

# The Role of Biomodification in Mineral Processing

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**Abstract:** Increasing environmental concern forces the reduction in the share of synthetic surfactants in the production of various industries, including mineral processing, by replacing them with more environmentally friendly compounds of biological origin. Several studies on the use of biosurfactants in mineral processing are currently available in the literature, but they contain limited information related to the physicochemistry of these processes. Therefore, this review aims to summarise publications from the last decade related to the role of microorganisms and their metabolic products in mineral surface modification applied in mineral processing. Theoretical principles of bacteria–mineral interactions are presented. Salt-type, sulphide, and oxide minerals were discussed with greater attention to the physicochemistry of biosurfactant–mineral interactions, such as the wettability and surface charge. The advantages and disadvantages of using bacterial cells and surface-active microbial compounds were proposed. The trends and challenges of biomodification in flotation and flocculation were discussed.

**Keywords:** adhesion; bioflotation; bioflocculation; biopretreatment; biosurfactants



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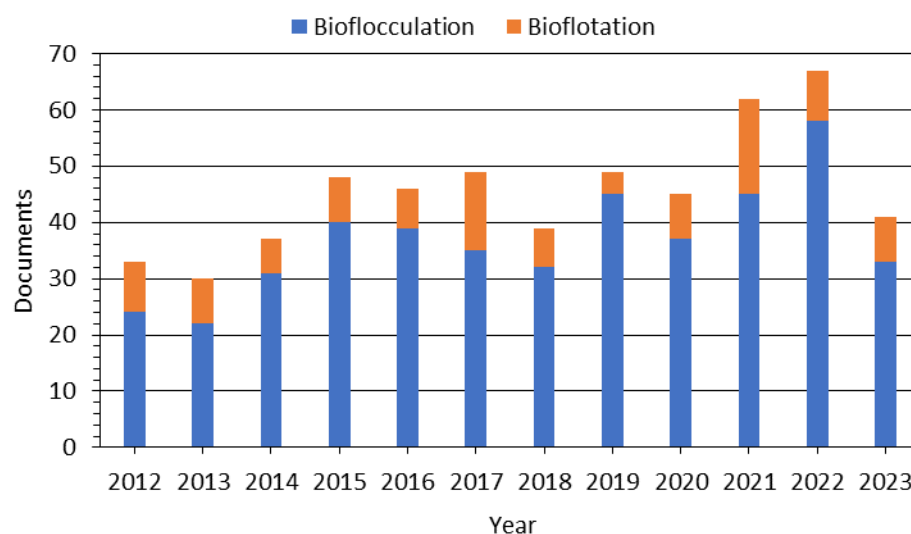
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## 1. Introduction

An important aspect related to new trends in the industry is the circular economy. Therefore, future technologies and production processes should be designed to minimise negative environmental impacts, including reducing the consumption of raw materials, energy, and greenhouse gas emissions. Compounds of microbial origin fit well into this trend. They have an advantage over their chemical and synthetic counterparts due to their simple preparation, lower toxicity, better environmental compatibility, high foaming ability, and specificity of action under extreme conditions such as pH, salinity, or temperature [1]. They may also be produced from renewable sources [2].

The increase in public awareness of environmental pollution has an impact on research on the application of biological methods for mineral separation. The number of publications related to mineral flotation and flocculation using agents of biological origin is increasing, as presented in Figure 1 and also reported by Oulakhir et al. [3], indicating a growing interest in the development of new ecological process approaches.

Bacteria, yeast, and fungi produce molecules with tension-active properties that act primarily as protective reagents, of which interaction with mineral surfaces leads to modification of their properties [4] by changing their hydrophobicity. Bio-modification can occur as a result of the adsorption and/or chemical reaction of metabolic products, adhesion of microbial cells to the mineral surface, or oxidation reactions in the case of sulphide minerals [5]. We can distinguish between direct interaction, when cell adhesion occurs, and indirect when biological products act as surface-active agents. Appropriate control of these processes offers the possibility of using microbes and bio-based compounds in flotation or flocculation [6].



**Figure 1.** Number of documents published by year based on the Scopus database (keywords: “bioflotation OR “bio-flotation”; “bioflocculation” OR “bio-flocculation”).

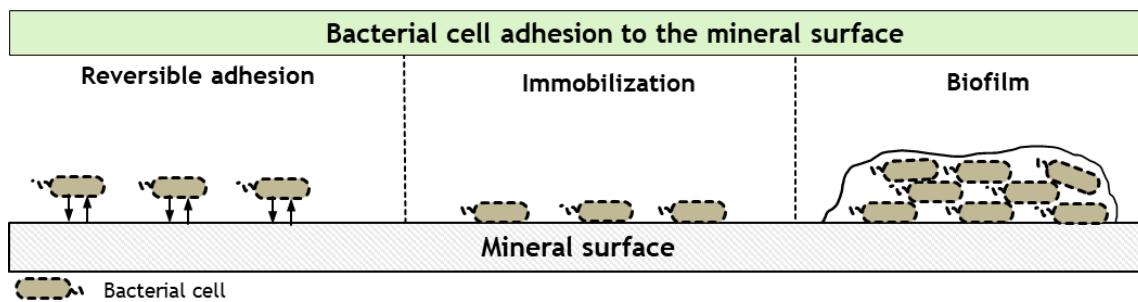
The main factors influencing the biomodification of the solid surface in mineral beneficiation have been described in detail [3,7] and include the particle size, the pulp density of the mineral suspension, bacterial cell concentration, the contact time of the bacteria with a mineral substrate, pH, the nutrient composition of the medium, surface potential, and surface charge.

Most of the recent literature on the application of biosurfactants in biobeneficiation addresses the characterisation of biological surfactants, adsorption mechanisms, and physicochemical characteristics of bioflotation, aspects of their industrial bacteria–mineral interactions [3,7–9], while the chemical and physical aspects of mineral surface alteration using microbial cells and their metabolites such as wettability and surface charge are less detailed. In this context, the objective of this present article was to provide a current overview of the interaction of microorganisms and their metabolites with mineral surfaces, emphasising the physicochemistry of these processes. Theoretical principles were also presented.

## 2. Adhesion of Microorganisms to the Mineral Surface

Contact between microbial cells and the rocks' surface and minerals is a common phenomenon in the surrounding world. The effects caused by the interaction cause significant, often irreversible changes in the properties of the solid surface, which are implemented in bioflocculation, bioagglomeration, bioflotation [10], and bioleaching [11]. The biological activity also leads to the formation of inorganic and organic acids that cause mineral erosion and bioweathering [12]. Biomodification can occur by adsorption of metabolic products produced by microorganisms or, in the case of chemolithotrophic bacteria, through cell adhesion and biocatalysed oxidation or reduction of the surface [13].

Bacterial cell adhesion is the first step that takes place when a cell comes into contact with a mineral (solid) surface. The following are responsible for forming the cell–solid interface: van der Waals forces, hydrogen bonds, and hydrophobic interactions. Variations in the bacterial cell attachment to the mineral surface depicted in Figure 2 include the following: (i) reversible adhesion, which occurs via weak van der Waals forces; (ii) immobilisation, when bacteria anchor to the surface with cell structures, that is, pili or exopolymers, which attach them irreversibly; and (iii) biofilm, when multilayered cells accumulate on the surface and produce extracellular polymeric substances (EPS) [14].



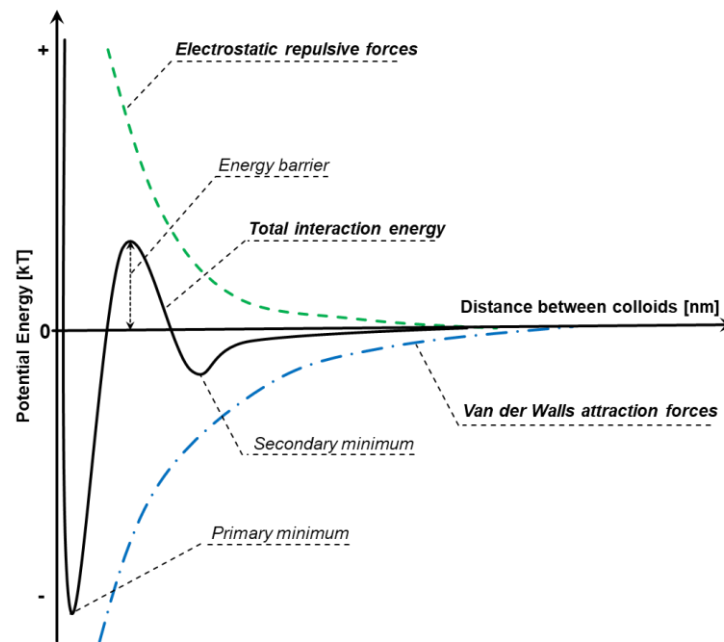
**Figure 2.** Visualisation of bacterial cell adhesion to the mineral surface.

