Abstract

The most significant environmental issue that has arisen in the mining industries over the last decade is that of acid mine drainage (AMD) from tailing dumps and waste mined rock piles. AMD is caused by the oxidation of sulphide minerals followed by leaching and flushing of the product into the receiving environment. Pyrite, chalcopyrite, quartz, and calcite are the most common sulfide and oxide gangue minerals present in tailing dumps originating from the mining operations all over the world. The mining operations results in the exposure of sulfide bearing minerals which has the capacity to produce acid mine drainage (AMD) for which control measures are necessary to protect the environment. Sulfide minerals such as pyrite (FeS_2) are oxidized to sulfate when water, containing oxygen, infiltrates the tailings. Paenibacillus polymyxa is a Gram-positive, neutrophilic, peri flagellated heterotroph indigenously associated with many mineral deposits. Extracellular polysaccharides, proteins, and organic acids such as oxalic acid, formic acid and acetic acid are the principal components of the biomass obtained from Paenibacillus polymyxa.

The current investigation was initiated to develop a bioremediation process to counter AMD problem and interrogate microbially-induced flotation and flocculation behavior of various sulphide and oxide minerals when present alone or in different combinations. The system initially considered consists of oxides such as quartz and calcite and sulphides such as pyrite and chalcopyrite. The prime objective with this system was to develop a process with Paenibacillus polymyxa cells and metabolic products, which would lead to a separation of unwanted pyrite and chalcopyrite from the oxides. After successful attempt with bacterial cells and metabolite, investigations were extended with major extracellular bacterial bioreagents present in metabolite such as proteins and polysaccharides. These bioreagents were observed to show different adsorption density onto different minerals. Selective separation with these extracellular bioreagents was also achieved. Studies were later extended to include the sulphide minerals such as sphalerite and galena and mixtures of quartz, calcite, pyrite, chalcopyrite, sphalerite, and galena. Besides the success of selective separation of desired minerals from different synthetic minerals system, detailed investigations were also carried out to examine the surface chemical behavior of various minerals in course of microbe-minerals interactions. Cell wall and their associated bioentites were isolated to examine their contribution towards microbe-mineral interactions.

Major objectives of this investigation are outlined as below.

1. The basic principles and mechanisms governing microbe-mineral interactions were examined from the viewpoint of desulphurization of AMD tailings and microbially-induced mineral flotation and flocculation. Detailed elucidation of surface chemical changes brought about by the microbe-mineral interactions is discussed with respect to surface chemical and bio dissolution studies. Besides bacterial cells the role of bacterial metabolite and associated extracellular proteins and polysaccharides in bringing about bacterial adhesion, modulation of surface properties of the minerals with respect to hydrophilicity and hydrophobicity and dissolution, along with development of probable mechanisms of microbe-mineral interactions are discussed.

2. Minerals were characterized to ascertain their purity through X-Ray diffraction and mineralogical analysis. SEM micrographic analysis was also carried out for the characterization of bacterial cells. Pure culture of bacteria was acquired through pour plating procedure. The investigation was commenced by characterizing both minerals and bacterium in the following ways

• Mineral samples were ground and sieved to obtain different size fractions. The size fractions were separated in two major groups, i.e. finer sizes, and coarser sizes. Finer particles were further ground to obtain very fine colloidal particles (< 5 microns). These fines were used for adsorption, electrokinetic, flocculation and bio dissolution studies. Coarser particles were used for flotation studies. Surface area of the minerals was also determined.

• To ascertain the purity of the bacterium it was pour plated and a pure colony was isolated and further recultured for different studies. The cell count was studied, and a growth curve was established. Besides, pH of the bacterial culture was also examined at regular intervals as it was an imported parameter in major part of this investigation. Bacterial culture was grown in presence of different minerals in order to observe any possible changes in their effect onto different minerals and separation processes.

3. It is generally believed that the effect of bacterial cells onto a mineral substrate is directly dependent on the cell count associated per unit mineral surface area. Hence the investigation was initiated by studying the

measurement of adsorption density onto minerals as a function of time. The results obtained from these studies prompted to examine the adsorption behavior with respect to pH and also establishment of adsorption isotherms to understand the pattern they form in course of adsorption process. As an attempt to understand the interfacial reagents associated in adsorption process, studies were carried out with bacterial metabolite, extracellular product, and cell wall bioreagents. Adsorption of bioreagents were also compared with chemical reagents used in mineral processing. To determine the comparative affinity of proteins and polysaccharides onto individual minerals, adsorption studies were also carried out in different sequences. SEM micrographic analysis was also successful to ascertain the specificity of the bacterial cells towards different minerals observed in the earlier studies.

