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Preface

Because ideas have to be original only with regard to their adaptation to the problem at hand, I am always extremely interested in how others have used them… – *Thomas Edison*

The quote above is not only quite reflective of the purpose of this book but also very meaningful to the topic of this book because Thomas Edison himself had personally ventured in iron ore industry, which proved to be his greatest commercial failure. Discovering that beach sand contained relatively high grade of iron, he formed the Edison Ore-Milling Company in 1881 based on his patented methods of ore crushing and extracting the metal using a large electromagnet. He tried for many years to make that business a success. The demand was not there, and despite the new technological innovations that Edison brought to the industry, the company could not compete with the operations in the Midwest iron ore production. Eventually, the technology was sold to other mine owners. However, Edison was able to discover a market for the large quantity of waste sand produced from the ore-milling company and decided to set up his own cement company in 1899. The cement company later went on to supply the concrete for the construction of the Yankee Stadium in 1922.

I am not trying to introduce innovative ideas or concepts with guaranteed technical or economic feasibility. Everything in this book can be found either directly or extrapolated from other literatures up to date. A mere rearrangement and compiling of all the information related to the topics is made in this book to give the readers an opportunity to understand some of the basic concepts and principles of iron ore bioprocessing, and to encourage readers to adapt and develop their own ideas to solve their problem at hand.

The information explosion has indeed created a situation where it's now impossible for any one person to stay up to date with the changes in any topic area (unless that topic is perhaps so minute in its focus that only a few dozen are following it and contributing to the existing body of knowledge). The explosion of the shear amount of literature, and birth of interdisciplines and disciplines or subject areas in the past decades have been phenomenal. Biohydrometallurgy is one that is born of biotechnology and mining engineering. With the maturing of bioprocessing as a discipline,

it evolves from an interdisciplinary subject area of microbiology and hydrometallurgy to a discipline that covers the mining engineering and engineering science aspects of biotechnology, green chemistry, biomass, or renewable resource engineering. As such, books in the area are needed to cover the needs of educating the new generation of fine bioprocessing engineers, not just by converting well-versed mining engineers and engineering-savvy biologists to bioprocess engineers. With this book, I hope to fill this gap and bring the maturity of bioprocess engineering. Yet, some of the chapters in this book are more oriented toward industrial application aspects that are good references for process engineers and researchers who are looking for new process developments.

The key aspect that makes iron ore bioprocessing special is that iron ore bioprocessing as an emerging field has potential to solve particular problems facing iron ore industry such as economic utilization of low-grade iron ore resources. Potential solutions stemmed from bioengineering functions and biological and chemical conversions concerning the sustainable use of these resources are critical to revitalize the iron ore mining industry. Thus, the mechanism, rate, dynamic behavior, transformation performance, and manipulations of bioprocessing systems are the main topics of this book.

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Disclaimer

This book is designed to provide information and resources to readers who are interested in the subjects discussed. While the author wishes to acknowledge the contributions of all of his peers, colleagues, and professional contemporaries whose works may have been quoted in this book, at times it is not easy to fulfill this responsibility, and the author is grateful to all those who have made this book possible. Included in this book are references to technologies and equipment that have been used or have potential to be used in iron ore bioprocessing; no guarantee is provided that the information is current, and discussion of any particular piece of technologies or equipment does not constitute an endorsement.

Contents

Introduction

1   Historical Background

Although mining is one of the oldest technologies known, it has succeeded in escaping the major technological advances seen in that of agriculture and medicine. Many minerals and metals are mined today in exactly the same manner, as they were hundreds of years previous. The crude ores are dug from the earth and crushed, and the mineral is extracted either by extreme heat or due to the addition of toxic chemicals. But due to the environmentally unfriendly aspect of these mining techniques, new methods, which are kinder and more environmentally friendly, are being used which uses microorganisms, which can help to leach out the metals.

The origin of extraction of metal values from ores with the aid of microorganisms can be traced back to ancient time. Historical records indicate that the process has been exploited empirically mainly for nonferrous metal extraction without any recognition of a biological contribution to the process (Ehrlich, 2004). Prior to electrolysis, the recovery of the copper from copper sulfate was by cementation (precipitation). It is thought that this process was known in Pliny time, but few written records of this have survived. It is known that the Romans used to place scrap iron into the river, and over a period of a few months, the copper precipitated around the iron. The pure copper was then recovered by smelting, but what the Romans did not realize was microorganisms played a major biological contribution to this process by generating the copper in the water.

In China and India, natural recovery of copper and other base metals such as zinc from solutions emanating from rocks was known almost 2000 years ago. The Chinese were aware of a process (cementation) as documented by King Lui-An (177–122 BC). Copper was precipitated from solution by dipping iron into the blue vitriol solution—a process identified as early as 150 BC in China. Therefore, presumably, the recognition of a natural copper leaching process can be identified as early as that date. Observations were later made on the natural leaching of copper and the formation of "gall springs" during the East Han Dynasty (206 BC–220 AD)

in China. The "Gall-Copper Process" was recorded as being used during the Song Dynasty (960–1271 AD). The Chinese implemented the commercial production of copper from copper sulfate when the Changshan cementation plant started operation in 1096 with an annual production of 190 ton of copper per year.

In ancient Roman, natural leaching of metals from rocks was recorded as early as 20–70 AD. Gaius Plinius Secundus (better known as Pliny the Elder, 23–79 AD), who had a passion for observing the wonders of nature, reported the "*vitreolus quasi vitrum*"—a glass-like substance—found on rocks and the practice of copper extraction as copper sulfate was widely used in Spain in his treatise on natural history. Another similar recording of bioleaching comes from Cyprus, reported by Galen, a naturalist and physician 162 AD who reported on the in situ leaching of copper. Surface water was allowed to flow through permeable rock, and as it percolated through the rock, the copper minerals dissolved, so the result was a high concentration of copper sulfate in solution. This solution was allowed to evaporate with the resultant crystallization of copper sulfate.

The German physician and mineralogist Georgius Agricola (1494–1555) referred to copper leaching from ores and leachates from mines in his work *de re metallica.* The techniques he described for the recovery of copper are based on the leaching of copper-containing ores (Schiffner, 1977). A woodcut from his book illustrates the (manual) transport of metal-containing leachates from mines and their evaporation in the sunlight (Fig. 1).

Bioleaching and cementation were also described by Paracelsus the Great (1493–1541). It is referred in his writings that the copper deposits onto iron at a spring in the Zifferbrunnen in Hungary. Although he confused this deposition with that of transmutation, he assisted in the use of bioleaching, and by 1750, approximately 200 ton of copper per year were obtained in the Zifferbrunnen area of Hungary using this process of bioleaching. Similar process is also referred to by Basil Valentine, who wrote about 1500. The copper leaching and cementation process were in practice in the Lower Hartz until the middle of the sixteenth century, and it was in operation in Peru before the year 1637. The process was later patented and adopted at Rio Tinto as early as the sixteenth century.

Even though few of these earlier bioleaching operations were documented, it is known that copper leaching was well established at the Rio Tinto mine in Spain by the eighteenth century. Rio Tinto literally means "Colored River," a name given to the acidified river that issues from the Sierra San Cristobal Mountain on the river bed and on the abundant microbial mats, the dense floating masses made up of different microorganisms. The reddish-brown water of Rio Tinto is mainly due to the presence higher concentrations of ferric ions. Natural iron and copper dissolution from minerals through activity of native microorganisms were later recognized at Rio Tinto. The contribution of sulfur- and iron-oxidizing *Thiobacillus ferrooxidans* in generating ferric ion-containing sulfuric acid which can dissolve minerals such as copper sulfides was first reported scientifically in 1947. But it is now widely accepted and known that it was in fact the microorganism *Acidithiobacillus ferrooxidans* that contributed to the success of Rio Tinto (Fig. 2).

Fig. 1 Woodcut from the book *de re metallica* written by Georgius Agricola (1494–1555) illustrating the manual recovery of copper-containing mine effluents which are collected in wooden basins and concentrated in the sun

At Rio Tinto, the process of heap leaching of copper sulfides was carried out on an industrial scale in 1752. In this process, the ore is heaped and crushed onto open-air pads. The layers of ore were altered with beds of wood. Once the heap was constructed, the wood was ignited which resulted in the roasting of copper and iron sulfides. Water was then added to the top of the heap. The addition of water caused the copper and iron to dissolve which formed copper and iron sulfates. But due to the significant environmental damage caused by the production of sulfuric acid in this process, the process was stopped in 1888. This heap leaching process minus the roasting step continued at Rio Tinto until the 1970s. The reason for its success was unknown, but it was thought to be due to "some obscure quality either of the Rio Tinto ore or the Spanish climate."

Role of microbial processes in mineral dissolution was not recognized till late 1900s. Since the 1950s, research activities on the use of *Acidithiobacillus* picked up and commercial applications of bioleaching particularly in copper dump and heap leaching began to emerge. The run-of-mine lean-grade copper ores were stacked in waste dumps over 100 meters in height and leached using acidic solutions for

Fig. 2 Rio Tinto, a 100 km long river in Southern Spain that originates in the Sierra San Cristobal Mountain of Andalusia, famous for being very acidic (pH 2), and its deep reddish hue is due to iron dissolved in the water

economic copper extraction at the Bingham mines of Kennecott Copper Company during the late 1950s and early 1960s. Heap and in situ mining using native microorganisms were developed later.

In the 1940s in America, several million tons of sulfuric acid was discovered in the Ohio River, and this discharge was attributed to the weathering of subbituminous coal. Naturally enough, this pollution incident was unacceptable, and it led to widespread investigation by universities and several US government institutions, such as the US Bureau of Mines as to the source of the pollution. The cause of the sulfuric acid was due to the oxidation of pyrite, which is present in the subbituminous coal, but it was also noted that this oxidation occurred much more rapidly than could be contributed to by that of inorganic chemistry. Also, an important observation was that of the presence of sulfur-oxidizing bacteria. In 1950, a couple of years after the incident, a new species was identified that of *Thiobacillus ferrooxidans*. This organism is able to oxidize elemental sulfur and ferrous ions at a much higher rate than that achieved by inorganic chemistry. It is this catalysis of the oxidation of ferrous ions that makes *Thiobacillus ferrooxidans* and other iron- and sulfur-oxidizing microorganisms such important catalysts in the bioleaching process.

