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RECENT DEVELOPMENTS IN THE FLOTATION OF SULFIDE ORES OF BASE METALS - BIOFLOTATION

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Abstract

Bioflotation of sulfide minerals is being developed recently at laboratory scale. Different bacterial cells, cell parts and microbial metabolites have been successfully used as reagents (mainly biodepressants and biosurfactants) to separate by flotation pyrite, sphalerite, galena, chalcopyrite, arsenopyrite, molybdenite, pyrrhotite, etc. from mixtures of those minerals or from gangue minerals, such as quartz or calcite.

Influence of conditions for microorganisms' cultivation and of operating parameters (such as pulp density, pH and temperature, concentration of bioreagents and their nature, addition to the system of typical flotation reagents, such as xanthate or activators) has been widely studied. Changes in the system "mineral(s)/microorganism cells (cell parts or metabolites)" have been investigated by various methods, such as contact angle, electro-kinetic and zeta-potential measurements, Scanning Electron Microscopy/Energy Dispersive X-Ray Spectroscopy (SEM-EDS) and Fourier-transform infrared (FTIR) spectroscopy, sodium dodecyl sulfate - polyacrylamide gel electrophoresis (SDS-PAGE), etc. Different mechanisms of microbial action leading to minerals' surface biomodification have been raised, such as microbial cells attachment, occurrence of oxidation-reduction reactions on the interface

"mineral/microorganisms", adsorption and/or chemical reactions with microbial metabolite products. This paper briefly presents and discusses the above-mentioned issues.

Keywords: bioflotation, sulfide minerals, depression, activation

Introduction

Our society needs base metals for its sustainable development and this need will increase in the future. At the same time the rich ores deposits are practically depleted and the result is the need to process more and more low-grade ores. Economical extraction of valuable metals from such ores demands selective separation of minerals.

Flotation is widely applied for minerals separation. The process uses modifiers, collectors and depressants that modify the mineral surface properties, as well as frothers and dispersants to increase the flotation froth stability. Potassium and / or sodium cyanide is the most often used depressant for pyrite in complex sulfide mineral flotation systems. Cyanide is toxic and environmental regulations are stringent for final disposal of cyanide bearing effluents and waste. The other widely used depressant, namely sodium sulfide, also raises environmental concerns. Lime is typically used as a modifier to increase the pH to alkaline levels thus depressing pyrite.

Generally, the flotation selection of lower grade ores requires increased amount of reagents. This, besides related to the price of reagents and environment concerns, can lead to other negative economic results, such as depression of precious metals (gold, silver) that are lost with the gangue material. When lime is applied as pH modifier and the process is carried out with sea water, excess lime has to be used, due to the sea water buffering properties. In addition, the $Ca(OH)^+$ species that present at pH above 8, in combination with magnesium hydroxide species from sea water can readily adsorb onto the valuable mineral surface, such as molybdenite, considerably reducing its flotability [1].

The increased need of reagents, combined with the fact that some of them are not environmentally friendly and that a number of them lead to loss of valuable metals, means that the process is going to become increasingly unsustainable as ores become poorer. All mentioned facts lead to a necessity to develop alternative reagents.

Bioreagents possess the potential to mitigate the toxicity issues as they are generally easier to remediate, more biodegradable and as a

whole easier and at their mature technological development stage even cheaper to be produced than synthetic chemicals.

Bioflotation practically appeared in the eighties of the previous century as an answer of the above described problems. It is virtually very new branch, compared to over 150 years use of flotation. The bioflotation may be defined as process in which whole microorganisms or their parts or metabolites act as reagents (collectors, modifiers or surfactants) to facilitate the selective separation of minerals in an environmentally friendly manner [2-4].

Systematic studies on the bioflotation of sulfide ores practically began in the nineties of the previous century. Bacterial cells belonging to genera Acidithiobacillus, Leptospirillum, Bacillus, Mycobacterium, Staphylococcus, etc., and their metabolites such as extracellular polymeric substances (EPS), bio-surfactants, and nucleic acid have been used as reagents in the mineral flotation process [5].

The aim of this paper is to present the recent developments in the bioflotation of sulfide ores of base metals. Good examples of the use of whole cells or microbial metabolites are given. Mechanisms underpinning the good results of bioflotation are briefly discussed.

