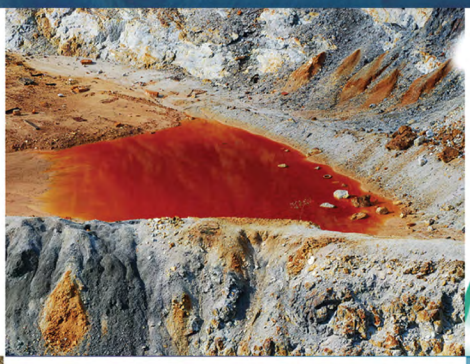
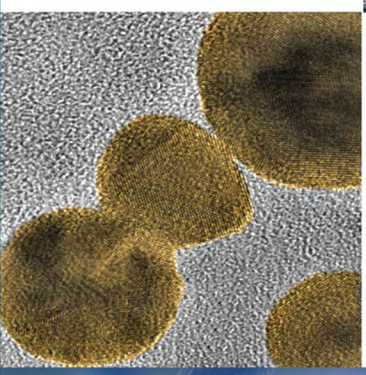
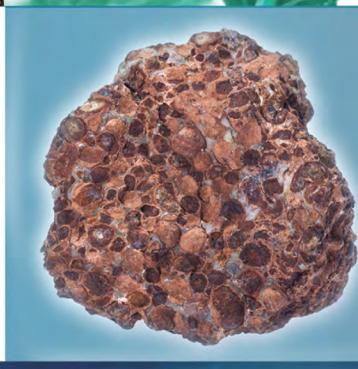
MICROBIOLOGY FOR MINERALS, METALS, MATERIALS AND THE ENVIRONMENT



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Contents

Preface

Microbiology for Minerals, Metals, Materials and the Environment is a generic book aimed at analysing and discussing the symbiotic relationship between microbiology and materials through an interdisciplinary approach. Minerals, metals and all other materials exhibit a close interlinking with different environmental microbes in different ways. For example, biogenesis and biomineralisation are now established to explain the role of indigenous microorganisms in the occurrence, transformation and transport of several metals and minerals across the Earth's crust as well as ocean depths. The biology–materials cycle in nature involves various biomediated processes such as materials processing, metals extraction from ore minerals, microbially influenced mobilisation and corrosion of metals and materials, and environmental degradation as well as bioremediation. Although the role of microorganisms in mineral dissolution from naturally occurring ore deposits was known centuries ago, relevance and applications of biology–materials interfaces in a truly interdisciplinary fashion were scientifically established for only 30–40 years. Several microorganisms and microbiological concepts find applications in different facets of metals and materials processing such as biogenesis and biomineralisation, bioleaching of ores, metal-containing wastes and secondary resources, biomineral beneficiation, desulphurisation of fossil fuels, microbially influenced metallic corrosion, and microbial aspects of environmental processing, such as acid mine drainage and biosorption and bioremediation.

A major objective of this book is to present the status and developments in various aspects of the above-mentioned themes. The book is aimed at a readership that includes teachers, students, researchers, scientists and engineers specialising in mining, metallurgy, materials processing, coal processing, corrosion engineering, secondary metals recovery, environmental pollution and protection. In this respect, personnel from the mining, metallurgical as well as materials processing industries would find the contents of this book very interesting and practically relevant. Each chapter is written by one or more experts from academic and R&D institutions as well as industries.

The book can be divided into thematic areas of applications of microbiology in mineral, metal, material and environment sectors. The chapters illustrated in the book cover different aspects such as biohydrometallurgy, biomineralisation, bioleaching, biobeneficiaton, biosynthesis and bioremediation. The basics and historical aspects of biohydrometallurgy, biomineralisation and biogenesis of commercially important ore minerals, microbially induced mineral flotation and flocculation processes are extensively illustrated. Other aspects that are covered include the applications of microbes for metal extraction (including mechanisms and methods) from primary ores/minerals and mining wastes, biomining and related concepts of microbial diversity

and various operations, and molecular biology of microbes involved in such systems (extremophiles). Selected chapters on specific systems include the importance and exploitation of microbes in the processing of enargite, uraninite and coal. The current industrial scenario of biohydrometallurgy is critically highlighted. The dissolution of minerals to metals may finally lead to the recovery/synthesis of products. Material synthesis by microbial intervention is depicted in detail highlighting the various mechanisms involved and essential role of microbes. Apart from minerals and ores, the recovery of metals from secondary resources/wastes such as e-wastes and urban mine wastes is also presented with respect to their types, composition, mechanisms of biodissolution and other allied aspects. Biogeochemistry of metals in the environment is an added attraction. The role of microbes in remediating (solid and liquid) wastes containing organic and inorganic contaminants as well as acid mine drainage are also presented in a few designated chapters. This also includes the role of microbes in influencing metallic corrosion. It would thus be useful to understand the linkage of microbiology with minerals, metals and materials, and grasp the importance of bacteria/microbes in this sector, microbial intervention for waste clean-up, and the role of microbes in extreme environments, and finally to establish connectivity of a non-biologist to biologist with a difference and a microbiologist to process metallurgy in its entirety.

The editors are thankful to all the authors for their valuable contributions. They also express their sincere thanks to CRC Press for bringing out this timely publication.

Abhilash
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K. A. Natarajan

Editors

Dr. Abhilash is a scientist in the biohydrometallurgy lab of the Waste Recycling and Utilisation Group of the Council of Scientific and Industrial Research-National Metallurgical Laboratory (CSIR-NML), Jamshedpur, India. He graduated in microbiology from Nagpur University and earned his post-graduate degree from Bangalore University specialising in mineral biotechnology. He earned his PhD (engineering) from Jadavpur University, India for his work titled 'Biohydrometallurgy of Indian Uranium Ores'. He worked as a lecturer in the Department of Biotechnology, SRN Adarsh College, Bangalore (affiliated to Bangalore University). Later on, Dr. Abhilash joined CSIR-NML as a project fellow and worked in the field of bioleaching of uranium from low grade Indian ores (a project under CSIR's Xth five-year plan) for 2 years. Later, he was selected as a junior scientist in the Metal Extraction and Forming Division of CSIR-NML to work in the area of biohydrometallurgy and other allied disciplines. He has been associated with various projects sponsored by the Government of India and industry under the designations of project leader and team member. In addition to these, he also served as an assistant professor in the Academy of Scientific and Innovative Research (AcSIR-NML).

He has been awarded three consecutive MISHRA awards for the best paper in extractive metallurgy by the Indian Institute of Mineral Engineers (IIME). He was also awarded the prestigious 'Young Scientist Award' at the 100th Indian Science Congress and Indian Nuclear Society for his work on uranium bioleaching. He was bestowed an award for his work on metal–microbe interactions by the Association of Microbiologists, India. He is a Fellow of the Society of Applied Biotechnology, India. He is also a member of various national and international professional bodies including the Engineering Section Committee of the Indian Science Congress Association.

Dr. Abhilash has published over 50 papers (out of which 20 are dedicated to uranium biohydrometallurgy) in SCI (Science Citation Index) journals and in National/International Conference Proceedings. He has presented over 30 papers under the oral/poster category at various national and international symposia/colloquia in India and abroad. He has authored the book *Chromium(III) Biosorption by Fungus*, Lambert Academic Publishing House, GmBH, and edited two compendium volumes. He is on the editorial board of international journals and also is a reviewer for prestigious journals in his

field of expertise. He has visited countries such as Egypt, Australia, Russia and China concerning ongoing projects, participated in conferences and fostered international collaboration. He has delivered keynote lectures in India, and also has guided a large number of students with their master's thesis. He is also actively engaged in interaction with school students and teachers to promote biotechnology education.

His areas of interest include microbial technology, environmental microbiology, bioleaching, bioremediation, biosynthesis, microbial corrosion and waste management (metal scraps, effluents, e-waste, etc.).

Dr. B. D. Pandey is currently the chief scientist and head of the Metal Extraction and Forming Division, Council of Scientific and Industrial Research-National Metallurgical Laboratory (CSIR-NML), Jamshedpur, and a professor at the Academy of Scientific & Innovative Research (AcSIR)-NML, India. He earned his PhD in metal extraction and separation by solvent extraction and metal complexation from the Banaras Hindu University, Varanasi, India. While working at CSIR-NML for over 32 years, he has contributed significantly in the area of extraction of non-ferrous metals from both primary and secondary resources, including the extraction of rare earths. He is a life member of professional bodies such as the Indian Institute of Metals and Indian Institute of Mineral Engineers (IIME) (India). He has received several medals and awards, such as the Best Mineral Engineer Award of IIME and Distinguished Researcher Award (International Conference on Non-ferrous Metals, India). He has led very active collaboration programs with the Korea Institute of Geo-science and Mineral Resources (KIGAM), Daejeon (as invited scientist during 2010–2011) under the Brain-pool scheme of Korea Government, Russian Institutes (IGIC, Moscow and Science Centre of Siberian Branch, RAS, Krasnoyarsk) and Paris Tech., France. He is on the editorial board of international journals such as *Hydrometallurgy* and *Metals & Materials International*, and has been the editor-in-chief of the *International Journal of Non-ferrous Metallurgy*. His areas of research include chemical beneficiation, hydro- and biohydro-metallurgy and bioremediation, sulphation roasting and waste processing, recycling and environmental management. He has published over 130 research papers in leading international journals in the above-mentioned areas and more than 85 papers in international conference proceedings. He is involved in teaching and managing the affairs of the Academic Committee as chairman, AcSIR-NML, Jamshedpur.

Dr. K. A. Natarajan is currently a NASI (National Academy of Sciences) senior scientist—Platinum Jubilee Fellow and emeritus professor in the Department of Materials Engineering, Indian Institute of Science, Bangalore, Karnataka, India. He earned his MS and PhD specialising in mineral beneficiation and hydrometallurgy from the University of Minnesota, USA. The Indian Institute of Science, Bangalore conferred on him the degree of Doctor of Science in 1992 for his pioneering research contributions in minerals bioprocessing. He is a Fellow of the Indian Academy of Sciences, Indian National Academy of Engineering and the National Academy of Sciences (India). He has received several medals and awards such as the National Metallurgist Award by the Ministry of Mines, Government of India; National Mineral Award by the Ministry of Mines, Government of India; Alumni Award of Excellence in Engineering Research by the Indian Institute of Science, Bangalore; Kamani Gold Medal of the Indian Institute of Metals; Hindustan Zinc Gold Medal and the Biotech Product and Process Development and Commercialization Award by the Department of Biotechnology, Government of India. He is on the editorial board of several international journals in the area of mineral processing. His areas of research include mineral processing, hydrometallurgy, minerals bioprocessing, corrosion engineering and environmental control. He has published over 300 research papers in leading international journals in the above areas. He was the chairman of the Department of Metallurgy, Indian Institute of Science, Bangalore during 1999–2004.

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Biomineralisation and Microbially Induced Beneficiation

K. A. Natarajan

CONTENTS

1.1 Introduction

A faster depletion of high-grade ore deposits has necessitated the development of alternative efficient beneficiation processes to treat low-grade ores. The beneficial roles of microbes in the field of mineral processing starting from mining, beneficiation and metal extraction to efficient waste disposal have now been well recognised. Physicochemical methods of mineral

beneficiation such as flotation, acid dissolution, magnetic and gravity separation are often energy-intensive, costly and environmentally unacceptable.