The process of bacterial cell adhesion to the mineral surface is complex, and the final step results in the formation of a biofilm. It is influenced by the following factors: the type of bacteria, their concentration, the structure of the mineral surface, its chemical composition, and hydrophobicity/hydrophilicity [15,16]. EPS involved in biofilm formation, is a collection of substances with the most important components composed of carbohydrates, proteins, lipids, and nucleic acids [17]. They promote the adhesion of microbial cells to the mineral surface and, at the same time, influence the wettability. By surrounding the bacterial cell, it plays a primarily protective role [18]. The production of extracellular biopolymers is influenced by the growth conditions of bacteria. The biopolymer conformation is determined by the ionic strength of the solution and may be colloidal or capsular, depending on whether strong or loose bonds occur between carbohydrates. An increase in the ionic strength of the solution results in a decrease in the hydrodynamic diameter of the biopolymer, which affects cell adhesion. This fact was confirmed by the poor adhesion of *Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans* cells to the silica surface in a strongly acidic environment [19]. The role of EPS in the adhesion of bacterial cells to mineral surfaces has been tested on TiO<sub>2</sub> or SiO<sub>2</sub> surfaces [20]. EPS was shown to reduce the surface energy of *Streptococcus mutans* and thus facilitated cell adhesion. For hydrophobic surfaces, exopolymers also increased the acid–base attraction. In aqueous solutions, the acid–base interaction was dominant between bacteria and solids. The adhesion to hydrophobic surfaces was driven by hydrophobic force, whereas binding to hydrophilic surfaces depended on hydrogen bonds and needed to overcome an additional repulsive hydration force.

After irreversible adhesion, bacteria accumulate on the solid surface, forming a biofilm, a highly heterogeneous structure of EPS and bacterial cells. *Acidithiobacillus ferrooxidans* 61, *L. ferrooxidans* ZC, and *Sulfobacillus thermosulfidooxidans* formed a monolayer biofilm on pyrite [21]. Biofilm formation was shown to involve molecular cell-to-cell communication and can determine the efficiency of bioleaching [22].

#### *Theoretical Models Used to Describe Biosurfactant-Mineral Surface Interactions*

Cell adhesion to solid surfaces, such as minerals, can be described using two theoretical approaches. The first is based on the Derjaguin–Landau–Verwey–Overbeek (DLVO) theory known from colloid chemistry to describe the stability of colloidal systems [23]. The second method, known as the thermodynamic approach, requires the determination of the free energy of the two interacting objects: the cell and the mineral [24]. Cell adhesion to the mineral surface is due to van der Waals, electrostatic interactions, and acid/base interactions [13]. When the cell has an electrical charge opposite that of the surface, strong electrostatic attraction determines adhesion. The DLVO theory sums up the energies of attractive and repulsive interactions. The magnitude of the total interaction energy changes with the distance between the interacting objects. The curve showing the change in total interaction energy has two minimums and one maximum (Figure 3). The first deep minimum corresponds to permanent adhesion; the second shallow minimum provides non-permanent adhesion. The height of the maximum interaction energy determines the possibility of cell adhesion to the mineral surface.



**Figure 3.** Potential energy profile according to the classical DLVO theory. Based on [25,26].

The classical DLVO theory is a simplified model that does not take into account many important factors affecting cell adhesion, such as acid–base interactions, surface hydrophobicity, or surface roughness. Therefore, the predictions of the extended DLVO theory (XDLVO) are more accurate [27,28]. The thermodynamic model of adhesion analyses the free energies at the bacteria–liquid  $\gamma_{(B/L)}$ , mineral–liquid  $\gamma_{(M/L)}$ , and bacteria–mineral  $\gamma_{(B/M)}$  phase boundaries. If the total free energy of adhesion ( $\Delta G_{adh}$ ) is less than zero, the adhesion of the cell to the surface is thermodynamically preferred [24]. Since bacterial cells have a variety of shapes, it is important to know how the shape and position of the cell affect the van der Waals interaction forces. Hamaker's microscopic approach made it possible to calculate the magnitude of the cell–surface interaction forces. The results of the calculations indicate that a horizontally aligned cell was more strongly attracted than a vertically aligned cell [29]. There are several methods to quantify the strength of adhesion. However, atomic force microscopy (AFM) appears to be the most accurate [30]. The measurement range of the AFM is from 10 pN to 1  $\mu$ N. The only difficulty with this method is the precise placement of a single cell at the end of the cantilever tip. The AFM technique allows for the planimetry of the mineral surface before and after cell adhesion. AFM studies have allowed for precise cell localisation and identification of convenient sites for cell attachment on heterogeneous surfaces [31].

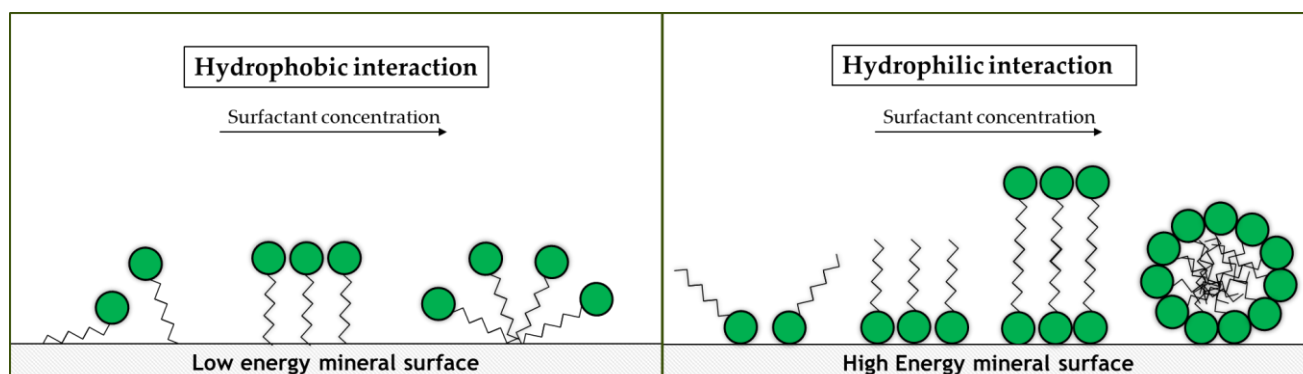
### 3. Adsorption of Microbial By-Products on the Mineral Surface

Whole microbial cells and bioproducts produced extracellularly or as a part of the cellular membrane that reduces surface and interface tension are called biosurfactants. In comparison to synthetic surfactants, they are much more complex. In terms of chemical structure, in addition to the whole cells used in the modification of mineral surfaces, the following groups can be distinguished, which are most commonly described in biobeneficiation processes: (i) glycolipids (rhamnolipids, sophorolipids), (ii) lipopeptides and lipoproteins (surfactin), (iii) polymeric (emulsan, liposan), (iv) fatty acids, phospholipids and neutral lipids [32]. The separation of biosurfactants from the broth is complicated. Crude biosurfactants can be obtained via acid precipitation or biomass separation by centrifugation. Unfortunately, these two methods did not eliminate exopolymers from the broth suspension. Dolman et al. [33] demonstrated that the application of membrane separation and foam formation improves the economics of purification.

The fixation of biosurfactants is the transfer of molecules from the bulk solution to the solid surface. The interaction of the surfactant of natural origin with the mineral surface, presented in Figure 4, is determined by many interactions caused by electrostatic, hydrogen, and hydrophobic forces or covalent bonding. The free energy of biosurfactant adsorption  $\Delta G_{\text{ads}}$  can be expressed as follows:

$$\Delta G_{\text{ads}} = \Delta G_{\text{elec}} + \Delta G_{\text{chem}} + \Delta G_{\text{C-C}} + \Delta G_{\text{C-S}} + \Delta G_{\text{H}} \quad (1)$$

where the lower reference means electrostatic force (elec), chemical bonding (chem), long hydrocarbon chain interaction (C-C), hydrophobic interaction (C-S), and hydrogen bonding (H).



**Figure 4.** Visualisation of the interaction of microbial surface-active compounds with the mineral surface.

The presence of microbial cells and their bioproducts on the mineral surface influences their physicochemical properties. The arrangement of lipopolysaccharide proteins and fatty acids on the cell surface contributes to the charge and hydrophobicity of bacteria [34]. For example, the *Mycobacterium phlei* bacterium owes a negative charge to the accumulation of fatty acids in the cell wall [6].