Use of whole cells as flotation reagents

Initial bioflotation investigations tested the bacterium *Thiobacillus ferrooxidans* (*T. ferrooxidans)* as a means for pyrite depression in the desulphurisation of coal [6].

Later it has been found that *T. ferrooxidans* sets off surface modification of sulfide minerals to enhance their hydrophilicity or hydrophobicity for the flotation of sphalerite and galena [7]. Higher bacterial growth was observed in the sphalerite suspension than in the galena suspension. It was found that the variables influencing the surface modification (and, thus, flotation response of the minerals) comprise the cell concentration used for bacterial conditioning, the period of biotreatment and the pulp density. Cell protein estimations suggested that more cells were attached on galena than on sphalerite. Flotation enhancement of galena obtained through sulfuric acid treatment was retarded by biotreatment at all cell concentrations. During the conditioning with sulfuric acid solution elemental sulfur is generated

$$ZnS \dashrightarrow Zn^{2+} + S + 2e^{-}$$

$$PbS \dashrightarrow Pb^{2+} + S + 2e.^{-}$$

It renders the mineral surfaces hydrphobic and hence increases their flotability. *T. ferrooxidans* is known to oxidize such elemental sulfur to form sulfate. At the pH of 2, the zinc sulfate formed on sphalerite surface is soluble, while lead sulfate formed on galena surface is insoluble. Availability of oxidized insoluble products on the surface of the sulfide mineral impedes the action of the collector leading to considerably decreased flotation recovery of galena observed after the biopretreatment. Such a decrease occurred for sphalerite only at cell concentrations enough high for bacterial attachment to the mineral surface, leading for a possibility for a differential flotation of sphalerite and galena [8].

Further, attempts were made to use *T. ferrooxidans* as pyrite depressant in sulfides ores flotation [9]. Bioconditioning of pyrite with *T. ferrooxidans* produced significant modification of the mineral surface. The flotability of pyrite was considerably reduced, depending on the bacterium concentration, conditioning pH, and the composition of the culture medium in which the bacterium was cultivated. Decrease in the pyrite floatability was attributed to the adhesion of *T. ferrooxidans* on its surface thus rendering the mineral hydrophilic and finally resulting in its depression, hydrophobic sulfur oxidation, and the formation of jarosite on the surface of pyrite.

Additional studies on mineral surfaces characterization using zeta-potential, contact angle, FTIR and FT-Raman spectrometry showed that a monolayer was formed on the pyrite and chalcopyrite surface in the presence of *T. ferrooxidans* [10]. The *T. ferrooxidans* adsorption began on pyrite at a much lower equilibrium cell population than on chalcopyrite. In the presence of *T. ferrooxidans* cells, the xanthate flotation of pyrite was completely depressed, whereas chalcopyrite flotation was unaffected, thus suggesting that chalcopyrite could be selectively floated from pyrite, at a neutral pH region, in the presence of *T. ferrooxidans* cells.

Nagaoka and co-authors studied the flotability of 5 sulfide minerals (pyrite, chalcocite, molybdenite, millerite and galena) upon addition of *T. ferrooxidans* [11]. They found that the flotability of pyrite was significantly depressed to less than 20%, while the addition of the bacterium had little or no effect on the flotabilities of the other minerals, even when they were present in relatively large

amounts. Their flotabilities remained in the range of 70 - 94%. Due to pyrite depression 84-95% of pyrite was removed from mineral mixtures, while 73-100% of non-pyrite sulfide minerals were recovered. The inhibition of pyrite flotability was assigned to bacterial attachment to its surface. The number of cells adhering to pyrite was significantly larger than the number adhering to other minerals.

The effect of *T. ferrooxidans* on the froth flotation of Iranian Sarcheshmeh copper ore was studied [12]. Pure strains of *T. ferrooxidans* were used to cause surface chemical changes in pyrite and chalcopyrite, and thus impact their flotation behavior. Pyrite was depressed in the presence of *T. ferrooxidans* - the recovery of was 50% lower than in the absence of any bacteria, and xanthate as collector. The flotability of chalcopyrite and other sulfide minerals were unaffected at natural pH.

