Bio-Beneficiation: Relevance to Mineral Processing

Hadi Abdollahi, Sina Ghassa, Ehsan Ebrahimi, Amirhossein Mohammadzadeh, Morteza Shahbaznejad and Roozbeh Saneie

Abstract

The increasing demand for various minerals and the limited high-grade mineral resources, concerning economic and environmental issues, has led to increasing research attention to the bio-beneficiation of low-grade mineral resources. Bio-beneficiation can be defined as employing microorganisms (including bacteria, fungi, algae, and yeast) in mineral processing and related industries. The high potential of microorganisms and their metabolites, especially extracellular polymeric substances (EPS), has been substantiated in bio-beneficiation processes. The bio-beneficiation is generally divided into two main categories: including bioflotation and bioflocculation. The bioflotation uses the microorganisms and their products (such as biomass and EPS) as flotation reagents. Microorganisms and their biomass can be applied as collectors, depressants, dispersants, and frothers in minerals floatation. Bioflocculation is another application of biotechnology in minerals processing. Almost all mineral processing techniques should be carried out in a wet environment. Therefore, dewatering and water recycling are essential steps in mineral processing plants. Microorganisms can be used as flocculants to decrease the dewatering time or minerals separation with high efficiency. Comprehensive information about flocculation, classification of flocculants, and the application of EPS as bioflocculant for selective bioflocculation of different minerals are provided in this chapter. The mechanism of the bioflocculation process and the effect of different parameters on the bioflocculation of various minerals have been investigated. Moreover, recent research on the application of bioflocculation in the selective separation or removal of minerals using different types of microorganisms has been briefly reviewed. Employing microorganisms for both bioflotation and bioflocculation can be a great strategy to save the environment and decrease process costs.

Keywords

Bioflocculation · Bioflotation · Mineral processing · Bio-beneficiation

1 Introduction

Nowadays, microorganisms are widely used in bioleaching of sulfide minerals, recycling mineral processing plant tailings, and treating hazardous wastes (Johnson & Hallberg, 2005; Olson et al., 2003). With rising demand for minerals and declining high-grade resources, researchers have increasingly focused on the beneficiation of low-grade mineral resources to meet the demand of global markets. Also, considering the resources and economic issues, today's industrial approach is further decreasing the effect on the environment of mineral processing activities, resulting in considerable developments in the application of biotechnology in mineral processing procedures. In bio-beneficiation, which includes bioflocculation and bioflotation processes, microorganisms and their metabolites may be used as collectors, regulators, flocculants, depressants, and surface modifiers for the flotation or flocculation of selected minerals (Chapelle, 2000). The role of microorganisms and their metabolites in the bio-beneficiation industry for the selective removal or separation of gangue or valuable minerals has been fully established. The effectiveness of selective separation of minerals in the refining processes has been substantiated. Fundamentally, in bio-beneficiation, the role of microorganisms is to alter the physicochemical properties of minerals' surfaces to

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S. Panda et al. (eds.), *Biotechnological Innovations in the Mineral-Metal Industry*, Advances in Science, Technology & Innovation, https://doi.org/10.1007/978-3-031-43625-3_6

achieve the highest separation efficiency (Groudev, 1987). Although biohydrometallurgical methods have been extensively explored and commercialized for ores like low-grade sulfides—particularly copper, uranium, lead, and zinc—as well as precious metals like gold and silver, bio-beneficiation methods are still evolving and require deeper investigations. (Hanumantha Rao & Forssberg, 2001; Holmes & Smith, 1995; Smith et al., 1991). The primary purpose of this chapter is to present recent reports on the applications of bio-beneficiation in mineral processing, address the mechanism of bioflocculation and bioflotation, and the factors affecting the process.

2 Absorption and Modification of Mineral Surfaces by Microorganisms

The absorption of microorganisms on solid surfaces is essential in nature for their growth. In most natural and artificial systems, the metabolic activity of microorganisms, especially bacteria, is associated with solid surfaces such as soil, minerals, and tissues. Surface biofilms can be employed in minerals' bioprocessing (Somasundaran et al., 2005). Production of metabolites by microorganisms and accumulation of their EPS is of significant importance in microorganism-mineral interactions (Deo & Natarajan, 1998). Interactions of microorganisms and minerals have the following results:

- Alteration in the chemical properties of the minerals' surface
- Reaction of bacterial cells and metabolic products with minerals
- Production of surface-active chemicals.

These interactions lead to surface modification, alteration of minerals' surface properties, selective dissolution of different components from the mineral's surface, and bioaccumulation of soluble metal ions. In addition to metabolic products, cell components of microorganisms such as bacterial cell walls and membranes can participate in these surface reactions (Somasundaran et al., 2005).

3 Bio-Beneficiation Mechanisms

Attachment between microorganisms and mineral particles is due to biological interactions like growth, metabolism, EPS secretion (Grossart et al., 2006; Simon et al., 2002), which may lead to the minerals particles' surface modifications (Kiørboe, 2001; Tang et al., 2014). In particular, through bioflocculation processes, the application of EPS has been proved (More et al., 2014; Sheng et al., 2010; Tang & Maggi, 2016). In fact, in the process of bioflocculation, the extracellular polymeric substances excreted by microorganisms are used as bioflocculants. Investigations have shown that increasing the content of EPS increases the capacity of bioflocculation (Badireddy et al., 2010). The collision between particles is the most important subject in particle aggregation; only a successful collision can cause flocs to agglomerate together. This effective collision can be created by different forces, such as electrostatic forces, Van der Waals forces, hydrophilic and hydrophobic interactions, and the polymer bridging through chemical, physical, and biological processes. Since the role of EPS and microorganisms in bioflocculation is not understood well yet, bioaccumulation mechanisms in bioflocculation remain unclear (Lai et al., 2018). However, several mechanical theories, including Derjaguin, Landau, Verwey, and Overbeek (DLVO) and extended DLVO (XDLVO) theory, cationic bridging theory (Sobeck & Higgins, 2002), and polymer bridging or adsorption bridging theory (Bolto & Gregory, 2007), have been suggested as the most important possible mechanisms for bio-beneficiation.

3.1 DLVO and XDLVO Theory

The (DLVO) theory was proposed to define the stabilization of colloidal suspensions for the first time (Derjaguin et al., 1987; Lai et al., 2018). According to this theory, the flocculation of dispersed particles in water can be explained quantitatively. The total surface energy of a particle is calculated by considering Lifshitz–Van der Waals (W^{LW}) and electric double-layer energy (W^{WE}) (Rijnaarts et al., 1999), as shown in (Fig. 1a) and (Fig. 1b). Particle aggregation occurs when particles defeat a potential barrier. DLVO theory can also define the mechanism of bioflocculation. The total interaction energy (W^{TOT}) from extended DLVO (XDLVO) theory can be represented by Eq. (1) (Chia et al., 2011; Lai et al., 2018):

$$W^{TOT} = W^{LW} + W^{EL} + W^{AB}, \tag{1}$$

where W^{EL} is a practical function of the distance between two particles with an amplitude of the thickness of electric double layers, W^{LW} decreases with increasing distance between particles, and W^{AB} is the energy due to Lewis base acid interactions, and based on thermodynamic parameters, it can be attractive or repulsive (Lai et al., 2018). Liu et al. (2008) examined the formulas of this theory, the sizes, and contributions of W^{EL}, W^{LW}, and W^{AB} in detail (Liu et al., 2008). Their research shows that Van der Waals attraction forces dominate at small distances between particles, while at middle distances, electric double-layer repulsion and Lewis acid–base interactions may predominate. Either

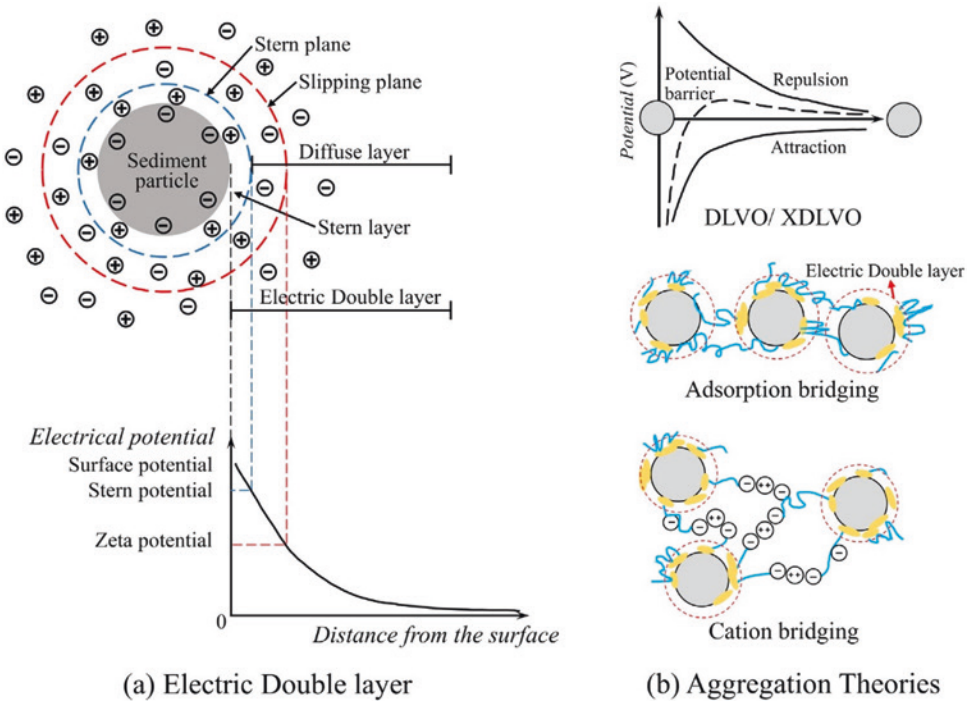

Fig. 1 **a** Electrical potential of double layer, **b** Three possible mechanisms for bio-beneficiation (Lai et al., 2018)

DLVO or XDLVO theories were used extensively in bio-flocculation and bioflotation (Li et al., 2012, 2014; Liu et al., 2007, 2008, 2010).

3.2 Polymer Bridging (Adsorption Bridging) Theory

Polymers are heavy molecules with long chains in their structure that have many binding sites for attachment to particles. EPSs produced by microorganisms are known as natural biopolymers. In general, polymers can adsorb on the surface of particles' by formation loops and tails, improving the attachment between particles and bridging them together (Bolto & Gregory, 2007; Joon & Schlautman, 2015). As shown in (Fig. 1b), this mechanism is known as polymer bridging or the adsorption bridging in bio-beneficiation. Hocking et al. (1999) suggested that the longer polymers enable to interact with more than one particle. Generally, the flocs formed by the polymer bridging mechanism are stronger than the flocs produced by other mechanisms because, in the case of polymer bridging, the flocs are highly flexible that do not easily disappear in the face of shear stress (Gregory & Barany, 2011). The degree of the covered surface area by the adsorbed polymer is an important factor in the bridging chance because, in the polymer bridging mechanism, the particles connected by the bridge must have an unoccupied surface to connect the polymer parts (Biggs et al., 2000). At high adsorption levels, the available adsorption sites will be insufficient, while at low adsorption levels, the connection of the formed bridges to each other will be insufficient, both of which are unsuitable for the polymer bridging adsorption flocculation mechanism (Lai et al., 2018). Bolto and Gregory (2007) indicated that the optimal bridging flocculation occurs at a level lower than saturation surface coverage of the adsorbed polymers (Bolto & Gregory, 2007).