The utility of microorganisms and bioreagents in mineral beneficiation has been well demonstrated (Chandraprabha and Natarajan, 2010; Natarajan, NPTEL Course, 2013). For example, microbially induced flocculation or flotation of minerals, remediation of toxic chemicals discharged from mineral processing operations, degradation of cyanide and so forth have been proven as promising alternatives. Different from bioleaching, microbially induced beneficiation involves selective removal of undesirable mineral constituents from an ore through interaction with microorganisms or their metabolic products and enriching it with respect to the desired value minerals. Unlike conventional techniques, microbial processes would be more energy efficient, cost effective and environmentally benign. Conventional beneficiation techniques involve the use of various toxic reagents, which could be safely replaced by environment-friendly biological reactants.

Microbially induced mineral beneficiation utilises microbe–mineral interactions to bring about an efficient removal of undesirable constituents in an ore through

a. Selective dissolution or
b. Conferment of surface hydrophobicity or hydrophilicity to bring about selective flotation or flocculation.

Consequences of microbe–mineral interactions relevant to microbially induced mineral beneficiation are the following:

a. Adhesion to mineral substrates resulting in biofilm formation,
b. Bio-catalysed oxidation and reduction reactions and secretion of biopolymers.
c. Interaction of cells and bioreagents with ore matrix.
d. Surface chemical changes on minerals or dissolution.

Several types of autotrophic and heterotrophic bacteria, fungi, yeasts and algae as well as their metabolic products can be used in mineral beneficiation processes.

Microbiological, surface-chemical as well as chemical factors involved in microbially induced beneficiation are discussed in this chapter with special reference to clays, bauxite, iron ores and sulphide minerals. Biomineralisation and biogenetic aspects of clay, bauxite, iron and sulphide ore deposits are analysed to understand the role of indigenous microorganisms. The different types of native mining organisms with respect to their role in mineral beneficiation are identified.

Microbially induced mineral beneficiation processes are illustrated with respect to surface properties of bacterial cells, their adhesion behaviour towards minerals and the surface chemical changes on the minerals consequent to microbe–mineral interaction.

1.2 Microbiological Aspects of Cell Adhesion to Mineral Substrates

Since microbial adhesion to mineral substrates and the formation of biofilms are important events in biobeneficiation processes, it is essential to understand the basic microbiological characteristics of different microorganisms. The structure and architecture of the bacterial cell wall play a prominent role in adhesion to mineral surfaces and resulting consequences. The mechanisms and consequences of adhesion differ depending on the type of the organism (Chandraprabha and Natarajan, 2010; Natarajan, NPTEL Course).

Bacteria are classified into two categories with reference to cell wall structure, namely, Gram-positive and Gram-negative. The chemical composition and structural features of cell walls are different in these two types. Gram-positive bacteria possess a well-defined, rigid outer cell wall, 15–30 nm thick, and an inner, closely held, cell-limiting plasma membrane. The wall constitutes 15%–30% dry weight of the cell. A major polymeric component is peptidoglycan and it may also contain one or more secondary polymers such as teichoic and teichuronic acids. On the contrary, the cell walls of Gram-negative bacteria contain a more general architecture having an outer membrane placed above a thin peptidoglycan layer. The bilayer membranes contain proteins, phospholipids, and lipopolysaccharides and also separate the external environment from the periplasm. In between the outer and the plasma membranes, a gel-like matrix (the periplasm) exists in the periplasmic space. The plasma membrane and the cell wall (outer membrane, peptidoglycan layer and periplasm) together constitute the Gram-negative envelope. In bacterial cells, the functional groups of polymers confer surface charges. The bacterial adhesion mechanisms are governed by surface charges and the nature of membrane polymers.

Bacterial cells possess a net negative surface charge at neutral pH due to the presence of peptidoglycan and teichoic acids. In this respect, microorganisms can be considered as living colloidal particles. The cells acquire charge through the ionisation of surface polymeric groups, which is pH dependent. Competition between electrical and chemical forces may control surface charge neutralisation. An electrical double layer is established at the mineral–solution–bacteria interface. Similar to mineral particles, the surface chemical properties of microorganisms can be characterised by zeta potential and the isoelectric point (IEP). Increasing solution ionic strength may favour bacterial adhesion to a mineral substrate. Bacterial cell dispersion and flocculation are also governed by electrical forces as in the case of mineral particles. The DLVO theory can be applied to explain the mineral–cell–solution interfaces. Bacterial surface hydrophobicity is also controlled by its cell membrane polymers. Hydrophobicity is conferred by hydrophobic molecules such as lipids present on cell surfaces. Hydrophobic bacterial

cells are known to adhere to surfaces due to repulsion from the polar water molecule. Electrostatic repulsive forces decrease higher surface hydrophobicity. Since the bacterial surface charge enhances the possibilities for polar interactions with water molecules, the more charged the cell surfaces, the less hydrophobic they become. A decrease in the cell negative charge may then enhance cell surface hydrophobicity. Cells having higher hydrophobicity and lower electrophoretic mobility are more adherent. Bacterial adhesion mechanisms are controlled by cell surface charges and hydrophobicity. The chemical composition and architecture of the cell wall outer layers play a significant role. The surface proteins and polysaccharides may be involved in bacterial adhesion to minerals. Many bacterial cells possess both hydrophilic and hydrophobic surface regions. Cell surface hydrophobicity can be modified depending on the environmental conditions. Since interfaces offer a better environment for bacterial nutrition and growth, bacterial adhesion at mineral–solution interfaces is necessitated under environmental conditions.

Organic and inorganic reagents generated by bacterial metabolism are useful in mineral beneficiation. Different types of mineral acids, fatty acids, polymers and chelating agents are generated by bacteria, fungi and yeasts. A large variety of fatty acids, polysaccharides and proteins produced by microorganisms have been tested in the flotation of various ores. The microbial products can function as flotation collectors, depressants or dispersants.

1.3 Biomineralisation and Biobeneficiation of Clays

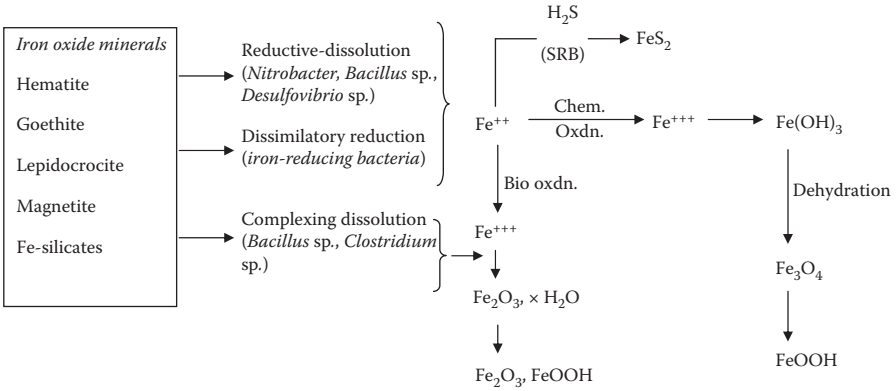
Clay formations portray a unique subsurface microbial habitat. Microorganisms are often trapped during deposition of clay layers (Shelobolina et al., 2005). Clays are biogenic and the plasticity of clays is due to microbial activity. Clay minerals are biologically altered and iron-rich diagenetic minerals in the form of iron hydroxide nanocrystals and biogenic clays are deposited around bacterial cells. Iron is present in cream-colored kaolins as ferric oxides and hydroxides (hematite and goethite) and as a structural replacement in kaolinite. In grey kaolins, which have not yet been oxidised, iron is present mainly as its sulphide (pyrite).

Clay minerals are abundant in soils and sediments. Together with microorganisms, they provide catalytic surfaces in sedimentary environments, which are important in many biogeochemical cycles. Microbial clay mineral oxidation and reduction can occur at temperatures and pH values existing in soils. Similar to iron oxide minerals, organic and electron transfer agents enhance the bioavailability of clay-bound ferric ions for reduction. Geochemical evidence attests to the fact that ferric iron in

clay minerals can be rapidly reduced. Iron-rich clay minerals serve as the primary electron acceptor available for microbial ferric reduction. Various types of aerobic and anaerobic bacterial species have been isolated from different clay deposits such as smectite, hard kaolin, soft grey kaolin and soft tan kaolin. The abundance of organic matter in clay samples suggested indirect iron oxidation by heterotrophic bacteria. There were significantly higher numbers of iron oxidisers than ferric-reducers (Shelobolina et al., 2005).

Another group of microorganisms that could influence the iron chemistry of clays (Shelobolina et al., 2005) is aerobic heterotrophic microorganisms producing ferric-specific chelating agents called siderophores. Iron is an essential micro-nutrient for many microorganisms. In oxygen-rich neutrophilic environments, in the absence of organic or inorganic chelators, iron availability is limited by the solubility of its hydroxides. In response to low iron availability, aerobic microorganisms generate siderophores for iron mobilisation. An example of a siderophore-producing bacterium is *Pseudomonas mendocina*, which can gather smaller concentrations of iron from kaolinite. Bacterial siderophores can increase the release of iron, silicon and aluminium from kaolinite. Aerobic heterotrophs have been located in large numbers in several kaolin samples.

Microbial iron mineral transformation and mobilisation in kaolin clays are schematically illustrated below:



Microbial activity can result in the following changes in the composition of iron minerals:

a. Quantity of magnetically separated magnetite significantly increases.
b. Newer thermodynamically unstable ferric hydroxides such as ferrihydrite and lepidocrocite are synthesised.
c. Formation of iron sulphides (pyrite).

d. Changes in morphology, structure and composition of thermodynamically stable goethite and hematite. For example, acicular goethite and chemical hematite.

Consequences of microbial adhesion on clay minerals need to be understood. Electrokinetic properties of clays are modified by microorganisms and their metabolic products. Significant surface chemical changes are thus brought about on clay minerals by bacterial interaction. The surface-chemical properties of clays such as hydrophobicity, flocculation and dispersion can be controlled through microbial interaction. The dispersion and flocculation of clays can be controlled by interaction with proteins, amino acids and polysaccharides, which are bacterial metabolic products. The formation of polymeric hydrous oxides of iron and aluminium due to microbial weathering result in changes in electrokinetic properties. The possible role of extracellular slime layers in bacterial adhesion need to be understood. Microbial cells readily attach (adhere) onto clay particles through electrostatic and chemical interaction mechanisms.

The need for clay beneficiation in the light of the mineralogical changes due to biomineralisation is analysed below.

Kaolin clays contain coarse and fine impurities that need to be removed. Coarse impurities, more than half the crude volume of clays consist of quartz, feldspar, mica or tourmaline. Sedimentary kaolin contains titania mineral impurities such as anatase and rutile. Titania minerals contain lattice-substituted iron imparting a yellowish brown color. Along with titania, significant amounts of iron oxides and iron sulphides also occur in clays. Iron oxide minerals such as hematite and goethite impart a dull yellowish brown color. Iron sulphide minerals such as marcasite and pyrite occur in kaolins containing organic matter, which impart a gray colour. Even in small amounts, iron impurities reduce the brightness and whiteness of raw materials derived from kaolin. Bright, white pigments are generally preferred over dull, yellow pigments manufactured from kaolin clays. Also, kaolin used in paper and ceramic industries needs be whitened through iron removal.

Most of the high-quality clay deposits occurring on the top layers of the Earth's crust have already been mined. The naturally whiter clays contained the more easily removable hematite and/or goethite, and most of their dark organic matter had already been oxidised. We are now forced to mine deeper regions of clay deposits where the as-yet un-oxidised kaolin is grayer, containing more organic matter and iron sulphides.