The heterotrophic *Paenibacillus polymyxa (P. polymyxa*) bacteria was also studied in the sulfide minerals flotation [13]. A pure strain of *P. polymyxa* and mineral-adapted strains were used to cause surface chemical changes on pyrite and chalcopyrite. The adaptation was achieved by a repeated subculturing of *P. polymyxa,* carried out in the presence of pyrite and chalcopyrite until their growth characteristics became alike to the growth at the nonexistence of mineral. The results from Hallimond tube flotation with xanthate applied as collector reviled that pyrite was depressed when the tests were carried out after interaction with chalcopyrite-adapted *P. polymyxa*. Chalcopyrite was not depressed.

In further studies *P. polymyxa* was adapted also to galena and sphalerite. It was found that for the all mentioned minerals the mineral-adapted cells became more hydrophilic as compared to unadapted cells [14].

Santhiya and coauthors [15,16] studied the influence of cells of *Bacillus polymyxa* (*B. polymyxa*) on sphalerite and galena flotation. Flotation tests showed that galena was nearly entirely depressed after interaction with the cells both in the absence and in the presence of the collector. When collector and activator were added to sphalerite, which was initially interacted with the cells, its floatability was restored at and beyond pH 8.5. The selective flotation tests carried out with a synthetic mixture of galena and sphalerite confirmed that

sphalerite could be preferentially floated in presence of galena, the latter being depressed by the bacterial cells. Selective flocculation tests demonstrated that galena could be flocculated from sphalerite, which was dispersed in the presence of cells of *B. polymyxa* at pH 9–9.5. Adsorption experiments and FTIR spectroscopic data showed that a higher amount of the *B. polymyxa* cells was adsorbed onto galena compared to sphalerite. The adsorption density of the cells onto galena was practically independent of pH while that onto sphalerite was found to constantly decrease with increasing pH. Additionally, bio-dissolution studies carried out exposed the release of lead/zinc species from galena/sphalerite, correspondingly. The biosorption experiments proved interaction of cells of *B. polymyxa* with those metal ions. The highest amount of exopolysaccharides was found in the cells that had interacted with galena, while the cells that had interacted with sphalerite possessed the least. The cells interacted with sphalerite possessed the highest amount of protein while the cells interacted with galena possessed the lowest amount. The adsorption of xanthate onto galena was decreased in the presence of the cells whereas the xanthate adsorption on activated sphalerite was unchanged in the pH range 9-11. The cell surface hydrophobicity tests confirmed that the cells that interacted with sphalerite were more hydrophobic compared to the cells that interacted with galena. The elevated exopolysaccharides and lesser protein contents together with the hydrophilic nature of the cells interacted with galena were considered as the main factors causing the selective flocculation and depression of galena. The higher floatability and dispersion of sphalerite-interacted cells were assigned to the higher amount of protein and lower exopolysaccharides contents leading to bigger hydrophobicity.

Interaction with *B. polymyxa* was also successfully applied for selective separation of pyrite and galena from mixture of the two minerals [17].

Chemical changes on galena and sphalerite surfaces before and after interaction with *Thiobacillus thiooxidans* (*T. thiooxidans*) were studied [18]. The adsorption density of bacterial cells onto the two minerals was found to be independent of pH. FTIR studies showed that adsorption was due to hydrogen bonding between minerals and microbial cells. Higher number of cells was adsorbed onto galena

compared to sphalerite, causing galena depression. The sphalerite was made hydrophobic after interaction with the cells. Thus selective flotation and flocculation was realized leading to separation of galena from sphalerite after bacterial interaction.

Acidithiobacillus ferrooxidans (A. ferrooxidans) (formerly known as *T. thiooxidans*) was studied for its ability to ensure selective separation of pyrite from other associated ferrous sulfides [19]. It was found that due to the interaction with bacterial cells, pyrite was depressed even in the presence of collector (potassium isopropyl xanthate - PIPX) while chalcopyrite showed significant flotability. The separation achieved was significant both at acidic and alkaline pH. This selectivity was observed when the minerals were interacted with both bacterial cells and collector simultaneously. It was found that the initial interaction with collector followed by conditioning with cells improved the flotation of chalcopyrite [20]. Thus, with this sequence of interactions the pyrite recovery was considerably decreased while the recovery of chalcopyrite was above 80%. The bacterial cells were able to effectively depress collector interacted pyrite even when the minerals were conditioned together.