3.3 Cation Bridging Theory

This theory was applied in by Tezuka (1969) primarily (Tezuka, 1969). Interactions between EPS and solid particles play a critical role in the bioflocculation of colloids. The bioflocculation process is also influenced by the surface characteristics of EPS. In the compound of EPS, there are many functional groups with negative charges (Sheng et al., 2010). Consequently, the presence of cations (solid particles with positive surface charge) can act as a bridge that connects functional groups in EPS, leading to particle aggregation, resulting in bioflocculation of solids, as presented in (Fig. 1b) (Sobeck & Higgins, 2002; Wilén et al., 2008). Based on the cation bridging theory, the effectiveness of bioflocculation depends on EPS molecular structure, the weight of the molecule, the surface charge of EPS, and the positive charge density on a solid surface, so the presence of polyvalent cations can increase the efficiency of bioflocculation (Lai et al., 2018). DLVO and polyvalent cation bridging theories can examine the impact of cations on bioflocculation. The electric double layers are compacted

when there is a high concentration of cations in a solution (Mietta, 2010) and thus, based on DLVO theory, increase the flocculation capacity. The polyvalent cation performs like a link between the negative charge of biopolymer and solid particles, thereby improving flocculation (Higgins & Novak, 1997a; Sobeck & Higgins, 2002). However, the actual effects of cations, especially the polyvalent cation in this mechanism, are not recognized well yet, and more profound researches are required. In general, the mechanism of the bioflocculation process is complex but can be described by XDLVO theories, adsorption bridging, and cationic bridging theory. Nevertheless, each has its limitations.

XDLVO theory evaluates the energy between particles, but the zeta potential of particles surface and charge redistribution by EPS are not factored into the equation in the XDLVO theory. In contrast, these factors could significantly impact the bioflocculation process (Sobeck & Higgins, 2002). The adsorption action between particles and EPS can be described by adsorption bridging theory, and the cation bridging theory clarifies why polyvalent cations can help bioflocculation. However, these two theories are conceptual models, and there is a limit to the quantitative evaluation of these theories (Lai et al., 2018).

3.4 Extracellular Polymeric Substances (EPS)

The metabolism of microorganisms leads to the secretion of extracellular polymeric substances (EPS). Wingender et al. (1999) proposed the "EPS" as a comprehensive and common term to denote various biomolecules produced by microbes, which include proteins, nucleic acids, polysaccharides, lipoproteins, and other biopolymer substances (Wingender et al., 1999). Wingender et al. (1999) suggested that all extracellular polymers that do not bind directly to peptidoglycan must be considered as EPS (Wingender et al., 1999). EPS are mainly macromolecular with high molecular weights (Liu & Fang, 2003; Wingender et al., 1999). Outside of the cell, EPS is categorized into two groups (Laspidou, 2002; Wingender et al., 1999):

1. Bound EPS, which includes coatings, capsular polymers, compact gels, polymers, and attached organic compounds
2. Soluble EPS, which contains soluble macromolecules, colloids, and sludge.

Bound EPS is completely limited to cells, while soluble EPS binding to cells is weak and easily dissolved in solution. In general, these two types of EPS can be detached by centrifugation. The bound EPS structure is generally represented by double layers (Fig. 2b) (Lin et al., 2014). The inner layer comprises tightly bound EPS (TB-EPSs), which have a special shape and are firmly placed adjacent to the cell surface, and the outer layer contains loosely bound EPS (LB-EPSs).

Macromolecules and polymer compounds produced by microorganisms are used as bioflocculants in bioflocculation processes. The volume of EPS production by microorganisms is mainly affected by the growth stage of microbes. Different food sources such as carbon, nitrogen, and other nutrients and environmental factors affect EPS production. Operating factors such as pH, temperature, metal ions, and oxygen content of the culture medium (Nichols et al., 2005; Sheng et al., 2010) also directly affect the amount of EPS and bioflocculants produced by microorganisms (Ye et al., 2011). Badireddy et al. (2010) suggested that bioflocculation capacity improves with the secretion of EPS. In the exponential growth stage of the microorganism, due to the low level of EPS content, the bioflocculation capacity is low, while the bioflocculation capacity significantly increases in the growth stabilization stage, with increasing EPS secretion (Badireddy et al., 2010). More et al. (2014) suggested that the optimal amount of EPS excreted by several microorganisms in the bioflocculation of kaolinite was in the range of 1–5000 mg/l depending on the composition

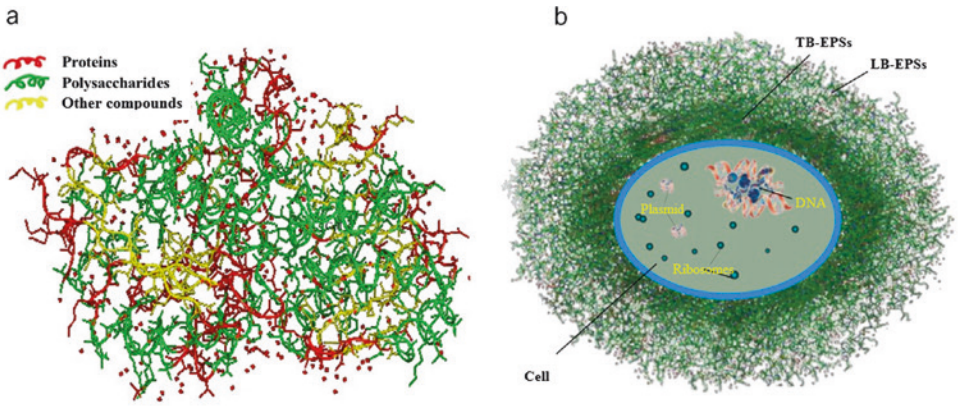

Fig. 2 **a** EPS compounds, **b** Different types of EPS sections: tightly bound EPS (TB-EPS), loosely bound EPS (LB-EPS), spatial patterns, and their position (Lin et al., 2014)

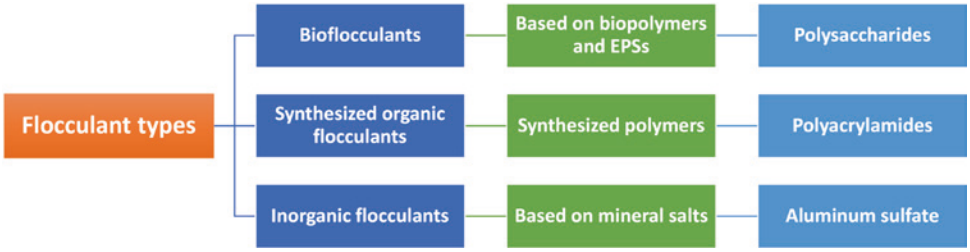

Fig. 3 Flocculant types with examples

of the EPS. It was indicated that the bioflocculation capacity should be determined by the EPS dosage and the characteristics of the EPS compounds (More et al., 2014; Tsuneda et al., 2003). Carbohydrates and proteins are regularly the main constituents of EPS (Fig. 2). (Fr/olund et al., 1995; Frølund et al., 1996). In addition, nucleic acids, humic substances, uronic acids, and some other components may be found in EPS (D'Abzac et al., 2010a, 2010b; Dignac et al., 1998; Frølund et al., 1996).

Numerous functional groups exist in the EPS compounds that significantly alter the surface properties of the flocs. The macromolecules in the EPS compound can interact with ultrafine particles by surface adsorption, hydrogen bonding, complex formation in surface, hydrophilic and hydrophobic interactions, protein-polysaccharide interactions, and electrostatic interaction, all of which indicate specific roles of EPS functional groups in bioflocculation (Higgins & Novak, 1997b; Parikh & Chorover, 2006; Wilén et al., 2003). The phosphate, carboxylate, and amine functional groups are involved in bacterial adsorption to the surface of the mineral (Parikh & Chorover, 2006). According to Lurie et al. (1997), humic compounds and other functional groups with extremely negative charge densities might cause upper repulsive forces between molecules (Lurie & Rebhun, 1997). In a study, Badireddy et al. (2010) showed that functional groups include carbohydrates and alcohols, which improve the bioflocculation process, while functional groups comprise carboxylate, carbonyl, acetal and in some cases reduce bioflocculation efficiency. Therefore, functional groups can play an essential role in bioflocculation by altering particle surface characteristics and floc interactions (Badireddy et al., 2010).

4 Bioflocculation

4.1 Flocculation

With the increasing complexity of ore compositions, excessive crushing and grinding of these resources for liberation in mineral processing plants have led to the formation of large volumes of fine particles. One of the most common methods for the separation of fine, very fine, and colloidal minerals is their selective flocculation using flocculants. The fine particles that must be separated from the liquid might vary in size, morphology, and chemical nature. Colloidal particles are larger than molecules, but they are so small that the gravitational forces are far less than the repulsive electric force between them. Under such conditions, the stability of Brownian motion keeps the particles suspended and dispersed in the liquid. Flocculation occurs when predominantly high molecular weight polymeric compounds cause particle aggregation and floc formation. The particular compounds used for this purpose are called "flocculants" (Hughes, 1990). Flocculants are divided into three main categories (Fig. 3) (Salehizadeh & Shojaosadati, 2001): (a) inorganic flocculants, such as aluminum sulfate salts, polyaluminum chloride, iron chloride, and iron sulfate, (b) synthesized organic flocculants, such as polyacrylamide and its derivatives, and polyethylenimine, (c) biological or bioflocculants, such as chitosan, sodium alginate, gelatin, and flocculants based on extracellular polymeric substances (EPS); bioflocculants are produced by microorganisms and generally containing polysaccharides, proteins, glycoproteins, and amino acids.

4.2 Biotechnology for Flocculation

Most synthetic flocculants are significantly toxic and harmful to animals, sea organisms, and humans (Campbell, 2002). For example, acrylamide monomer, a small amount of which can cause contamination, is carcinogenic and dangerous to humans (Gao et al., 2009). It was proved that aluminum salts might cause Alzheimer's in humans (Salehizadeh & Shojaosadati, 2001). Due to the mentioned concerns, today, the bioflocculants are preferable for flocculation process in various industrial plants such as water and wastewater treatment, decolorization of solutions, mining and mineral processing, pharmacy and serology, food productions, and many industries because most bioflocculants are non-toxic and fully environmentally friendly (Chen et al., 2014; Salehizadeh & Shojaosadati, 2001; Virk-Baker et al., 2014; Zhuang et al., 2012). Different types of microorganisms, including bacteria, fungi, microalgae, and their metabolites, have been applied as bioflocculants with different structures and properties. These biopolymers are active materials that are biodegradable and environmentally

friendly with significant flocculation capabilities (Ben et al., 2018). Microbial bioflocculants produced during the growth of bacteria are different in the composition of polysaccharides, proteins, cellulose, sugar, and polyaminoacids. The type and amount of nutrients and culture medium conditions for the growth of microorganisms have an essential effect on the amount of bioflocculant produced. Thus, the amount of bioflocculant produced is directly affected by carbon and nitrogen sources in the culture medium, operating temperature, pH, inoculation ratio, and aeration rate (Salehizadeh & Yan, 2014). The absorption of bacteria on the minerals is very important for the surface modification of minerals in bio-beneficiation processes. Most bioflocculation and bioflotation studies have shown that the initial absorption of bacteria is mainly controlled by the physicochemical of the bacterial cell surface properties, which are associated with the arrangement of protein membranes and polysaccharides (Raichur et al., 1996).