The wettability of a surface is a specific characteristic of the surface related to surface energy and plays an important role in adhesion. It can be empirically determined using the value of the contact angle. Surface hydrophobicity can be defined when the static water contact angle  $\theta > 90^\circ$ . When  $\theta < 90^\circ$ , the surface is considered hydrophilic [35]. The action of the biosurfactant as a collector can be tracked by changing the contact angle, as shown in the example of hemimorphite ( $\text{Zn}_4\text{Si}_2\text{O}_7(\text{OH})_2\text{H}_2\text{O}$ ) flotation [36]. The initial value of the contact angle of the hemimorphite was  $54^\circ$ , and after the adsorption of the biosurfactant (sodium N-lauroylsarcosinate), this value increased to  $85^\circ$ , allowing the flotation of this mineral. Under the same conditions, the contact angle of the silica changed from  $24^\circ$  to  $30^\circ$ , enabling selective bioflotation of the hemimorphite.

It should be noted that the action of biosurfactants does not necessarily favour cell adhesion to the mineral surface. The amphiphilic structure of the surfactant molecule can cause biofilm destruction and prevent bacterial cell attachment. For example, lipopeptide biosurfactants have such properties and, therefore, could be used in place of antibiotics. Wood et al. [37] demonstrate that the supernatant of *Pseudomonas aeruginosa* containing rhamnolipids effectively dispersed the biofilm formed by *Desulfovibrio vulgaris* (sulfate-reducing bacteria), *Escherichia coli*, and *Staphylococcus aureus*.

The dissociation of surface groups located on the cell wall causes the cell to acquire an electrical charge, which determines the formation of an electrical double layer that surrounds the cell. A measurable parameter that determines the electrical properties of a cell is the zeta potential. Biosurfactants or biopolymer adsorption influences the electric potential of minerals and, therefore, affects the flotation behaviour of mineral particles. Didyk-Mucha [38] showed that the adsorption of biosurfactants produced by *Streptomyces* sp. on serpentinite and magnesite increased the negative values of the zeta potential throughout the tested pH range (1–10). A smaller difference was observed for magnesite,

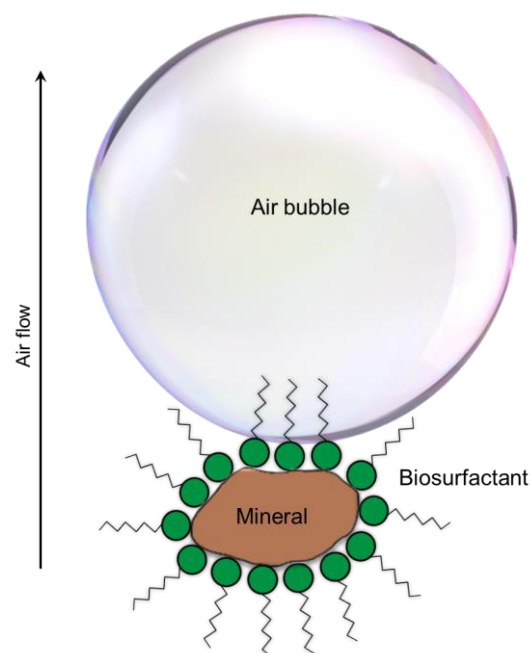
which corresponded well to the results of lower nickel ion adsorption, indicating that fewer biosurfactant molecules could adsorb on the mineral surface than in the case of serpentinite.

Didyk-Mucha also investigated the effect of biosurfactant adsorption on magnesite, serpentinite, and silica [39]. Biosurfactants were produced by *Streptomyces* sp. S4 and were used without purification as bacterial culture broth. It was shown that surface-active compounds strongly influence minerals' surface charge, increasing the negative zeta potential due to the reconstruction of a double electrical layer. In the case of serpentinite, the surface increased negative zeta potential values and changed the isoelectric point (IEP) from pH 4.4 to 1.7, suggesting that at alkaline pH, biosurfactant adsorption could be initiated by the interaction between positively charged ions on the crystal lattice of the mineral surface. Serpentinite and silica had positive zeta potentials above the IEP. Therefore, physical adsorption takes place. Below the IEP, the adsorption occurs because of van der Waals interactions. During bacteria growth, the surface tension of the solution systematically decreases to the level of 27 mN/m. The biosurfactant adsorption isotherms in the minerals under investigation corresponded to the Somasundaran–Fuerstenau model, which is one of the most common forms of adsorption isotherm. In such a model, when the biosurfactant concentration is close to or above the CMC, micelles are formed, the biosurfactant monomer becomes constant, and the main adsorption force is the hydrophobic interaction between the hydrocarbon chains [40].

In other works, the biosurfactants of *Bacillus circulans* and *Streptomyces* sp. served as modifying agents for serpentinite and quartz. The adsorption of natural surfactants onto the mineral surface caused an electrical double-layer change, leading to an increase in negative zeta potential and a shift of the IEP toward lower pH. The presence of hydrocarbon groups on mineral surfaces was also observed [41].

#### 4. Bioflotation

Flotation in mineral processing is a method used to separate and concentrate ores where the difference in the wettability of the components is used [42]. Bioflotation occurs when microorganisms or their metabolism products act as modifying reagents, increasing surface hydrophobicity and facilitating the selective separation of minerals [5]. The single act of flotation is presented in Figure 5.



**Figure 5.** Diagram of mineral particle–biosurfactant–air bubble behaviour in flotation.

The action of bacteria on the mineral surface applied in flotation is a complex phenomenon, as it is necessary to consider the effects of cell adhesion to the surface as well as the adsorption of microbial bioproducts. The hydrophobicity of the cell surface varies depending on the proportion of fatty acids to biopolymers. Bacteria will attach to the mineral surface if the charge and hydrophobic interactions between the bacteria cell and the mineral surface cause adhesion [43].

#### 4.1. Bacterial Cell Application

Bacteria pretreatment of sulphide mineral suspension depresses the minerals as a result of the bio-oxidation of the sulphide surface. The cell wall has a membrane composed of phospholipids and glycopospholipids. These two molecules are hydrophilic because of the presence of phosphate and OH groups. The adhesion of bacterial cells to the mineral surface makes it hydrophilic, thus decreasing its floatability. Sulphide minerals occur in the form of a mixture in exploited ores. Contact between sulphides and chemolithotrophic bacteria, such as *A. ferrooxidans*, facilitates mineral separation. Preliminary studies of the bioflotation of chalcopyrite, sphalerite, and pyrrhotite have already shown that the density of bacterial cells adhering to pyrrhotite was higher than that of chalcopyrite. Biooxidation products such as sulphur ( $S^0$ ) and iron ( $Fe^{3+}$ ) play an important role in the biomodification of sulphide minerals using chemoautotrophic bacteria and can be used to improve their selective bioflotation [44]. The result of the biooxidation of mineral surfaces was that chalcopyrite is less reactive than sphalerite and pyrrhotite. Existing differences can be used for the separation of sulphide minerals. For the chalcopyrite–pyrrhotite mixture, the biomodification of the surface caused an increase in the degree of hydrophobicity of chalcopyrite, resulting in easy separation of these two minerals. Bleeze et al. [45] used *L. ferrooxidans* to modify a mineral surface. Flotation tests showed that the bacteria had a depressive effect on both minerals and exhibited a selective attachment to pyrite over chalcopyrite within the first 7 days of incubation. It was observed that cell adhesion to pyrite was facilitated via EPS and led to biofilm formation. SEM micrographs showed the absence of EPS on the chalcopyrite surface, which explained weaker cell–mineral interaction. Chalcopyrite was separated from pyrite after conditioning the minerals for 74 h with bacterial culture grown under different conditions (*Leptospirillum* HH medium, chalcopyrite and pyrite) and EPS. The selective depression of pyrite in the presence of EPS supernatant extracted from chalcopyrite-grown microorganisms resulted in a recovery of 95.8% Cu.

In the work of Sanwani [46] *Bacillus pumilus* and *Alicyclobacillus ferrooxydans* cultured together with pyrite caused a systematic decrease in the wetting angle, resulting in pyrite depression. The bioflotation of pyrite and chalcopyrite was also studied by Nasrollahzadeh [47]. Halophilic bacteria such as *Halobacillus*, *Alkalibacillus*, and *Alkalibacillus almallahensis* were tested. The results showed that a mixture of these bacteria had a depressing effect on pyrite, allowing 72.3% chalcopyrite concentrate to be obtained.