Various methods of beneficiation are practised for iron removal from clays. Physico-chemical methods of clay beneficiation for removal of impurities such as iron and titanium oxides are the following:

a. High intensity superconducting magnetic separation.
b. Froth flotation.
c. Chemical treatment using hydrosulphides to reduce and solubilise iron oxides.

d. Wet high intensity magnetic separation.
e. Iron leaching by oxalic acid.
f. Beneficiation and dewatering (flocculation, deflocculation).
g. Selective flocculation, reductive leaching and dewatering.

All the above abiotic methods are energy-intensive, costly and not environment-friendly. A biotechnological alternative to clay beneficiation would prove to be cost-effective, energy-efficient and environment-friendly (Styriakova and Styriak, 2000; Groudev, 2001; Kostka et al., 2002; Lee et al., 2002; Mandal and Banerjee, 2004; Gao et al., 2010; Ajayi and Adefila, 2012).

Development of a flexible biotechnology for kaolin beneficiation, either through microbial ferric reduction or ferrous oxidation, can be considered depending on the iron mineralogy. For commercial quality kaolin, ferric-reduction of hematitic and goethitic iron by using indigenous ferric-reducing bacteria would enhance the quality.

Selective bioflocculation can be considered to remove titania. In some selective flocculation methods, kaolin containing titania (and other fine impurities) is interacted with flocculants to promote settling of titania impurities, leaving the product kaolin to be recovered from the supernatant in a dispersed form.

Clay-slimes are generated during the beneficiation of phosphate and potash ores. The use of microorganisms for dewatering such clay-slimes and enhancing settling rates would prove beneficial. Biopolymer-producing microbes can flocculate phosphatic clay-slimes. Dilute phosphatic clay-slimes can be flocculated with indigenous organisms. Polymers isolated from *Leuconostoc mesenteroides* and *Xanthomonas* sp. were found to flocculate phosphatic clay-slimes.

Microorganisms also play an important role in the dissolution of silicate structures. *Bacillus* spp. are active and can remove both free and bound iron occurring in kaolin. Biodegradation of iron oxyhydroxides and partial destruction of mica structure can be brought about by microbial interaction.

Microbiological reducing and solubilising agents can also be used. Biotechnological methods for leaching kaolin using fungi such as *Aspergillus niger* can also be considered.

Iron removal from quartz sands, kaolins and clays can be achieved in a number of ways such as:

a. The use of bacterial and fungal metabolites at high temperatures.
b. *Bacillus cereus* isolated from kaolin deposits.
c. Fungal strains of the type *Aspergillus*, *Penicillium* and *Paecilomyces* spp.
d. High amounts of oxalic acid produced by *A. niger*.

Passive 'in situ' beneficiation methods at mine sites also become possible. Stimulation of activity of natural microflora present in clay deposits through nutrient additions is an 'in situ' biobeneficiation approach. Bacterial growth and production of organic acids decrease pH leading to transformation of iron minerals in clay deposits.

Microbial removal of pyrite from low-grade clay using sulphur and iron-oxidising bacteria such as *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* has been demonstrated. About 82%–90% of the pyrite was removed in 5–12 days for pulp densities up to 70%. With the refined clay no red colour due to the presence of pyrite was developed after firing and its whiteness was similar to that of high-grade clay.

1.3.1 Microbially Influenced Iron Removal from Some Indian Clays

Iron is present in several Indian clays in the form of Fe_2O_3 and FeS_2 which need to be removed to achieve whiteness and desired qualities for use in ceramic, paper, paint and other industries. Using cell cultures and acidic metabolites of *A. thiooxidans* and *Bacillus* sp. the efficient removal of iron from two types of clay samples (one containing iron as hematite, another as pyrite) was demonstrated. Microbially influenced selective flocculation–dispersion as well as reductive dissolution of iron oxides from clays were also demonstrated successfully.

A few examples are illustrated below:

China clay samples containing pyritic iron having the following chemical composition were used to demonstrate biobeneficiation.

SiO_2	44%
Al_2O_3	32%
Fe_2O_3	5.2%
TiO_2	2.3%
CaO	0.3%
LOI	20%

Note: Brightness (as received) = 45–46.

Kaolinite was the major mineral with small amounts of pyrite and quartz.

The above china clay deposits are rather unique in the sense that iron is present as a sulphide (pyrite). Due to weathering of the sulphides, the clay samples in contact with water generated acidity and equilibrated at a pH of 2–2.5. Indigenously occurring acidophilic chemolithotrophs such as *Acidithiobacillus* were isolated from the clay samples. For this purpose, clay samples were inoculated into 9 K medium and Basal medium containing sulphur at a pH of 2 to promote the growth of *A. ferrooxidans*, *A. thiooxidans* and also *Leptospirillum ferrooxidans*. Variations of cell population along with changes in concentrations of ferric and sulphate ions were monitored as a

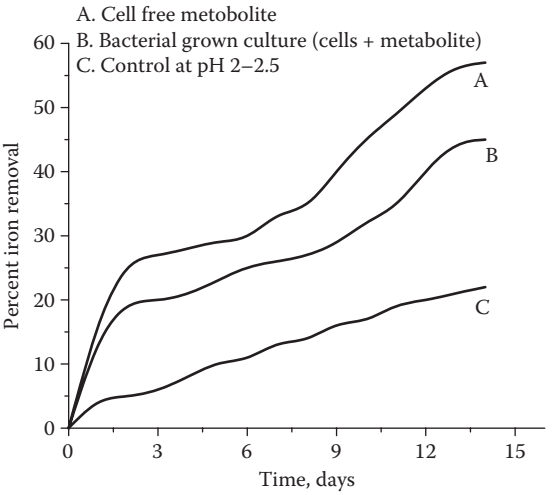


FIGURE 1.1
Iron removal from pyritic clays through microbial dissolution.

function of time. Several subculturings were carried out to attain higher cell populations of isolated acidophiles.

All the above isolated acidophilic bacteria could be used to remove the iron (as pyrite) from the clay samples. Among the clay isolates, *A. ferrooxidans* and *A. thiooxidans* were used to study iron removal. Typical results are illustrated below:

a. With cultures of *A. ferrooxidans* grown for different periods of subculturing up to 70%–80% of iron could be removed after a period of two to three weeks.
b. Similar results were obtained with cultures of *L. ferrooxidans.*

Iron removal from clays using different acidic metabolites from *A. thiooxidans* are illustrated in Figures 1.1 and 1.2.

Bioleaching of iron (pyrite) using metabolites (pH 0.5) of *A. thiooxidans* is illustrated in Table 1.1.

Brightness measurements of samples treated with pH 0.5 metabolite of *A. thiooxidans* are given in Table 1.2.

1.3.2 Microbially Induced Selective Flocculation of Hematite from Kaolinite

Microbially induced selective flocculation of hematite from kaolinite was also demonstrated with respect to interaction with *B. subtilis* (Poorni and Natarajan, 2013, 2014). The growth of bacterial cells in the presence of kaolinite resulted

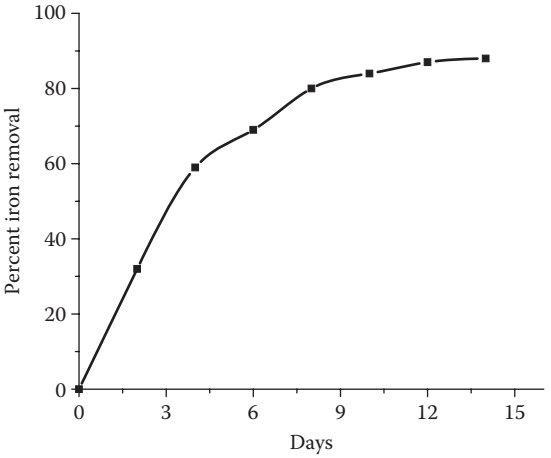


FIGURE 1.2
Pyrite removal from pre-acid washed clay fines with metabolite of *A. thiooxidans.*

in enhanced production of extracellular proteins whereas that of hematite promoted significant secretion of exopolysaccharides. Bacterial cells were successfully adapted to grow in the presence of both of these minerals and the advantage of using hematite-grown and kaolinite-grown cells and their metabolic products in the selective flocculation of hematite and effective dispersion of kaolinite was demonstrated. Bacterial cells and extracellular polysaccharides exhibited higher surface affinity towards hematite, rendering it hydrophilic, while significant protein adsorption on kaolinite led to enhanced

TABLE 1.1
Pyritic Iron Removal Using *A. thiooxidans*

	Interaction Time (h)	Percent Iron Removed
Growing culture	1	55
	12	68
	24	99
Cell free extract	1	50
	12	75
	24	98

TABLE 1.2
Brightness of Clay Samples

	Brightness (% ISO)
Untreated pyritic kaolin clay	58
12 h interaction	66
24 h interaction	73

TABLE 1.3

Selective Bioflocculation for Hematite–Kaolinite Separation

	Percent Settled			
			Mixture	
Conditions	**Kaolinite Only**	**Hematite Only**	**Kaolinite**	**Hematite**
Without bacterial interaction	40	60	40	65
Bacterial cells adapted to hematite	10	95	10	90
Kaolin-adapted bacterial cells	05	70	10	60
Bioproteins from hematite-adapted cells	45	80	25	82
Bioproteins from kaolinite-adapted cells	10	75	10	80
Exopolysaccharides from hematite-adapted cells	04	95	20	95
Exopolysaccharides from kaolinite-adapted cells	08	90	20	75

Source: Adapted from Poorni, S., Natarajan, K.A., *International Journal of Mineral Processing* 125; 2013: 92–100.

surface hydrophobicity. Bacterial interaction promoted selective flocculation of only hematite, while kaolinite was fully dispersed. Mineral-specific proteins were generated on growing *B. subtilis* in the presence of kaolinite.

Typical selective bioflocculation results are illustrated in Table 1.3.

At neutral pH, maximum settling (flocculation) for hematite was achieved using exopolysaccharides extracted from hematite-grown cells. A maximum dispersion of kaolinite was achieved after interaction with kaolinite-adapted cells and proteins and polysaccharides separated from adapted cells. Higher-surface affinity of kaolinite for proteins and of polysaccharides for hematite is responsible for the above observed flocculation–dispersion behaviour.

An efficient separation of hematite from kaolin samples could be obtained using cells of *B. subtilis* preadapted to kaolinite as well as the proteins and polysaccharides extracted from such cell cultures. The developed bioprocess could be compared with existing chemical alternatives such as selective flocculation and flotation using toxic chemicals such as amines and polyacrylamides. Efficient separation of iron oxides from clays is not possible through such methods including high-intensity magnetic separation and selective flocculation–dispersion. A bioprocess, on the other hand, would be more process efficient, cost effective and environment friendly.

1.4 Biomineralisation and Biobeneficiation of Bauxite

Significant chemical and biological weathering of silicates can occur in bauxite deposits. Strongly oxidised conditions to gradually reduced environments

are encountered during the bioevolution of bauxites. Bauxite deposits contain water, organic carbon, sulphur and iron that can be used as energy sources by indigenous microbes. The role of microorganisms in the formation and evolution of bauxite has now been well established (Laskou and Eliopoulos, 2007, 2013).

Biomineralisation from oxidised to reduced environments involves the following stages:

a. Rock weathering due to excretion of microbial metabolites (organic and inorganic acids, ligands)
b. Microbial weathering of Al-silicates release Al, Fe and Si followed by precipitation of biogenic carbonates, sulphides and iron oxides
c. Aerobic and anaerobic microbes participate in sulphate formation and sulphide precipitation.