A. ferrooxidans was studied also for its ability to ensure effective separation of arsenopyrite from pyrite [21]. It was found that the adhesion of the bacterium to the surface of arsenopyrite was very slow compared to that to pyrite, causing a difference in surface modification of the minerals after their interaction with cells. *A. ferrooxidans* cells were able to efficiently depress pyrite flotation in presence of collectors, such as PIPX and potassium amyl xanthate. It was found that the flotability of arsenopyrite after conditioning with the cells was not significantly affected and the mineral rendered its good flotability in the presence of the same collectors. The activation of pyrite by copper sulfate was decreased when the minerals were conditioned together, while the copper activated arsenopyrite was able to retain its hydrophobicity in presence of cells due to poor attachment kinetics of cells to the mineral surface, thus resulting in better selectivity.

Further studies of the same authors included chalcopyrite [22]. It was found that the collector was able to render good flotability to chalcopyrite even after interaction with bacterial cells. Thus, by con-

ditioning with the cells and collector prior to flotation, it was possible to successfully depress pyrite from chalcopyrite and arsenopyrite.

Use of *A. ferrooxidans* instead of NaCN as depressant in the flotation of lean lead-zinc ore, bearing pyrite in high amounts, was studied [23]. *A. ferrooxidans* adapted to ore were used. The results showed that pyrite was significantly depressed in both galena and sphalerite concentrates, using *A. ferrooxidans* as depressant.

Completely new research shows that bacterium *A. ferrooxidans* can be used to depress pyrite in seawater flotation of copper sulfide at natural pH [24]. It has been found that biodepression of pyrite was improved by increasing the pH from 4 to 8, with a decrease in recovery from 92% to 36%. The improved depression is assigned to the increased density of bacteria attached on pyrite, from 2.58×10^8 bacteria/g to 1.99×10^9 bacteria/g at pH 4 and 8, respectively. It has been found that the collector produces a smaller increase in the hydrophobicity (contact angle) of pyrite when the mineral is preconditioned with *A. ferrooxidans* than when it is used alone in seawater. This implies that the bacteria prevent the action of the collector. The increased consumption of lime when seawater is used in flotation is avoided.

Leptospirillum ferrooxidans (L. ferrooxidans) is another studied microorganism [25]. The adhesion of *L. ferrooxidans* cells on pyrite and chalcopyrite minerals was studied by using adsorption, zeta-potential and diffuse reflectance FTIR measurements. The FTIR spectra of minerals treated with bacterial cells showed the presence of all the cell functional groups thus proving cells adsorption. The bacterial cells adsorption on chalcopyrite was higher compared with pyrite, which was in agreement with their greater depression effect on chalcopyrite flotation and pronounced flocculation behaviour in comparison with pyrite. The higher affinity of *L. ferrooxidans* to chalcopyrite was assigned to mineral's higher surface defects and consequently higher accessibility of iron available on mineral's surface as an energy source for the bacteria. Hallimond tube flotation tests, carried out under previously established optimum conditions showed that the flotation recoveries of both minerals were decreased in the presence of cells but the depression of chalcopyrite was much higher than that of pyrite. The depression of minerals was found to depend on cell concentration. At significantly higher *L. ferrooxidans*

concentration chalcopyrite showed floatability under the same conditions.

The behaviour of chalcopyrite and pyrrhotite in microflotation tests was studied using pure minerals and mixtures in the presence of *L. ferrooxidans* [26]. The results indicated that for chalcopyrite, the flotation rate significantly increased in the presence of bacteria in the first 10 min and use of thionocarbamate as collector.

B. subtilis was used for selective separation of galena and sphalerite [27]. The experiments showed that sphalerite can be preferentially (with a high selectivity index) floated from galena in the presence of the insoluble fraction of lysed *B. subtilis* cells initially adapted to sphalerite. Thermolysed sphalerite adapted cells showed enhanced selective recovery of sphalerite when compared to the intact sphalerite adapted cells.