A mixed-bacterium consortium of *Halobacillus* sp., *A. almallahensis*, and *Alkalibacillus* sp. caused the pyrite depression and flotation of chalcopyrite. According to Bafti [48], the microorganisms mentioned above and *Marinobacter* sp. were able to replace industrial pyrite depressants at pH 7–8 (bioflotation and chemical collector). The recovery of chalcopyrite was lower than that obtained using standard flotation. The mixed microbial culture was also applied to chalcopyrite and galena separation. At a basic pH of 9.3, bacteria increased the hydrophilicity of sulphide minerals, resulting in poor flotation efficacy [49]. In the work of Consuegra et al. [50], halophilic bacteria such as *H. boliviensis*, *Halobacillus* sp., *Halomonas* sp., *Marinobacter* spp., and *Marinococcus* sp. were tested as mineral depressants. Sodium isopropyl xanthate was used as a collector. Only hydrophilic bacteria (*Halomonas* sp. and *Halobacillus* sp.) adhered to the pyrite, showing the highest reduction in the floatability of the pyrite (68%). A chalcopyrite depression was observed for *H. boliviensis* (from 40 to 9%) and *Halomonas* sp. (14%). The mechanism of bacterial cell adhesion to pyrite was considered hydrophobic. Electrokinetic studies showed that the zeta potential of pure

pyrite was between  $-20$  and  $-70$  mV and that of chalcopyrite between  $-30$  and  $-60$  mV, respectively, for pH 2 and pH 10. At pH 4–8, the presence of bacteria on the mineral surface shifted the zeta potential towards negative values.

The problem of removing pyrite from coal has important environmental implications. For this reason, research was being conducted into the separation of pyrite from coal, and one of the methods was bioflotation. As shown by Holda and Mlynarczykowska, the grain size of the feedstock and the density of bacterial cells play an important role in the separation of pyrite from coal [51]. Using the 53–75  $\mu\text{m}$  coal particle size and *A. ferrooxidans* suspension (concentration of  $0.5 \times 10^9$  cells/ $\text{cm}^3$ ), 70% of the pyrite was recovered. The results of pyrite separation were much worse for the 38–53  $\mu\text{m}$  grain class.

El-Midany and Abdel-Khalek conducted studies on the removal of pyrite and ash from coal with the bacteria *Bacillus subtilis* and *Paenibacillus polymyxa* via bioflotation [52,53]. The results show that with coal containing 3.3% sulphur and 6.65% ash, the sulphur content can be reduced to 0.9% and the ash content to 1.95% via bioflotation. Flotation tests were carried out around pH 3 using a 3% coal suspension. *B. subtilis* had a higher affinity for coal than *P. polymyxa*, with an average of 140 cells/ $\text{cm}^2$  and 50 cells/ $\text{cm}^2$  on the mineral surface.

In the modification of oxide minerals, the common soil bacteria *Bacillus mucilaginosus* was applied for the biopretreatment of pyrolusite and quartz, while laurylamine was used as a typical cationic surfactant [54]. Surface modification was due to bacterial products adsorption, not cell adhesion. Quartz had a higher affinity for metabolites compared to pyrolusite. Therefore, the separation of quartz from pyrolusite by flotation can be effective if the solid material is biopretreated [54].

*Rhodococcus ruber* was used for hematite flotation. Under acidic conditions, the positively charged hematite surface became negative after contact with microorganisms as a result of the electrostatic interaction between oppositely charged surfaces. The highest floatability of hematite was achieved under acidic conditions, as biomass attachment was found to be stronger in such environment. For example, at pH 3, the recovery of hematite was around 65% using 150 mg/l of biosurfactant, and particle size  $-53 + 38 \mu\text{m}$  [55].

The non-pathogenic strain of *R. opacus* with hydrophobic properties (contact angle around  $70^\circ$ ) was used as a bioreagent to separate apatite from quartz. The highest flotability of apatite was achieved at pH 5. The flotation process carried out under these conditions gave apatite recovery equal to 92% and 52% for apatite and quartz after 7 min of flotation. It was also observed that, with decreasing particle size, the flotation rate of apatite decreased. Quartz flotation yielded higher values when particle size decreased [56]. Electrokinetic studies showed that within pH 3–10, both minerals and *R. opacus* exhibited a negative zeta potential. The negatively charged surface of the bacteria was due to the domination of anionic groups on the bacterial cell wall. The contact of microorganisms with apatite slightly increased the negative surface charge (pH 5–12), while for quartz, the effect was the opposite. The surface tension of bacteria suspension decreased significantly below pH 7 and with increasing cell concentration. The contact angle increased after bacteria pretreatment, enhancing the hydrophobicity of the mineral surface ( $\sim 45^\circ$  for apatite,  $\sim 20^\circ$  for quartz). The difference in the wettability of the samples was visible in bioflotation experiments. At pH 5 and 0.15 g/l of biomass, 60% of apatite and 14% of quartz were recovered [57].

The kinetic study of the bioflotation process with the application of bacterial cells showed that for the hematite–quartz mixture, hematite flotation can be described using the first-order kinetic equation [58].

In many bioflotation processes, bacterial cells play the role of collectors, especially when the cell surface is hydrophobic. This type of bacteria can include *R. opacus*, *R. ruber*, *R. erythropolis*, *B. subtilis*, and *M. phlei*. The adsorption of these bacteria cells onto the mineral surface makes it hydrophobic and able to flotation. Similar to *R. ruber*, *R. erythropolis*, a Gram-positive, non-pathogenic bacterium found in soil and bottom sediments, was used for hematite flotation. Flotation tests conducted in a modified Halimond tube showed that the maximum bioflotability of hematite was 83.86 % at pH 6 [59].



The bioflotation of the hematite–pyrolusite mixture at pH 3 in the presence of *P. polymyxa* floated hematite with a manganese reduction of 65%. The flotation of natural Bahariya Oasis iron ore in the presence of bacteria cells yielded a hematite recovery of 72.46% [60]. In the work of Yang [61], nine bacteria strains were isolated from soil. Four of them, *S. marcescens* strain PW114, *S. marcescens* strain S20, *Acinetobacter* sp. MSG8, and *Stenotrophomonas* sp. MB-1-6-5 were used as a biocollector for hematite separation, but only the latter one was non-pathogenic to humans. Using 60 mg/l of bacteria at pH 6, the recovery rates for all bacteria testes were greater than 75%. The addition of *Serratia marcescens* strain S20 during hematite flotation increased the mineral hydrophobicity and particle size. The FTIR spectra revealed four new groups on the hematite surface after contact with microorganisms. Adsorption occurred primarily via chemical interactions between carboxylic groups and hydrophobic association [62].

*R. opacus* cells with a highly hydrophobic surface were tested as collectors in the flotation of a malachite–silica mixture and for the enrichment of copper oxide ore [63]. Laboratory-scale flotation studies have shown that the process using *R. opacus* provides a more than 90% yield of malachite at pH 7. Optimal malachite bioflotation conditions were faced with cell–mineral interaction energies calculated from the DLVO theory. It was shown that the best bioflotation conditions correlate well with the conditions for the strongest interactions (adhesion).

*Pseudomonas songnenensis* was shown to improve apatite flotation in phosphate ore at pH 6.5, but it also did not have a significant change in calcite recovery [64]. Furthermore, another bacteria, *S. aureus*, was found to preferentially adsorb on apatite, increasing its hydrophobicity and allowing selective separation from quartz at pH 6–7 [65]. Similar observations have been reported for apatite and quartz conditioned with *Bacillus cereus*. In addition to the higher floatability of apatite, bacteria decreased the isoelectric point of this mineral from 4.7 to 1.8 and had no significant effect on quartz [66]. Another strain, *Bacillus licheniformis*, and its metabolites were tested in barite and quartz separation [67]. Bacterial cells improved barite hydrophobicity, resulting in barite recovery that yielded up to 87% at pH 3. Quartz recovery was highest at pH 9 and conditioning with microbial metabolites.

Flotation tests of the synthetic mixture of galena and sphalerite showed that galena can be selectively floated in the presence of lysed *B. subtilis*, preadapted to sphalerite, with a high selectivity index [68].

#### 4.2. Application of Microbial Surface-Active Compounds

Bacteria interact with the sulphide surface, i.e., in bio-oxidation, which can be realised indirectly if they use enzymes or directly if they do not. This process was observed during the bioweathering of copper sulphide minerals [69]. In addition to the bio-oxidation process, which can alter the flotation properties of sulphides, the adsorption of organic polymers produced by bacteria can also affect the mineral behaviour in flotation. Govender and Gericke [70] used both microorganisms and EPS extracted from bioleaching consortia as collectors for chalcopyrite flotation. Moreover,  $1 \times 10^6$  cells/g was the optimal concentration, and its further increase resulted in a decrease in recovery. Mineral floatability increased from 27% to 39% for EPS concentration of  $1.7 \times 10^{-3}$  to  $3.5 \times 10^{-2}$  mg/g, respectively. At higher values, the flotation recovery decreased. The experimental tests indicated that free EPS was more efficient as a flotation reagent than cells with bound EPS adhered to the surface. Higher recoveries of chalcopyrite (35–58%) were observed compared to pH 4 (18–32%). Flotation at elevated temperatures with EPS as a collector led to an increase in recovery (38% for 37°C and 77% for 70°C).

The separation of sphalerite from galena is a major problem in the enrichment of sulphide Zn–Pb ores. Vasanthakumar and colleagues proposed using DNA obtained from *Bacillus* species as a collector for sphalerite flotation [71,72]. At the same time, the extracted DNA was used as a galena depressant.