4.3 Application of Microorganisms in Bioflocculation

4.3.1 Paenibacillus polymyxa

Paenibacillus polymyxa is a neutrophilic, heterotrophic, gram-positive, periflagellated bacterium found with some oxide minerals. It releases EPS which contains polysaccharides, proteins, organic acids such as formic acid, acetic acid, and oxalic acids (Murphy, 1952). The application of *P. polymyxa* to the beneficiation of different minerals has been reported in several works.

Oxide Minerals

Interaction between *Paenibacillus polymyxa* with oxide minerals causes chemical changes on minerals and bacteria surface (Deo & Natarajan, 1998; Vijayalakshmi & Raichur, 2002), for instance, based on Deo et al. (1998) investigations, after biotreatment by *P. polymyxa*, quartz and kaolinite became hydrophobic, while hematite and corundum converted hydrophilic. The EPS content of bacteria causes surface-chemical changes. As shown in (Fig. 4), adhesion of biopolymers extracted from *P. polymyxa* on minerals surfaces follows this order: extracellular proteins show high adhesion on kaolinite and quartz, while extracellular polysaccharides show high adhesion on corundum and hematite surface (Deo & Natarajan, 1998).

Extracted bioflocculants from bacterial EPS such as polysaccharides can selectively flocculate fine particles of hematite and corundum through a polymer bridging mechanism. Based on (Table 1), the settling rates of quartz and kaolinite saw to be reduced. In contrast, the settling rate of hematite and corundum enhanced due to interaction with *P. polymyxa* cells or the metabolite (Deo & Natarajan, 1998). Thus, selective bio-beneficiation of oxide minerals like hematite and corundum was accomplished by selective dispersion of kaolinite and quartz particles.

Coal

Paenibacillus polymyxa also is used to remove ash and quartz from coal samples by bioflocculation (Vijayalakshmi & Raichur, 2002). Vijayalakshmi et al. (2002) investigated the application of the *P. polymyxa* to remove ash from coal samples. As presented in (Fig. 5), the high ash coal and the bacterium have a very similar ZPC in the pH range of 2–3, and both show a negative charge over a wide range of pH values. The maximum adhesion of *P. polymyxa* on the coal samples occurs at pH 2, which is very close to the ZPC, while the minimum number of bacteria is attached to the quartz surface at this pH (Vijayalakshmi & Raichur, 2002). Settling studies in the presence of the *P. polymyxa* proved that coal samples flocculated at a higher rate than quartz, thus representative selective separation of coal from quartz by bioflocculation (Fig. 6). Similar results were demonstrated for removing ash from coal samples. According to results, almost 55–60% of the ash was removed from coal samples in a bioflocculation using *P. polymyxa* (Vijayalakshmi & Raichur, 2002).

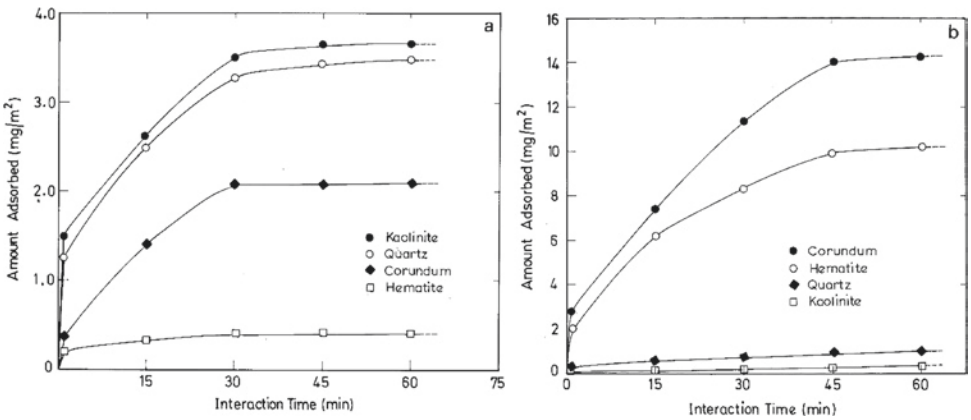

Fig. 4 *P. polymyxa* adsorption density **a** protein and **b** polysaccharides on the surface of different minerals (Deo & Natarajan, 1998)

Table 1 Bioflocculation of minerals interacted with cells and metabolite of *P. polymyxa* (5 min at 5% pulp density, size <38 μm) (Deo & Natarajan, 1998)

Mineral	pH	Weight settled(%) in 1 min		
		Control	Bacterial cells	Metabolite
Quartz	4–5	58	10	17.1
	7	45	18	–
	12	38	11	–
Kaolinite	4–5	85	40	1.65
	7	70	20	–
	12	62	18	–
Corundum	4–5	85	97	90
	7	82	96	–
	12	70	90	–
Hematite	4–5	84	99	92
	7	80	95	–
	12	70	98.8	–

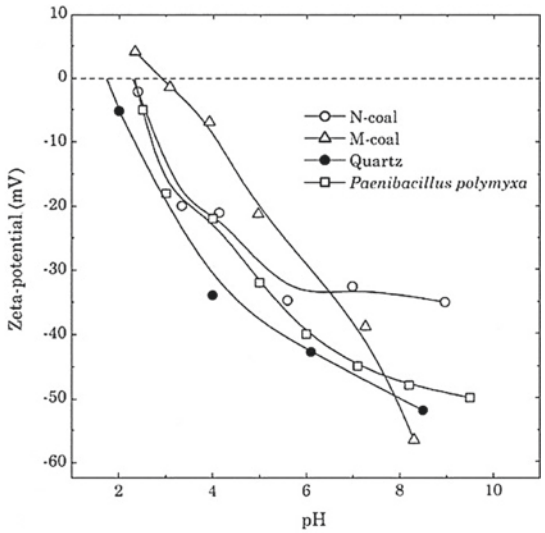

Fig. 5 Zeta potential of coal samples and the *P. polymyxa* (Vijayalakshmi & Raichur, 2002)

Sulfide Minerals

Santhiya et al. (2002) studied on adsorption of *P. polymyxa* and its EPSs on galena and sphalerite. Considering their results, selective bioflocculation and separation of galena from sphalerite in the presence of *P. polymyxa* at pH = 9–9.5 proved (Santhiya et al., 2001a, 2002). Adsorption studies showed that the amount of adsorbed bacteria cells onto galena against sphalerite was significant, and the adsorption density of the *P. polymyxa* cells onto the galena surface was not affected by pH. In contrast, the adsorption of the bacterial cells onto the sphalerite surface reduced with a rise in the amount of pH (Santhiya et al., 2001a). For galena-interacted cells, polysaccharides were the dominant bioflocculant. In contrast, for sphalerite-interacted cells, the protein was the main bioflocculation agent (Santhiya et al., 2002). The surface hydrophobicity investigations approved that the sphalerite was more hydrophobic while the galena was more hydrophilic after interaction with *P. polymyxa* cells. Thus, in addition to the hydrophilic character of galena-interacted cells, high polysaccharides and fewer protein concentrations could be the main reason for selective flocculation and separation of galena from sphalerite. Almost 95% of galena is separated from sphalerite through selective bioflocculation presented in (Table 2) (Santhiya et al., 2001a).

Patra et al. (2004) studied on adsorption of *P. polymyxa* and its EPSs on pyrite and sphalerite. According to their studies, selective bioflocculation and separation of pyrite from sphalerite were verified after interaction with either bacterial cells or extracellular proteins in the pH range of 8–9 (Patra & Natarajan, 2004a). Adsorption investigations showed that the amount of adsorbed bacteria cells onto the pyrite surface was significant compared to sphalerite. Patra et al. (2006) also examined selective bioflocculation and pyrite removal from galena in the presence of *P. polymyxa*, and it EPSs in the pH range of 6–7 after interaction with either bacterial cells or extracellular proteins. Adsorption investigations showed that the adsorption of bacterial cells onto pyrite was significant against galena. Selective flocculation results at pH = 8.5–9 verified that 91% of pyrite could be selectively separated from galena (Patra & Natarajan, 2006).

Patra & Natarajan (2004a, b) achieved selective separation of chalcopyrite and pyrite from oxide gangue minerals like quartz and calcite through bioflocculation after interaction with cells of *P. polymyxa* or proteins separated from its EPS (Patra & Natarajan, 2003, 2004b). Based on the results, *P. polymyxa* cells had a great affinity for chalcopyrite compared to quartz. Adhesion of *P. polymyxa* follows this order (Patra & Natarajan, 2004b): Chalcopyrite >> Calcite > Quartz.

In the case of quartz biotreatment, the author indicated that extracellular bacterial protein could be responsible for hydrophobicity increasing in quartz surface, which helped dispersion of quartz, while polysaccharides promoted quartz flocculation. They found that the dispersion performance of calcite interacted with *P. polymyxa* cells was similar to quartz. The author proved that extracellular protein was a predominant factor in the selective separation of quartz from chalcopyrite.

4.3.2 *Acidithiobacillus* Group

Acidithiobacillus group are gram-negative, acidophilic, mesophilic, bacteria such as *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans,* which are widely used in bioleaching and bio-beneficiation of

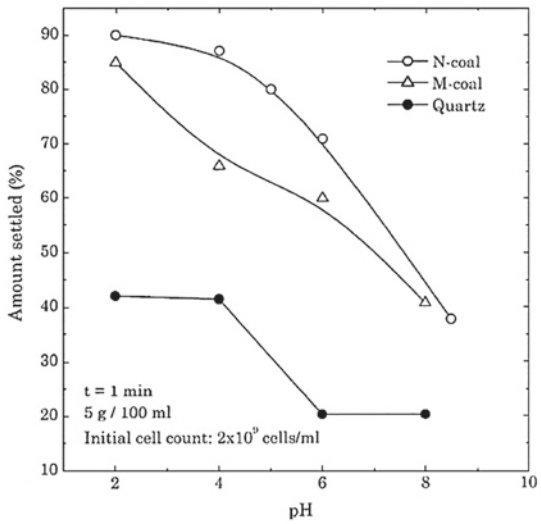

Fig. 6 Bioflocculation of coal and quartz by *P. polymyxa* (Vijayalakshmi & Raichur, 2002)

Table 2 Selective bioflocculation sphalerite from Galena using *P. polymyxa* cells (Santhiya et al., 2001a)

Experimental conditions	ZnS (%)		PbS (%)	
	Dispersed	Flocculated	Dispersed	Flocculated
Blank at pH 9–9.5	70.5	27.8	27.0	71.7
	70.3	29.5	27.0	72.3
With *Bacillus polymyxa* at pH 9–9.5	93.7	4.9	5.6	94.2
	94.3	4.6	5.8	94.9

sulfide minerals. Santhiya et al. (2000) examined selective bioflocculation and separation of galena from sphalerite by *Acidithiobacillus thiooxidans* (Santhiya et al., 2000). According to their studies, in the presence of *Acidithiobacillus thiooxidans*, 95% of galena flocculated while sphalerite dispersed (Santhiya et al., 2000). The effect of pH on the settling of galena and sphalerite is shown in (Fig. 7). In the presence of *Acidithiobacillus thiooxidans* cells, sphalerite sedimentation reduced from about 40% at pH = 3 to about 5% in the pH range of 10–11. In contrast, the percentage of galena flocculation improved from about 20% at pH = 2.5 to about 95% at pH = 11. Thus, galena can be separated from sphalerite in the presence of *Acidithiobacillus thiooxidans* in the pH range of 9–11 (Santhiya et al., 2000).