Bacillus pumilus SKC-2 and *Alicyclobacillus ferrooxydans* SKC/SAA- 2, capable to produce biosurfactants and oxidize iron and sulfur, were studied with respect to their ability to act as bio-collector or depressant in sulfide bioflotation processes [3]. It was found that both bacteria strains were able to change the surface chemical properties of pyrite due to biosurfactant production and their adhesion on the pyrite surface. It was established that due to their capability of oxidizing iron and sulfur, the bacteria were also usable as depressants. SEM-EDS and FTIR data also proved the ability of the studied bacterial strains to change the pyrite surface properties to more hydrophilic or more hydrophobic in dependence of time of incubation.

Very recently halophilic bacteria have been studied as potential pyrite bio-depressants in Cu-Mo bioflotation [28]. Halophilic bacteria are a group of bacteria adapted to grow well at high salt concentrations (sea water), which are usually detrimental to the growth of bacteria. Halophilic bacteria are also known to produce EPS. Such bacteria are assumed to be beneficial if can be used in flotation processes in regions where sea water is applied in flotation. Microflotation experiments (using a Hallimond tube) have been carried out with pyrite and chalcopyrite with sodium isopropyl xanthate as collector and bacterial cells as depressant of pyrite as substitute for lime. Biodepression of pyrite is observed therefore at the natural pH of sea water (8.00-8.22) when *Halomonas boliviensis*,

Halobacillus sp. and *Halomonas sp*. were used (from around 68% to below 10% depending on the bacterium used.) It was supposed that the mechanism of adhesion to pyrite by halophilic bacteria was hydrophobic in nature (i.e. hydrophobic extracellular moieties interact with mineral's surface and depress it). Chalcopyrite flotation was unaffected under the same conditions as the pyrite flotation and in fact, it was enhanced by *Halobacillus sp*.

Use of microorganism metabolites as flotation reagents

The idea to use not only whole microbial cells in mineral separation but also to gain knowledge about the impact of specific bacterial metabolic products become essential in the beginning of our century. This was evoked by the need to understand the role of extra cellular polysaccharides and proteins in regulating microorganisms' attachment to minerals.

In one of the pioneering works the interaction of galena and sphalerite minerals with the metabolite obtained from *B. polymyxa* has been studied [29]. The carbohydrate component of the metabolite showed the highest adsorption on sphalerite at pH 6-7. For galena the adsorbed quantity increased with pH increase. The adsorbed amount of the bacterial protein on both minerals was reduced with pH increase. It was found that the adsorption affinity of carbohydrate and protein was higher for galena compared to sphalerite. Bioflotation tests exhibited selective depression of galena from its mixture with sphalerite. Entire depression of galena was observed in the pH range 3-11, in the absence or presence of PIPX, following conditioning for 15 min with *B. polymyxa* metabolite. Sphalerite was selectively floated at 3.3 (the natural pH of the metabolite) without application of any collector or activator. Sphalerite was floated in the pH range of 5-10.5 in the presence of $CuSO_4$, PIPX and metabolite. Bioflocculation studies showed that under appropriate conditions galena was selectively flocculated from sphalerite. Co-precipitation tests confirmed complexation of lead and zinc species with the metabolic products that present in the bulk solution.

B. polymyxa was also studied as a reagent in selective separation of pyrite from quartz and calcite via microbiologically induced flotation and flocculation [30]. It was shown that pyrite can be separated from quartz and calcite through either selective

flocculation or flotation after interaction with cells of *B. polymyxa* or bioproteins separated from the bacterial metabolite. This was due to the fact that cells of *B. polymyxa* exhibited higher affinity towards pyrite compared to quartz and calcite and the bacterial cell adsorption onto minerals followed the order pyrite>calcite>quartz. This phenomenon lead to the same order of the minerals' settling after the interaction with bacteria. Similar behavior was observed also with extracellular bacterial proteins. The study showed that through flotation pyrite could be efficiently separated from quartz after interaction with bacterial cells or extracellular bacterial protein. The efficiency of selective separation was increased by addition of small amount of amine collector to enhance the recovery of quartz.