Bacterial consortia occurring in bauxite deposits dissolve primary rock-forming minerals and serve as nucleation sites for the precipitation of secondary minerals. For example, the presence of various types of microorganisms in gray-colored bauxites has been well documented. Many metals can be enzymatically enriched and dispersed by organisms (Papassiopi et al., 2010).

Two stages of the transformation of bauxite ores can be envisaged:

a. Reduction of ferric to ferrous leading to the formation of pyrite in a variety of forms and size.
b. Subsequent pyrite oxidation and formation of goethite.

Various morphological forms of pyrite could be identified commonly associated with fossilised bacteria in grey-red bauxites. The coexistence of fine-grained spherical pyrite and pyrite pseudomorphs after iron oxides suggest that pyrite is the product of bacterial activity and has formed from iron oxides. Bacteria facilitate nucleation and growth of minerals.

The role of microorganisms in bauxite mineral formation can be understood in relation to the following:

a. Bacterial metabolism involving redox reaction with sulphur and metals such as iron and aluminium.
b. Bacterial communities catalyse mineral formation since they derive energy for growth through selective oxidation–reduction of metals and serve as nucleation sites for precipitation of secondary minerals.
c. Both aerobes and anaerobes participate in the above biomineralisation processes.

To demonstrate the role of microorganisms in bauxite mineralisation, studies were carried out using Indian west coast bauxites. Indigenous microbes

from several deposits were isolated, identified and their role in mineralisation analysed (Phalguni et al., 1996).

Typical microorganisms isolated from some Indian west coast bauxite deposits are listed in Table 1.4.

Various autotrophic and heterotrophic bacteria and fungi isolated from west coast Indian bauxite ore deposits were found to participate in the formation and concentration of bauxite. *A. ferrooxidans* generates sulphuric acid and the pH changes cause weathering of the aluminosilicates and precipitation of iron oxyhydroxides. Biological and chemical weathering causes mobilisation of aluminium by the settling of alumina in swamps, where it precipitates as aluminohydroxides. *Bacillus* spp. mediate the release of alkaline metals, such as sodium, potassium and calcium from the bauxite deposits. Magnesium and iron from bauxite also can be released by microbial action. Aluminium and silica remain in the bed leading to bauxite mineralisation. Fungi such as *Cladosporium* are alumina solubilisers and iron reducers. *Pseudomonas* anaerobically reduce ferric oxides, while *Bacillus coagulans* and *Paenibacillus polymyxa*, through the excretion of polysaccharides, flocculate iron oxides, alumina and calcite. Biogenic origin of the bauxite mineralisation thus becomes evident.

TABLE 1.4

Mine Isolates and Their Functions

	Isolates	Salient Characteristics Relevant to Biomineralisation
Fungi	*Aspergillus niger* *Aspergillus* spp.	Soil fungus, utilising carbohydrate sources from degrading plant waste, secretes organic acids such as citric, oxalic and gluconic acids.
	Cladosporium *Cladosporoides*	Fungus capable of reducing iron and dissolving aluminium.
Bacteria	*Bacillus* spp. *Bacillus circulans* *Paenibacillus polymyxa*	Bacteria secrete exopolysaccharides and organic acids such as acetic and citric acids and can grow anaerobically reducing ferric iron.
	Bacillus coagulans	Spore forming, Gram-positive bacteria, acidophilic, thermophile with optimum temperature of 35–45°C; and generate acid conditions of pH 4–5.
	Pseudomonas spp. *Pseudomonas putida* *Pseudomonas stutzeri* *Acidithiobacilli*	Capable of anaerobic ferric iron reduction.
		Autotrophic acidophile, capable of oxidising ferrous iron and precipitating iron oxyhydroxides. Also generates sulphuric acid and forms sulphates.
	Sulphate-reducing bacteria	Precipitates sulphide minerals.

Source: Adapted from Natarajan, K.A., Modak, J.M., Anand, P. *Minerals and Metallurgical Processing* 14(2); 1997: 47–53.

TABLE 1.5

Biobeneficiation of Bauxite Ore Using Mine Water as Inoculum

Days	Percent Ca Removed	Percent Fe Removed
4	55	34
15	88	40

Source: Adapted from Natarajan, K.A., Modak, J.M., Anand, P. *Minerals and Metallurgical Processing* 14(2); 1997: 47–53.

Beneficiation of low alumina bauxites (<50% Al_2O_3) was studied in detail using mine-isolated native organisms (Phalguni et al., 1996; Modak et al., 1999; Vasan et al., 2011).

Initial screening tests were performed to establish the feasibility of using mine isolates in the removal of calcium and iron. Both these impurities as present in Indian western bauxites need to be removed to produce a commercially acceptable raw material for the abrasive and refractory manufacturers. Typical results are shown in Tables 1.5 and 1.6. The mine water contained a consortia of heterotrophs capable of removing both calcium and iron.

Isolates capable of efficient removal of calcium included the fungus, *A. niger* and a number of neutrophilic bacteria such as *B. cirulans, P. polymyxa* and *Pseudomonas* spp.

It becomes readily evident from the above discussions that many types of microorganisms indigenously present in bauxite deposits are capable of bringing about different reactions such as reduction of ferric ions, reductive dissolution of iron oxides and dissolution of calcium carbonate. It then becomes possible to use such mine-isolated microorganisms to achieve selective removal of calcite, iron oxides as well as silica and silicates (clays) from the bauxite ores.

Paenibacillus polymyxa, a neutrophilic, organic-acid-producing bacterium was found to be present in the mine waters and bauxite deposits. The bacteria, when grown in a Bromfield medium containing sucrose, ammonium sulphate, magnesium sulphate and yeast in the presence of calcium carbonate, were found to undergo calcium-dependent metabolism utilising calcium

TABLE 1.6

Beneficiation of Bauxite Ore Using Different Microorganisms Isolated from Indian West Coast Bauxite Mines

Isolates	Percent Ca Removed	Percent Fe Removed
Apergillus niger	78	15
Bacillus circulans	88	21
Paenibacillus polymyxa	86	23
Pseudomonas spp.	83	33

Source: Adapted from Natarajan, K.A., Modak, J.M., Anand, P. *Minerals and Metallurgical Processing* 14(2); 1997: 47–53.

to produce amylases to synthesise a dipicolinate. Profuse excretion of extracellular polysaccharides and organic chelating acids led to the dissolution of calcium carbonate.

The use of the above bacteria in the removal of iron oxides and calcium carbonate from low-grade bauxites was studied to establish an environmentally benign and cost-effective biobeneficiation process (Modak et al., 1999; Vasan et al., 2011).

To demonstrate calcium removal using *P. polymyxa,* a column bioreactor was designed which could be operated either in the fluidised-bed mode (for fine particles) or in a drain mode (coarser particles). Pulp density of the bauxite ground slurry could be controlled and fed from the top of the column reactor as total recycle slurry. Air vents on top facilitated aeration and spillage control. A uniform suspension of the particles using controlled air passage was ensured. Depending on bacterial growth cycles, two types of operations, namely, cascade and uncascade were carried out. Since the cell growth ceases after 24 h, the contact time was maintained at 24 h, when the slurry was drained off to a storage tank. After settling the suspended slurry, the leach liquor was decanted. Fresh bacterial culture was added and the solids pumped back to the reactor. The entire operation was repeated 4–5 times as recycle mode in the cascade operation sequence. In the uncascade mode, no such reinoculation or recycle of the slurry was permitted. The entire operation was carried out continuously for 4–5 days at a desired pulp density. Higher calcium removal could be observed when the reactor was operated in the cascade mode and typical results are presented below:

a. More than about 90% of calcium removal was achieved in the cascade mode.
b. Bioleaching of calcium carbonate took place in two stages, namely, (i) a rapid indirect mode in the presence of acidic bacterial metabolites containing exopolysaccharides and (ii) a slower direct mode, involving bacterial attachment to particles and enzymatic dissolution.
c. Typical compositions of the raw feed and leached residue are given in Table 1.7.

TABLE 1.7

Metallurgical Analysis of Ground Bauxite Ore and Bioleached Residues

	Percent Composition			
	Al_2O_3	Ca	Fe_2O_3	SiO_2
Ground bauxite ore	58.6	2.0	2.3	3
Biotreated residue	59	0.3	1.8	2

Source: Adapted from Modak, J.M., Vasan, S.S., Natarajan, K.A. *Minerals and Metallurgical Processing* 16; 1999: 6–12.

Calcium solubility in the bacterial metabolite was found to be very high even when compared to hydrochloric acid leaching.

The following mechanisms are proposed to explain the enhanced $CaCO_3$ removal through indirect mechanism.

a. Bacteria produce levan from sucrose (soluble capsule) which is a homopolysaccharide.
b. $CaCO_3$ solubility in culture increases from 70 mg/L in growth media to 300 mg/L in full-grown culture.
c. Fully grown bacterial culture contains organic acids such as formic acid, acetic acid, butyric acid and lactic acid.
d. Ca-organic acid salts have high solubility in water. Calcium forms stable complexes with many of these acids.
e. Compared to mineral acid, grown culture shows significantly higher calcium solubility.
f. Al_2O_3 solubility is very limited. Al-organic acid complexes are not known.

The mechanism of direct leaching of calcium carbonate on the other hand may involve:

a. Adhesion of cells to bauxite minerals.
b. Ca-dependent metabolism resulting in the formation of polysaccharides (in the capsule and biofilm).
c. Formation of soluble Ca–polysaccharide complexes resulting in dissolution of complex.

The following changes in calcium carbonate substrate due to adhesion of *P. polymyxa* could be observed:

a. Higher than 90% surface coverage in 24–30 h.
b. Presence of polysaccharides in the bacterial capsule.
c. Negative shift in zeta potential.
d. Decrease in surface hydrophobicity and enhanced settling rate in aqueous solutions.

The use of *P. polymyxa* in the dissolution and flotation beneficiation of bauxite ores has also been reported (Zhou et al., 2010).

The following observations are noteworthy:

a. pH change from 7 to about 4 (18 h) due to organic acid generation by bacterial growth.

TABLE 1.8

Calcium Removal from High Calcium Bauxite by Selective Bioflocculation Using Adapted Cells of *P. polymyxa*

Deslimings	Percent Ca Removal
1	57
2	63
3	67
4	71
5	73
6	79

b. Bacterial metabolites (attached bacteria + metabolites) complexed iron, silica and calcium.

c. Flotation of alumino-silicates (kaolinite) and dissolution of iron hydroxides could be achieved through bacterial interaction.

Yet another biological approach to remove calcium and iron from bauxites is through selective bioflocculation (Deo, 1998). Unlike in biodissolution, flocculation involves only interfacial changes brought about by bacterial interaction and is therefore a very fast process. Typical results are given in Tables 1.8 and 1.9.

The use of *P. polymyxa* is thus very effective in the removal of both calcium and iron from bauxite ores. Silicate bacteria such as *B. mucilaginosus* and *B. circulans* can remove SiO_2 from bauxite. The screening of silicate bacteria and bioleaching of silicon from bauxite (*Desiliconisation*) using *B. mucilaginosus* to control Al_2O_3:SiO_2 ratio has been attempted. The isolated strains adapted (grown) in hematite, olivine and calcite showed different leaching capabilities. Bioremoval of iron from bauxite amounted to 92% using microbial isolates. An interaction with *P. polymyxa* released iron and silicon. Owing to bacterial adhesion and generation of acidic metabolites iron could

TABLE 1.9

Iron Removal from High Iron Bauxite by Selective Bioflocculation Using Adapted Cells of *P. polymyxa*

Deslimings	Percent Fe Removal
1	23
2	40
3	51
4	69
5	85
6	96

be efficiently leached out. Iron-reducing bacteria can also be effectively used to bring about reductive dissolution of iron oxides (Groudev and Groudeva, 1983; Groudev, 1987; Karavaiko et al., 1989).