Natarajan et al. (2003) studied the selective separation of pyrite, chalcopyrite, and sulfur from quartz through selective flocculation/dispersion after biotreatment with *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* (Natarajan & Das, 2003). According to Natarajan et al. (2003), after biotreatment with *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* cells, the

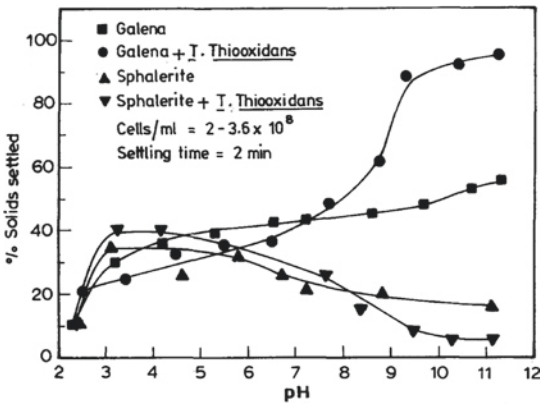

Fig. 7 Bioflocculation of galena and sphalerite as a function of pH in the absence and presence of *Acidithiobacillus thiooxidans* (Santhiya et al., 2000)

settling rates of pyrite and elemental sulfur improved. As a result, selective flocculation and separation of pyrite and sulfur from quartz were demonstrated (Natarajan & Das, 2003).

4.3.3 Bacillus subtilis

Bacillus subtilis is a gram-positive, neutrophilic, aerobic, periflagellated, and capsulated bacterium usually found in soil (Brock et al., 2006). The application of *Bacillus subtilis* for beneficiation of different minerals has been described in several approaches.

Iron Removal (Iron Oxides) from Kaolin Clays

Poorni and Natarajan (2013) used *Bacillus subtilis* and its EPSs to remove iron oxides such as hematite from kaolinite via selective bioflocculation. As the results indicated, in the presence of hematite, *Bacillus subtilis* secretion of extracellular polysaccharides (ECP) increased while kaolinite promoted the secretion of extracellular proteins (EP). Moreover, extracellular polysaccharides showed great affinity to the hematite surface, which caused the zeta potential of hematite to shift in the negative direction. In contrast, extracellular proteins showed great affinity to the kaolinite surface, which affected the zeta potential of kaolinite in a positive direction (Fig. 8). After biotreatment with *Bacillus subtilis*, hematite converted to more hydrophilic, and kaolinite exhibited higher surface hydrophobicity. As shown in (Table 3), almost 90% of iron could be removed from the kaolin clays after biotreatment with the ECP extracted from *Bacillus subtilis* (Poorni & Natarajan, 2013a).

Pyrite Removal from Galena

Sarvamangala et al. (2013) studied *Bacillus subtilis* and its EPSs to separate pyrite from galena. Adsorption investigations showed that the amount of adsorbed bacteria cells onto pyrite was significant compared to galena (Fig. 9).

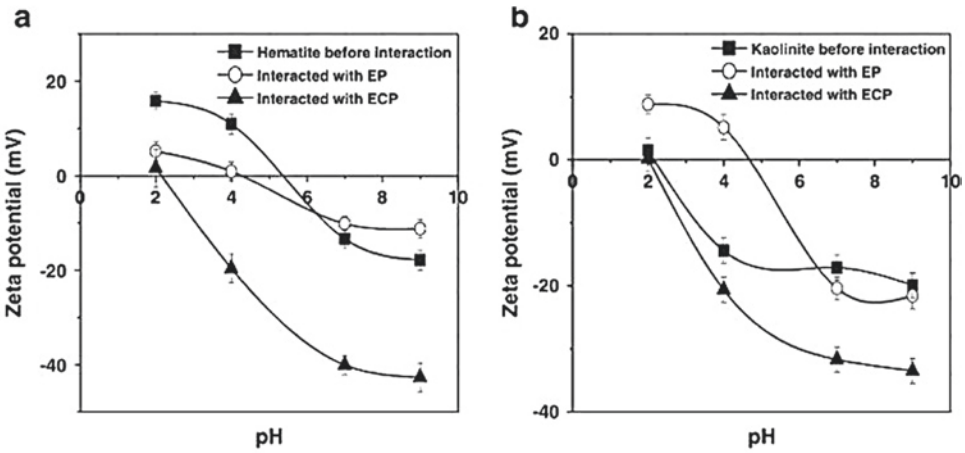

Fig. 8 Effect of extracellular protein (EP) and extracellular polysaccharide (ECP) of *Bacillus subtilis* on zeta potential of **a** hematite and **b** kaolinite (Poorni & Natarajan, 2013a)

Table 3 Settling behavior of hematite and kaolinite under different conditions in the presence of *Bacillus subtilis* (Poorni & Natarajan, 2013a)

Interaction conditions	Percent settled %			
	Hematite alone	Kaolinite alone	1:1 mineral mixture	
			Hematite	Kaolinite
Control (no bacterial interaction)	60	45	65	50
Solution-grown cells	70	35	75	40
Cell-free extract from solution-grown cells	75	20	70	35
Hematite-grown cells	95	12	95	10
Cell-free extract from hematite-grown cells	80	20	65	30
Kaolinite-grown cells	70	04	60	10
Cell-free extract from kaolinite-grown cells	60	20	70	30
EP from hematite-grown cells	80	50	82	25
EP from kaolinite-grown cells	75	08	80	10
ECP from hematite-grown cells	98	05	95	20
ECP from kaolinite-grown cells	90	10	75	20

After biotreatment with *Bacillus subtilis,* pyrite converted to more hydrophilic, and galena showed higher surface hydrophobicity. Furthermore, in the presence of galena, *Bacillus subtilis* secreted hydrophobic proteins, which enhanced dispersion of galena, while in the presence of pyrite; the bacteria secreted polysaccharides that enhanced the settling of pyrite. Therefore, selective bioflocculation and separation of pyrite from galena are evidenced in (Table 4) (Sarvamangala et al., 2013).

4.3.4 Bacillus licheniformis

Bacillus licheniformis is a rod-shaped, gram-positive, aerobic bacterium that usually can be isolated from natural sources, such as soil (Waldeck et al., 2006).

Quartz Removal from Kaolinite

Ghashoghchi et al. (2017) used *Bacillus licheniformis* cells and extracellular polymeric substances (EPS) to bioflocculation of kaolin and quartz. It was indicated that extracellular protein secreted from *B. licheniformis* was more effective in quartz agglomeration, while extracellular polysaccharides secreted from *B. licheniformis* were more effective in kaolin flocculation. In the best state, the sedimentation of kaolin increased by 40% using bacterial cells and metabolites at pH = 7 and 3. Also, the sedimentation of quartz using the same bioflocculants was improved by about 50% at pH = 1–3 (Ghashoghchi et al., 2017). In (2019), Hosseini et al. (2020) also investigated the bioflocculation of quartz and kaolinite in the presence of *Bacillus*

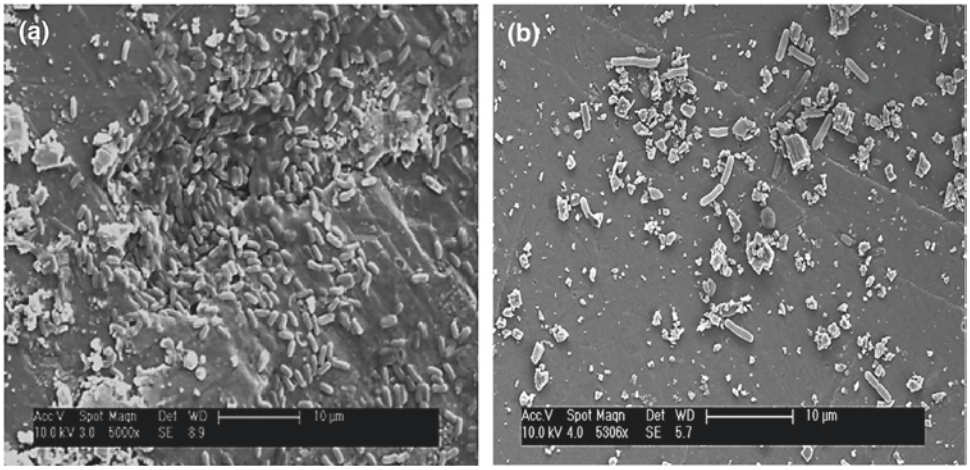

Fig. 9 SEM images of *B. subtilis* attached to **a** pyrite and **b** galena (Sarvamangala et al., 2013)

licheniformis cells and metabolites in basic conditions. The adsorption of bacterial cells and EPS on kaolinite was three times higher than adsorption on quartz. In the presence of bacterial cells and EPS, sedimentation of kaolinite was less than quartz at most of the pH values, and the selective bioflocculation occurred at pH = 11 and 12. They indicated that in the presence of *Bacillus licheniformis* cells, 98.3% of the kaolinite was selectively separated from quartz (Hosseini et al., 2020).

Removal of Iron Oxides from Kaolinite and Quartz

Differential bioflocculation in the presence of *Bacillus licheniformis* and extracellular polymeric substances (EPS) was investigated by Hosseini et al. (2019) to separate kaolinite and quartz from hematite and goethite (Fig. 10). The best separation of kaolinite and quartz from iron oxides was observed at pH = 7. Based on this study, the application of bacterial protein was the best bioflocculant to remove hematite from kaolinite. However, the recovery of kaolinite was low. Thus, the authors suggested bacterial polysaccharides as bioflocculant, which resulted in 77.6% separation of hematite from the mixture with a kaolinite recovery of about 59.4%, respectively (Hosseini et al., 2019).

Table 4 Bioflocculation of pyrite and galena using *Bacillus subtilis* (Sarvamangala et al., 2013)

Interaction conditions	Percent settled %			
	Pyrite alone	Galena alone	1:1 mineral mixture	
			Pyrite	Galena
No bacterial interaction (control)	60	45	65	50
After interaction with cells	70	35	75	40
After interaction with cell-free metabolite	75	20	70	35
After interaction with EP (35 mg/g)	95	12	95	10

4.3.5 Other Microorganisms

In 1994, Schneiderl et al. (1994) studied the flocculation of hematite by the *Candida parapsilosis yeast*. The results revealed that *C. parapsilosis* and substances released from it improve flocculation of fine hematite suspensions (Schneider et al., 1994). Then in 1996, Raichur et al. (1996) used *Mycobacterium phlei* as a bioflocculant to remove sulfur and ash contents of coal by flotation and flocculation (Raichur et al., 1996). In 1999, Haas et al. (1999) investigated the application of *Corynebacterium xerosis* bacterium for fine fluorite flocculation. They indicated that *C. xerosis* cells improved the aggregation of fine fluorite particles (Haas et al., 1999). Padukone and Natarajan (2011) utilized *Saccharomyces cerevisiae yeast* and its metabolites for selective bioflocculation of quartz from calcite. Yeast cells showed a better affinity to the calcite surface. After biotreatment, the quartz surface was more hydrophobic, and calcite was hydrophilic. So this situation facilitated the selective separation of quartz from calcite (Padukone & Natarajan, 2011). The application of *Bacillus firmus* and its metabolites to bioflocculation of clay minerals was investigated by Karthiga Devi and Natarajan (2015). They also showed bioflocculant produced from *Bacillus firmus* could be used as absorbents to remove toxic Cr (VI) ions from aqueous solutions (Karthiga Devi & Natarajan, 2015). Selim & Rostom (2018) used *Bacillus cereus* to bioflocculation and separation of hematite from its mixture with silica. *Bacillus cereus* showed a higher affinity to hematite mineral surface compared with silica surface (Selim & Rostom, 2018). Camarate et al. (2021) used *Candida stellata yeast* for the selective separation of ultrafine hematite from quartz via the bioflocculation process (Camarate et al., 2021). The application of various microorganisms in minerals bioflocculation is reviewed in (Table 5).