Other use of biological systems by the metallurgical industry was in the leaching of uranium in the 1950s. In the1980s, bioleaching was used to treat refractory gold ores. Recently, commercial processes have been developed for leaching base metal sulfides such as copper, zinc, nickel, and cobalt. Cyanide destruction using bacterial processes was pioneered in Lead, South Dakota, at the Homestake Mine.

Table 1 *Chronological development of biotechnologies* and their application in mining

Events	Time
Yeasts employed to make wine and beer	Before 6000 BC
Copper bioleaching and cementation reported by Chinese King Lui- An	177–122 BC
Copper extraction as copper sulfates reported by Pliny	23–79 AD
In situ leaching of copper reported by Galen (Cyprus)	162 AD
"Gall-copper process" recorded as being used in the Song Dynasty, China	960–1271 AD
H_2SO_4 by pyrite heap bioleaching (Georgius Agricola, De Re Metallica 1556, as per Andy Carter in Wardrop)	1556
Copper mined with aid of microbes, Rio Tinto, Spain	Before 1670
Antoni van Leeuwenhoek discovers bacteria (Bardell, 1982)	1680
The first recorded use of the word biology	1802
Louis Pasteur discovers the bacterial origin of fermentation	1862
Heaps of low-grade ore, left for 1–3 years for "natural decomposition" (Salkield, 1987)	1890's
Large-scale sewage purification systems employing microbes are established	1910
Karoly Ereky, a Hungarian agricultural engineer, first uses the word biotechnology	1919
Thiobacillus ferrooxidans was identified and later reclassified as *Acidithiobacillus* (Colmer & Hinkle, 1947)	1947
Commercial heap leaching of low-grade uranium ores first practiced in the USA	1950s
Mining of uranium with the aid of microbes begins in Canada	1962
Discovery of the first iron- and sulfur-oxidizing archaea (Brierley & Brierley, 1973)	1965
Lo Aguirre, first industrial gold heap bioleaching plant (Readett, 2001)	1980
Fairview: first commercial refractory gold (agitated tank) bioleaching plant	1986
Forced aeration on heap bioleaching systems developed	1993
Bioleaching of chalcopyrite concentrate developed and evaluated on commercial scale	1995
First commercial cobalt bioleaching from pyrite concentrates, Uganda	1999
Discovery of the first mesophilic, acidophilic, chemoautolithotrophic iron-oxidizing archaea—*Ferroplasma acidiphilum* in a bioleaching pilot operation (Golyshina et al., 2000)	2000
High-temperature heap bioleaching, transitional primary/secondary copper ore (Mintek)	2006
Positive feasibility study results reported on bioleaching high phosphorus iron ore deposit in Nigeria	2014

Chronological development of biotechnologies together with its applications in mining is shown in Table 1 from the earliest event to the latest event. These discoveries, inventions, and modifications are evidence of the evolution of biotechnology and its applications in mining since before the Common Era.

2   Purpose

Biotechnology embraces a wide range of techniques, and none of these will apply across all industrial sectors. Nonetheless, the technology is so versatile that many industries including iron ore mining that have not used biological sciences in the past are now exploring the possibility of doing so. The application of knowledge obtained from life science research has given rise to emergent biotechnological processes. The biological applications in the food, pharmaceutical, and nonferrous industries are well substantiated. The maturity of such processes has led to the development of more sophisticated tools for the R&D activities. Tools associated with improving efficiency of R&D activities to take ideas from the point of discovery to commercialization have exploded. The main purpose of this book is to explore the possibilities and stimulate the research in the area of iron ore bioprocessing which would eventually lead to automation of the implementation of bioprocessing in iron ore mining industry.

In order to understand the process of iron ore bioprocessing or biomining, a number of considerations must be understood and answered, such as what microorganisms are involved in the extraction of the metals from the rocks and where in nature do they occur? What biochemical functions do these microorganisms perform and what do they require in the need of nutrient and environmental conditions in order to maintain their activity? What are the constraints of the commercial exploitation of such biological techniques? And what impact will the new tools of genetic engineering have on the future of iron ore bioprocessing?

In addition to answering the abovementioned questions, it is an imminent task to bridge the gaps between iron ore, biotechnology industry, academia, and policymakers. Achieving the goal of developing bioprocessing technologies for iron ore requires joint efforts by academia, government, and industry. The development of bioprocessing processes requires a multidisciplinary effort that addresses key challenges necessary to unleash its commercial potential for that application. Biotechnology, including genetic engineering (recombination DNS technology and its applications), has become increasingly important as a tool for creating a value-added products and for developing biocatalysts making collaborative work imminent.

The strong development of industrial biotechnology is of immediate interest to the economically important iron ore and biotech industries. From the collaboration of these two industries, entirely new activities can be created in the form of bioprocessing of iron ores. Industrial biotechnology may also contribute significantly to the future biotechnology worldwide.

Furthermore, efforts to increase public awareness about industrial biotechnology are needed, with the added benefit that this is likely to improve the consumer's perception of biotechnology as a whole, in view of the clear link between industrial biotechnology and the sustainable development of our society.

3 Scope

Iron ore mining has long been considered as capital and energy-intensive and associated with negative impacts on the environmental. Conventional iron ore crushing, milling, and concentrating processes consume large amount of energy. Uncontrolled release of gases, solids, and wastewater has been a long-standing problem in many mining operations. Another major problem is the depletion of high-grade mineral deposits and the consequent need to mine at greater depths with higher costs.

Bioprocessing of iron ore has received increasing attention because the technology has the potential to ease some of the problems experienced by the mining industry. The bioleaching of ores and concentrates may be an energy-saving alternative. Besides, bioprocessing of iron ores has potential environmental benefits. The controlled leaching of waste rock can result both in the recovery of valuable metals from the sites and the protection of environment pollution. In many instances, it may be possible to use microorganisms to leach the desired mineral out of deep or low-grade deposits without removing them from the ground, thus saving the costs of bringing vast tonnage of ore and waste rock to the surface.

The term "iron ore bioprocessing" initially proposed by decision-making entities of the US DOE and Europe Union is now gaining momentum worldwide; it covers the field on industrial biotechnology with positive environmental aspects linked to the application of industrial biotechnology. This new biotechnology is being developed into a main contributor to the so-called green mining area, in which renewable resources such as microorganisms or their components of cells and products can be transformed into or replace a wide variety of chemical substances such as flotation and flocculant reagents, solvents, adsorption agents, dewatering, and water treatment additives as well as biofuels such as bioethanol and biodiesel.

This development is now mainly driven by the laws of market economy in view of the better overall "efficiencies" obtained by biotechnology production processes. In the new future, a number of societal and technological changes are expected to reinforce this trend even further, such as the depletion of high-grade iron ore reserves, the increased demand for sustainability and efficiency in iron ore production, and changes in environmental policy.

In this book, basic concepts in iron ore mining and biotechnology will be introduced first and then the implementation of industrial iron ore bioprocessing will be discussed with respects to its technical, ecological, and economic advantages and disadvantages comparing to conventional mining methods. Since the microorganisms are the major agents or starting materials for iron ore bioprocessing, microbiological aspects of bioprocessing will be focused in terms of engineering design and process requirement, economics, and environmental considerations. Considering the fact that iron ore industry is far behind nonferrous industry on adopting the idea of bioprocessing, separate chapters will be dedicated to discuss on laboratory research techniques, methods of feasibility studies, scaling-up, and technology transfer.

Another important scope of iron ore bioprocessing is to use biological systems to assist the production of iron ore products (sintering feed, lump ores, concentrates, and pellets) and to upgrade existing iron ore products (such as removal of sulfur and phosphorus). Potential applications in these aspects will be explored with main focus on biocatalysis (the use of enzymes to catalyze chemical reactions) and in bioleaching technology (direct use of microorganisms), in combination with breakthroughs in molecular genetics, directed evolutions, and enzyme engineering and metabolic engineering of microorganisms and cells.

4   Key Concepts

"Iron ores" are minerals and rocks from which metallic iron can be economically extracted. The ores are usually rich in iron oxides and vary in color from black, dark gray, gray to silver gray, bright yellow, brown to reddish-brown, and deep purple to rusty red. The iron usually presents itself in the form of magnetite (Fe_3O_4, 72.4% Fe), hematite (Fe_2O_3, 69.9% Fe), goethite (FeO(OH), 62.9% Fe), limonite (FeO(OH).n(H_2O)), or siderite ($FeCO_3$, 48.2% Fe). Ores containing very high quantities of hematite or magnetite (greater than about 60% iron) are known as "natural ore" or "direct shipping ore," meaning they can be fed directly into ironmaking blast furnaces. Iron ore is the raw material used to make pig iron, which is one of the main raw materials to make steel; 98% of the mined iron ore is used to make steel.

"Iron ore processing, concentrating, or beneficiation" includes all the processes that will increase (upgrade) the iron content of an ore by removing impurities. Beneficiation, a slightly broader term, includes these processes as well as those that make an ore more usable by improving its physical properties (e.g., pelletizing and sintering). Many of the iron ore mines employ some form of beneficiation to improve the grade and properties of their products. The operation of the concentrators has also increased the iron ore resources available at these mines.

Pelletizing is a treatment process used for very fine or powdery ores. Pellets are an ideal blast furnace feed because they are hard and of regular size and shape.

Sintering is a process used to agglomerate iron ore fines in preparation for blast furnace smelting and is usually carried out at iron and steelmaking facilities. It involves the incorporation of crushed limestone, coke, and other additives available from iron and steelmaking operations. These additives include wastes extracted from furnace exhaust gases, scale produced during rolling mill operations, and coke fines produced during coke screening.