Legawiec et al. [73] used mono and dirhamnolipid mixtures for dolomite destabilisation. At the critical micelle concentration (CMC) of 50 mg/dm<sup>3</sup>, the most effective

destabilisation of the suspension was observed, indicating its possible application as a depressant in mineral processing. Rhamnolipids (RLs) produced by *P. aeruginosa* MA01 were also found to have a depressing effect on coal flotation [74]. It was shown that RLs depressed coal flotation by physical interaction with the solid via chemical bonding between the carboxyl group in the RLs structure with those on the coal surface. Merma et al. [75] presented the optimisation of hematite and quartz flotation with *R. erythropolis* biosurfactant using an artificial neural network. The biosurfactant molecules preferred to adsorb onto hematite particles more than quartz, and the correlation between a model and the experimental data reached a value near 100% and showed greater selectivity for hematite.

Bacterium *B. subtilis*, capable of producing surfactin, can substitute oleate in calcite flotation, allowing for 80% recovery compared to 50% in classical flotation (pH 8.5–9.5). Only 360 g/t of metabolite was used instead of 4000 g/t for a chemical collector. In the case of surfactin, one-third of the conditioning time was needed (5 min.) [76].

Surfactin was also applied in magnesite–quartz flotation. Bioflotation studies have shown that magnesite can be selectively floated from an ore containing magnesite and quartz. The silicate content was reduced from 19.7% SiO<sub>2</sub> to 4.77% [77]. In another work, the usability of surfactin as a collector of magnesite was studied in terms of surface tension and adsorption properties [78]. Surfactin reduced the surface tension of water to a greater extent than oleate. The contact angle of the magnesite surface increased with increasing biosurfactant concentration. The highest surface hydrophobisation was obtained at pH 8 and 9 (contact angle 85°, 2 × 10<sup>−4</sup> M of surfactin), while pH 7 had the lowest. According to the zeta potential, the addition of surfactin negatively charged the surface in the tested pH range (4–11). In bioflotation studies, approximately 33% magnesite weight yield was obtained at 150 g/t surfactin dosage (4 min conditioning time, room temperature).

As presented, natural surfactants, such as bacterial cells and their metabolites may have a positive, neutral, or negative impact on minerals. They can be used as collectors, frothers, and depressants. Table 1 shows a summary of the research carried out on mineral surface modification with potential use in mineral processing.

**Table 1.** List of research with key results conducted on the mineral surface modification for mineral processing.

Mineral/Ore	Particle Size	Biosurfactant	Form of Application	Role	Surface Modification Effect	Ref.
Pyrite Chalcopyrite	38–75 $\mu\text{m}$	<i>Leptospirillum ferrooxidans</i> (No data on pathogenicity)	Bacterial culture/ EPS	Depressant	The bacterial cells had a depressive effect on both minerals. The presence of only EPS led to greater separation via selective suppression of pyrite under acidic conditions. The best separation efficiency (95.8%) was achieved for the EPS supernatant extracted from bacteria grown on chalcopyrite.	[45]
Pyrite	37–74 $\mu\text{m}$	<i>Bacillus pumilus</i> SKC-2 <i>Alicyclobacillusferrooxydans</i> SKC/SAA-2 (No data on pathogenicity)	Bacterial culture	Surface modifier/depressant	Decrease in bacterial cell and pyrite surface tension in time from 67.5 mN/m to 51.6 mN/m for <i>B. pumilus</i> and 55.7 mN/m for <i>A. ferrooxydans</i> . A decrease in surface tension changed the contact angle values, that is, the hydrophobicity of the pyrite surface, which could be attributed to bacterial cell adhesion and/or metabolic product interactions.	[46]
Pyrite Chalcopyrite	–74 $\mu\text{m}$	<i>Halobacillus</i> sp., <i>A. almallahensis</i> <i>Alkalibacillus</i> sp. <i>Marinobacter</i> sp. <i>Alkalibacillus salilacus</i> (No data on pathogenicity)	Bacterial culture	Collector/ depressant	Bioflotation with <i>Halobacillus</i> sp., <i>A. almallahensis</i> , and <i>Alkalibacillus</i> sp. gave recovery of pyrite depression of 30.9, 30.3, and 34.0%, respectively, and flotation of chalcopyrite of 52.9, 68.6, and 55.7%, respectively, which indicated the high selectivity of these bacteria in flotation. The application of three types of bacteria (33.3% of each type) resulted in pyrite depression better than other tests (27.5%). Chalcopyrite recovery yielded 72.6%.	[47]
Sulfide copper ore	–2 mm	<i>Halobacillus</i> sp. <i>Alkalibacillus almallahensis</i> <i>Marinobacter</i> sp. <i>Alkalibacillus</i> sp. (No data on pathogenicity)	Cells	Depressant	Pyrite depression (chemical collector was used), pH 7–8; Bacteria were able to replace industrial depressants such as sodium metabisulfite and pH regulators used in industry such as lime. Chalcopyrite recovery was lower than that of standard flotation. The use of collectors (gas oil, Z11, and C7240) together with halophilic bacteria was required for successful flotation.	[48]

Table 1. Cont.

Mineral/Ore	Particle Size	Biosurfactant	Form of Application	Role	Surface Modification Effect	Ref.
Chalcopyrite Galena	38–108 $\mu\text{m}$	Mixed culture of microbes/ Sodium ethyl xanthate	Cells	Surface modifier/ depressant	<p>A reduction in electronegativity of zeta potential of chalcopyrite and galena after 1 h contact with microbial culture was observed.</p> <p>The adhesion occurred even when both the mineral and the bacteria were negatively charged, suggesting hydrophobic interactions or enhancing adsorption due to the high affinity for the hydroxide film on the oxidised tested mineral surface.</p> <p>Microorganisms exhibited negative zeta potentials within the pH range 2–12 due to the presence of negatively charged functional groups such as <math>-\text{COOH}</math>, <math>-\text{NH}_2</math>, and <math>-\text{OH}</math>. The application of microbial community, induced hydrophilicity on the mineral surface, for which polysaccharides were responsible, causing poor flotation. Chalcopyrite pretreatment with bacterial culture inhibited effective adsorption of the chemical collector to the sulphide surface. When the collector was first adsorbed, the bacteria did not influence the mixed potentials of the mineral surface.</p> <p>In the case of galena, bacteria inhibited the interaction of the collector via mineral surface passivation. When the collector was first adsorbed, bacteria also decreased the mixed potential of the minerals, probably as a result of its continuous oxidation.</p>	[49]
Pyrite Chalcopyrite	100–200 $\mu\text{m}$	<p><i>Halomonas boliviensis</i></p> <p><i>Marinobacter</i> spp. <i>Halobacillus</i> sp.</p> <p><i>Marinococcus</i> sp.</p> <p><i>Halomonas</i> sp.</p> <p>(No data on pathogenicity)</p>	Cells	Depressant	<p>Bacterial adhesion to pyrite was observed for <i>Halobacillus</i> sp. and <i>Halomonas</i> sp. and chalcopyrite only for <i>H. boliviensis</i> and <i>Halomonas</i> sp.</p> <p>Sodium isopropyl xanthate was used as a collector, and bacteria as depressants. The biodepression of pyrite was observed when halophilic bacteria were used as replacements for lime. Pyrite microflotation was reduced from around 68% to less than 10% depending on the bacterium used; <i>H. boliviensis</i>, <i>Halobacillus</i> sp., and <i>Halomonas</i> sp. were the best pyrite depressants in the microflotation experiments.</p> <p>Chalcopyrite depression was observed from 40% to 9% with <i>H. boliviensis</i> and 14% with <i>Halomonas</i> sp.</p> <p>The mechanism of halophilic microorganisms' adhesion to pyrite was considered to be hydrophobic. In the pH range of 4–8, the adhesion of bacteria to minerals resulted in a change in the zeta potential towards more negative values.</p> <p>The zeta potential of pyrite was approximately between <math>-20</math> and <math>-70</math> mV and chalcopyrite <math>-30</math> to <math>-60</math> mV for pH 2 and pH 10, respectively.</p>	[50]

Table 1. Cont.