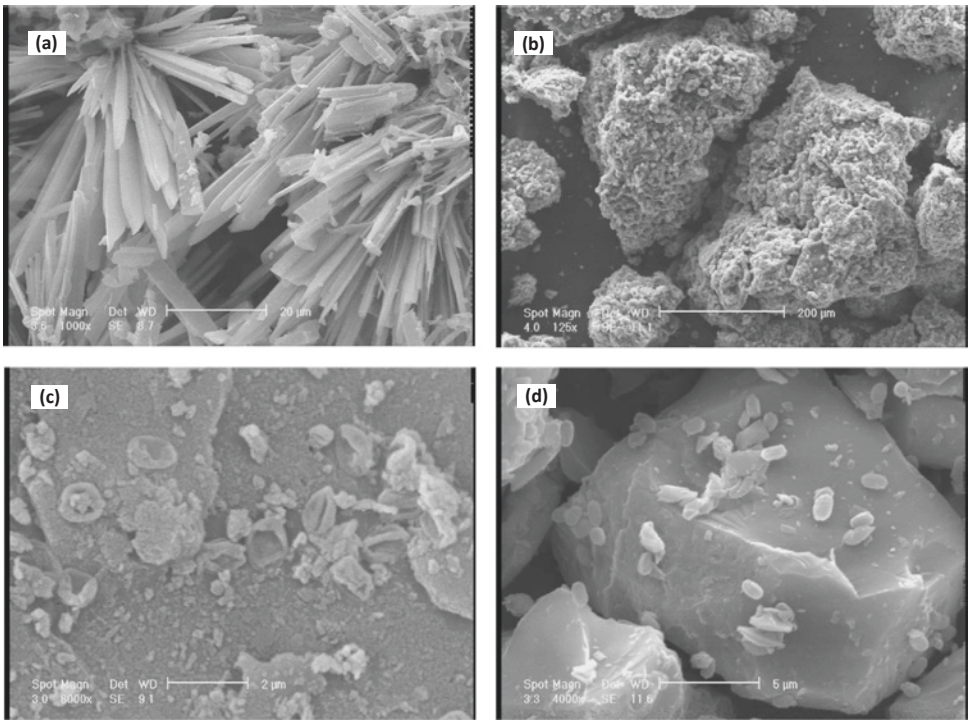

Fig. 10 **a** Polysaccharide crystal formation on hematite. **b** Formation of kaolinite flocs after biotreatment with extracellular proteins. Attachment of *Bacillus licheniformis* on **c** goethite and **d** quartz particles (Hosseini et al., 2019)

5 Bioflotation

Froth flotation is used widely in mineral processing for selective separation of valuable minerals from gangue minerals. In this method, minerals with hydrophobic surfaces attach to the air bubble and selectively separate. The process is carried out in a wet environment, and different chemicals (including depressants, pH-adjusting reagents, dispersants, activators, collectors, and frothers) are used to modify the minerals surfaces (Gaudin, 1975).

To separate minerals with high recovery, conditioning should be carried out in several steps, and the following steps should be passed:

1. A mineral–water slurry with a pulp density of 25 to 35 should be made.
2. The pulp pH should be adjusted using acids and bases.
3. The dispersants are added to the pulp for spreading the particles.
4. Depressants are added to the pulp to make selected particles hydrophilic. The activators also can be added to the pulp in this stage. Activators are used for modifying the mineral's surface for better collectors' adsorption.
5. The collectors were added to the pulp to make targets minerals surface hydrophobic.
6. The frother is added to the pulp to produce stable bubbles.

After conditioning, hydrophobic minerals will be attached to the air bubbles due to Archimedes' force.

In the bioflotation method, microorganisms act as one of the mentioned chemical reagents. In other words, microorganisms can be used as depressants, dispersants, collectors, frothers, and even flocculants. Various microorganisms, such as autotrophic or heterotrophic bacteria, fungi, yeasts, and algae, can be used for this purpose. The applications of these microorganisms will be reviewed in the following sections.

5.1 Application of Microorganisms in Bioflotation

5.1.1 Acidithiobacillus ferrooxidans

Acidithiobacillus ferrooxidans is a gram-negative chemolithoautotrophic bacteria that has been used successfully in bioleaching and bio-beneficiation processes in recent years. This bacterium derives its energy from the oxidation of (Fe^{+2}) to (Fe^{3+}) and sulfur (S^0) to sulfuric acid (H_2SO_4) (Chandraprabha et al., 2004a; Dwyer et al., 2012; Pecina-Treviño et al., 2012).

For the first time, Misra et al. (1996) examined *Acidithiobacillus ferrooxidans* as pyrite depressants for coal desulfurization. This microorganism was used as an

Table 5 Application of microorganisms in minerals bioflocculation

Microorganism	Mineral bioflocculation	Bioflocculant	Optimal pH bioflocculation	Contact time (min)	Bioflocculation efficiency	References
Paenibacillus polymyxa	High-ash coals	Protein–Polysaccharide and 50 ml suspension bacterium	2–4	25–30	90%	Vijayalakshmi and Raichur (2002)
	Hematite, corundum, calcite from quartz	Protein–Polysaccharide	Corundum: 7 Hematite: 7 Calcite: 12	5	90–99.6%	Deo and Natarajan (1997)
	Hematite, corundum, from quartz and kaolinite	Protein–Polysaccharide	Corundum: 4–5 Hematite: 7 kaolinite:12	5	Corundum–quartz: 90–97% Hematite–quartz: 50–90% Hematite–kaolinite: 30–55%	Deo and Natarajan (1998)
	Hematite, corundum from quartz	Protein–Polysaccharide	4–7	15	–	Deo and Natarajan (1999)
	Sphalerite and galena	Protein–Polysaccharide	9–9.5	15	Galena: 94.9% Sphalerite: 4.6%	Santhiya et al., (2001a)
	Chalcopyrite from quartz	25 mg/l of extracellular bacterial proteins	8	20	95% of quartz could be removed 81% of calcite could be removed	Patra and Natarajan (2004b)
Acidithiobacillus thiooxidans	Galena and sphalerite	Bacterial cells ($2–3 \times 10^8$ cell/ml)	11	120	95% of galena flocculated	Santhiya et al., (2000)
Acidithiobacillus ferrooxidans	Pyrite, chalcopyrite from quartz	Bacterial cells (1.8×10^8 cell/ml)	2	2	90% of quartz separation from pyrite, chalcopyrite is possible	Somasundaran et al., (2005)
Bacillus subtilis	Fine coal	Bacterial suspensions	2	5	99%	Vijayalakshmi and Raichur (2003)
	Pyrite	100 ml of bacterial cell suspension	6.0–7.5	3	98%	Sarvamangala et al., (2013)
					85%	
					80%	
	Galena				18%	
					26%	
					27%	
	Pyrite from galena				85% for pyrite and 21% for galena	
					74% for pyrite and 25% for galena	
					72% for pyrite and 26% for galena	
	Hematite and kaolinite	100 ml of bacterial cell suspension	7	5	95% for hematite and 10% for kaolinite	Poorni and Natarajan (2013a)
	Hematite and kaolinite	100 ml of bacterial cell suspension	7–8	5	95% for hematite and 20% for kaolinite	Poorni and Natarajan (2013b)
	Hematite and kaolinite	100 ml of bacterial cell suspension	7	5	95% for hematite and 15% for kaolinite	Poorni and Natarajan (2014)

(continued)

Table 5 (continued)

Microorganism	Mineral bioflocculation	Bioflocculant	Optimal pH bioflocculation	Contact time (min)	Bioflocculation efficiency	References
Bacillus licheniformis	Quartz	20% v/v of bacterial metabolite	1	8	92%	Ghashoghchi et al., (2017)
	Kaolinite	0.24 mg/ml polysaccharide. 20 g/l solid concentration	9	8	45%	
	Hematite and kaolin	0.24 mg/mL extracellular polysaccharides	7	8	59.4%	Hosseini et al., (2019)
	Hematite and quartz				69.2%	
	Goethite and kaolin				56.2%	
	Goethite and quartz				64.8%	
	Hematite	Bacterial cells (3.6×10^8 cell/ml)	5	8	100%	Sadeghizadeh et al., (2017)
	Goethite	Bacterial cells (3.6×10^8 cell/ml)	7	8	95%	
	Kaolinite from quartz	Bacterial cells (19×10^8 cell/ml)	12	6	98.3%	Hosseini et al., (2020)
	Kaolinite clays suspension	6ml from a 5 g/L bioflocculant solution	7 – 7.2	5	96%	Karthiga Devi and Natarajan (2015)
	Cr^{4+} removal	6ml from a 2 g/L bioflocculant solution	7	60–120	85%	
Bacillus firmus	Cr^{4+} removal	6ml from a 2 g/L bioflocculant solution	7	60–120	77%	
	Kaolinite clays suspension	6ml from a 5 g/L bioflocculant solution	9	5	92%	
Bacillus cereus	Hematite from quartz	10 ml of bacterial solution (10×10^{11} cell/ml). 10 g/L solid concentration	4.5	10	82%	Selim and Rostom (2018)
Corynebacterium xerosis	Fine fluorite particles	40 mg/L bacterial cell concentration	7	3	96%	Haas et al., (1999)
Yeast Cell Rupture	Hematite	cell as biofloculant (60 kg/t) or soluble cell fraction (1.2 kg/t)	7	3	98%	Schneider et al., (1994)
Mycobacterium phlei	Fine coal	250 ppm of bacteria solution	3.5	3	93%	Raichur et al., (1996)
Saccharomyces cerevisiae	1:1 mixture of quartz and calcite	100 ml of the metabolite from quartz-grown cells	7	3	92% for calcite and 21% for quartz	Padukone and Natarajan (2011)

alternative for cyanide that is extremely toxic. Bacteria had changed the surface properties of pyrite by attaching it to this mineral and making it hydrophilic. This research showed that the pyrite floatability using sodium isopropyl xanthate (PIX) collector was reduced from above 90% to less than 45% after bacterial treatment by increasing pH from 1 to 7 which means that pyrite can be depressed with *Acidithiobacillus ferrooxidans* at low pHs. Furthermore, the results indicated that pyrite depression is dependent on the bacterial counts and type and concentration of salts in culture media (Misra et al., 1996).

Amini et al. (2009a, b) also studied the effect of this type of bacteria on pyrite depression in coal flotation and compared results with sodium cyanide (Amini et al., 2009a). Again, the results showed that bacteria have a higher ability to depress the pyrite than sodium cyanide. The pyrite recovery decreased to less than 14% after ore treatment with *Acidithiobacillus ferrooxidans* (Amini et al., 2009b).

Nagaoka et al. (1999) conducted experiments on the *Acidithiobacillus ferrooxidans* depression ability on sulfide minerals, including pyrite (FeS_2), molybdenite (MoS_2), chalcocite (Cu_2S), millerite (NiS), and galena (PbS). They stated that in the absence of bacteria, all sulfide minerals showed high floatability. After bacterial contact with minerals surfaces, the floatability rate of pyrite decreased dramatically from 96 to 19%. The bacterial treatment also affected the flotation of millet and galena by reducing these minerals recoveries from 96 to 84% and 91% to 82%, respectively. On the other hand, bacterial treatment had almost no effect on the flotation of chalcocite and molybdenite. Since the ability of *Acidithiobacillus ferrooxidans* to inhibit pyrite flotation was confirmed, bioflotation was employed to separate pyrite from a mixture of minerals. The results once again demonstrated that pyrite could be successfully depressed after bacterial treatment (Nagaoka et al., 1999).