Pig iron is an intermediate step in the production of steel and is produced by smelting iron ore (commonly in lump, pellet, or sinter form) in blast furnaces. The removal, by oxidation, of impurities in pig iron such as silicon, phosphorus, and sulfur and the reduction in the carbon content results in the production of steel.

"Bioprocessing" is a broad term encompassing the research, development, manufacturing, and commercialization of products prepared from or used by biological systems, including food, feed, biopharmaceutical, and cosmetics. Graduates will

have the technical competence and hands-on experience to immediately contribute to the biomanufacturing and pharmaceutical industries. The Food, Bioprocessing and Nutrition Sciences Department draws from its expertise in training professionals with a breadth of knowledge in microbiology, biotechnology, engineering, and biochemistry. In addition to a strong technical background, students earning a B.S. in bioprocessing science will understand other subject matter relevant to the bioindustries, including quality control and assurance, validation procedures, as well as ethical and regulatory issues. This unique collection of courses is designed to provide bioprocessing students with a special skill set specific to bioprocessing and pharmaceutical manufacturing needs.

"Bioleaching" is defined as being "the dissolution of metals from their mineral source by certain naturally occurring microorganisms" or "the use of microorganisms to transform elements so that the elements can be extracted from a material when water is filtered through it" (Atlas & Bartha, 1997). Additionally the term biooxidation is also used (Hansford & Miller, 1993). There are, however, some small differences by definition (Brierley, 1997): Usually, "bioleaching" is referring is referring to the conversion of solid metal values into their water-soluble forms using microorganisms. In the case of copper, copper sulfide is microbially oxidized to copper sulfate and metal values are present in the aqueous phase. Remaining solids are discarded. "Biooxidation" describes the microbiological oxidation of host minerals which contain metal compounds of interest. As a result, metal values remain in solid residues in a more concentrated form. In gold mining operations, biooxidation is used as a pretreatment process to (partly) remove pyrite or arsenopyrite. This process is also called *"biobeneficiation"* where solid materials are refined and unwanted impurities are removed. The term "biomining," "bioextraction," or "biorecovery" is also applied to describe the mobilization of elements from solid materials mediated by bacteria and fungi. *"Biomining"* concerns mostly applications of microbial metal mobilization processes in large-scale operations of mining industries for an economical metal recovery.

"Biohydrometallurgy" covers bioleaching or biomining processes (Rossi, 1990). Biohydrometallurgy represents an interdisciplinary field where aspects of microbiology (especially geomicrobiology), geochemistry, biotechnology, hydrometallurgy, mineralogy, geology, chemical engineering, and mining engineering are combined. Hydrometallurgy is defined as the treatment of metals and metal-containing materials by wet processes and describes "the extraction and recovery of metals from their ores by processes in which aqueous solutions play a predominant role" (Parker, 1992). Rarely, the term "biogeotechnology" is also used instead of biohydrometallurgy (Farbiszewska et al., 1994).

"Biocatalysis" is defined as a biological material or a material of (nonhuman) biological origin, which possesses the ability to catalyze one or more reactions, sometimes in the presence of cofactors or coenzymes.

"Biosorption" and *"bioaccumulation":* Biosorption can be defined as the selective sequestering of metal soluble species that result in the immobilization of the metals by microbial cells. Metal sequestering by different parts of the cell can occur via various processes: complexation, chelation, coordination, ion exchange,

precipitation, and reduction. Biosorption is a process with some unique characteristics. It can effectively sequester dissolved metals from very dilute complex solutions with high efficiency. This makes biosorption an ideal candidate for the treatment of high-volume low-concentration complex wastewaters. Bioaccumulation refers to the accumulation of substances, such as pesticides, or other organic chemicals in an organism. Bioaccumulation occurs when an organism absorbs a toxic substance at a rate greater than that at which the substance is lost. Thus, the longer the biological half-life of the substance, the greater the risk of chronic poisoning, even if environmental levels of the toxin are not very high.

"Bioconcentration" is a related but more specific term, referring to uptake and accumulation of a substance from water alone. By contrast, bioaccumulation refers to uptake from all sources combined (e.g., water, food, and air).

Though biosorption and bioaccumulation are used synonymously, they are very different in how they sequester contaminants. Biosorption is a metabolically passive process, meaning it does not require energy, and the amount of contaminants a sorbent can remove is dependent on kinetic equilibrium and the composition of the sorbents cellular surface. Contaminants are adsorbed onto the cellular structure. Bioaccumulation is an active metabolic process driven by energy from a living organism and requires respiration. Bioaccumulation occurs by absorbing contaminants which are transferred onto and within the cellular surface. Both bioaccumulation and biosorption occur naturally in all living organisms; however, in a controlled experiment conducted on living and dead strains of bacillus sphaericus, it was found that the biosorption of chromium ions was 13–20% higher in dead cells than living cells. In terms of environmental remediation, biosorption is preferable to bioaccumulation because it occurs at a faster rate and can produce higher concentrations. Since metals are bound onto the cellular surface, biosorption is a reversible process, whereas bioaccumulation is only partially reversible.

"Bioflotation" and *"bioflocculation"*: Bioflotation utilizes microorganisms to replace or to interact with chemical reagents to increase the gas between surface properties of similar minerals and to enhance the separation selectivity. It represents one of the growing trends to enhance the selectivity of conventional flotation processes. Bioflocculation is defined as a process, whereby flocculation is mediated by the presence of microorganisms or bioflocculants (which are biodegradable macromolecular flocculant secreted by microorganisms) (Gao et al., 2005). In a broader sense, it is the removal of colloidal particles from solution by flocculating substances of biological origins.

"Biofilm" is any group of microorganisms in which cells stick to each other on a surface. These adherent cells are frequently embedded within a self-produced matrix of extracellular polymeric substance (EPS). Biofilm extracellular polymeric substance, which is also referred to as slime (although not everything described as slime is a biofilm), is a polymeric conglomeration generally composed of extracellular DNA, proteins, and polysaccharides. Biofilms may form on living or nonliving surfaces and can be prevalent in natural, industrial, and hospital settings. The microbial cells growing in a biofilm are physiologically distinct from planktonic cells of the same organism, which, by contrast, are single cells that may float or swim in a liquid medium.

Microbes form a biofilm in response to many factors, which may include cellular recognition of specific or nonspecific attachment sites on a surface, nutritional cues, or in some cases, by exposure of planktonic cells to subinhibitory concentrations of antibiotics. When a cell switches to the biofilm mode of growth, it undergoes a phenotypic shift in behavior in which large suites of genes are differentially regulated.

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Iron Ore Processing, General

1 Introduction

Iron is the world's most commonly used metal–steel, of which iron ore is the key ingredient, representing almost 95% of all metal used per year. It is used primarily in structural engineering applications and in maritime purposes, automobiles, and general industrial applications (machinery).

Making iron and steel from iron ore requires a long process of mining, crushing, separating, concentrating, mixing, agglomeration (sintering and pelletizing), and shipping to steel mills. Iron ore processing is characterized by a constant adaptation to changing raw materials and market conditions. It is the link between the mined raw materials and a marketable product. As a lot of high-grade reserves are exploited, a steady deterioration of raw material quality can be observed. At the same time, the customers' requirements for product purity and consistent quality increase. Over the last few decades, processing and beneficiation techniques for iron ore have become more important in order to achieve a maximized utilization of ore resources and to produce competitive products according to international standards.

2 Iron Ore Mining

There is a long and rich history of iron ore mining and processing. Prior to the industrial revolution, most iron was obtained from widely available goethite or bog ore. Prehistoric societies used laterite as a source of iron ore. Historically, much of the iron ore utilized by industrialized societies has been mined from predominantly hematite deposits with grades greater than 60% iron. These deposits are commonly referred to as "direct shipping ores" or "natural ores," meaning they can be fed directly into ironmaking blast furnaces. Iron ore is the raw material used to make pig iron, which is one of the main raw materials to make steel; 98% of the mined

iron ore is used to make steel. Indeed, it has been argued that iron ore is "more integral to the global economy than any other commodity, except perhaps oil."

Increasing iron ore demand, coupled with the depletion of high-grade hematite ores in the United States, after World War II led to development of lower-grade iron ore sources, mainly the utilization of magnetite and taconite. (Taconite is a rock whose iron content, commonly present as finely dispersed magnetite, is generally 25–30%).

2.1 Iron Ore Minerals

Iron ores are rocks and minerals from which metallic iron can be economically extracted. The ores are usually rich in iron oxides and vary in color from dark gray, bright yellow, deep purple, to rusty red. Iron ores occur in igneous, metamorphic (transformed), or sedimentary rocks in a variety of geologic environments. Most are sedimentary, but many have been changed by weathering, and so their precise origin is difficult to determine. The most widely distributed iron-bearing minerals are oxides, and they consist mainly of hematite (Fe_2O_3), which is red; magnetite (Fe_3O_4), which is black; goethite or bog-iron ore ($FeO(OH)$), which is brown; and siderite ($FeCO_3$), which is pale brown. Hematite and magnetite are by far the most common types of ore. The iron can also be found in the form of sulfide minerals such as pyrite, but not commonly used for making iron and steel. Detailed description for each iron mineral type is listed in Table 1.

The iron ore processing industry produces usable concentrations of iron-bearing material by removing nonferrous rock (gangue) from low-grade ore. In the United States, predominant iron ore is taconite which is a hard, banded, low-grade ore. Ninety-nine percent of the crude iron ore produced in the United States is taconite. If magnetite is the principal iron mineral, the rock is called magnetic taconite; if hematite is the principal iron mineral, the rock is called hematic taconite.