Microbial removal of silicon from low-grade bauxite has been reported. Silicate bacteria isolated from rock samples and soil were used. Silicon leaching is attributed to the formation of mucilaginous capsules consisting of exopolysaccharide oxides (Groudev and Groudeva, 1983; Groudev, 1987; Karavaiko et al., 1989). Continuous leaching was carried out in bioreactors at room temperature with a residence period of 5–7 days. Typical leaching results indicated reduction of silica from about 25% to about 9% with enrichment of alumina from about 44% to 64%.

Bauxite beneficiation using 'silicate' bacteria (such as *Bacillus circulans*) has also been demonstrated. Up to 75% of silica could be removed.

In situ microorganisms also find application in environmental control with respect to red mud disposal. Microbiology and microbial ecology of red mud samples can be established. The use of bacteria to reduce red mud alkalinity has also been established. Acid producing and alkaline bacteria can be readily isolated from red mud storage piles. Native bacteria proliferation as well as inoculation from the outside can be practiced to bring down alkalinity of red mud. The genera of red-mud isolated microbes include *Bacillus*, *Lactobacillus*, *Micrococcus*, *Pseudomonas*, *Flavobacterium* and different fungi.

Reclamation of bauxite residues through afforestation has also been tried. Bioremediation of bauxite residues has been reported in Western Australia by adding organic substrate to stimulate the growth of indigenous microorganisms (Jones et al., 2012). Native organisms generate organic acids and neutralise red mud. Inoculation of appropriate microbes can also be done from the outside. Bioextraction/microbial uptake of trace metals from bauxite red mud also has been attempted (Jones et al., 2012).

Some examples are:

a. Microbial selenate reduction.
b. Bioleaching of heavy metals from red mud using *A. niger.*
c. Microbial dispersion and flocculation to settle particles and water harvesting.

We have so far analysed two ore mineral systems, namely, clay and bauxite, both of which are basically aluminium silicates containing iron oxides, calcium carbonate and silica as impurities. Biomineralisation features of the above two mineral systems, namely, kaolin clay and bauxite appear similar and complimentary. From a beneficiation angle also, there are complimentary aspects pertaining to the removal of iron. A close correlation among the clay and bauxite systems could then be made with iron ore mineralisation and beneficiation.

For example, clay beneficiation deals with iron removal. Bauxite beneficiation dealt with iron oxide removal from alumina. In iron ore beneficiation, separation of alumina, silica and aluminium silicates from iron oxide becomes paramount. The role of different microorganisms such as yeasts, anaerobic bacteria (SRB) and two *Bacillus* spp. on iron ore beneficiation with respect to silica and alumina removal is discussed below.

1.5 Biomineralisation and Biobeneficiation of Iron Ores

The following questions need to be answered in order to understand the relevance of iron ore biogenesis and biomineralisation with respect to beneficiation.

What types of microorganisms inhabit iron ore deposits?

Why they are there and what roles do they play?

What mineral-related biochemical and surface chemical reactions they can perform?

Microorganisms play a significant role in the natural iron cycle. Many iron-based redox reactions promote bacterial growth through iron dissolution and precipitation. Biogenic iron oxides occur as nanocrystals having different morphology and mineralogy. Direct bacterial nucleation processes can result in the formation of various iron minerals. Acidophilic and neutrophilic, aerobic bacteria promote oxidation of ferrous iron to ferric form, while anaerobic bacteria reduce ferric to ferrous ions. Iron oxides such as goethite, lepidocrocite, magnetite and hematite are found in sediments. Iron oxide particulates occur closely associated with bacterial cell walls having exopolymers. Iron-oxidising microbes can be isolated from iron-rich seepages. The formation of intracellular magnetite has been reported in magnetotactic bacteria. Banded iron formations represent biogenic iron mineralisation (Bonneville et al., 2004; Fortin and Langley, 2005; Gilbert et al., 2005; Roberts et al., 2006; Liu et al., 2006; Williams and Cloete, 2008; Arakaki et al., 2008; Natarajan, 2013).

The following aspects of biomineralisation are significant with respect to the identification of microorganisms relevant to iron ore beneficiation.

- Biotic and abiotic mechanisms are involved in the formation of iron ore minerals such as iron oxides, clays, silica, alumina, calcite and apatite.
- Biogenic iron oxides exist in close association with microorganisms that inhabit the ore body. For example, in natural sediments, iron oxide particulates occur in close vicinity of bacterial cell walls

containing secreted biopolymers and extracellular biogenic iron minerals. Iron oxidising and reducing bacteria are associated with biofilms formed on iron oxide minerals.

- A wide range of microorganisms existing under acidic to neutral pH as well as oxic and anoxic environments bring about oxidation and reduction of iron. A few examples are *Gallionella* sp., *Leptothrix* sp., *Acidithiobacillus, Leptospirillum ferrooxidans* and *Thermoplasmales* (archea).
- FeOOH sheaths are formed by *Lepthothrix* spp. in iron ore mines and generation of exopolysaccharides in the capsule is considered as a protection mechanism against metal toxicity.
- Extracellular polymers such as polysaccharides and bioproteins secreted by several iron bacteria would be useful in iron ore beneficiation. Examples are magnetotactic bacteria, *Bacillus* spp.
- Biogenic iron minerals carry biosignatures. Banded iron formation is a case in point. Nanocrystals of lepidocrocite on and away from the cell wall of *B. subtilis* have been identified.
- Diverse groups of magnetotactic bacteria (MTB) present in iron ore deposits synthesise intra- and intercellular magnetic minerals such as magnetite and magnetosomes.
- Microorganisms isolated from iron ore deposits will be useful in iron ore beneficiation (e.g. removal of phosphorous, alkalis, silica, clays and alumina). Since phosphorous promotes bacterial growth, iron ore particles having higher phosphorous contents were seen to be colonised by bacterial cells. Microbial mobilisation of phosphorous occurs in iron ore deposits. Microorganisms such as *Acidithiobacillus, Clavibacter* and *Aspergillus* isolated from iron ores may act as good phosphate solubilisers, since they generate inorganic and organic acids.
- *Shewanella oneidensis,* an iron-reducing bacterium produces mineral-specific proteins and exhibits a high affinity towards goethite under anaerobic conditions. *S. oneidenisis* can recognise goethite under anaerobic environments.
- Microbial ferric iron reduction by *Shewanella putrefaciens,* a facultative anaerobe would be useful in iron ore beneficiation since they attach preferentially to magnetite and ferrihydrite.

Isolating, characterising, and establishing the usefulness of native microorganisms in iron ore processing hold the key.

The advantages of a biotechnological approach to iron ore processing are many as listed below (Sarvamangala et al., 2012):

- Use indigenous organisms that are adapted to mine environments.
- Cost effective, energy efficient and environment friendly.

- Conventionally used costly toxic chemicals can be replaced by biodegradable, biosynthesised reagents.
- Fast interfacial processes bring about rapid surface chemical changes on minerals
- Three approaches are possible:
  - Selective biodissolution
  - Selective microbially induced flotation
  - Microbially induced selective flocculation
- Diverse areas of applications such as dephosphorisation, desiliconisation, desulphurisation as well as silica, alumina and clay removal from iron ores.

Biobeneficiation of iron ores with respect to separation of silica, alumina, phosphorous (apatite) and clay (aluminium silicate) from hematite is illustrated below using four types of microorganisms, namely, *Saccharomyces cerevisiae* (yeast) SRB (*Desulfovibrio desulfuricans*), *Bacillus subtilis* and *Paenibacillus polymyxa.* All the tested organisms are closely associated with iron ore deposits. The developed biobeneficiation processes are specially suited to treat iron ore fines and slimes (Natarajan and Padukone, 2012).

1.5.1 Yeast-Mediated Separation of Quartz from Hematite

The utility of *S. cerevisiae* in the separation of silica from hematite has been investigated. *S. cerevisiae* is a unicellular and chemoorganotrophic eukaryote occurring associated with iron ore deposits.

Adsorption density of yeast cells was found to be significantly higher on hematite compared to quartz. The growth of yeast cells in the presence of quartz resulted in the generation of higher amounts of mineral-specific proteins, which exhibited higher surface affinity to quartz. Exopolysaccharides were increasingly generated when the yeast cells were adapted or grown in the presence of hematite, which exhibited a higher affinity towards yeast exo-polysaccharides (Natarajan and Padukone, 2011, 2012).

Yeast-mediated separation of quartz from hematite was demonstrated through microbially induced flotation and selective flocculation as illustrated in Table 1.10.

Unadapted yeast cells and their metabolites did not bring about significant flotation separation of quartz–hematite mixtures. Yeast cells grown in the presence of quartz and metabolic products separated from adapted cells promoted efficient selective flotation of quartz from hematite. Similarly, quartz-adapted yeast cells and metabolites promoted the dispersion of quartz particles while significantly flocculating hematite. Quartz can be effectively separated from hematite through selective bioflocculation, also using quartz-adapted yeast cells or metabolites.

TABLE 1.10

Quartz–Hematite Separation through Yeast-Induced Flotation

	Percent Flotation			
			Mixture	
Conditions	**Hematite**	**Quartz**	**Hematite**	**Quartz**
No biointeraction	8	12	14	15
Quartz-grown yeast cells	8	95	10	93
Hematite-grown yeast cells	10	12	9	14

Source: Adapted from Natarajan, K.A., Padukone, S.U. *Minerals and Metallurgical Processing* 29(2); 2012: 81–87.

1.5.2 Use of *B. subtilis* in Iron Ore Beneficiation

B. subtilis is a Gram-positive, neutrophilic, aerobic bacterium found associated with iron ore deposits. The use of *B. subtilis* for the separation of silica and alumina from hematite was investigated as discussed below (Sarvamangala and Natarajan, 2011):

Profuse and significant cell adhesion of *B. subtilis* on hematite were observed, unlike adhesion on quartz and corundum. When bacterial cells were grown in the presence of various iron ore minerals such as hematite, silica (quartz) and alumina (corundum), varying amounts of extracellular proteins and polysaccharides were secreted. The presence of quartz during bacterial growth promoted a higher secretion of proteins while that of hematite enhanced secretion of polysaccharides.

Bacterial interaction conferred increased surface hydrophobicity on quartz, while hematite was rendered more hydrophilic. Interaction with bacterial cells and cell-free metabolites significantly enhanced the flocculation (settling rate) of hematite at neutral pH, while quartz particles were increasingly dispersed. Dispersion of corundum was also facilitated by bacterial interaction, though not to the same extent as observed in quartz.

Flotation behavior of various minerals after interaction with *B. subtilis* is illustrated in Table 1.11.

TABLE 1.11

Flotation of Some Iron Minerals in Presence of *B. subtilis*

	Percent Flotation		
	Control	**With Cells**	**With Cell-Free Extract**
Hematite	10	5	15
Corundum	12	70	30
Quartz	15	92	90

Source: Adapted form Sarvamangala, H., Natarajan, K.A. *International Journal Mineral Processing* 99; 2011: 70–77.