In a series of research, Chandraprabha et al. (2004) investigated the effect of *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* on floatability pyrite, chalcopyrite, and arsenopyrite (Chandraprabha & Natarajan, 2006; Chandraprabha et al., 2004a, 2004b, 2005). In 2004, they stated that bacterial cells showed a greater tendency to bind with pyrite compared to arsenopyrite. The binding kinetics of *Acidithiobacillus ferrooxidans* on pyrite was high, and it reached equilibrium within 15 min, while the existence of insoluble arsenic on the surface of arsenopyrite prevented the initial binding of cells. They also examined the selective separation of pyrite and chalcopyrite in this research. In the kinetics and binding studies of *Acidithiobacillus ferrooxidans*, it was observed that the binding kinetics were fast for both pyrite and chalcopyrite and equilibrated in about 15 min. However, the binding rate for pyrite was higher. Moreover, the interaction of all three minerals with the cells caused the isoelectric points (IEP) of the minerals to reach higher pH values. However, this change was much more significant for pyrite than arsenopyrite and chalcopyrite. After minerals interaction with bacteria, the bacterial cell surface charges and minerals IEP showed similar changes, which were more significant for the pyrite reciprocal cells. In addition, selective separation of pyrite from arsenopyrite was carried out using potassium isopropyl xanthate as a collector at natural and acidic pH. The results stated that pyrite was depressed by sodium isopropyl xanthate as the collector after 5 min contact with bacterial cells. In similar conditions, there was no significant change in arsenopyrite recovery after interaction with bacterial cells for 5 min, in the presence of sodium isopropyl xanthate and copper sulfate ($CuSO_4$) as the activator. They stated that copper ions increase the recovery of both pyrite and arsenopyrite minerals; however, flotation improvement is more significant for arsenopyrite. This occurs because As^{3+} forms a stable arsenide complex with Cu^{2+} and Cu^{3+}, while Fe^{2+} and Fe^{3+} cannot form a stable formation as arsenide. In addition, the galvanic effect between pyrite and arsenopyrite reduces the adsorption of xanthates on the pyrite surface and increases on arsenopyrite, which improves the separation process between these two minerals. The results also showed that the *Acidithiobacillus ferrooxidans* could selectively remove pyrite from a mixture of pyrite and chalcopyrite. When pyrite and chalcopyrite are contacted separately with bacterial cells, pyrite was depressed, but chalcopyrite showed different behaviors in the flotation process using the xanthate (PIPX) collector. While if both minerals have interacted with bacteria simultaneously, the dissolution of copper from chalcopyrite activates the pyrite surface and disrupts the selective separation process. The dissolution of chalcopyrite in the presence and absence of cells and collectors was also investigated. The results indicated that the concentration of copper in the flotation environment was higher when pyrite and chalcopyrite were treated simultaneously. This clearly shows that the copper ions which were released from chalcopyrite migrate to the pyrite surface and activate it (Chandraprabha & Natarajan, 2006; Chandraprabha et al., 2004a, 2004b, 2005).

Hosseini et al. (2005) used *Acidithiobacillus ferrooxidans* for the bioflotation of two sulfide copper ores (sample A with higher copper grade and less iron and sample B with lower copper grade and more iron) as well as pyrite and chalcopyrite. According to the results, in all samples except chalcopyrite, the binding of *Acidithiobacillus ferrooxidans* increased with increasing cell numbers. The highest binding rate was for pyrite, then the B-sulfide ore sample, A-sulfide ore sample, and then chalcopyrite. Therefore, it was evident that the *Acidithiobacillus thiooxidans* is not interested in binding to copper sulfide while it binds selectively to pyrite. They said that because bacteria get their energy from the oxidation of iron and sulfur, they can be adsorbed on the

surface of pyrite and prevent the adsorption of xanthate collectors. Bacteria do not absorb chalcopyrite due to the presence of copper, which is toxic to bacteria, and therefore, the collector can be absorbed on it and make it hydrophobic (Hosseini et al., 2005).

Rao et al. (1992) also showed that *Acidithiobacillus ferrooxidans* change the surface's chemical and flotation behavior of non-ferrous sulfides (sphalerite and galena) by direct microbial adhesion on the mineral surface. The initial cell concentration and duration of mineral interaction were the most effective mineral flotation parameters. The formation of insoluble lead sulfate causes galena to be depressed therefore not be floated, while in sphalerite, the formed zinc sulfate is soluble and does not depress. Only in high concentration may be the sphalerite depression occur. In addition, selective bioflotation of lead and zinc sulfides can be achieved with *Acidithiobacillus ferrooxidans* under acidic conditions (pH = 2) (Rao et al., 1992).

The pyrite depression in the bioflotation of high-grade pyrite and low-grade lead–zinc ore by *Acidithiobacillus ferrooxidans* was done by Mehrabani et al. (2010). In their research, the concentration effect of four parameters of PAX (collector), copper sulfate (activator), and bacteria and sodium cyanide (depressants) was investigated in three different levels. The optimal points of the process were to minimize the flotation rate of pyrite, which was achieved in four experiments. In these experiments, the concentration of collector and frother was lowest. In the results of these experiments, in the presence of bacteria, the recovery of pyrite decreased from 38.11 to 23.52%.

In comparison, the recovery of sphalerite increased from 65.91 to 74.03% and zinc grade from 15.22 to 20.84%. Also, under optimal conditions, the results obtained for pyrite recovery when using bacteria and sodium cyanide as depressants were similar. However, when using bacteria, the zinc grade increased by 3%, and the iron grade decreased by 4% (Mehrabani et al., 2010, 2011).

In 2018 and 2020, San Martin et al. studied the effect of *Acidithiobacillus ferrooxidans* on pyrite recovery in seawater. In the bacterial preparation of pyrite with *Acidithiobacillus ferrooxidans* for 15 min, at pH > 4, pyrite recovered in fresh water, pyrite decreased at pH > 8 in fresh. Thus, *Acidithiobacillus ferrooxidans* can act as a pyrite depressant even when inhibited by bacterial activity. At the same time, in seawater (pH = 8), pyrite is depressed but does not affect molybdenite or chalcopyrite (San Martín et al., 2018, 2020).

5.1.2 Acidithiobacillus thiooxidans

Acidithiobacillus thiooxidans is a gram-negative acidophilic mesophile and, along with *Acidithiobacillus ferrooxidans,* is the most-used bacteria for bioleaching. This bacterium is characterized as a sulfur-oxidizing bacterium (Jerez, 2019; Johnson, 2009). *Acidithiobacillus thiooxidans* produces sulfuric acid with a sulfur-oxidizing with sulfur-oxidizing enzymes and sulfite-oxidizing enzymes with sulfite as the critical intermediate (Chandraprabha & Natarajan, 2006), according to the Eqs. (2) and (3):

$$S^0 + O_2 + H_2O \rightarrow H_2SO_3 \tag{2}$$

$$H_2SO_3 + \frac{1}{2}O_2 \rightarrow H_2SO_4. \tag{3}$$

Since *Acidithiobacillus thiooxidans* utilize elemental sulfur or reduced sulfur, it can alter the surface of sulfide minerals. Three researchers reported using these bacteria for flotation of galena, sphalerite, pyrite, and chalcopyrite (Chandraprabha & Natarajan, 2006; Santhiya et al., 2000, 2001b).

Chandraprabha and Natarajan (2006) showed that the isoelectric point (IEP) for pure pyrite was standing at pH 3.25, and at the lower pHs, the electronegative character decreased. However, after bacterial treatment for 1 h and 12 h, IEP increased to 3.5 and 4.2, respectively. On the other hand, IEP for pure chalcopyrite IEP was on pH 2.4. Moreover, it could be shifted to 3 and 3.4 after 1 and 12 h interaction with bacteria, respectively. Adhesion kinetics of bacteria on chalcopyrite and pyrite surface was similar, and its equilibrium was reached after 80 min. However, the cell concentration on the pyrite surface was more than chalcopyrite (6.125×10^8 cells/ml compared to 6.25×10^8 cells/ml). In micro-flotation tests, recovery of both minerals was dropped after bacterial treatment. However, pyrite depression was more significant. While the recovery of pyrite flotation using 1 mM PIPX was 41% after bacterial treatment, the chalcopyrite recovery was 76% (Chandraprabha & Natarajan, 2006). In differential flotation tests with pyrite: chalcopyrite ratio of 1:1, the pyrite and chalcopyrite recoveries were 41% and 69%, respectively, after bacterial treatment and flotation at pH = 4.5, using 0.5 mM PIPX as the collector. The recoveries improved to 32% and 72% by increasing the pH to 6.5. Nevertheless, better separation was achieved by changing the conditioning sequence (addition of collector followed by interaction with cells). Thus, recoveries for pyrite and chalcopyrite were improved to 21% and 86%, respectively, at pH 4.5 and 19.3%, and 84.6% at pH 6.5 (Chandraprabha & Natarajan, 2006).

Santhiya et al. (2000) studied bacterial cell attachment on galena and sphalerite surfaces in three different pH (acidic, neutralized, and basic). The observations indicated that more cells were attached to the galena surface in comparison with sphalerite (about 10^9 cells/ml for galena compared to 5×10^7 for sphalerite). This difference in attachment concentration resulted from different solubility of these minerals in an oxidation acidic environment which is made in the presence of acidophilic microorganisms. The highest absorbed cell in

minerals surface obtained with a bacterial count in culture media was 10^9 cells/ml. Electrostudies were performed at pHs between 2 and 2.5 for 1 h and 24 h bacterial treatment. Both minerals before interaction had an isoelectric point at pH = 2.2. After 1 h and 24 h interactions between minerals and bacteria, sphalerite IEPs shifted to pH = 2.6 and pH = 3.2, and galena IEPs shifted to pH = 3.1 and pH = 3.6, respectively, which means that bacterial treatment causes a more significant shift in galena IEP. As mentioned, these changes occurred due to the attachment of more bacterial cells to the galena surface. Microflotation tests were performed using PIPX as the collector and $CuSO_4$ as an activator for sphalerite. The result showed galena recovery is higher than 95% for all pHs and sphalerite recovery is higher than 90% in the pH range of 4.5–8. After 2 h interactions of minerals with cells at the pH of 2–2.5, the recovery of sphalerite stays at 90%, while galena gets fully depressed. In (1:1) differential flotation tests, both mineral recoveries stayed above 95% without cell interactions. However, after 2 h cell interactions at pH = 2–2.5, sphalerite recovery was higher than 90%, while most of the galena depressed (~90%) (Santhiya et al., 2000).