About 98% of the demand for taconite comes from the iron and steel industry. The remaining 2% comes mostly from the cement industry but also from

Table 1 Common iron ore minerals

Minerals	Chemical formula	Iron content (wt %)	Specific density (g/cm³)	Crystal system/color
Hematite	Fe_2O_3	69.9	4.90–5.30	Hexagonal-rhombohedral, red, black, gray
Magnetite	Fe_3O_4 or $FeO \cdot Fe_2O_3$	72.4	5.16–5.18	Cubic, black, strongly magnetic
Martite	Fe_2O_3	70.0	4.80–5.30	Cubic, black, often tarnished
Goethite	$FeO(OH)$	62.9	4.30	Rhombohedral, yellow, red, brown, black
Siderite	$FeCO_3$	48.2	3.83–3.88	Hexagonal-rhombohedral, gray, brown, white, yellow
Pyrite	FeS_2	46.5	4.95–5.10	Cubic, brass yellow

manufacturers of heavy medium materials, pigments, ballast, agricultural products, and specialty chemicals. Ninety-seven percent of the processed ore shipped to the iron and steel industry is in the form of pellets. Other forms of processed ore include sinter and briquettes. The average iron content of pellets is 63%.

2.2   Mining Methods

Iron ore mining methods vary by the type of ore being mined. Currently, there are four main types of iron ore deposits, depending on the mineralogy and geology of the ore deposits. These are magnetite, titanomagnetite, massive hematite, and pisolitic ironstone deposits.

For taconite iron ores, mining iron ore begins at ground level. Taconite is identified by diamond drilling core samples on a grid hundreds of feet into the earth. Taconite rock comprises about 28% iron; the rest is sand or silica. These samples are analyzed and categorized so that mining engineers can accurately develop a mine plan.

To uncover taconite reserves, the mine area is first "stripped" of the overburden or glacial drift, comprised primarily of rock, clay, and gravel. The overburden is loaded by large hydraulic shovels into production trucks, which haul it to contour dumps. These dumps are environmentally designed to match the surrounding area.

Once the taconite rock is exposed, large drilling rigs drill blast holes 16″ in diameter by 40′ deep, in some cases. Nearly 400 of these holes are drilled in a blast pattern. Before the blast, the holes are filled with a special mixture of blasting agents. Once prepared, the mine site is cleared of workers and equipment, and the blast is detonated. Each of the holes is detonated just a millisecond apart, resulting in a pile of crude taconite that is broken apart to a minus 6′ × 6′ size.

After blasting, hydraulic face shovels and larger loaders load the taconite into 200-ton or 240-ton production trucks, which haul it to crushers. The taconite is ground to a fine powder and mixed with water. A series of magnets is run over the mixture. The magnets grab the iron particles, and the rest is discarded. For every ton of iron retained, two tons of waste, or tailings, is discarded.

Generally for most types of iron ores, the most appropriate mining method is selected based on technical, economic, and environmentally accountable considerations after a mineral deposit has been discovered, delineated, and evaluated. The first step in selecting the most appropriate mining method is to compare the economic efficiency of extraction of the deposit by surface and underground mining methods.

Surface Mining

Extraction of iron ore by operations exclusively involving personnel working on the surface without provision of manned underground operations is referred to as surface mining. While an opening may sometimes be constructed below the surface

and limited underground development may occasionally be required, this type of mining is essentially surface-based. Surface mining can be classified into two groups on the basis of the method of extraction; mechanical extraction, or aqueous extraction.

Mechanical extraction methods employ mechanical processes in a dry environment to recover minerals, encompassing the specific mining methods of:

1. Opening pit mining
2. Open cast mining
3. Quarrying of dimension stone
4. Highwall/auger mining.

Open-pit and open cast mining employ a conventional mining cycle of operations to extract iron ores: Rock breakage is usually accomplished by drilling and blasting for consolidated materials and ripping or direct removal by excavators for unconsolidated soil and/or decomposed rock, followed by materials handling and transportation.

Dimension stone quarrying is quite similar to open-pit mining, but rock breakage without blasting is almost exclusively employed to cut prismatic blocks or tabular slabs of rock. The high labor intensity and cost associated with cutting stone make quarry the most expensive surface mining method.

Highwall mining is a coal mining method for recovery of outcropped coal by mechanical excavation without removal of overburden. A continuous miner with single or multiple augers/cutting heads is operated underground and controlled remotely by crew located outside. Augering can be regarded as a supplementary method for open cast mining in cases when coal seams in the highwall would otherwise remain unmined (unless recoverable by underground methods) or when rugged terrain would preclude economic stripping by conventional surface methods. Quarrying of dimension stone and highwall mining are not very commonly used for iron ore mining because they are either labor-intensive or costly.

Aqueous extraction in most cases involves the use of water or a liquid solvent to flush minerals from underground deposits, either by hydraulic disintegration or physicochemical dissolution. This extraction includes placer mining and solution mining. Placer mining is intended for the recovery of heavy minerals from alluvial or placer deposits, using water to excavate, transport, and/or concentrate minerals. Solution mining is employed for extracting soluble or fusible minerals using water or a lixiviant.

Underground Mining

Underground mining is carried out when the rocks, minerals, or precious stones are located at a distance far beneath the ground to be extracted with surface mining. To facilitate the minerals to be taken out of the mine, the miners construct underground rooms to work in. The mining company selects the best feasible way to get the minerals extracted out. Most mining is carried out using; Continuous mining that

employs a continuous mining mechanism to cut the coal deposits from the walls. This means there is less of blasting and drilling and utilizes fewer miners down in the mines. It is safer than the yesteryear techniques of mining that is being described on our coal mine tour page.

This kind of mining is done when the rock or mineral is on the side of a mountain. This makes it an easy and cheaper way to mine. Minerals that are mined with draft mining are gold, coal, etc. With slope mining, the coal or mineral bed is located very deep and parallel to the ground. It is called a slope mine because the shafts are slanted. Shaft mining has a vertical man shaft, a tunnel where men travel up and down in an elevator. Shrinkage stoping is a flexible mining method for narrow ore bodies that need no backfill during stoping. Longwall mining consists of multiple coal shearers mounted on a series of self-advancing hydraulic ceiling supports. Retreat mining is the last phase of a common type of coal mining technique referred to as room and pillar mining. Retreat mining is a process that recovers the supporting coal pillars, working from the back of the mine toward the entrance, hence the word retreat. Room and pillar mining advance inward, away from the entrance of the mine. Other underground mining methods include hard rock mining, bore hole mining, drift and fill mining, long hole slope mining, sublevel caving, and block caving.

Typically, there are two methods for underground mining depending on the hardness of the excavated ore, i.e., hard and soft.

Underground hard rock mining refers to various underground mining techniques used to excavate hard ores such as those containing metals like gold, copper, zinc, nickel, and lead or gems such as diamonds. In contrast, soft rock mining refers to excavation of softer minerals such as coal or oil sands.

Accessing underground ore can be achieved via a decline (ramp), vertical shaft, or adit. Declines can be a spiral tunnel which circles either the flank of the deposit or circles around the deposit. The decline begins with a box cut, which is the portal to the surface. Depending on the amount of overburden and quality of bedrock, a galvanized steel culvert may be required for safety purposes.

Shafts are vertical excavations sunk adjacent to an ore body. Shafts are sunk for ore bodies where haulage to surface via truck is not economical. Shaft haulage is more economical than truck haulage at depth, and a mine may have both a decline and a ramp. Adits are horizontal excavations into the side of a hill or mountain. They are used for horizontal or near-horizontal ore bodies where there is no need for a ramp or shaft. Declines are often started from the side of the high wall of an open cut mine when the ore body is of a payable grade sufficient to support an underground mining operation, but the strip ratio has become too great to support open cast extraction methods.

Levels are excavated horizontally off the decline or shaft to access the ore body. Stopes are then excavated perpendicular (or near perpendicular) to the level into the ore.

One of the most important aspects of underground hard rock mining is ventilation. Ventilation is required to clear toxic fumes from blasting and removing exhaust fumes from diesel equipment. In deep hot mines, ventilation is also required for

cooling the workplace for miners. Ventilation raises are excavated to provide ventilation for the workplaces and can be modified to be used as escape routes in case of emergency. The main sources of heat in underground hard rock mines are virgin rock temperature, machinery, auto-compression, and fissure water although other small factors contribute like people breathing, inefficiency of machinery, and blasting operations.

Cut and fill mining is a method of short-hole mining used in narrow ore zones. An access ramp is driven off the main level to the bottom of the ore zone to be accessed. Using development mining techniques a drift is driven through the ore to the defined limit of mining. Upon completion, the drift (or "cut") is filled back to the access ramp with the defined type of backfill, which may be either consolidated or unconsolidated. Another drift is driven on top of filled cut. This process continues until the top of the stope is reached.

Drift and fill are similar to cut and fill, except it is used in ore zones which are wider than the method of drifting will allow to be mined. In this case, the first drift is developed in the ore and is backfilled using consolidated fill. The second drift is driven adjacent to the first drift. This carries on until the ore zone is mined out to its full width, at which time the second cut is started atop of the first cut.

Room and pillar mining: Room and pillar mining is commonly done in flat or gently dipping bedded ore bodies. Pillars are left in place in a regular pattern while the rooms are mined out. In many room and pillar mines, the pillars are taken out starting at the farthest point from the stope access, allowing the roof to collapse and fill in the stope. This allows a greater recovery as less ore is left behind in pillars.

Block caving such as is used at the Northparkes Mine in NSW, Australia, is used to effect with large-sized ore bodies which are typically composed of low-grade, friable ore. The method works best with cylindrical, vertical ore bodies. Preproduction mining development work consists of driving accesses underneath the ore body. This includes the formation of "drawbells" by undercutting and blasting. Initially, blasted ore is removed via the extraction level underneath the drawbells until a sufficient area of unsupported ore is formed that the ore body begins to fracture and cave on its own. The eventual aim of the block caving method is that the friable ore needs no blasting and continues to fracture and break up on its own, flowing down the drawbells to the extraction level, where it is removed from the ore chute mouths with loaders and sent off for processing. Eventually the fracturing will propagate to the surface, resulting in subsidence. One of the main hazards associated with block caving is that fracturing can potentially stop before it reaches the surface unbeknownst to the people in control of the mine. If fracturing stops propagating upward and extraction continues, a large void can be formed, resulting in the potential for a sudden and massive collapse and catastrophic windblast throughout the mine.