Mineral/Ore	Particle Size	Biosurfactant	Form of Application	Role	Surface Modification Effect	Ref.
Galena Sphalerite	105–150 $\mu\text{m}$	<i>Bacillus subtilis</i> (NCIM 2063) (No data on pathogenicity)	Cells	Surface modifier	Adaptation of bacteria to the mineral increases the flotation recovery of that mineral compared to that without adaptation. Selective flotation tests on a synthetic mixture of galena and sphalerite confirm that sphalerite can be preferentially floated from galena in the presence of the insoluble fraction of thermolysed cells of <i>B. subtilis</i> initially adapted to sphalerite, with a high selectivity index. Thermolysis disrupted the structure of the bacterial cells, releasing molecules responsible for surface modification. The amphipathic DNA molecule hydrophobised sphalerite surface, whereas other macromolecules present after cell disruptions bound to galena, leading to its depression.	[68]
Galena Sphalerite	105–150 $\mu\text{m}$	<i>Bacillus megaterium</i> (No data on pathogenicity)	Cells/ EPS/eDNA *	Surface modifier/Collector	Cells and metabolites of <i>B. megaterium</i> aid in the selective flotation of sphalerite. Sphalerite-adapted cells yield the highest selectivity of separation. Dissolved metal ions, protein, and DNA components modulated the bacterial surface charge. At pH 2–10, the surface charge of the unadapted <i>B. megaterium</i> cells was negative and increased with increasing pH. The zeta potential of sphalerite and galena, adapted cells exhibited less negative values compared to the unadapted bacteria, probably due to neutralisation of negative charges on the bacterial cell surface by the free cations leached out from the mineral. Bacteria secreted a larger amount of eDNA in the presence of either sphalerite or galena compared to in their absence. The magnitude of the negative zeta potential of both sphalerite and galena after adaptation was found to decrease compared to that of the unadapted mineral, as a result of the adsorption of proteinaceous secretions on the mineral surfaces. Selective flotation of sphalerite (over 80%) was achieved using thermolysed cells, a soluble fraction of thermolysed cells, and secreted proteins of bacteria adapted to sphalerite.	[71]
Apatite Calcite	–150 $\mu\text{m}$	<i>Pseudomonas songnenensis</i> (No data on pathogenicity)	Cells	Collector	Bacterial cells improved the flotation of apatite minerals in phosphate ore; Tested pH 3–11. Maximum floatability 98% at pH 6–7 in the presence of $4 \times 10^7$ cells/ml. Low affinity of a bacterial cell to the calcite surface; No significant effect on flotation. The flotation of a binary mixture contained 25% $\text{P}_2\text{O}_5$ and 20% $\text{CaCO}_3$ , resulting in a concentrate of 32.7% $\text{P}_2\text{O}_5$ and 6.8% $\text{CaCO}_3$ in the presence of $4 \times 10^7$ cells/ml at 25 °C and pH 6.5. Natural phosphate ore flotation contained 21.2% $\text{P}_2\text{O}_5$ and 25.6% $\text{CaCO}_3$ , which produced a concentrate of 31.5% $\text{P}_2\text{O}_5$ and 9.1% $\text{CaCO}_3$ .	[64]

Table 1. Cont.

Mineral/Ore	Particle Size	Biosurfactant	Form of Application	Role	Surface Modification Effect	Ref.
Apatite Quartz	Not specified	<i>Bacillus cereus</i> (Pathogenic)	Bacterial culture	Collector	An isoelectric point (IEP) occurred at pH 4.7 for apatite and pH 2.1 for quartz. The IEP of the treated apatite with <i>B. cereus</i> decreased from 4.7 to 1.8. There was no significant change in the IEP value of quartz. The adsorption of bacteria onto apatite was attributed to electrostatic forces, hydrogen bonding, and chemical interaction. The higher floatability of apatite compared to that of quartz was due to the higher affinity of <i>B. cereus</i> for apatite.	[66]
Barite Quartz	63–90 $\mu\text{m}$	<i>Bacillus licheniformis</i> (PTCC1320) (No data on pathogenicity)	Cells/ purified culture medium/ medium containing metabolites	Collector	Bacteria adhered better to the barite surface, which was attributed to a strong electrostatic effect between the cell and the mineral as a result of the opposite charges revealed in zeta potential measurements. Bacterial cell adsorption decreased with an increase in pH due to the negative charge of the minerals and cells and the strong repulsive electrostatic forces. <i>B. licheniformis</i> cells enhanced barite hydrophobicity as a bio-collector, resulting in the separation of quartz impurities from barite minerals. Under the optimal treatment and separation conditions ( $6.55 \times 10^3$ cells/ml, 20 min, pH = 3), a maximum of 87% barite and 15% quartz flotation recoveries were obtained after 3 min of aeration. Flotation experiments in 1:1 mineral mixtures (0.5 g of each mineral) led to barite and quartz recoveries of approximately 76% and 4% and a product grade of 96.3% with a separation efficiency of 72%. Barite flotation recovery was highest at pH 3 for bacterial cells (87%), and quartz for pH 9 and metabolites (16%).	[67]
Dolomite Apatite	Not specified	<i>Corynebacterium diphtheriae</i> (Pathogenic) <i>Pseudomonas aeruginosa</i> (Pathogenic)	Bacterial culture	Surface modifier	Preferential adsorption of <i>P. aeruginosa</i> was observed on dolomite. <i>Corynebacterium</i> caused an increase in the mean particle size diameter from 5 to $\sim 12 \mu\text{m}$ , while <i>Pseudomonas</i> increased to $\sim 30 \mu\text{m}$ . The best grade (0.7% of MgO and 31.8% of $\text{P}_2\text{O}_5$ ) with high recovery (>80%) was obtained for bacteria-collector interactions at pH = 11, 3 kg/t dodecyl- <i>N</i> -carboxyethyl- <i>N</i> -hydroxyethyl-imidazoline, and a concentration of <i>P. aeruginosa</i> of $4 \times 10^7$ cells/ml. The collector–bacteria interaction improved the flotation selectivity.	[79]

Table 1. Cont.

Mineral/Ore	Particle Size	Biosurfactant	Form of Application	Role	Surface Modification Effect	Ref.
Hematite Pyrolusite Iron ore	~74 $\mu\text{m}$	<i>Paenibacillus polymyxa</i> (No data on pathogenicity)	Cells	Collector	The adsorption of bacterial cells was pH dependent and decreased at pH 6–10. Bacteria cells showed higher adhesion to pyrolusite, but the FTIR results showed that chemical adsorption occurred on hematite, which made it more hydrophobic than pyrolusite. Conditioning with a bacteria suspension changed the IEP to higher values. Bioflotation of a binary hematite-pyrolusite mixture at pH 3 for 10 minutes in the presence of $5 \times 10^{10}$ cells/ml, floated hematite with a manganese reduction of 65%. Flotation of a natural iron ore containing 8.79% $\text{MnO}_2$ , 0.49% $\text{SiO}_2$ and 67.90% $\text{Fe}_2\text{O}_3$ at pH 3, conditioning with bacteria for 10 min. gave a concentrate that contained 3.7% $\text{MnO}_2$ , 0.5% $\text{SiO}_2$ and 71.30% $\text{Fe}_2\text{O}_3$ , with a hematite recovery of 72.46%.	[60]
Hematite	<30 $\mu\text{m}$	<i>Serratia marcescens</i> (Pathogenic) <i>Acinetobacter</i> sp. MSG8 (Pathogenic) <i>Stenotrophomonas</i> sp. MB-1-6-5 (Non-pathogenic)	Cells	Surface modifier/ collector	With 60 mg/L of a single strain at pH 6, the hematite recovery rates for tested species were all greater than 75%. <i>Stenotrophomonas</i> sp. MB-1-6-5 was non-pathogenic to humans and could be of practical use as a bio-collector for hematite flotation. The surface area of the cells belonging to this strain was strongly hydrophobic (contact angle $69 \pm 1^\circ$ ) and highly negatively charged (zeta potential was $-27.8$ mV; pH 6). Bacteria adsorption on the hematite surface might occur via chemical adsorption, where interactions of phosphate groups with the hematite surface and hydrophobic associations among hydrophobic hematite particles play a crucial role.	[61]
Hematite	<30- $\mu\text{m}$	<i>S. marcescens</i> (Pathogenic)	Cells	Collector	Bacteria (contact angle $69 \pm 1^\circ$ ; zeta potential $-4.5$ for pH 3 to $-36.3$ mV for pH 10) were used as a bio-collector during hematite flotation. At a bacteria cell concentration of 60 mg/L, the recovery of hematite was close to 80%. The addition of bacteria cells during hematite flotation increased the hydrophobicity of hematite and the hematite particle size. Bacteria adhesion to hematite occurs mainly via chemical adsorption, including chemical interactions between carboxylic groups and the hematite surface and hydrophobic associations among hydrophobic hematite particles. Hydrophobic agglomerates are formed.	[62]

Table 1. Cont.