Santhiya et al. (2001) investigated galena IEP at pH = ~2.2. After bacterial treatments for 1 h and 24 h, the IEP shifted to pH = 3.0 and pH = 3.5, respectively. While IEP for pure sphalerite was ~2.3, it was shifted to 2.6 and 3.3, after 1 h and 24 h interaction with cells. Adsorption of bacteria on galena and sphalerite surfaces was measured for 24 h. The results showed that after 1 h, equilibrium is reached. The optimum adsorbed cell for galena was significantly higher than sphalerite, and it had more change in IEP after interaction with cells. It was interpreted that the sphalerite is more soluble in an oxidation environment than galena, making attachment more difficult. In differential tests, it was reported that galena and sphalerite recovery reached 95% and 98% in the presence of collector and activator, respectively. However, galena and sphalerite recovery stayed at 31% and 94% after bacterial treatment. In the absence of collector and activator recovery, 94% of galena were depressed, while 94% of sphalerite recovered, which shows the high potential of *Acidithiobacillus thiooxidans* for the separation of sphalerite and galena with high efficiency (Santhiya et al., 2001b).

5.1.3 Aspergillus niger

Aspergillus niger is heterotrophic fungi that produce large amounts of organic acids such as citric, gluconic, and oxalic acids. These products are able to complex and mobilize metals from non-sulfide minerals. As the tolerance of this microorganism is high and can produce critical lixiviants, they became one of the essential fungi in the bioleaching of metals and bioprocessing of minerals (Donati & Sand, 2007; Muddanna & Baral, 2019).

Gawel et al. (1997) investigated the potential of *Aspergillus niger* for magnesite depression in the presence of sodium oleate as the collector. The effects of bacterial pretreatment in 1, 7, and 14 days were studied. The results indicated that by increasing the bio-pretreatment time, the adsorption of sodium oleate on the magnesite surface decreased, and flotation recovery dropped. With 14 days of bio-pretreatment, recovery dropped from about 55% to 35%. This decrease probably happened because active surface sites were blocked by *Aspergillus niger* products (Gawel et al., 1997).

5.1.4 Bacillus subtilis

Bacillus subtilis is a mesophilic bacterium and is able to tolerate extreme conditions as it can form a protective endospore (Vasanthakumar et al., 2017). The produced EPS affects the aqueous phase conformation and adsorb on the mineral's surface. The adsorption would alter substrata's physicochemical properties (Sarvamangala et al., 2013).

Bacillus subtilis can be employed to decrease the sulfur and ash content in coal (Abdel-Khalek & El-Midany, 2013; El-Midany & Abdel-Khalek, 2014). Abdel-Khalek and El-Midany reduced coal ash and sulfur content from 6.65% and 3.3% to 1.95% and 0.92%, respectively. The zero points of charge (ZPC) of pure coal and bacterial-treated coal were found at pH = 3, but interacted coal had a more positive surface charge in higher pH values (Abdel-Khalek & El-Midany, 2013). El-Midany and Abdel-Khalek compared *Bacillus subtilis* with *Paenibacillus polymyxa* influence in coal flotation. Pure coal ZPC was found at pH = 2.5. After interaction with each bacteria, the ZPC point did not change and stayed the same, but its positivity in higher pH values increased (El-Midany & Abdel-Khalek, 2014).

In adsorption tests, both bacteria had maximum adsorption at pH 3, but *Bacillus subtilis* had faster kinetics and higher adsorption amounts. FTIR spectra analysis indicated that the main forces are hydrogen bonding and long-term hydrophobic, which means the adsorption nature is physical (El-Midany & Abdel-Khalek, 2014).

Bacillus subtilis shows high performance compared to *Paenibacillus polymyxa* for ash and sulfur removal. While the coal sample had 3.3% sulfur and 6.65% ash contents, *Bacillus subtilis* and *Paenibacillus polymyxa* decreased this amount to 0.92% sulfur and 1.95% ash content and 1.12% sulfur and 2.64% ash content, respectively (El-Midany & Abdel-Khalek, 2014).

5.1.5 Bacillus megaterium

Bacillus megaterium is a rod-shaped, neutrophilic, gram-positive, mesophilic bacterium, producing biotechnologically relevant vitamins and proteins that have become important in this industry. It can widely be found in numerous environments, such as soil to seawater, sediment. Its

cell surface consists of components like peptidoglycan, teichoic and teichuronic acids, lipoproteins, lipopolysaccharides, surface proteins, polysaccharides, and polypeptides, which is essential in the microbe–mineral interactions (Vasanthakumar et al., 2013, 2014).

Vasanthakumar et al. (2014) studied the effect of *Bacillus megaterium* products on sphalerite and galena flotation as bio-collector. It was found that extracellular DNA is an essential parameter in sphalerite selective flotation since single-stranded DNA had a more significant bio-collector capacity compared to double-stranded DNA. With the addition of combined single-stranded DNA and non-DNA components, about 95% of sphalerite was recovered. It was found that the presence of calcium and phosphate components in the nutrient media is valuable for sphalerite selectively separating (Vasanthakumar et al., 2014).

Vasanthakumar et al. (2013) investigated the effect of the adaptation of *Bacillus megaterium* on the flotation and ZPC of sphalerite and galena. Adapted bacteria had less negative surface charge than un-adapted cells. Sphalerite-adapted cells obtained better flotation recovery in case of selectivity as un-adapted and galena-adapted ones (Vasanthakumar et al., 2013).

5.1.6 Paenibacillus polymyxa (P. polymyxa)

The main components of *P. polymyxa* EPS are polysaccharides, proteins, and organic acids (Patra & Natarajan, 2004a). These metabolic products are biodegradable, effective at extreme temperatures, and low toxic (Subramanian et al., 2003).

Patra et al. (2008) (Patra & Natarajan, 2006, 2008) investigated the role of extracellular *P. polymyxas* protein and polysaccharide in flotation. The results indicated that fractionated protein groups could selectively alter pyrite and chalcopyrite surfaces to hydrophilic while galena, quartz, and sphalerite hydrophobicity increased. Extracellular bacterial protein and extracellular polysaccharide absorbed with higher density in pyrite surface compared to galena, and galena could selectively be floated in the presence of extracellular polysaccharide. Also, Patra et al. (2004) (Patra & Natarajan, 2004a) selectively floated pyrite from sphalerite using *P. polymyxa*. The adsorption studies revealed higher adsorption density on pyrite surface at different pH, especially at pH = 9. ZPC of pyrite and sphalerite before and after interaction with cells (by 1×10^7 cells/ml) for 1 h, 2 h, and 24 h had no significant difference. Selective flotation of sphalerite at pH = 9 in the presence of extracellular bacterial protein was achieved, and recovery was 96%. Extracellular bacterial protein proved to work as a bioflocculant for pyrite, enhancing its depression.

Subramanian et al. (2003) investigated the role of *P. polymyxas* metabolite in the sphalerite-galena system. They observed a higher adsorption density of metabolites on the galena surface compared to sphalerite. ZPC of both minerals before and after interaction with *P. polymyxas* metabolite was measured. For sphalerite, the ZPC of pure sphalerite was around pH = 2.2, and after interaction with metabolite, it shifted to more basic pH values. In contrast, in galena's case, the ZPC remained the same after interaction with metabolite at about pH = 2.5. Results of micro-flotation showed that galena depressed after metabolite interaction, while 90% of sphalerite was recovered with the addition of $CuSO_4$ and PIPX. In differential flotation tests with only *P. polymyxas* metabolite, sphalerite was selectively floated at pH of 3.2–3.4 with 90% recovery, while 96% of galena was depressed. At the pH range of 9–9.5 with the addition of 10^{-6} M $CuSO_4$ and 10^{-4} M PIPX before metabolite interaction, about 95% of sphalerite recovered, while 95% of galena was depressed.

Patra et al. (2008) (Patra & Natarajan, 2008) investigated the effect of different types of EPS groups driven from *P. polymyxa,* using micro-flotation tests with a Halimond tube. Four different types of extracellular proteins (EP), comprised of various kinds of amino acids (protein groups), were studied in adsorption and flotation tests. The adsorption studies on different minerals are shown in (Figs. 11 and 12). As results indicate, quartz had 65%, 80%, 30%, and 40% recovery with groups A, B, C, and D proteins. All protein groups depressed pyrite and chalcopyrite, and galena had the highest recovery with group D. The results indicated that selective depression of pyrite and chalcopyrite in the presence of galena, quartz, and sphalerite could be achieved.

5.1.7 Bacterial Consortium

Govender et al. (2011) investigated the effects of different mixtures of bacteria and their EPS on chalcopyrite flotation. After parameter optimization, the efficiency of both produced EPS and bioleaching bacteria was investigated and compared. Five types of EPS are extracted from different bacteria, including *Acidithiobacillus caldus*, *Leptosprillium sp*, *Sulfobacillus sp*, *Ferroplasma sp*, *Acidianus sp, Metallosphaera sp, Sulfolobus sp*. The EPS constituents are mostly carbohydrates and proteins with a small amount of humic acid and uronic acid. The best recovery for chalcopyrite EPS flotation was achieved with 3.5×10^{-2} mg/g EPS concentration. The optimum cell concentration to be used for bioflotation was 1×10^{-6}. The flotation process was conditioned for 20 min with EPS or bacterial cell at pH = 9; after that, 1×10^{-5} M of SIBX was added, and conditioning continued for 5 more min. Obtained results are shown in (Fig. 13), as it is obvious that produced free EPS is more successful (Govender & Gericke, 2011).

Patra et al. (2008) (Patra & Natarajan, 2008) investigated the role of different EPS groups drove from *P. polymyxa* in micro-flotation tests with Halimond tube. The results

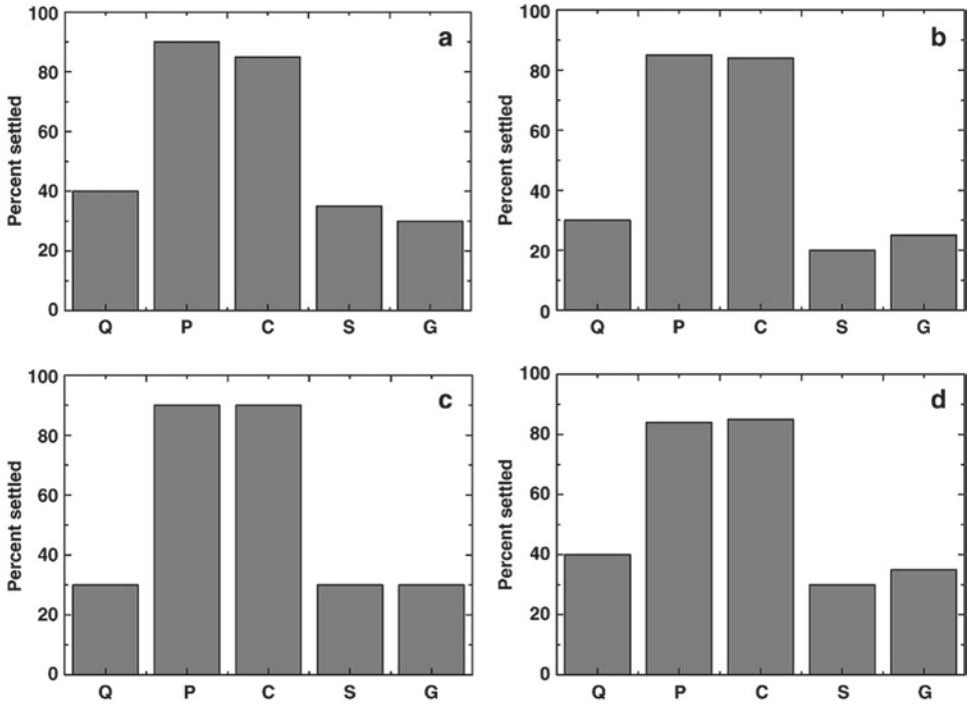

Fig. 11 Adsorption densities of protein fractions on various minerals **a** A group 0–20%, **b** B group 20–40%, **c** C group 40–60%, **d** D group 60–90% (Q: Quartz, P: Pyrite, C: Chalcopyrite, S: Sphalerite, G: Galena) (Patra & Natarajan, 2008)