3   Iron Ore Handling

Iron ore handling, which may account for 20–50% of the total delivered cost of raw materials, covers the processes of transportation, storage, feeding, and washing of the ore *en route* to or during its various stages of treatment in the mill.

Since the physical state of iron ores in situ may range from friable, or even sandy materials, to monolithic deposits with hardness of granite, the methods of mining and provisions for the handling of the freshly mined materials will vary extremely widely. Iron ore that has been well broken can be transported by rails, trucks, and conveyor belts, but large lumps of hard ore may need individual blasting.

Iron ores mined by open pit tend to be very heterogeneous, the largest lumps often being about more than 1 m across. The broken ore from the pit, after blasting, is loaded directly into trucks, holding up to 300 tons of ore in some cases, and is transported directly to the primary crushers. Storage of such ore is not always practicable, because its "long-ranged" particle size can cause segregation during storage. The fines can also work their way down through the voids between the larger particles. Sophisticated storage and feed mechanisms are therefore often dispensed with the trucks depositing their loads directly into the mouth of the primary crusher.

The operating cycle on an underground mine is complete different. Drilling and blasting are often performed on shift, the ore broken in this time being hoisted to the surface during the other two shifts of the working day. The ore is transported through the passes via chutes and tramways and is loaded into skips, holding as much as 30 tons of ore, to be hoisted to the surface. Large rocks are often crushed underground by primary breakers in order to facilitate loading and handling at this stage. The ore, on arrival at the surface, having undergone some initial crushing, is easier to handle than that from an open-pit mine and storage, and feeding is usually easier, and indeed essential, due to the intermittent arrival of skips at the surface.

3.1   Iron Ore Transportation

The conveyor belt is the most widely used method of handling loose bulk materials. Belts are in use with capacities up to 20,000 ton/h and single flight lengths exceeding 5000 m, with feasible speeds up to 10 m/s. The standard rubber conveyor belt has a foundation of sufficient strength to withstand the driving tension and loading strains. This foundation, which may be of cotton, nylon, or steel cord, is bound together with a rubber matrix and completely covered with a layer of vulcanized rubber.

The carrying capacity of the belt is increased by passing it over troughing idlers. These are support rollers set normal to the travel of the belt and inclined upward from the center so as to raise the edges and give it a trough-like profile. There may be three or five in a set, and they will be rubber-coated under a loading point, so as

to reduce the wear and damage from impact. Spacing along the belt is at the maximum interval which avoids excessive sag. The return belt is supported by horizontal straight idlers which overlap the belt by a few inches at each side.

Dry ore can be moved through chutes, provided they are of sufficient slope to allow easy sliding, and sharp turns are avoided. Clean solids slide easily on a 15–25 degree steel-faced slope, but not for most ores, a 45–55 degree working slope is used. The ore may be difficult to control if the slope is too steep.

Feed chutes must be designed to deliver the bulk of the material to the center of the belt and at a velocity close to that of the belt. Ideally, it should be the same, but in practice this condition is rarely obtained, particularly with wet or sticky materials. Where conditions will allow, the angle of the chute should be as great as possible, thereby allowing it to be gradually placed at lesser angles to the belt until the correct speed of flow is attained. The material, particularly if it is too heavy, or lumpy, should never be allowed to strike the belt vertically. Baffles in transfer chutes, to guide material flow, are now often remotely controlled by hydraulic cylinders.

The conveyor may discharge at the head pulley, or the load may be removed before the head pulley is reached. The most satisfactory device for achieving this is a tripper. This is an arrangement of pulleys by which the belt is raised and doubled back so as to give a localized discharged point. It is usually mounted on wheels, running on tracks, so that the load can be delivered at several points, as over a long bin or into several bins. The discharge chute on the tripper can deliver to one or both sides of the belt. The tripper may be moved by hand, by head, and tail ropes from a reversible hoisting drum or by a motor. It may be automatic, moving backward and forward under power from the belt drive as shown in Fig. 1.
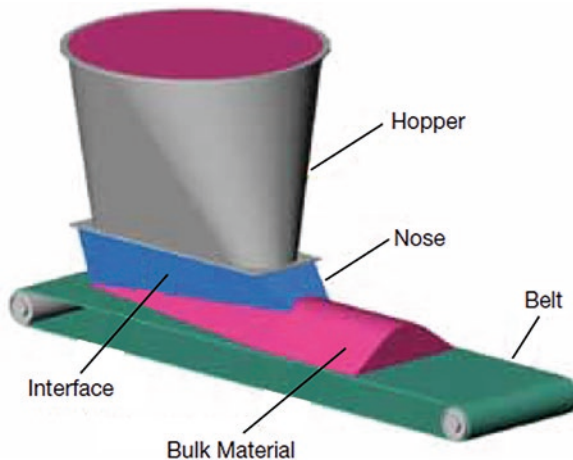

Fig. 1 Typical design of belt feeder

3.2   *Iron Ore Storage*

The necessity of iron ore storage arises from the fact that different parts of the operation of mining and milling are performed at different rates, some being intermittent and some continuous, some being subject to frequent interruption for repair, and others being essentially batch processes. Thus, unless reservoirs for materials are provided between the succeeding different steps, the whole operation is rendered spasmodic and, consequently, uneconomical.

The amount of storage necessary depends on the equipment of the plant as a whole, its method of operation, and the frequency and duration of regular and unexpected shutdowns of individual units.

At most iron ore mines, grinding and concentration circuits are most efficient when running continuously. Mine operations are more subject to unexpected interruption than mill operations, and coarse crushing machines are more subjected to clogging and breakages than fine crushers, grinding mills, and concentration equipment. Consequently, both the mine and the coarse-ore plant should have a greater hourly capacity than the fine crushing and grinding plants, and ore storage reservoirs should be provided between them. Ordinary mine outages, expected or unexpected, will not generally exceed more than 24 h, and ordinary coarse crushing plant repairs can be made within an equal period if a good supply of spare parts is kept on hand. Therefore, if a 24-h supply of ore that has passed the coarse crushing plant is kept in reserve ahead of the mill, the mill can be kept running without stop if the shutdowns were less than 24 h at mine and coarse crushing plants. It is wise to provide for a similar mill shutdown, and, in order to do this, the reservoir between coarse crushing plant and mill must contain at all times unfilled space capable of holding a day's tonnage from the mine. This is not economically possible, however, with many of the modern very large mills; there is a trend now to design such mills with smaller storage reservoirs, often supplying less than a two-shift supply of ore, the philosophy being that storage does not do anything to the ore, and can, in some cases, have an adverse effects by allowing the ore to oxidize. Particularly, iron ore fines or concentrates cannot be left exposed to rainy or cold winter conditions as it will be wet or become frozen and be difficult to handle.

Storage has the advantage of allowing blending of different ores so as to provide a consistent feed to the mill. Both tripper and shuttle conveyors can be used to blend the material into the storage reservoir. If the unit shuttle back and forth along the pile, the materials are layered and mix were reclaimed. If the units form separate piles for each quality of ore, a blend can be achieved by combining the flow from selected feeders onto a reclaim conveyor.

Depending on the nature of the material treated, storage is accomplished in stockpiles, bins, or tanks.

Stock piles are often used to store coarse ore of low-value outdoors. In designing stockpiles, it is merely necessary to know the angle of repose of the ore, the volume occupied by the broken ore, and the tonnage.

Although material can be reclaimed from stockpiles by front-end loaders or by bucket wheel reclaimers, the most economical method is using the reclaim tunnel system. Because the reclaim tunnel system requires a minimum of manpower to operate. It is especially suited for blending by feeding from any combination of openings. Conical stockpiles can be reclaimed by a tunnel running through the center, with one, or more, feed openings discharging via gates, or feeders, onto the reclaim belt. The amount of reclaimable material, or live storage, is about 20–25% of the total. Elongated stockpiles are reclaimed in a similar manner, the live storage being 30–35% of the total.

For continuous feeding of crushed ore to the grinding section, feed bins are used for transfer of the coarse material from belts, rail, and road trucks. They are made of wood, concrete, or steel. They must be easy to fill and must allow steady fall of the ore through to the discharge gates with no "hanging up" of material or opportunity for it to segregate into coarse and fine fractions. The discharge must be adequate and drawn from several alternative points if the bin is large. Flat-bottomed bins cannot be emptied completely and must retain a substantial tonnage of deadlock. This, however, provides a cushion to protect the bottom from wear, and such bins are easy to construct. This type of bin, however, should not be used with easily oxidized ore which might age and mix with the fresh ore supply. Bins with sloping bottoms are better in such cases.

Ore slurry storage on a large scale is not as easy as dry ore. Conditioning tanks are used for storing suspensions of fine particles to provide time for chemical reactions to proceed. These tanks must be agitated continuously, not only to provide mixing, but also to prevent settlement and clogging. Surge tanks are placed in the slurry flow line when it is necessary to smooth out small operating variations of feed rate. Their content can be agitated by stirring, by blowing in air, or by circulation through a pump.

4   Iron Ore Beneficiation

"Beneficiation" of iron ore includes concentration, generally by physical removal of unwanted gangue; also, considered beneficiation is the regulation of product size, or other steps such as agglomeration to improve its chemical or physical characteristics prior to processing. Processing of the concentrated product into iron or steel typically involves the use of pyrometallurgical techniques.

4.1   Gravity Separation

Gravity separation uses differences in specific gravity (SG) between various minerals to achieve a separation and is normally a wet process although examples of dry gravity separators exist. Gravity separation works best when there is a large

difference in the SG in the minerals to be separated, and the particle size is similar and not excessively fine. The gravity separation methods are particularly suitable for beneficiation of iron ores because common iron oxides are usually sufficiently heavy than the lighter waste minerals with minimum metal loss in tailing. The choice of separator is generally determined by ore characteristics and includes conventional jigs, spirals, pinched sluices, centrifugal jigs, centrifugal concentrators, shaking tables (wet and dry), and mozley gravity separators (MGS).