The same authors used successfully cells and metabolic products of *B. polymyxa* in flocculation and flotation to remove chalcopyrite from quartz and calcite [31]. The adsorption studies showed that the cells of *B. polymyxa* exhibited higher affinity towards chalcopyrite and their adsorption density onto this mineral was the highest when compared to quartz and calcite. Extracellular bacterial protein and extracellular bacterial polysaccharides were isolated and their effects on minerals were studied through flocculation and flotation. Selective separation of chalcopyrite from quartz and calcite was realized through interaction with whole cells, as well as with extracellular protein. The protein flocculated chalcopyrite thus facilitating its settling and dispersed quartz thus decreasing its settling rate. It was found that chalcopyrite could be efficiently separated through flotation from quartz, after interaction with bacterial cells or extracellular bacterial protein. Further increase in the efficiency of selective separation was achieved by addition of small amount of amine collector to enhance the quartz recovery. The studies revealed that through microbially induced flocculation and flotation, both pyrite and chalcopyrite could be removed from oxide gangue minerals, like silicates and calcite, leading to their desulphurisation and consequently - environmental protection.

Cells of *P. polymyxa* and their metabolite products were also successfully utilized to separate selectively sphalerite from pyrite, through bio-flocculation and flotation [32]. It was suggested that the effective separation was due to significantly higher adsorption of cells of *P. polymyxa* onto pyrite compared to sphalerite - the phe-

nomenon observed beyond neutral pH range. Pyrite surface became more hydrophilic upon interaction with bacterial cells whereas sphalerite surfaces became hydrophobic at near neutral pH conditions. In addition, flocculation of pyrite and enhanced dispersion of sphalerite were observed in the pH range of 8–9 after interaction with either bacterial cells or extracellular bioproteins. All those phenomena facilitated the flotation selection with xanthate.

The same research group studied the affinity of different purified protein fractions of extracellular bacterial protein (EBP), isolated from *P. polymyxa*, to quartz, pyrite, chalcopyrite, sphalerite and galena [33]. EBP derived from *P. polymyxa* consisted of various kinds of amino acids (protein groups). Different protein fractions were separated from the EBP and characterized through controlled ammonium sulfate precipitation and SDS-PAGE. The obtained protein fractions exhibited varying adsorption capacity towards quartz, pyrite, chalcopyrite, galena and sphalerite. When bacterial cells were grown in the presence of the different minerals, mineral-specific proteins were squeezed. Protein fractions possessing significant affinity towards surfaces of different minerals were isolated and tested. Appropriate use of fractionated protein groups made pyrite and chalcopyrite hydrophilic while quartz, sphalerite and galena exhibited enhanced surface hydrophobicity after biotreatment. In a similar way protein preconditioning led to selective flocculation of pyrite and chalcopyrite, while galena, sphalerite and quartz were dispersed. Consequently, sphalerite, galena and quartz could be selectively separated from pyrite and chalcopyrite through prior interaction with bacterial proteins.

The above-described studies were limited mainly to use of pure cultures in microflotation tests. Govender and coauthors presented the first study demonstrating on a laboratory scale the potential use of free EPS, extracted from mixed bioleaching microbial consortia, as a viable flotation agent for bioflotation of sulphide minerals [34]. Analyses of the EPS extracted from different bioleach systems (operating at temperatures between 35 and 70 °C and using pyrite, sphalerite and chalcopyrite) showed that the EPS were composed mainly of carbohydrates, proteins and uronic acids. The EPS influence on the flotability of chalcopyrite was determined. The results from the micro-flotation tests indicated that free EPS were more

efficient as a flotation agent than the cells with bound EPS. Tests using free EPS achieved a chalcopyrite recovery of 77% when chalcopyrite was floated alone and 70% during the flotation of a mixture of pure chalcopyrite and pure pyrite, while the recovery was 32% when only sodium isobutyl xanthate was used. The results obtained suggested that free EPS, extracted from mixed bioleaching microbial consortia, can be used as flotation agent during bioflotation of sulphide minerals.