After interaction with *B. subtilis* cells and metabolites, hematite was found to be significantly depressed. Bacterial interaction however promoted the flotation of quartz and corundum. Efficient separation of quartz from hematite from their mixtures could be achieved after interaction with cells and metabolites of *B. subtilis.*

1.5.3 Biobeneficiation Using Anaerobic Bacteria

The use of an anaerobic SRB, namely, *Desulfovibrio desulfuricans* in the quartz–hematite separation was also demonstrated (Sabari Prakasan and Natarajan, 2010).

High amounts of mineral-specific extracellular proteins were secreted by quartz-grown *D. desulfuricans.* Secretion of extracellular polysaccharides was found to be the highest in the case of hematite-grown cells. Protein profiles of unadapted and mineral-adapted *D. desulfuricans* were then established. Quartz-adapted proteins exhibited the highest adsorption density on quartz. Hematite exhibited the highest affinity towards exopolysaccharides.

Flotation separation of hematite and quartz using *D. desulfuricans* was then established as shown in Table 1.12.

In the absence of bacterial interaction, no significant flotation of quartz and hematite was achieved. Higher surface hydrophobicity was exhibited by quartz due to adsorption of bacterially produced hydrophobic proteins. Flotation recovery of hematite decreased to 2% with hematite-grown cells due to its higher affinity towards polysaccharides, which rendered them increasingly hydrophilic. Selective separation of quartz from a binary mixture of quartz and hematite was also established. Efficient preferential flotation of quartz from hematitic could be achieved after interaction with quartz-adapted cells or metabolites of *D. desulfuricans.*

1.5.4 Biobeneficiation Using *P. polymyxa*

P. polymyxa is a Gram-positive, neutrophilic heterotroph occurring in iron ore deposits. The use of *P. polymyxa* and their metabolic products in iron

TABLE 1.12

Flotation Behaviour of Some Iron Ore Minerals before and after Interaction with *D. desulfuricans*

	Percent Flotation			
			Mixture	
	Hematite	Quartz	Hematite	Quartz
Without biotreatment	5	17	5	15
Hematite-adapted cells	2	39	5	37
Quartz-adapted cells and metabolites	9	75	9–12	76–85

Source: Adapted from Sabari Prakasan, M.R., Natarajan, K.A. *Colloids and Surfaces B: Biointerfaces* 78; 2010: 163–70.

TABLE 1.13

Flotation Behaviour of Iron Ore Minerals in the Presence and Absence of *P. polymyxa*

	Control	Cells Alone	Protein	Polysaccharides
Quartz (silica)	4	60	98	4
Corundum (alumina)	5	2	7	1
Hematite	4	3	4	1

Source: Adapted from Deo, N., Natarajan, K.A. *Minerals Engineering* 10; 1997: 1339–354; Deo, N., Natarajan, K.A. *International Journal of Mineral Processing* 55; 1998: 41–60.

ore beneficiation was studied (Deo and Natarajan, 1997, 1998, 1999, 2001; Somasundaran et al., 2000; Natarajan, 2009).

Flotability of different iron ore minerals under different biopretreatment conditions is illustrated in Table 1.13.

Interaction with cells of *P. polymyxa* and their metabolic products affected the flotation and flocculation–dispersion of various minerals as shown in Tables 1.13 and 1.14. Flotation and dispersion of quartz were promoted while hematite and corundum were depressed or flocculated after interaction with bacterial cells and metabolic products. Bacterial proteins promoted surface hydrophobicity and flotation (dispersion) of quartz while interaction with exopolysaccharides promoted flocculation and depression of hematite and corundum (Deo and Natarajan, 1997, 1998, 1999, 2001; Somasundaran et al., 2000; Natarajan, 2009).

Selective flocculation of 1:1 mixtures of hematite and alumina with silica (quartz) after interaction with cells of *P. polymyxa* indicated efficient separation of quartz from hematite and alumina. However, efficient separation of alumina (corundum) from hematite required prior adaptation of bacterial cells in the presence of corundum. Such preadapted cells secreted alumina-specific bioproteins (Deo and Natarajan, 1999). Corundum-adapted cells of *P. polymyxa* and their metabolic products were efficient in the separation of hematite from corundum through bioflocculation (Table 1.15).

Applicability of bioflotation and bioflocculation using *P. polymyxa* was also demonstrated using real iron ore samples. From an alumina-rich iron

TABLE 1.14

Settling of Minerals under Different Biopretreatment Conditions

	Percent Weight Settled at Neutral pH			
Mineral	**Control**	**Cells**	**Proteins**	**Polysaccharides**
Quartz (silica)	58	20	18	45
Corundum (alumina)	85	95	78	96
Hematite	90	98	85	95

Source: Adapted from Deo, N., Natarajan, K.A. *Minerals Engineering* 10; 1997: 1339–354; Deo, N., Natarajan, K.A. *International Journal of Mineral Processing* 55; 1998: 41–60.

TABLE 1.15

Selective Bioflocculation of Corundum-Hematite (1:1) Mixture Using Corundum-Adapted Cells and Bioproteins Separated from Adapted Cells

Deslimings	Percent Hematite Separation
1	50–55
3	67–70
5	83–90
6	95–98

Source: Adapted from Deo, N., Natarajan, K.A. *Minerals and Metallurgical Processing* 16(4); 1999: 29–34.

ore containing up to 15% aluminium oxides and silicates, a very significant reduction in alumina and silica could be brought about through bioflotation or bioflocculation using adapted cells of *P. polymyxa.* Similarly, a significant removal of silica could be obtained through bioflotation and bioflocculation from another silica-rich iron ore, containing up to 32% silica and 45% iron.

1.5.5 Bioremoval of Phosphorous and Clays from Iron Ores

The separation of phosphorus from hematite can be achieved by microbially mediated solubilisation in an environment-friendly and cost-effective manner. In the mining environment many microorganisms take part in the phosphorus cycle and solubilise mineral phosphates through the production of organic and inorganic acids. In nutrient-limited environments, organisms are capable of mobilising the phosphorus contained in minerals. Phosphorus mobilising bacteria *Burkholdaria caribensis* isolated from a Brazilian iron ore deposit could be used to liberate mineral phosphate from iron ore. The production of gluconic acid and exopolysaccharides solubilised the phosphorus contained in the ore. Selective bioleaching of phosphorus from high phosphorus iron ores can be achieved. Indigenous sulphur-oxidising bacteria from municipal wastewater could grow well in iron ore slurries containing phosphorous (Nautryal, 1999; Delvasto et al., 2009). High acidic conditions resulting from bacterial growth lead to efficient solubilisation of phosphorous. Fungal species such as *A. niger* could be used for phosphate solubilisation since the organisms produce chelating organic acids.

Selective flocculation of hematite–apatite mixtures using yeast cells (*Saccharomyces*) was attempted as a function of pH. At a pH in the range of 9–10, significant selective flocculation of hematite particles was observed, while apatite fines were dispersed (Natarajan and Padukone, 2011).

Kaolinite can be removed from hematite using selective bioflocculation using *B. subtilis*. Kaolinite-adapted cells and their metabolic products were found to be efficient in the separation of kaolinite from hematite. Kaolinite particles were dispersed while hematite was selectively flocculated (Poorni and Natarajan, 2013, 2014).

1.6 Biobeneficiation of Sulphide Minerals Using *Acidithiobacillus* Bacteria

The sulphur-bacteria cycle in mining environments constitutes iron-sulphur-sulphide oxidising acidophiles such as *A. ferrooxidans* and *A. thiooxidans* as well as SRB, which are sulphate-reducing neutrophilic heterotrophs. Biogenesis of various sulphide minerals such as pyrite, chalcopyrite, arsenopyrite, sphalerite and galena is due to participation and activity of bacteria in the sulphur cycle. Sedimentary mineral sulphides are of biogenic origin. Many sulphide minerals can be formed due to bacterial reduction of sulphates by SRB.

Biobeneficiation of sulphide-bearing ores such as those containing pyrite, chalcopyrite, arsenopyrite, sphalerite and galena can be achieved using *A. ferrooxidans, A. thiooxidans* as well as SRB. A few examples are illustrated below.

A. ferrooxidans has been shown to adhere selectively onto pyrite leading to significant changes in its surface chemical properties. Profuse attachment of *A. ferrooxidans* cells onto pyrite renders them hydrophilic, leading to significant depressions in subsequent flotations. Such a bio-modification can be beneficially used in desulphurisation of pyritic coals (Ohmura et al., 1993).

In a pyrite–chalcopyrite system as those occurring in copper ores, selective microbial depressions of pyrite would prove to be beneficial. *A. ferrooxidans* can be effectively used for selective pyrite removal from chalcopyrite through prior bacterial conditioning. For example, when a mixture of pyrite and chalcopyrite were conditioned with bacterial cells after the addition of small quantities of a xanthate collector, the selective depression of pyrite and significant chalcopyrite flotation could be obtained. Similarly, the selective flotation of arsenopyrite from pyrite could also be achieved through bacterial conditioning using *A. ferrooxidans.* Bacterial cells exhibited a significantly higher affinity towards pyrite compared to arsenopyrite. Even shorter periods of interaction with bacterial cells resulted in a significant depression of pyrite, while the flotability of arsenopyrite was promoted. Very good flotation separations between pyrite and arsenopyrite could be achieved through bacterial conditioning followed by the addition of a xanthate collector and copper activator (Chandraprabha et al., 2004a).

The settling behaviour of pyrite and arsenopyrite in the presence and absence of *A. ferrooxidans* is illustrated in Table 1.16. The settling rate (flocculation) of pyrite was promoted at even lower cell densities at acidic and neutral pHs. On the other hand, there was no significant change on the settling rate of arsenopyrite. The control of cell density is essential to achieve selective flocculation of pyrite from arsenopyrite, since higher cell densities would result in a decrease in selectivity (Chandraprabha et al., 2004b).

TABLE 1.16

Settling Behaviour of Pyrite and Arsenopyrite in the Presence of *A. ferrooxidans*

		Percent Settled	
Mineral	**pH**	**Cells**	**Control**
Pyrite	2.5	96	32
	6.5	95	16
Arsenopyrite	2.5	40	37
	6.5	24	20

Source: Adapted from Chandraprabha, M.N., Natarajan, K.A., Somasundaran, P. *Journal of Colloid and Interface Science* 276; 2004b: 323–32.

Good separation of pyrite from a ternary mixture containing chalcopyrite and arsenopyrite could also be achieved through bacterial conditioning. In the presence of copper sulphate, selective pyrite depression from arsenopyrite and chalcopyrite could be achieved by suitably conditioning with bacterial cells and a xanthate collector. For example, 85% chalcopyrite and about 80% arsenopyrite could be recovered with significant pyrite depression.

The use of *A. ferrooxidans* in the flotation separation of galena from sphalerite was also demonstrated (Yelloji Rao et al., 1992). Control of cell density and interaction time is critical in achieving selective galena—sphalerite separation as illustrated in Table 1.17.

The enhancement of the flotability of sulphide minerals in the presence of *A. ferrooxidans* could be attributed to surface sulphur formation through bio-oxidation. Bacterial oxidation over an extended period of time leads to reoxidation of the sulphur to sulphoxy compounds and to sulphate; a gradual build-up of such oxidised sulphate layer on mineral surfaces would impede flotation. Similarly, higher surface coverage of bacterial cells could also lead to depression.