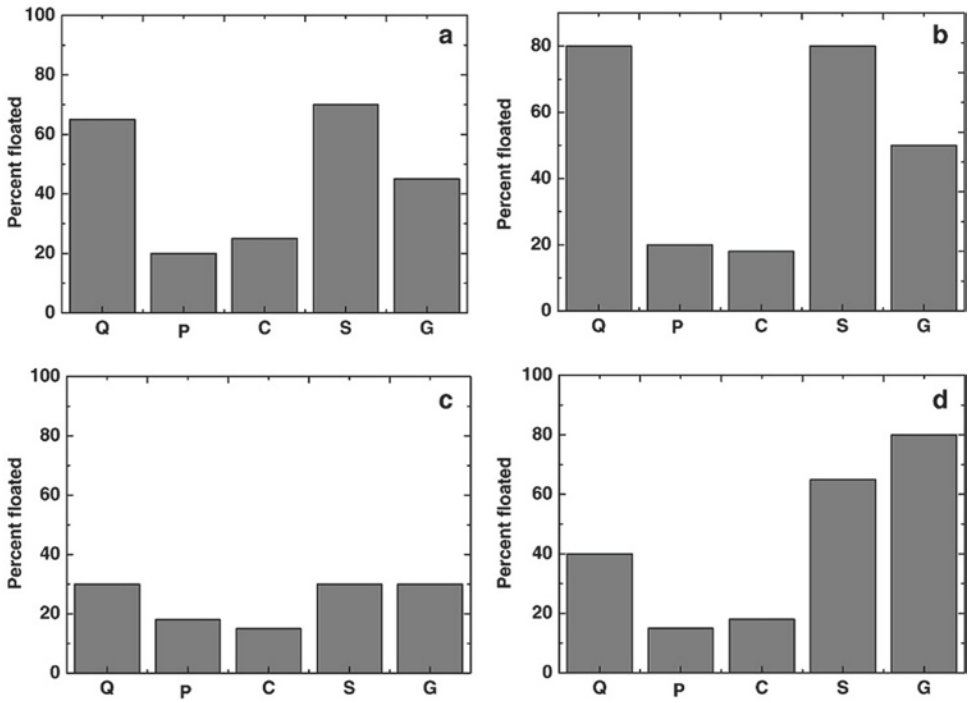

Fig. 12 Flocculation behavior of minerals after interaction with different protein fractions **a** A group 0–20%, **b** B group 20–40%, **c** C group 40–60%, **d** D group 60–90% (Q: Quartz, P: Pyrite, C: Chalcopyrite, S: Sphalerite, G: Galena) (Patra & Natarajan, 2008)

indicated that selective depression of pyrite and chalcopyrite in the presence of galena, quartz, and sphalerite could be achieved (Patra & Natarajan, 2008).

5.1.8 Leptosririllum ferrooxidans

Leptospirillum ferrooxidans is a chemolithotrophic acidophilic bacterium that can be found in abundance in acid mine drainage (AMD). This microorganism can bind selectively to sulfide minerals, having the ability to alter the properties of surfaces and alter their floatability. *Leptospirillum ferrooxidans* derive their energy from the oxidation of Fe^{2+} to Fe^{3+}.

In 2008 and 2011, Velinska et al. investigated the effect of *Leptospirillum ferrooxidans* on pyrite and chalcopyrite

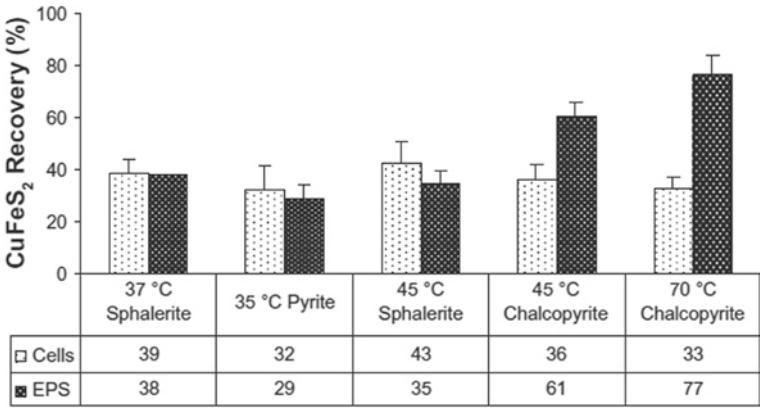

Fig. 13 Comparison of the efficiency of using bacterial cells with bound EPS and free

flotation. These researchers also investigated the thermodynamics and DLVO theory for bacterial attachment. The results indicated that bacterial treatment has a more significant effect on chalcopyrite than pyrite, both for bioflotation and bioflocculation. The DLVO theory also confirmed the results. At pH = 2, the ZPC of the chalcopyrite surface was lower due to the electrostatic repulsion forces associated with pyrite. In flotation experiments performed at pH = 4 and 0.5×10^{-4} mol/L xanthate as a collector, it was observed that the recovery of both minerals decreased in the presence of bacterial cells. However, this reduction was much higher for chalcopyrite in comparison to pyrite. Chalcopyrite flotation recovery was decreased from 95 to 25%, while pyrite recovery was reduced to 67% under similar conditions. These researches attributed the higher tendency of *Leptospirillum ferrooxidans* to bind the chalcopyrite surface rather than pyrite due to more defects and higher iron availability as a source of energy in chalcopyrite surface (Vilinska & Rao, 2008, 2011).

In another research, Bleeze et al. (2018) examined the effect of this microorganism in the separation of pyrite and chalcopyrite. The best separation of pyrite and chalcopyrite was obtained when *Leptospirillum ferrooxidans* were cultured in the presence of chalcopyrite before the addition of the PIPX collector. The results indicated that *Leptospirillum ferrooxidans* have an inhibitory effect on both minerals, while the presence of EPS in acidic conditions has a more inhibitory effect on pyrite than chalcopyrite (Bleeze et al., 2018).

Pacina et al. (2009) investigated the effect of *Leptospirillum ferrooxidans* on the flotation kinetics of chalcopyrite, pyrrhotite, and sphalerite. The results showed that the effect of this bacterium on the flotation rate of the studied sulfide minerals is directly related to the susceptibility of the ore to oxidation by the microorganism. The floatability of bacterially conditioned chalcopyrite increased by the addition of elemental sulfur to the treatment environment. The recovery of chalcopyrite was directly related to bacterial activity, while pH was not an adequate parameter. *Leptospirillum ferrooxidans* had a slightly depressing effect on pyrrhotite after more than 60 min conditioning. Also, as sphalerite had low sensitivity to oxidation, no change in flotation recovery was observed (Pecina et al., 2009).

Díaz-López et al. (2012) also studied the effect of *Leptospirillum ferrooxidans* on the flotation of chalcopyrite and pyrrhotite. The results indicated that the recovery of chalcopyrite in the presence of *Leptospirillum ferrooxidans* increased from 80 to 95%. However, in the case of pyrrhotite, the effect was different for each particle size, which made it a weak depressant because bacteria could not cover all the hydrophobic surfaces. In addition, chalcopyrite recovery was increased with a shorter contact time. In contrast, pyrrhotite conditioning needs high contact time. It was also stated that in both minerals, adsorption is a quick process that happens in the first 10–20 min, and that bacteria are more prone to pyrrhotite than chalcopyrite (Díaz-López et al., 2012).

5.1.9 Rhodococcus opacus

Rhodococcus opacus is a gram-positive bacterium that contains various organic compounds, including polysaccharides, carboxylic acids, lipid groups, and mycolic acids in its cell wall that exhibit amphoteric behavior at the cell surface.

Botero et al. (2007) investigated the effect of *Rhodococcus opacus* as a bio-collector of calcite and magnesite. The results showed that the bacterial treatment decreased the IEP for both minerals. In addition, the number of *Rhodococcus opacus* cells adsorbed on the surface of magnesite was 10 times higher compared to calcite. The interaction of *Rhodococcus apacus* with the surface of these two minerals was dependent on pH, and the optimal adsorption results of the two minerals were observed at a pH = ~7. The adsorption kinetics for both minerals was rapid and reached equilibrium after 5 min. The magnesite recovery at pH = ~5 and bacterial concentration of 100 ppm was 93%, while it was 55% at pH = ~7 and 220 ppm bacterial concentration (Botero et al., 2007).

In similar experiments, Merma et al. (2013) examined the effect of *Rhodococcus opacus* on quartz and apatite flotation. Bacteria altered the zeta potential of both minerals immediately after contact, which was more noticeable for apatite. It was also found that the bacterial cells' surface tension was influenced by pH and bacterial concentration. The lowest surface tension was found in the 0.15 g/l biomass and at a pH range of 3–7. However, bacterial cells were more inclined to the apatite surface. They also stated that the bioflotation of apatite and quartz particles depends on the pH and bacterial concentration. The best separation efficiency was obtained at pH = 5, and recoveries were 90% and 14% for apatite and quartz, respectively (Merma et al., 2013).

Kim et al. (2017) investigated the possibility of bioflotation of copper oxide (malachite) ores by *Rhodococcus opacus*. They observed that the agitator speed in the range of 800–1200 rpm did not influence malachite flotation. However, ionic strength and especially pH changes affect the recovery and grade of malachite. At pH = 7, the highest grade (98%) and recovery (93%) and pH = 11, the lowest grade (68%) and recovery (90%). The experimental results showed that bioflotation was appropriate for fine particle size and led to a higher degree of malachite freedom (5.17%) than the conventional process (1.43%) (Kim et al., 2017).

6 Future Prospective and Conclusion

Bio-beneficiation is the application of microorganisms (including bacteria, fungi, algae, and yeast) and their products (such as biomass and extracellular polymeric substances (EPS)) to facilitate the selective separation of gangue minerals from valuable minerals in mineral processing. Bio-beneficiation is divided into two main categories: bioflotation and bioflocculation. Microorganisms and their biomass can be used as collectors, depressants, dispersants, bioflocculants, and frothers. Fundamentally, the role of microorganisms in bio-beneficiation is to change the physicochemical properties of mineral surfaces to obtain the best separation efficiency. Several mechanisms have been proposed to explain mineral bio-beneficiation, including (DLVO) and extended DLVO (XDLVO) theory, cationic bridging theory, and polymer bridging or adsorption bridging theory. However, the actual role of microorganisms and their extracellular polymeric substances (EPS) in the bio-beneficiation process is not understood well yet. There is still a significant gap in the literature describing exact dominating bio-beneficiation mechanisms, so future studies in this area must be accomplished. Numerous studies on bioflotation and flocculation associated with the selective separation/removal of coal, oxide minerals, and sulfide minerals have been reported in this chapter, indicating the high potential of bio-beneficiation in mineral processing. Interactions of microorganisms and minerals cause alteration in the chemical properties of the minerals' surface, the reaction of bacterial cells and metabolic products with minerals, production of surface-active chemicals. So the intrinsic differences between minerals attaching to microorganisms and their products lead to their selective biological separation. Although biohydrometallurgical methods for various ores have been thoroughly studied and commercialized, bio-beneficiation has not yet been as widely used as biohydrometallurgical processes on industrial scales. So due to the general studies reviewed in this chapter, there is a noteworthy need for commercial-scale advancements in bio-beneficiation processes, and more attention should be directed to it.

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