4. The zeta potential values of bacterial cells and minerals considered in this investigation were determined. The effect of pH, interaction time and reagent concentration on the zeta potential values of minerals and bacterial cells after mutual interaction with each other, has been evaluated. Zeta potential of minerals after interaction with extracellular bioreagents as well as cell wall associated bio entities was also measured.

5. Settling behavior of individual minerals after interaction with bacterial cells as well all associated bioentites has been studied. These studies revealed different possibilities of selective separation of individual minerals from different synthetic mineral systems.

6. Micro flotation studies have been conducted on single mineral systems in order to assess the changes in their hydrophobicity and hydrophilicity, consequent to interaction with the bacterial cells, the metabolic products and also the cell wall associated bioreagents. These studied showed different

possibilities of selective separation of individual minerals from different systems. Additional surfactant was also used in these experiments to enhance the efficiency of separation processes.

7. Bio dissolution studies were also carried out with bacterial culture, metabolite, and metabolic products.
8. Bioreagents were isolated from the minerals surface subsequent to adsorption process. Presence of different bioreagents was assured through various self-developed techniques. It was interesting to note that specific group of proteins are involved in driving the adsorption processes for individual minerals. Hence investigation was extended to purify the proteins and their effect on the minerals was studied.
9. Extracellular bacterial protein after characterizing through SDS PAGE technique revealed that it consists of numerous groups of different proteins. Initially EBP was fractionated through ammonium sulphate precipitation procedure. Further purification of the proteins was attempted with different chromatographic techniques such as Ion exchange chromatography and FPLC chromatographic techniques. Purified protein fractions obtained from these experiments were used for adsorption studies. The results obtained from these experiments strengthen the fact that there are specific proteins available in EBP, which show selective affinity towards different minerals.
10. Since bacterial cells were washed thoroughly prior to their studies with minerals it was assumed that the cell wall bio entities may play a prime role. Hence proteins and polysaccharide associated with the cell wall was separated and their effect onto the minerals was studied. Partial characterization of proteins was attempted through SDS PAGE technique.
11. In order to gain better understanding of the mechanisms of microbe-mineral interactions, ruthenium red adsorption, protein assay and cell surface hydrophobicity tests have been conducted. Cursory experiments have been performed to characterize the secreted proteins and polysaccharides using SDS PAGE electrophoresis techniques, mass spectrometry and NMR techniques.
Major conclusions based on this work are summarized below.
Bacterial cells showed higher affinity towards pyrite, chalcopyrite and galena compared to sphalerite, calcite, and quartz. The adsorption isotherms studies showed that the adsorption patterns of bacterial cells onto the minerals were different. Pyrite and chalcopyrite showed highest adsorption of EBP whereas ECP adsorption was higher onto galena, pyrite, and chalcopyrite. S-Layer protein
adsorption was higher on pyrite and chalcopyrite. Plasma membrane protein was higher onto quartz and was quite reasonable onto chalcopyrite. Adsorption of Cell Wall polysaccharides was observed to be the highest onto galena. Adsorption of all the above bioreagents along with PIPX showed that pyrite and chalcopyrite hardly accommodated any PIPX onto its surface. Similar phenomenon was observed with adsorption of ECP onto galena. Electrokinetic studies showed shift in IEP values for quartz, calcite, and sphalerite. Since galena, pyrite and chalcopyrite gets flocculated with bacterial cells or associated bioreagents there was no shift in IEP observed in these cases. Galena, pyrite, and chalcopyrite was observed to get flocculated with cells and the effect was prominent with pyrite and chalcopyrite. Quartz was observed to show dispersion characteristics with cells and EBP. Other bioreagents were observed to show differences in the settling properties with individual minerals. This led to selective separation of

pyrite and chalcopyrite from their mixture with other minerals. Micro flotation of individual minerals also showed difference in floatability with bacterial cells and all the other bioreagents also. Pyrite and chalcopyrite were depressed with bacterial cells and hence were used to selectively separate from other minerals. Similar behavior was also observed with other bioreagents. Use of collector was observed to improve the efficiency in many cases. Characterization showed that EBP consisted of numerous proteins. Hence purification of protein was attempted to isolate individual or groups of proteins through Ammonium sulphate precipitation techniques, ION exchange and FPLC chromatography techniques. It was observed that proteins involved in adsorption, flocculation and flotation were mineral-specific proteins. Hydrophobicity studies revealed that bacterial cells develop both hydrophobic and hydrophilic characteristics when interacted with different minerals. Finally, ruthenium red adsorption onto mineral-adapted bacterial cells revealed that galena adapted cells produced the highest number of polysaccharides. Similarly, protein assay test showed that pyrite/chalcopyrite-adapted cells lead to higher amount of secreted proteins. Characterization of ECP showed that it consisted of a wide range of carbohydrate related functional groups.