TABLE 1.17

Effect of Initial Cell Density on the Flotability of Galena and Sphalerite

	Percent Flotation	
Cells/mL	**Sphalerite**	**Galena**
10^4	96	75
10^8	92	45
10^9	50	20

Source: Adapted from Yelloji Rao, M.K., Natarajan, K.A., Somasundaran, P. *Minerals and Metallurgical Processing* 9; 1992: 395–400.

Initial cell population as well as the duration of biotreatment are important factors influencing the flotability of sphalerite and galena. With an increase in cell population and duration of bacterial interaction, the flotability of sphalerite and galena was observed to decrease. Significantly higher numbers of cells were seen to be attached to galena unlike on sphalerite. Surface oxidation of galena lead to formation of oxidised sulphate layers that may hinder its flotation. Enhanced hydrophobicity of sphalerite is due to the presence of elemental sulphur due to bacterial oxidation.

Microbially induced flotation of sulphide minerals using *A. thiooxidans* has also been reported (Santhiya et al., 2000).

Electrophoretic mobilities of pyrite, chalcopyrite and arsenopyrite were observed to be affected after interacting with *A. thiooxidans.* For example, the isoelectric point of pyrite shifted from a pH value of about 3.2 to about 4.2, while that of chalcopyrite and arsenopyrite shifted from pH 2.4 to 3.2 after bacterial interaction. The highest surface adsorption of *A. thiooxidans* could be observed on pyrite, followed by chalcopyrite and arsenopyrite.

The differential flotation of pyrite–chalcopyrite mixtures after conditioning with xanthate followed by cells of *A. thiooxidans* resulted in 85% flotation recovery for chalcopyrite while only about 15% of pyrite could be recovered. In the presence of copper activation, chalcopyrite–arsenopyrite recoveries could be substantially increased to more than 90% with significant pyrite depression from a ternary mixture of the three sulphides.

Flotation behaviour of galena and sphalerite was also studied before and after interaction with *A. thiooxidans.* Flotation recovery of sphalerite was observed to be significantly enhanced while galena was depressed. Significant differences in surface adsorption of cells onto galena and sphalerite along with the nature of the surface reaction products are responsible for the observed flotation behaviour of galena and sphalerite. Bacterial interaction also promoted the dispersion of sphalerite particles while flocculating galena, especially at pH values near about 8–9.

$ZnSO_4$ formed on sphalerite surfaces due to bacterial interaction is soluble, unlike the case with lead sulphate on galena surfaces. Adsorption of *A. thiooxidans* was found to be significantly higher on galena along with formation of insoluble lead sulphate as a reaction product. Sphalerite surfaces exhibited lower cell adsorption and an increased presence of elemental sulphur.

1.6.1 Use of SRB in Mineral Processing

Unlike acidophilic, aerobic autotrophs, SRB such as *Desulfovibrio* spp. are anaerobic, neutrophilic heterotrophs reducing sulphate to sulphide (Solozhenkin and Lyubavina, 1985).

H_2S and other metal sulphides are generated during their growth. From a flotation view point, SRB could function as biological sulphidising agents. Many oxidised ores could be sulphidised through interaction with SRB. For

example, flotation of cerusstie was improved after pre-treatment with SRB. Selective flotation of lead and zinc from sulphide ores can be achieved after conditioning with SRB. Lead recovery in concentrate was enhanced due to bacterial treatment by about 95% with almost complete depression of sphalerite after SRB interaction. Selective separation of chalcopyrite and molybdenite after biotreatment with SRB has been attempted. Besides, they can also be used as desorbents for adsorbed xanthate from floated mineral surfaces.

SRB generate large volumes of H_2S, which can convert metals and their salts to sulphides. Through sulphate reduction, SRB can produce biogenic sulphides such as silver, cadmium and other metal sulphides. Bacterial interaction can be so controlled as to generate either a sulphide product (promotion of flotation) or H_2S gas (depressant). Bioconversion of iron oxides to ferrous sulphide, malachite to covellite, silver carbonate/chloride to argentite and smithsonite to sphalerite would facilitate easy flotation recovery of the otherwise non-floatable oxide/carbonate minerals using xanthate collectors. SRB has been increasingly used in the remediation of acid mine/drainage and in biomaterials processing. Dissolved metal ions in acidic and neutral effluents can be precipitated as sulphides and recovered after treatment with SRB. Biogenic metal sulphides can be produced through interaction with SRB from different industrial minerals.

1.7 Biobeneficiation Mechanisms

Microorganisms bring about selective mineral dissolution through direct and indirect mechanisms. Direct mechanism involves microbial adhesion to the mineral surfaces leading to enzymatic attack. Bacterial cells can attach and colonise on minerals forming biofilms, which consist of various microbial consortia along with their metabolic and metal-reacted products. For example, microorganisms such as *Acidithiobacillus* sp., *Bacillus* sp., *P. polymyxa*, SRB, fungi and yeasts used in this study were observed to adhere on minerals such as kaolinite, alumina, silica, calcite, hematite apatite, pyrite, chalcopyrite, sphalerite and galena. Mechanisms of bacterial adhesion involve electrostatic, chemical and hydrophobic forces. Since mineral particles as well as bacterial cells are charged in aqueous solutions, electrostatic forces may come into play in initial bacterial adhesion. Subsequent to surface adhesion, attached organisms secrete various biopolymers that serve as adhesives facilitating irreversible attachment. Such biopolymers contain amino acids, exopolysaccharides and organic acids. Spore-forming microbial cells when exposed to mineral and metal ion containing environments form protective capsules around the cell walls. For example, cells of *P. polymyxa* on growth in the presence of calcite were found to form polysaccharide-enriched capsules (Somasundaran et al., 2000). However, such cells did not exhibit

capsule formation when grown in the presence of silica. As discussed above, microorganisms when grown in the presence of different minerals secrete mineral-specific bioreagents such as proteins and exopolysaccharides. In the presence of hematite and calcite, *P. polymyxa* cells were found to secrete significant amounts of polysaccharides, while the presence of silica and kaolinite promoted the secretion of proteins. Similarly, fungi such as *A. niger* and bacteria such as *B. subtilis* and *P. polymyxa* secrete several organic acids such as citric, oxalic and gluconic acids promoting the dissolution of the mineral in the medium. Exopolysaccharides are known to form calcium-based complexes in the presence of calcite.

Indirect mechanism of mineral dissolution involves the role of microbial metabolites acting as mineral solvents. Both oxidative and reductive biodissolution can take place. A typical example of oxidative dissolution is solubilisation of sulphides such as pyrite in the presence of sulphur and iron oxidising bacteria such as *A. ferrooxidans* and *A. thiooxidans*. Microbial iron removal from pyritic coals using *Acidithiobacillus* bacteria falls into this category. Since the acidic metabolites of *A. thiooxidans* also contain reducing ions such as HS^-, thiosulphates and tetrathionates, the dissolution of minerals through reductive processes could also occur. The use of iron-reducing bacteria such as SRB, *Shewanella* and *Desulfuromonas*, on the other hand, bring about reduction of ferric to the bivalent state facilitating solubilisation of ferric oxides such as hematite in an acid medium or in the presence of chelating agents. Direct bioreduction of iron oxides exploiting the iron-reducing metabolisms of iron-reducing bacteria is a treatment option for removal of ferric oxides from kaolin clays and bauxite.

Other than selective biodissolution, interfacial changes brought about by microorganisms due to mineral adsorption can induce surface hydrophobicity or hydrophilicity leading to selective flotation or flocculation. Such microbially induced surface chemical changes occur at a faster rate (unlike biodissolution) facilitating rapid separation of different mineral constituents present in an ore matrix. All the bacterial and yeast species used in biobeneficiation brought about significant surface chemical changes on interacted minerals such as kaolinite, silica, alumina, calcite, apatite, hematite, pyrite, chalcopyrite, sphalerite and galena. Significant shifts in mineral IEP values and measured zeta potentials could be observed after microbial interaction. Microbial interactions enhanced surface hydrophobicity of silica and kaolinite while hematite and calcite were rendered more hydrophilic. Increased settling (flocculation) of hematite and calcite and dispersion of silica and kaolin clays could be observed after interacting with microbial cells and their metabolic products. Adsorption of bacterial proteins conferred enhanced surface hydrophobicity (as in the case of sphalerite, silica and kaolinite) and of exopolysaccharides rendered minerals such hematite, calcite and galena increasingly hydrophilic. During bioflocculation, mineral interface containing the biofilm is modified and inter-particle bridging through surface biopolymers takes place.

Acidophiles such as *A. ferrooxidans* and *A. thiooxidans* were observed to alter the surface chemistry of sulphide minerals such as pyrite, chalcopyrite, arsenopyrite, sphalerite and galena. Different adsorption behaviours of *Acidithiobacillus* cells led to selective flotation depression of pyrite and galena among the above sulphides.

1.8 Conclusions

Biomineralisation and biobeneficiation aspects of clays, bauxites, sulphide and iron ores are critically discussed. The role of native microorganisms in their genesis, mineralisation and mobilisation is clearly established. It is significant that native mining microorganisms isolated from the above ore deposits are capable of bringing about beneficiation-related surface chemical changes on kaolin, alumina, aluminium silicates, iron oxides, as well as sulphide minerals such as pyrite, chalcopyrite, sphalerite and galena. It becomes very clear that microbially induced beneficiation processes could become the most promising technologies for processing low-grade resources of complex multisulphides, iron ores, bauxite and industrial clay deposits. The most promising areas for potential commercialisation are iron removal from clays and bauxites, calcium and silica removal from bauxites, alumina, phosphorous and clay removal from iron ores as well as flotation of complex multisulphide ores. Biobeneficiation processes are especially suited to treat fines and slimes including processed tailings with a view to value addition and economical utilisation of waste resources. Ever-increasing demands for cost effective, energy efficient and environment-friendly process technologies for low grade and waste mineral resources will be a major incentive for adopting bio-beneficiation processes in place of existing physiochemical methods. Some attractive features of biobeneficiation processes are

a. Mineral-specific bioreagents are generated by different microorganisms when grown and adapted in the presence of minerals.
b. Harvesting of such mineral-specific biopolymers containing proteins and polysaccharides will pave the way for development of bioflocculants, biocollectors and depressants.

All the research studies on biobeneficiation have so far been confined to bench scale only and it becomes highly essential to carry out scaled-up and pilot-scale studies in close collaboration with concerned mining industries to establish techno-economic process viabilities of commercially relevant bioprocesses.

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2

Biomining of Base Metals from Sulphide Minerals

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CONTENTS

Biomining has a long history, although the early miners did not know that microbes were involved in the mining process. The use of microbes in ore processing has some distinct advantages over the traditional pyrometallurgical and hydrometallurgical processing.

The world-wide high-grade ore reserves are falling every day at an appalling rate as most of them are worked out because of high metal demand, traditional techniques such as pyrometallurgy and chemical processing are becoming more and more economically incompatible. Microorganisms bear a clear advantage over it, as not only they offer an economically viable option but also a clean technology (Siddiqui et al., 2009). They do not require the high amounts of energy used during roasting or smelting and do not produce sulphur dioxide or other environmentally harmful gaseous emissions (Rawlings, 2002). To some extent it holds the promise of reducing the capital costs (Devasia and Natarajan, 2004). Additionally, microbiological solubilisation processes are also applicable to recover metal values from industrial and mineral wastes, which can serve as secondary raw materials (Siddiqui et al., 2009).