A selective flotation of sphalerite from a sphalerite-galena mineral mixture has been realized using the cellular components of *Bacillus circulans* [35]. Some bacterial species are able to secrete nucleic acids onto their cell surface. This double stranded DNA (dsDNA) has been designated as extracellular DNA to distinguish it from genomic DNA that is intracellular. Thermolysed genomic DNA is designated as single-stranded DNA (ssDNA). Experiments showed that flotation recovery of sphalerite over 80 % could be achieved by using ssDNA as a bio-collector in an anionic buffer. However, when pure ssDNA is used in the flotation of sphalerite from a sphalerite-galena mixture, the flotation recovery was reduced significantly compared to that of sphalerite alone. Even an addition of several fold excess of ssDNA did not enhance the flotation, possibly due to the preferential adsorption of ssDNA on galena than on sphalerite. However, it was found that in the presence of a large excess of other polyanionic species in the thermolysed cell-free supernatant (which consists of all the cellular non-DNA components), ssDNA was left free to bind with sphalerite and facilitate its flotation, while the non-DNA components (teichoic acids and polysaccharides) preferentially bound with galena. Polysaccharides have been found to possess a depressing action in the sulfide minerals flotation. The studies pointed out that the ratio of the bio-depressant and bio-collector likely regulated the flotation recoveries of sphalerite from the mixture of minerals.

A selective separation of sphalerite from a mixture sphalerite/ galena was realized by applying cells and extracellular secretions of *Bacillus megaterium* (*B. megaterium)* after the microorganism adaptation to these minerals [36]. The highest flotation recovery of sphalerite (with a selectivity index (SI) of 24.5) was achieved at using the extracellular secretions acquired after thermolysis of bacterial cells adapted to sphalerite. The amount of extracellular proteins secreted by sphalerite-adapted cells was bigger than that of galena-

adapted or unadapted cells. The hydrophobic proteins present in the soluble fraction of the thermolysed sphalerite-adapted cells facilitated the flotation selectivity of sphalerite. It was found that the protein profile for the unadapted and mineral-adapted cells differred distinctly leading to variation in the yield and nature of EPS. The changes induced in the bacterial cell wall components after adaptation to sphalerite or galena with respect to the contents of phosphate, uronic acid and acetylated sugars were quantified. The role of the dissolved metal ions from the minerals as well as that of the constituents of extracellular secretions (such as phosphate, e-DNA, and surface proteins) in modulating the surface charge of the bacterial cells and of the minerals under study was confirmed by applying different enzymatic analyses. It was discovered that zinc was mainly bioaccumulated, while lead was mainly biosorbed onto the bacterial cells. The sphalerite-adapted cells showed an increase in the phosphate content, while the glucosamine content was increased after adaptation to galena. The availability of additional molecular weight protein fractions as well as the higher amount of extracellular proteins and phosphate content, secreted after adaptation to sphalerite, compared to galena, facilitated the selective separation of sphalerite from galena.

The same authors studied also the role of extracellular constituents of *B. megaterium* as flotation biocollector in sulphide minerals separation [37]. Experiments on the flotation of the individual minerals showed that sphalerite was preferentially floated compared to galena. A SI of 11.7 was attained in the presence of the soluble fraction of the thermolysed cells. It was higher than that found with the intact cells (SI of 6.5) and the insoluble fraction of the thermolysed cells (SI of 9.6). The results of the enzymatic treatment tests revealed that extracellular DNA played a very important role in the selective flotation of sphalerite. It was found that the ssDNA had a higher biocollector capacity in comparison to the dsDNA, leading to higher flotation efficiency. About 95 % recovery of sphalerite could be achieved from the mineral mixture by the combined use of the ssDNA and the non-DNA components of the bacterial cells, resulting to SI of 19.1.

Cells and metabolic products of *Bacillus subtilis* (*B. subtilis*) were used in microbially-induced flocculation and flotation in an attempt