Mineral/Ore	Particle Size	Biosurfactant	Form of Application	Role	Surface Modification Effect	Ref.
Quartz Hematite	75–106 $\mu\text{m}$	<i>Rhodococcus erythropolis</i> (No data on pathogenicity)	Cells/ Metabolites		<p>The high selectivity of biosurfactant for hematite gave flotation efficiency close to 100% and around 33% for quartz. The floatability of quartz was improved using a surfactant concentration within pH 3–5 (15–35%). Above pH 7, there was no significant effect (&lt;5%).</p> <p>Hematite flotation was significantly improved via surfactant concentration within pH 3–5 (&gt;85%). Above pH 7, the effect of collector addition floatability did not exceed 30%.</p>	[75]
Magnetite Phlogopite	37–74 $\mu\text{m}$	<i>Paenibacillus amylolyticus</i> (No data on pathogenicity)	Bacterial culture	Surface modifier	<p>Bacteria exhibited a higher adsorption to magnetite. Cell adhesion caused the magnetite surface to be more hydrophobic and the phlogopite to be more hydrophilic. For magnetite, flotation efficiency (pH 6.6) decreased with increasing bacteria concentration and incubation time. The dose of bacterial culture above 3.0 mL had little impact on phlogopite.</p> <p>Pretreatment with bacteria increased mineral recovery. The maximum difference in floatability between magnetite and phlogopite was observed when the minerals were pretreated for 10 days. The flotation separation was driven by the selective adsorption of bacterial cells and metabolic products (including proteins and polysaccharides) mechanism.</p>	[80]
Kaolin Quartz	(38 $\mu\text{m}$ )	<i>B. licheniformis</i> (PTCC1320) (No data on pathogenicity)	Cells/ Bacteria culture both/ Metabolites	Flocculant	<p>About 40% improvement in kaolin settling was observed using bacterial cells and metabolite at pH = 7 and 3, respectively.</p> <p>Quartz sedimentation was &gt; 50% at pH = 1–3. Polysaccharide was more effective in kaolin flocculation, and protein was more influential in quartz agglomeration. All biosurfactants were more likely adsorbed on quartz. Application of bacteria culture broth (metabolites) caused quartz sedimentation to be 90% at pH 1. When polysaccharides were used, quartz sedimentation yielded 82% at pH 5. In the case of protein, 78% of the quartz was flocculated at pH 1.</p> <p>Increasing the pH value decreased the reagent adsorption and mineral flocculation, probably as a result of repulsive forces between the particles, as they became progressively negative with increasing pH.</p> <p>Electrostatic interactions are the most important driving forces in the surfactant adsorption to both minerals. In the case of polysaccharides and protein adsorption at neutral to alkaline pH, it might be due to chemical, van der Waals, hydrogen bonding, or depletion forces.</p>	[81]



Table 1. Cont.

Mineral/Ore	Particle Size	Biosurfactant	Form of Application	Role	Surface Modification Effect	Ref.
Bauxite Kaolinite	58–75 $\mu\text{m}$	<i>Paenibacillus mucilaginosus</i> BM-4 (No data on pathogenicity)	Cells/ polysaccharides/ proteins	Surface modifier	<p>The interaction of bacteria with kaolinite increased the contact angle between the mineral and the water from 36.5° to 64.1°, increasing its hydrophobicity.</p> <p>The contact of bacteria with bauxite contact angles between water and bauxite declined the contact angle from 34.2° to 24.3°, enhancing mineral hydrophilicity. Therefore, the tested bacteria could be used as the collector of kaolinite and the inhibitor of bauxite, respectively. The floatability of bauxite was significantly depressed by strain, while those of kaolinite were enhanced.</p> <p>The bioflotation test of the mixture of bauxite and kaolinite (mass ratio 5:1) showed that the Al/Si ratios improved from 3.05 to 8.60 after bacterial conditioning due to the depression of the bauxite. 83.0% of <math>\text{Al}_2\text{O}_3</math> was recovered from mixed minerals.</p> <p>Kaolinite flotation recovery improved from 50.1% to 65.3% to 77.3% due to the adsorption of bacteria. For bauxite, it decreased from 48.9% to 25.7%–27.8% after adsorption with <i>P. mucilaginosus</i>.</p>	[82]
Talc Chlorite minerals	–0.105 mm	<i>B. subtilis</i> (No data on pathogenicity)	Bacterial culture	Collector	<p>The talc surface was covered by biofilm, while there was no significant bacterial cell adhesion to the chlorite surface.</p> <p>The maximum separation efficiency of talc from chlorite was achieved at pH 4. A talc concentrate with 98% quality and approximately 95% recovery was prepared from a binary talc/chlorite mixture containing 85% talc under optimum conditions of <math>8 \times 10^5</math> cells/ml, pH 4, 35 °C, and a contact time of 10 min.</p> <p>The results show that the negativity of zeta potential decreased strongly after bacterial treatment, whereas the surface of the chlorite mineral was less affected by bacteria adhesion IEP shifted to 4.15 instead of 4.85 before treatment).</p> <p>Both minerals had a negative value of the zeta potential, and adhesion occurs due to the presence of polysaccharides, hydrophobic and ionic moieties, hydrogen bonds and chemical interactions.</p> <p>The maximum difference in the floatability of the talc and chlorite treated with <i>B. subtilis</i> was obtained in the presence of <math>8 \times 10^5</math> cells/ml at pH 4, 35 °C, and a contact time of 10 min.</p>	[83]

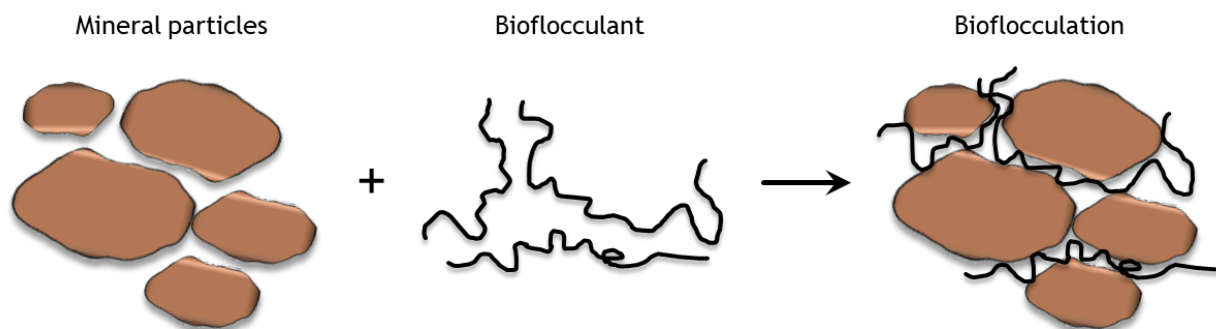
Table 1. Cont.

Mineral/Ore	Particle Size	Biosurfactant	Form of Application	Role	Surface Modification Effect	Ref.
Coal	Not specified	Rhamnolipid	Purified surfactant obtained from the bacterial culture of <i>Pseudomonas aeruginosa</i> MA01	Depressant	Rhamnolipid had a negative effect on coal flotation selectivity, both in the absence and in the presence of a chemical collector. The potential mechanism responsible for the depression process involves the van der Waals bonding between surfactant molecules with aromatic functions on the surface of coal particles in the absence of a chemical collector or physical bonds with the collector's hydrocarbon rings.	[74]
Coal	~44 $\mu\text{m}$	Surfactin lipopeptide	Surfactant solution	Collector	Coal modification by surfactin shifted the zeta potential of the solid toward more negative values with increasing concentration of surfactant. Two types of interactions were proposed: polar interactions (hydrogen bonding) between surfactin and coal-oxygenated groups and non-polar chain interactions (via van der Waals forces) with the hydrophobic carbonaceous surface. Physisorption via hydrophobic interaction was proposed. The best flotation recovery (74%) was achieved at pH 3 and 10, using 15 mg/L of biosurfactant.	[84]

\* Extracellular DNA.

## 5. Bioflocculation of Minerals

Flocculation is the accumulation of particles in aggregates. It involves preferential adsorption of organic flocculants in certain solids, leaving the remaining particles suspended. The application of substances of biological origin to this process is known as bioflocculation (Figure 6) [85]. In industrial processes, synthetic flocculants are most commonly used, the biodegradation of which is a difficult and lengthy process. For this reason, bacteria-produced flocculants are more environmentally friendly [86]. The adsorbed flocculant macromolecule shows a specific spatial conformation, which forms trains, loops, and tails. The main mechanism of flocculation is "bridging" as a result of the association of fine mineral particles by long tails. Extracellular polymeric substances produced by bacterial cells play a fundamental role in the bioflocculation process. In general, substances of this type can be divided into two groups: water-soluble EPS and EPS that are strongly bound to the host cell. The latter group includes polysaccharides, proteins, lipids, humic substances, and nucleic acids [85]. The functional groups in the macromolecules of bioflocculants play a special role since they are responsible for adsorption onto the mineral surface. The presence of such functional groups can ensure the selective adsorption of the flocculant on the selected mineral, which creates conditions for the separation of that mineral from a mixture of other minerals. Such a process is referred to as selective flocculation.