Conventional Jigs

Jigging is one of the oldest methods of gravity separation in which its feed is kept in motion by water pulsing vertically through it. The heavier grains move downward to the bottom of the bed and are removed. Since the weight of the grain is governed by its size as well as its specific gravity, it is necessary for the feed to be in size within close limits. Many kinds of jigs are currently in use according to the size and the nature of the ore.

Jigs are commonly used as a primary stage to recover coarse liberated minerals sized 2 mm and above. Feed slurry is distributed into the hutch which consists of a moving slurry bed located above a screen and is subjected to a pulsating motion and upward hutch water flow which alternately causes dilation and compaction. The pulsation can be caused by a diaphragm (in the case of the Russell jig) or by mechanical movement of the screen (as in the ILPJ). Separation is also assisted by a ragging material of intermediate SG, with the result being that high SG (concentrate) particles pass through the ragging and screen and are removed via the hutch, while low SG particles (tailings) discharge over the lip of the hutch (Fig. 2).

Spirals

Spirals are popular due to their low maintenance and simplicity and higher separation efficiency than pinched sluice devices. Spirals are commonly used to separate sand-sized particles with moderate SG differential in the range −2 mm +75 μm, although varieties exist that can separate reasonably efficiently down to 63 microns.
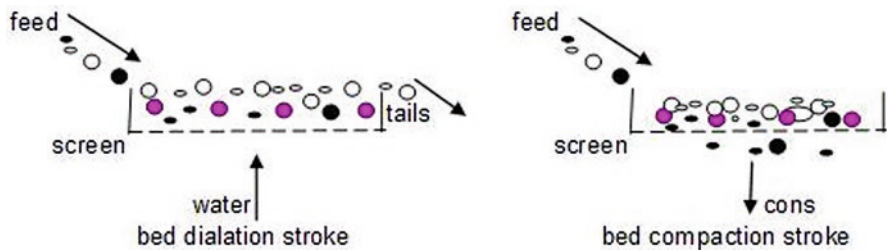
Fig. 2 Principle of conventional jig

Fig. 3 Principle of spiral
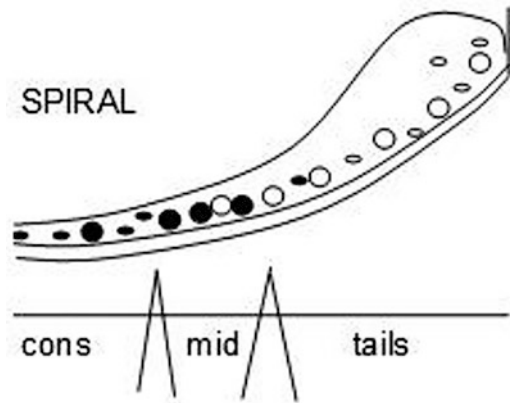
Below, this size range efficiency falls off rapidly, and enhanced fine gravity separators are generally required.

Spirals are usually made of fiberglass onto which smooth urethane surface is molded to form a trough in the shape of a spiral as the name suggests. Between individual spiral types, the profile of the trough and the pitch as well as the diameter and height and number of turns can vary according to duty. Feed slurry is introduced at the top and is subjected to a combination of gravitational and centrifugal forces imparted by its motion down the spiral. This causes high SG minerals to move toward the center of the trough and water and low SG minerals toward the outside. The segregated slurry discharging from the spiral at the bottom can thus be separated by cutters into high SG (concentrate) and low SG (tailings) together with intermediate SG (middling).

The choice of spiral depends on the duty: rougher/scavenger or cleaner; the feed grade: low/medium/high; and the particle size: sand/fine. Typically, rougher/scavenger spirals where the feed type favorable for easy separation and overall throughput is large are high capacity designs with triple or quadruple to reduce floor area. Cleaner and fine spirals where overall throughput is less and separation is more difficult are typically operated at lower feed rates, and as twin starts to assist access for operational requirements. Cleaning spirals are also available with wash water (Fig. 3).

Pinched Sluices

Pinched sluices were commonly used to separate sand-sized particles with moderate SG difference prior to the appearance of the modern-day spiral in the 1980s. They operate on the principle of stratification of particles of differing SG on an inclined surface, area of which becomes smaller "pinched" as the slurry flows down the slope, with high SG grains on the bottom and low SG grains on the top. High SG (concentrate) grains are removed through a slot, while low SG (tailing) discharges off the end. Reichert cones normally have multistage cons cleaning and water addition to maintain optimum density (Fig. 4).
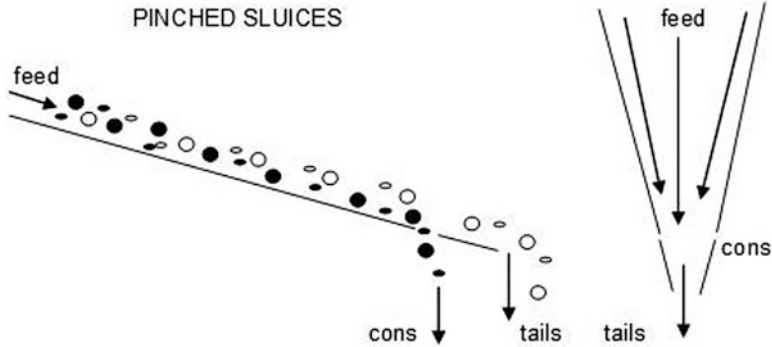
Fig. 4 Principle of pinched sluices

Centrifugal Jigs

An example of a centrifugal jig is the Kelsey jig, manufactured by Mineral Technologies. This incorporates the pulsing action, water injection, and the use of a ragging medium and internal screen of a conventional jig, but in addition centrifugal spinning motion to enhance the gravity separation. This enables sand-sized minerals of fairly similar SG as well as fine minerals to be separated more efficiently. Because of the additional processes used, the Kelsey jig is significantly more expensive and complex to operate and maintain. The feed to a Kelsey jig must be screened at a size less than the internal screen to avoid pegging, and the tailings must be screened in order to recover ragging as oversize. In order to maintain operational and mechanical efficiency, the Kelsey jig must have the internal screen cleaned daily and some of the moving parts checked and greased. This can now be done automatically, but the feed must still be stopped. The Kelsey jig is offered in two models: the J1300 and J1800, the latter having greater capacity (Fig. 5).

Centrifugal Concentrators

The Falcon C and Knelson CVD are derivatives of the SB types (semi-batch) used extensively in the gold industry, but provide continuous discharge of concentrate to enable higher-grade feeds to be processed without having to shut down. The recently developed Falcon U/F is a batch machine and spins at extremely high (up to 600G) rates in order to target very fine particles. They all utilize the centrifugal force generated by spin to enhance gravity and enable SG separation on finer particles to be achieved. Feed slurry enters centrally and is distributed outward at the base of the cone by centrifugal force and then flows up the inclined surface of the bowl, segregating in the process, with high SG particles on the outside closest to the bowl surface and low SG particles on the inside which discharge over the lip at the top of the bowl.

The Falcon C spins at high rates and can generate a G force of up to 200 and features a positioning valve for continuous concentrate discharge, while the Knelson
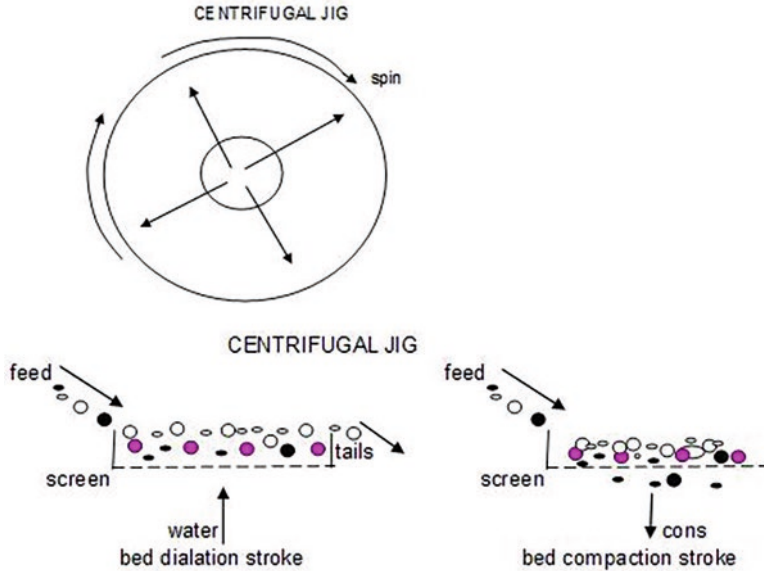
Fig. 5 Principle of centrifugal jig
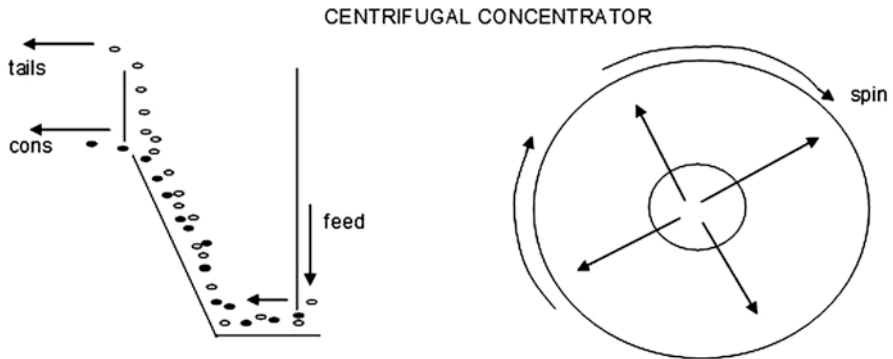
Fig. 6 Principle of centrifugal concentrator

CVD operates at lower G force (up to 150G), but has an injection water system which fluidizes the bed and assists separation. All three separators have different bowl profiles (Fig. 6).