2.1 Biomining Processes for Base Metals

There are two main types of biomining processes for the commercial-scale microbially assisted metal base metal recovery. These are irrigation-type and stirred-tank-type processes (Figure 2.1). Irrigation processes involve the percolation of leaching solutions through the crushed ore that have been stacked in columns, heaps or dumps. There are also several examples of the irrigation of an ore body *in situ*, that is, without bringing the ore to the surface. Stirred-tank-type processes employ continuously operating, highly aerated stirred-tank reactors. One feature of both types of processes is that, unlike most other commercial fermentation processes, neither is sterile, nor any attempt is made to maintain the sterility of the inoculum (Rawlings, 2002).

2.1.1 Bioleach Heap Technology: The Most Commercialised Irrigation-Based Biomining Expertise

Irrigation-based processes can be categorised based on the type of resources to be processed such as dump leaching, heap leaching and *in situ* leaching. Heap leaching deals with the newly mined materials (intermediate-grade oxides and secondary sulphides deposited in the form of a heap on an impervious natural surface or a synthetically prepared pad leached with circulation, percolation and irrigation of the leaching medium) (Pradhan et al., 2008). Primary sulphides such as chalcopyrite are also suitable for this type of leaching. Bioleach heap technology has emerged as the predominant technology route for the recovery of metals from low-grade ores. In terms of revenue generated, it is the most significant industrial application of biomining (Rawlings, 2002). The technology has been in use since the 1960s for the acid

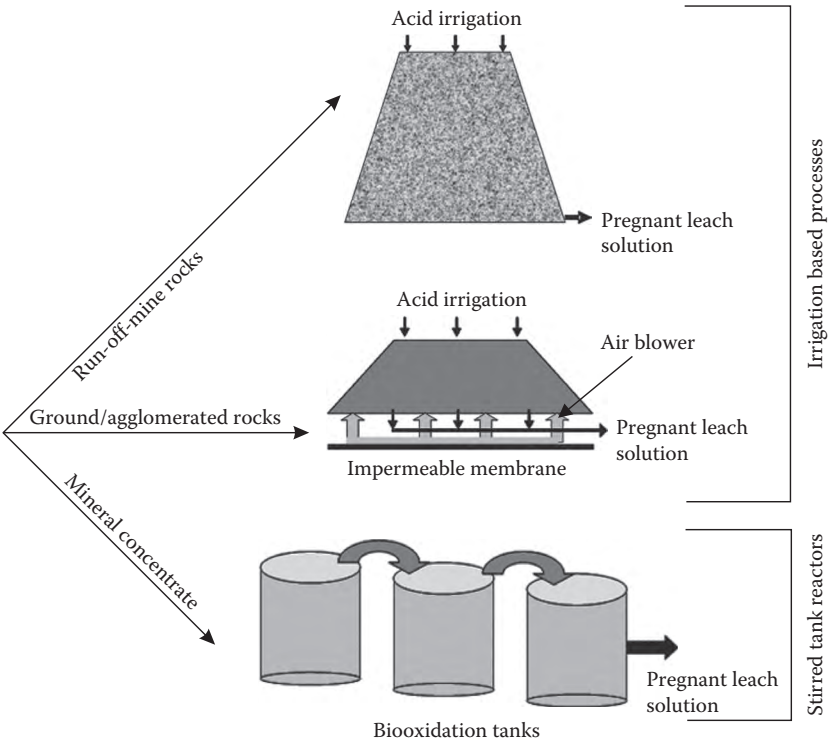

FIGURE 2.1
Major process options used in biomining. (Modified from Johnson, D. B. 2010. In: *Geomicrobiology: Molecular and Environmental Perspective*, 401–426. Netherlands: Springer.)

leaching of copper oxide minerals, and since the 1970s for the cyanide leaching of gold and silver.

The static bioleaching techniques are based on the principle of circulating acidic water and air through heaps of ore coarsely fragmented to activate the growth of microorganisms that amplify the oxidation of the sulphide minerals (Morin et al., 2006). This process involves stacking crushed ore into piles constructed on an impermeable layer fitted with a solution drainage system, or arranged on a slope to facilitate drainage. In many cases, the ore is agglomerated through tumbling with acid and/or irrigation solution prior to stacking.

2.1.2 Fundamental Principles of Bioleach Heap Technology

Although heap leaching appears to be a very simple process in concept, the sub-processes taking place within the ore bed are rather complex and their interactions not yet fully understood.

To unravel the processes underlying heap bioleaching, it is useful to distinguish between the phenomena taking place at different scales within the heap (Petersen and Dixon, 2007a). Beginning at the heap scale, we can distinguish a number of transport effects. More specifically, these include:

1. *Solution flow:* In unsaturated, coarsely granular packed beds, the solution generally flows along tortuous pathways but remains stagnant in pores and crevices between particles. This strongly influences heap performance in terms of reagent delivery and product removal from the reaction sites within the ore particles.
2. *Heat flow:* Heat of reaction, which is significant in sulphide leaching, is transported downwards through the heap as sensible heat with the flowing solution and upwards as latent heat with the flow of humid air. Depending on air and solution flow rates, heaps can assume certain temperature profiles, and a judicious manipulation of these variables may even allow a certain degree of control.
3. *Gas flow:* Although gas flow is usually well distributed in aerated heaps, ensuring ample supply of oxygen throughout, the supply of CO_2 may be limited under certain conditions. In non-aerated heaps, O_2 availability may also be limited, and gas distribution patterns are complex.

The next level, at the meso-scale, represents a cluster of particles within a heap bed. Here, two processes contribute to the overall rate of leaching:

1. *Diffusion transport:* Diffusion is the main mode of transport for dissolved constituents from and to the moving solution into pore spaces between particles, and into cracks and fissures within particles. The effect of pore diffusion on the overall kinetics is determined by the length of the diffusion path, which can be significant for systems with a poor solution distribution (Petersen and Dixon, 2007b).
2. *Microbial population dynamics:* This encompasses the complex interactions of a variety of microorganisms in the liquid phase and on the mineral surface. It includes the growth behaviour of each strain as a function of temperature and concentration of dissolved constituents (acid, Fe^{2+} and Fe^{3+} iron, O_2, CO_2 as the carbon source, etc.), and any synergies between these and the concomitant iron and sulphur oxidation reactions.

Finally, the smallest scale at which sub-processes of heap bioleaching need to be analysed is that of the individual mineral grain. Here, leaching is governed by the electrochemical interactions between the mineral grains and reagents in solution.

2.1.2.1 Microbiology of Bioleach Heap

A wide variety of microorganisms are found in natural leaching environments such as bioheap. The majority of known acidophilic microorganisms have been isolated from such natural environments. Understanding the microbiology of a bioheap is important for the advancement in commercial bioheap applications. It will also enable better control of conditions to improve upon the leaching rates, metal recoveries and cost of production. Limited comprehension is available on what actually occurs in a full-scale microbiologically operated bioheap, despite the commercial achievement in the copper ore bioheap leaching (Pradhan et al., 2008). Although oxidative dissolution of simple and complex sulphide ores and concentrates may be mediated by pure cultures of iron-oxidising acidophiles, as has often been described in laboratory studies, axenic cultures are never found in actual biomining operations. A consortia of microorganisms with synergistic (and sometimes complimentary) metabolic physiologies have been identified in all commercial-scale systems that have been examined (Johnson, 2010).

Microbial diversity is far greater in heaps and dumps, which are highly heterogeneous and uncontrolled environments, than in stirred tanks where conditions are far more homogeneous. Both operate essentially as 'inorganic' systems in that, inorganic nutrients are added to stimulate microbial activity, not organic carbon. This, together with the primary energy sources available being the sulphide minerals themselves, means that the dominant prokaryotes present are invariably chemo-autotrophic iron and sulphur oxidisers. However, organic carbon derived from living (as exudates) and dead (as lysates) primary producers can accumulate in leach liquors, and can support the growth of mixotrophic and heterotrophic acidophiles. Hence, it is possible, as noted by Johnson and Hallberg (2009), to divide microorganisms in biomining operations into three groups: (i) 'primary acidophiles', iron-oxidising prokaryotes that generate ferric iron and are responsible for initiating mineral dissolution; (ii) 'secondary acidophiles', sulphur-oxidising acidophiles that generate sulphuric acid from reduced sulphur produced during mineral dissolution and help to maintain the required pH conditions that are conducive for the biooxidation of sulphide minerals and (iii) 'tertiary acidophiles', heterotrophic and/or mixotrophic microorganisms that degrade soluble organic carbon wastes originating from the autotrophs, thereby detoxifying the environment of some highly sensitive primary and secondary prokaryotes into organic matter.

Okibe and Johnson (2004) evidently demonstrated the importance of microbial consortia and their interactions for optimising sulphide mineral dissolution in laboratory studies. As recently reviewed by Kondrat'eva et al. (2012), the microbial analysis of commercial-scale bioprocessing operations has shown, in all cases reported so far, that bacteria and/or Archaea that fulfill these primary, secondary and tertiary roles are present. Recently, Soto et al. (2013) have extensively studied and analysed the parameters influencing

the microbial oxidation activity in the industrial bioleaching heap at the Escondida mine in Chile. In their study, the industrial run of mine (ROM) bioleaching heap of the Escondida mine is monitored monthly from pregnant leach solution (PLS) to assess the concentration of microorganisms, microbial activity and physicochemical parameters; this study has generated a huge amount of information. 'Knowledge Discovery in Databases' (KDD) was used to obtain a better description of the iron microbial activity and the dissolution rate of sulphide ores occurring in the leaching cycle. Thus, such data-mining tools have been useful to propose and confirm hypotheses on data obtained from industrial bioleaching systems. Industrial heap bioleach operations process very low-grade ores, often <1% of the mineral of interest. Understanding the dynamics of microbial interactions on low-grade ores, containing substantial proportions of gangue minerals, becomes essential for the effective exploitation of more recalcitrant and complex ore bodies and informs industrial operations. In this study, they have successfully studied the interaction of thermophilic microorganisms with metal sulphides in a simulated heap environment. Such an approach was also investigated by Tupikina et al. (2013) to determine the effect of acid stress on the persistence and growth of thermophilic microbial species after the mesophilic colonisation of low-grade ore in a heap-leach environment. Microbial analysis of selected PLS and ore samples provided useful information on the effect of pH on microbial colonisation. Their study indicated the low temperature during the initial phase of operation that negatively affected the persistence of the thermophilic microorganisms in the ore bed. Furthermore, their subsequent growth, on reaching thermophilic conditions, was negatively affected as a function of decreasing pH.

2.1.2.2 Current Applications and New Developments of Bioleach Heap Technology

A substantial number of heap-leaching metal-recovery processes have been in operation for many years (Rawlings et al., 2003). 'Thin layer' leaching, where crushed and acid-cured ore is stacked 2–3 m high and then rinsed, was first applied at the Lo Aguirre Copper Mine in Chile in 1980, and is regarded as the first instance of heap bioleaching. A further significant milestone in heap bioleaching was the introduction of forced aeration for the heap bioleaching of secondary copper sulphide ores at the Girilambone Copper Mine in Australia in 1993 (Gerick et al., 2009).

As estimated by Brierley (2008a), heap bioleaching of copper accounts for some 7% (about 10^6 t/year) of the total global annual production of approximately 1.7×10^7 t of copper. This does not include copper recovered using dump bioleaching processes. It is estimated that if dump bioleaching is included, some 20%–25% of the world's copper production is attributable to bioleaching. Examples of very large copper-leaching operations are those by Sociedad Contractual Minera El Abra and the Codelco Division Radimiro