**Figure 6.** Scheme of a single act of bioflocculation.

*B. subtilis* produces mainly proteins and polysaccharides, which act as flocculants to selectively act on kaolinite [86]. The adsorption of proteins on kaolinite makes its surface more hydrophobic. On the contrary, the adsorption of polysaccharides on the surface of hematite results in its surface becoming hydrophilic. Polysaccharides cause the selective flocculation of hematite, while kaolinite remains dispersed. *Bacillus licheniformis* cells and the biopolymers produced by this cell were used to selectively flocculate kaolin in a mixture with quartz. *B. licheniformis* (PTCC1320) improved kaolin settlement by approximately 40% when bacteria cells and metabolites were used for pH 7 and 3, respectively, and quartz sedimentation by more than 50% at pH 1-3 [81]. *B. cereus* isolated from Egyptian iron ore deposits was used to selectively flocculate a suspension of hematite and silica [87].

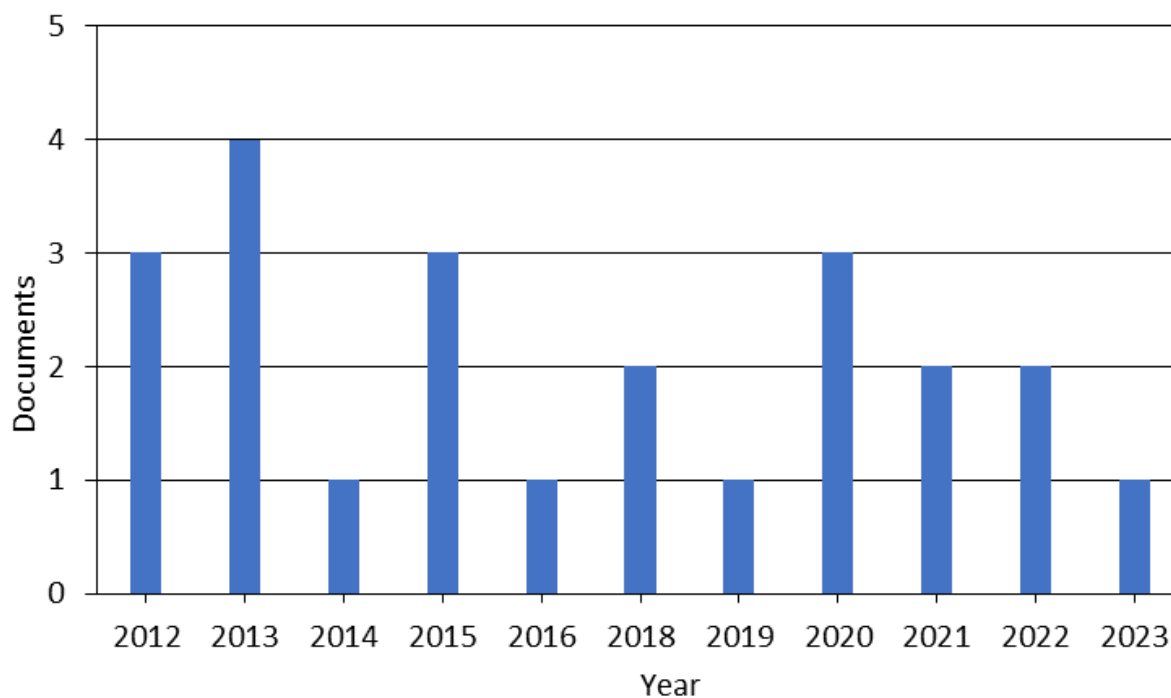
As a result of the flocculants produced by the bacteria, selective separation of hematite from its mixture with silica was realised. The concentrate obtained via flocculation contained 2% silica and 98% hematite. Using selective bioflocculation, it was possible to remove more than 80% of the silica from the hematite mixture. The selective action of bioflocculants was used to separate silica and clay minerals from a fine-grained aqueous coal suspension [88]. The carbon flocculation efficiency was 83% under pH 2 conditions, using an 80 mg/L dose of flocculant. The authors showed that the hydroxyl groups of the bioflocculant were responsible for the bridging mechanism. The presence of these groups promotes chemical bonding between the bioflocculant molecule and the kaolin surface groups. Carbon particles are selectively flocculated as a result of hydrophobic interactions between carbon and the bioflocculant. The rich assortment of microorganisms in suspension can produce a variety of bioflocculants. Their main action is to accelerate

the sedimentation of mineral particles [89]. In addition to mineral processing, a common application of the bioflocculation process is wastewater treatment.

## 6. Summary

Until now, large-scale production of most active microbial surface agents has not reached a satisfactory economic level because a high-cost input is required for downstream processing to recover and purify microbial surfactants. Therefore, new strategies are needed for the commercialisation of biosurfactant production. Such obstacles can be overcome by isolating potential microorganisms that can use renewable substrates to increase the quality and quantity of surface-active compounds. The possibility of using waste materials, including crop residues, animal fat, dairy, distillery, and by-products of food and agro-industries as better substrates for production has been reported [2,90]. Helmy et al. [91] have also reviewed several alternative strategies for commercial production.

Based on the articles from the last decade, a significant part of the research conducted used bacterial culture or cell suspension as collectors or depressants in flotation. The number of publications on the use of rhamnolipids as potential biological compounds to modify the surface of minerals has decreased compared to previous years (Figure 7).



**Figure 7.** Number of documents published by year based on the Scopus database (keywords: “rhamnolipid” AND “flotation”).

New studies are emerging targeting surfactants other than those of microbial origin, e.g., plants. Furthermore, despite extensive research on this matter, technology has not yet been developed to allow the use of bioflotation on a larger scale. The current review shows that much of the literature considers the use of bacterial cells, testing newer strains of microorganisms. Because of this, another important aspect that should be taken into account is that the strains should be non-pathogenic to humans. Among the literature reviewed, only a few authors included such information.

The role of physicochemical interactions at the biosurfactant-mineral interface is essential in realising effective and eco-friendly mineral processing. Based on the presented literature, it might be stated that microorganisms are more likely to be applied for surface modification in flotation, whereas microbial by-products in flocculation. In most cases, in the pH range of 4–8, the surface of bacterial cells exhibited a negative initial surface

charge, and its contact with the minerals caused a further increase in negative zeta potential. When considering technological applications, it is also necessary to take into account the advantages and disadvantages related to the use of bacterial cells and surface-active compounds (Table 2).

**Table 2.** Pros and cons of using microbial surface-active compounds and microbes.

Bacterial Culture/Cells	Microbial Surface-Active Compounds
ADVANTAGES	
<input type="checkbox"/> Ease of suspension preparation	<input type="checkbox"/> Dramatic reduction in surface tension
<input type="checkbox"/> Low cost of producing	<input type="checkbox"/> Higher temperature tolerance
<input type="checkbox"/> Easy bioremediation	<input type="checkbox"/> High salinity tolerance
	<input type="checkbox"/> Eco-friendly
DISADVANTAGES	
<input type="checkbox"/> Pathogenicity of microorganisms	<input type="checkbox"/> Antimicrobial properties
<input type="checkbox"/> Low concentration of biosurfactants	<input type="checkbox"/> High production and isolation costs
<input type="checkbox"/> Low selectivity of action	
<input type="checkbox"/> High flocculation potential	

However, even an analysis of the pros and cons cannot indicate which method of mineral surface modification is better. It should be remembered that both processes occur simultaneously, further complicating the choice.

## 7. Conclusions

Based on the reviewed literature, it is possible to indicate which microorganisms can potentially be used for the selective separation of particular minerals on an industrial scale.

1. Pyrite depression was caused by bacteria such as *A. ferrooxidans*, *A. ferrooxydans*, *L. ferrooxidans*, *Halobacillus*, *Alkalibacillus* and *A. almallahensis*, *H. boliviensis*, *Halobacillus* and *Halomonas* sp., and *B. pumilus*, *Marinobacter* sp. and allowed separation of pyrite mixtures with coal and chalcopyrite.
2. Hematite surface biomodified using *B. subtilis*, *P. polymyxa*, *S. marcescens* PW114, *S. marcescens* S20, *Acinetobacter* sp. MSG8, *Stenotrophomonas* sp. MB-1-6-5, *R. ruber*, *R. erythropolis*, and *M. phlei* become more hydrophobic, increasing their floatability.
3. The interaction of quartz with the metabolites of *B. mucilaginosus* and *B. licheniformis* increases its hydrophobicity, facilitating flotation.
4. Hydrophobisation of the apatite surface is possible with the use of *R. opacus*, *B. cereus*, and *P. songnenensis*.
5. *B. licheniformis* enhances the hydrophobicity of barite.
6. Galena modified with lysed *B. subtilis* can be floated from sphalerite.
7. Dolomite destabilisation was achieved using rhamnolipids produced by *P. aeruginosa*.

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