to separate pyrite from galena [38]. Bacterial cells showed an enhanced affinity towards pyrite compared to galena at neutral pH range. Extracellular (EP) and intracellular bacterial proteins were isolated from *B. subtilis* before and after interaction with the minerals and their profiles were established. It was found that the presence of galena during bacterial growth encouraged increased generation of EP that rendered hydrophobic the galena surfaces. The presence of pyrite resulted in enhanced production of exopolysaccharides that render pyrite more hydrophilic. Similar changes in galena and pyrite surfaces were observed also after interaction with cell free extract and EP. Such microbially-induced mineral surface chemical changes can be used to achieve a selective separation through selective flotation/flocculation of minerals without use of any conventional toxic collectors. The settling rate of pyrite was significantly increased after interaction with bacterial cells due to its nearly complete flocculation caused by the adsorption of exopolysaccharides. Settling rates for pyrite were found to be 85, 74 and 72% as a result of interaction with cells, cell free extract and EP respectively. Dispersion of galena was facilitated after interaction with cells, cell free extract and EP due to predominant adsorption of secreted hydrophobic proteins. Galena flotation yield was 92, 73 and 75% after conditioning with bacterial cells, cell free extract and EP respectively. Significant depression of pyrite was observed under similar conditions. Mineral-specific proteins (EP ones) were expressed when bacterial cells were grown in presence of galena. Generation and separation of such mineral-specific proteins points the way for development of suitable bioreagents for selective mineral separation.

Conclusions

Bioflotation of sulfide minerals has been developing during the recent 20-30 years as an answer of the scientific community to the need to extract metals in an environmentally friendly manner. Flotation separation of minerals like pyrite, sphalerite, galena, chalcopyrite, arsenopyrite, molybdenite, pyrrhotite, etc. has been studied. *A. ferrooxidans* (formerly known as *T. Ferrooxidans), T. thiooxidans, P. polymyxa, B. polymyxa, L. Ferrooxidans, B. Megaterium,* and *B. Subtilis* have been and are among the most used microorganisms in the sulfides ores beneficiation. Recently bacteria showing dual action

of biosurfactants and biodepressants, such as *Bacillus pumilus* SKC-2 and *Alicyclobacillus ferrooxydans* and halophilic bacteria (*Halomonas boliviensis*, *Halobacillus sp*. and *Halomonas sp.*) have also been investigated. Studies have included not only whole cells but also cells' parts and / or metabolites.

The interaction between microorganisms and minerals have been studied by means of different methods such as adsorption and flocculation, contact angle, electro-kinetic and zeta-potential measurements, SEM-EDS and FTIR spectroscopy, SDS-PAGE, etc.

Different mechanisms of microbial interaction with minerals' surface leading to essential changes in the surface characteristics of the minerals have been proposed but generally they may be classified as (*a*) microbial cells attachment to minerals surfaces, (*b*) proceeding of oxidation-reduction reactions on the interface mineral / microorganisms, (*c*) adsorption and/or chemical reactions with microbial metabolite products.

In order to go insight of the proposed mechanisms and to be able to direct them in a preferred course, systematic studies on both sides (biochemical and geochemical) of the mineral - bacteria interactions have to be carried out. Different scientific questions have to be answered, such as [39]: (a) Do and how bacteria exploit the energetic heterogeneities of the minerals surface to adhere and/or oxidize/reduce the mineral? (*b*) what is the influence of the mineral particle on the microbe adhesion and the rate of the biotically induced reactions with the participation of the mineral surface? (*c*) How the potential of the mineral (regulated either by pH or potentiostatically) influences the attachment of the whole bacteria cells or of the metabolites and the eventual heterogeneous charge transfer reaction? (*d*) How the presence of the bacteria and the potential of the mineral impact the collector adsorption, its forms and surface equilibria?

Influence of conditions for microorganisms' cultivation on their properties as bioreagents has been studied. It has been established that growth of bacterial cells in the presence of various minerals, i.e., bacteria preadaptation, may involve changes in cell morphology, qualitative and quantitative changes in the secretion of proteins and polysaccharides and surface changes in their levels. Such changes could be used to bring about increased selectivity and desired

mineral separation from multi-mineral mixtures. Currently these procedures are still expensive for an industrial application but represent an interesting perspective. This could pave the way for synthesis of specific environmentally friendly flotation reagents that can replace existing flotation agents.

Influence of operating parameters (such as pulp density, pH and temperature, concentration of bioreagents and their nature, addition to the system of typical flotation reagents, such as xanthate or activators) has been widely studied. However, to date, microbial flotation tests have been primarily carried out at laboratory scale by applying mainly modified Hallimond tube or Microflot tests. Experiments on larger scale are indispensably needed.

Nevertheless, there are more than enough evidences on the possibility to expand the eco-friendly bioflotation process in mineral processing industry.

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