Shaking Tables (Wet and Dry)

Shaking tables have been used for many years in gravity separation, and although limited in capacity, they produce a high upgrade, can handle a wide range of SGs, are very visual and operator-dependent and are still used for cleaning stages to produce final concentrates.

Feed slurry is distributed at the head of the table via a launder, together with wash water, and spreads out across the inclined surface on the basis of particle SG, with high SG grains moving along the top of the flowing film to discharge off the far end as concentrate, while low SG grains move down the inclined slope of the table with the majority of the water to discharge at the bottom as tailings. The particle separation is assisted by the backward and forward motion (stroke) of the table, the tilt (both longitudinally and laterally), wash water applied along the length of the table, and riffles (Fig. 7).

Dry shaking tables or air tables have specialized dry gravity separation applications such as fine quartz and zircon and consist of a table covered in cloth through which low-pressure air is forced through by fans located underneath and controlled by dampers so that particles are partially fluidized and segregate according to SG and size, under the influence of stroke and tilt and discharge separately with the aid of splitters. The forces acting on the particles on air tables reverse the pattern of wet tables with the low SG grains discharging at the top of the table (Fig. 8).
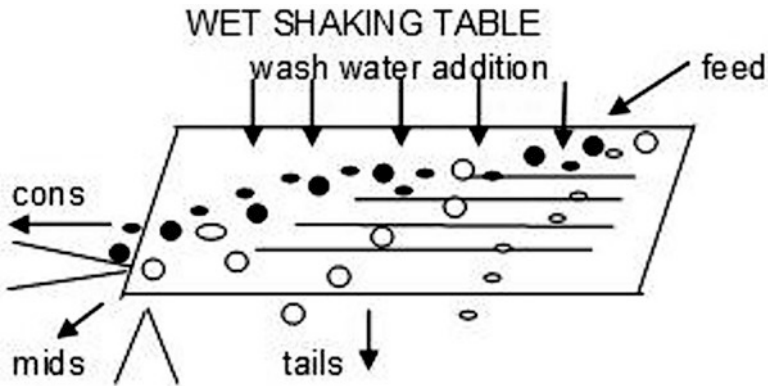
Fig. 7 Principle of wet shaking table
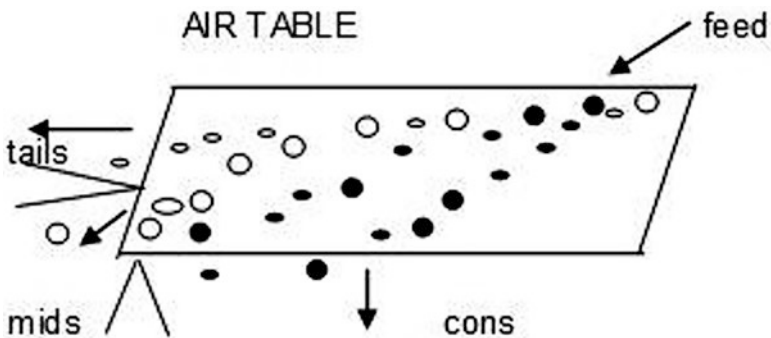
Fig. 8 Principle of dry shaking table
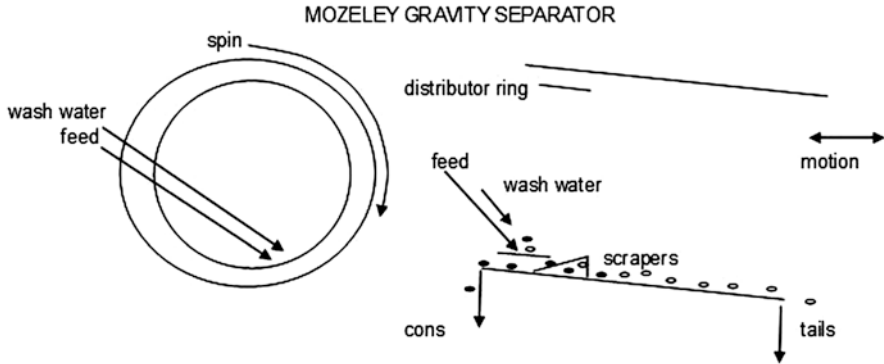
Fig. 9 Principle of Mozley gravity separator

Mozley Gravity Separators (MGS)

The Mozley gravity separator combines the centrifugal motion of a spinning drum to enhance fine gravity separation with the shaking motion of a table, making it suitable for upgrading of low tonnage rate fine particle slurries.

Feed slurry enters the MGS and is distributed onto a perforated feed ring mounted internally near the top end of an inclined spinning drum, together with wash water. The diluted slurry is thus subjected to centrifugal and shaking forces which cause the high SG particles to move up the inclined drum and low SG particles to move down the drum slope to discharge as tailings. Discharge of the high SG (concentrate) particles is assisted by internal scrapers which rotate at a speed slightly faster than the drum (Fig. 9).

4.2   Flotation

In iron ore flotation process, air is bubbled through a suspension of fine iron ore in water to which a small quantity of flotation reagent is added. This reagent modifies the surface of either the iron oxide or the principle gangue component, normally silica, so that these particles attach themselves to the air bubbles and are carried to the surface, where they are removed as froth.

Iron ore flotation processes can be classified by two very inclusive terms, anionic and cationic flotation methods according to the type of collector used in the flotation process. Either of the methods has been successfully utilized on industrial scales to recover the economic iron minerals in the froth product or in the underflow product. Based on these two methods, a variety of flotation routes have been developed to remove undesired constituents from iron minerals. The flotation routes of iron ore can be further classified into five major groups, i.e., cationic flotation of iron oxide, cationic flotation of quartz, anionic flotation of iron oxide, anionic flotation of

quartz, and combination. Despite the variety of flotation routes developed for iron ores, currently, the reverse cationic flotation route is by far the most widely used flotation route in the iron ore industry. The two anionic flotation routes, i.e., direct anionic flotation and reverse anionic flotation routes, are also being used in the iron ore industry.

The three types of flotation reagents are collectors, frothers, and modifiers. The function of the collector reagent is to promote affinity between mineral particles and air bubbles by formation of a water repellent coating on the surface of the mineral. Flotation reagents which ionize to yield a negatively charged ion are called anionic collectors. Examples of such collectors are fatty acids, resin acids, soaps, and alkyl sulfates or sulfonates. Those flotation reagents which ionize to give a positively charged organic ion are called cationic reagents. The most important types of cationic collectors in use are fatty amines and ammonium salts. Table 2 shows some of the common flotation reagents which are being used in the flotation of iron ores. Approximate consumption rates in pounds per ton of feed ore are also given.

Anionic Flotation

The earliest attempts to float iron oxide minerals involved the use of anionic collectors such as oleic acid or sodium oleate. Such a process was used at the Cuyuna Range plant which processed manganiferous iron ore in 1931. One of the successful applications of anionic flotation process was the iron ore flotation plant at Marquette county, Michigan. Anionic flotation of iron oxides has been accomplished in both acid and basic circuits. The acid circuits are, of course, unsuitable for ores containing a large amount of calcite or other acid-consuming minerals.

The anionic flotation of silicates through the activation of silica by calcium ions was tested by several laboratories, in particular those of Hanna Mining and the U.S. Bureau of Mines, in the form of discontinuous tests as well as pilot circuits (at 900 kg/h). This type of flotation implies a depression of the iron-bearing minerals which are collected at the bottom of the cell. The reagents which are used most commonly are gums, various starches in the form of gels or causticized, and dextrins. The silica is floated in a basic environment by a fatty acid after activation by a calcium salt, usually calcium chloride.

The nature of gangue minerals associated with iron oxides has an important bearing on amenability to flotation. Gangue minerals which break down and slime easily often consume large quantities of reagents. Desliming is an extremely important operation in most iron ore flotation processes. Hydrocyclone desliming methods are often used to control slime and reduce reagent consumption.

Most flotation processes using fatty acid-type anionic collectors are very sensitive to water quality. Sodium, calcium, and magnesium cations in process water can be absorbed on quartz or other silicate gangue minerals, thus decreasing their tendency to float. Adding water softening facilities and using ion exchanger to selectively remove unwanted ions from flotation pulp can be an effective way to improve flotation efficiency and reduce reagent cost.

Table 2 Common reagents used for iron ore flotation

Function	Reagents	Addition methods	Application	Appx. cons. lbs/ton ore
Collectors	Anionic reagents (fatty acids)			
	Tall oil	Liquid emulsion	Collectors for iron ores, can be precipitated by hard water containing calcium and magnesium ions	0.5–3.0
	Refined oleic acid	Liquid emulsion		
	Sodium soap of fatty acid	5–20% solution		
	Alkyl sulfates and sulfonates	5–20% solution	Collectors for iron ores	0.5–2.0
	Cationic reagents			
	Primary/ secondary amines	In kerosene	Quartz and silicates removal in reverse iron ore flotation	
	Amine acetates	5–10% solution	For floating quartz, silicates	
	Quaternary ammonium salts	5–10% solution	For floating quartz, silicates	
	Ether amines and diamines	Undiluted	For floating quartz, silicates	
Modifiers	Starch	Aqueous solution	Disperses hematite and/or slime control	0.1–2.0
	Lime or slaked lime(CaO or Ca(OH)$_2$)	Slurry	pH regulator	1.0–20.0
	Soda ash (NaOH)	Dry or in aqueous solution	Disperses gangue slimes; pH regulator	0.5–3.0
	Sulfuric acid (H$_2$SO$_4$)	10% solution	pH regulator	0.5–4.0
	Sodium silicate (Na$_2$SiO$_3$)	5–10% solution	Disperses siliceous gangue slimes; embrittles froth	
	Tannic acid	5% solution	Depresses CaCO$_3$, (CaMg)CO$_3$	
Frothers	Fuel oil	Undiluted	Fine-textured froth, often used with ores containing slimes	0.1–4.0
	Aliphatic alcohols, MIBC	Undiluted		0.2–4.0
	Pine oil	Undiluted	Provides most viscous stable froth	0.5–2.0
	Cresylic acid	Undiluted	Less viscous but stable froth, acts as collector too	
	Polypropylene glycols	Aqueous solution	Fine, fragile froth, inert to rubber	0.01–0.1
	DF (Dow Froth) 200/250/450	Aqueous solution		

Cationic Flotation

The commercial use of cationic collectors to float siliceous gangue minerals dates back to as early as 1930s when the Valley Forge Cement Company began using dodecyl amine hydrochloride to float mica and talc from argillaceous limestone. Up to date, the reverse cationic flotation route has been widely used in the iron ore industry to float siliceous waste materials and to upgrade lower-grade concentrate.

