrophobocity and lower surface charge are related to higher dispersion and flotation tendencies Therefore, in a flotation process, proteins can aid in the selective separation of sulphide minerals such as pyrite and chalcopyrite (Patra and Natarajan, 2004).

Uronic acids are considered to be replicate units of acidic polysaccharides or mucopolysaccharides and is a constituent in biofilms and in bacterial aggregation (Mojica *et al*., 2007). Studies have indicated a good correlation between concentration of uronic acid concentration and high protein levels with increased flocculation and flotation activity (Punal *et al*., 2003). From this study it is suggested that EPS containing higher amount of proteins and acidic polysaccharides result in higher recoveries of chalcopyrite.

As the results for the flotation of chalcopyrite using free EPS extracted from the 70 °C chalcopyrite system looked most promising, the experiment was repeated in triplicate, using a mixture of pure chalcopyrite and pure pyrite (ratio of 1:1). The biochemical analysis of all the EPS fractions were similar to those previously obtained (Figure 2). The microflotation results using these EPS fractions indicated that recoveries of 65, 71 and 73 % could be obtained. The floated chalcopyrite fractions from the triplicate experiments were pooled and yielded an average chalcopyrite recovery of 70 % based on

dry mass calculations. In order to confirm these results the pooled fractions were analysed using ICP. The ICP analysis indicated that 65 % of the floated fraction was chalcopyrite which is comparable to the 70 % obtained when based on dry mass calculations.

4. SUMMARY AND CONCLUSIONS

Many of the bioflotation studies concerning bioleaching bacteria and their EPS as potential flotation bioreagents used pure cultures. However, during this study the effect of mixed microbial cultures and free EPS extracted from bioleaching systems, operating at temperatures varying between 35 and 70 °C using pyrite, sphalerite and chalcopyrite, on the floatability of chalcopyrite was determined. Key process parameters such as the collector concentration, bacterial cell concentration, EPS concentration, flotation time and pH were optimized. The results from the test work indicated that free EPS was more efficient as a flotation agent than the cells with bound EPS. Biochemical analysis of the free EPS extracted from these bioleach systems, indicated that the EPS consisted mainly of carbohydrates, proteins and uronic acids. The results indicated that chalcopyrite recoveries of 77 % (chalcopyrite floated alone) and 70 % (separation of chalcopyrite from pyrite) could be achieved using EPS extracted from a thermophile culture grown on chalcopyrite. This study, to our knowledge, is the first to successfully demonstrate on a laboratory scale the potential use of free EPS, extracted from mixed bioleaching microbial consortia, as a possible flotation agent during bioflotation of sulphide minerals. Future work will include demonstration of the process on a larger-scale.

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