In a typical cationic flotation process, the ferriferous minerals need to be depressed first by reagents such as starch or dextrin, and then, the silica is collected by a cationic reagent normally without requiring activation by calcium ions, until at basic pH, the silica, and the silicates have a negative surface charge. The collectors are part of a large group of amines R-NH$_2$. At first used in the form of acetate or chlorhydrate of alkylamine, these reagents were difficult to administer, specifically at the level of production: difficult dissolution once the alkyl chains were of the stearyl or oleyl type. Only the amines of the lauryl type showed a certain degree of ease of dissolution. The froth remained difficult to manipulate if the desliming was insufficient.

New Development of Iron Ore Flotation

Microbially Induced Flotation and Flocculation

It has been widely reported that the microorganisms, both living and dead, and products derived from the microorganisms can function as flotation and flocculation agents. They can act as flotation collectors, depressants, and activators depending on their interactions and adhesion of relevance to minerals, which typically change surface chemistry of minerals; modify the mineral surface (hydrophobicity or hydrophilicity); and selective dissolve minerals.

Up to date, much of the focus of the fundamental bioflotation and flocculation research has been associated with the beneficiation of nonferrous sulfide minerals. Very little research effort has been expended to determine its application for the beneficiation of oxide minerals such as iron ores until recently. Deo and Natarajan (1999) reported that bacteria such as Bacillus polymyxa and their metabolic products, such as exopolysaccharides, and bioproteins interact effectively with oxide minerals and bring about significant surface chemical changes. Detailed electrokinetic studies have shown that interaction of the above bacteria with quartz, hematite, calcite, kaolinite, and corundum results in significant shifts in isoelectric points. Bacterial cells adsorb on the mineral surfaces to different degrees of coverage and along with metabolite products form a biofilm. The adsorption tendency of the bacterial cells follows the sequence as reported earlier (Deo & Natarajan, 1998), i.e., kaolinite > calcite > corundum > hamatite > quartz. Such an adsorption was also found to be almost independent of pH between 2 and 10 indicating nonelectrostatic forces being responsible for bacterial mineral adhesion.

Sarvamangala et al. (2012) reported that quartz surfaces are rendered more hydrophobic after bacterial interaction, while hematite and corundum become more hydrophilic. Similar tests indicated that kaolinite behaves similar to quartz, while calcite surfaces are rendered more hydrophilic after bacterial interaction. Such microbially induced mineral surface chemical changes can be beneficially used to bring about selective flotation/flocculation of minerals.

Table 3 shows the percentage weight flotation of quartz and hematite before and after interactions with cell and cell-free metabolites. It was observed that quartz flotation was enhanced greatly in the presence of microorganism either in forms of cells or cell-free metabolites. Such an enhancement in hydrophobicity of quartz is due to mineral-induced proteins secreted by various microorganisms into the cell-free metabolite during growth in the presence of quartz. It should be noted that under all conditions whether interacted with cells or cell-free metabolites, hematite flotation was significantly impaired.

de Mesquitaa et al. (2003) conducted microflotation tests on synthetic mineral mix of hematite and quartz using only R. opacus cells as flotation reagent. The test results indicated that a recovery of about 70% of hematite can be obtained with a concentrate grade of 49% for a head grade of 35% (total Fe). The results demonstrate the potential of using R. opacus cells as a collector at neutral pH in direct flotation systems of hematite where iron grade is low.

Natarajan (2003) studied the settling rates of various minerals at different pH values in the presence of bacterial cells and bacterial metabolite. It was found that the settling rates of hematite, calcite, and corundum particles were increased after interaction either with the bacterial cells or the metabolite; however, those quartz and kaolinite settling rates were seen to be significantly decreased after similar treatment. Therefore, selective flocculation of calcite, hematite, and corundum and dispersion quartz and kaolinite can be facilitated by bacterial interaction. Various polysaccharides such as starches and dextrans are used as depressants for iron oxides in iron ore flotation. Selective flocculation of iron oxide with dispersion of silica can be achieved by addition of the above polysaccharides. Biopolymers such as those containing exopolysaccharides can interlink the mineral particles through polymer bridging and flocculate the mineral fines selectively. Increased affinity of bacterial cells and excreted polysaccharides toward hematite and corundum result in their selective flocculation facilitating their rapid settling in an aqueous solution.

Results of selective flocculation tests carried out with mineral mixtures substantiate the above conclusion. It is possible to separate efficiently silica and silicates from alumina, calcite, and iron oxide through bioflocculation. However, it is difficult to separate alumina from hematite efficiently using ordinary bacterial cells since bacterial interaction brings about similar surface chemical changes on both of them. Tests with hematite–corundum mixtures have shown that both the minerals settled down faster in an aqueous medium after bacterial interaction without any selectivity. However, it has been observed that through the use of corundum-adapted strains of B. polymyxa, efficient separation of alumina from iron oxides can be achieved.

Table 3 Flotation of minerals after microbial interactions at neutral pH (7.0)

Minerals/microorganisms	Floatability (wt%)								
	Control	B. polymyxa	P. polymyxa	B. subtilis	S. cerevisiae (adapted)	D. desulfuricans (adapted)	R. opacus	B. polymyxa	P. polymyxa
Quartz	4–19	60–80	60	96	95	78	6	60–80	60
Hematite	4–7	2–4	8	4	8	9	72	2–4	8
Kaolinite	38	80–90	–	–	–	–	–	80–90	–
Corundum	5	2–20	–	–	–	–	–	2–20	–
Calcite	8	7–8	–	–	–	–	–	7–8	–
Ref.	Deo and Natarajan (1998)	Deo and Natarajan (1999)	Deo and Natarajan (1998)	Poorni et al. (2013)	Natarajan et al. (2012)	Sabari Prakasan and Natarajan (2010)	Mesquita et al. (2003)	Deo and Natarajan (1999)	Deo and Natarajan (1998)

The use of genetically modified microorganism may also provide enhanced selectivity over the use of indigenous bacteria. Farahat et al. (2008) recently reported the potential application of molecular cloning in bioflotation. In their study, a genetically modified strain of Escherichia coli (E. coli) was used as a collector and surface modifier for quartz. The modified strain carries and expresses the silica-induced protein gene, which gives the cell a positive surface charge under acidic conditions, allowing it to adhere strongly with the negatively charged quartz. Under acidic conditions, the adsorption of the bacteria onto mineral surface renders the mineral hydrophobic, allowing recovery by flotation. Using the bacteria alone, the authors demonstrated that 60% recovery could be achieved by bacterial conditioning with the silica-induced protein—E. coli strain. Using sodium dodecyl sulfate for anionic flotation of quartz, recoveries of up to 85% were achieved with the modified E. coli strain.

The laboratory experimental investigations showed that the microorganism and the iron oxide particles below 10 microns can coagulate effectively. Furthermore, it has been shown that bioflotation is suitable for the separation of iron oxide minerals. However, successful development of a bioflotation method for iron ore entails many steps. Identification of potential microbes is a major part of the development process. Factors such as difficult adaptation of nonindigenous microbes, biofilm formation, and lack of cheap carbon sources have hindered the scaling-up to larger scale of this technology. Although problem associated with large scale and rapid bacteria culturing may be overcome by the use of genetically modified strains, its application may be considerably limited by the costs associated with the modification of the microorganism.

With the depletion of high-grade iron ore, the removal of alumina containing minerals and phosphorus from low-grade iron ore could be the first area of application of bioflotation in iron ore industry. The flotation behavior of kaolinite is generally opposite to that of quartz, and special care has to be taken for the successful removal of the clay minerals. The surface properties of gibbsite are similar to that of iron oxides which makes it refractory to treatment using conventional flotation methods. The mineralogy of phosphorus in iron ore depends on the type of iron minerals. In magnetite, phosphorus is often found in the form of discrete apatite minerals which can be removed by flotation using anionic collectors. In hematite and goethite ores, phosphorus tends to be incorporated in the lattice of iron minerals and has to be rejected by chemical methods.

Reverse Cationic Flotation

Reverse cationic flotation is currently the most widely used flotation route in the world. However, the iron metal loss in the desliming stage, which is an essential step in reverse cationic flotation, and the high reagent cost of amine collector are the common problems of reverse cationic flotation.

Reverse anionic flotation rejects quartz by first activating it with the use of lime and then floating it using fatty acids as collectors. The advantages of reverse anionic

flotation, in comparison to reverse cationic flotation, include its relatively lower sensitivity to the presence of slimes and the lower reagent cost for the fatty acid collectors, which are the main components of waste from the paper industry. This was demonstrated in an investigation by the U.S. Bureau of Mines and has been generally accepted in the literature thereafter.

In recent years, reverse anionic flotation has been successfully applied in China's iron ore industry and achieved excellent results. In 1998, three reverse anionic flotation circuits were built in China's major iron ore area, Anshan. In 2003, these circuits processed 6.77 million tons of iron ore, with a feed grade of 29.9% Fe, a concentrate grade of 67.5% Fe, a tailings grade of 8.31% Fe, and a Fe recovery of 82.1%.

A significant advantage of reverse anionic flotation circuits is the omission of desliming prior to flotation attributed the higher tolerance to slimes using reverse anionic flotation to the increased surface charges on the oxides at higher pH (pH 11–12). The electrostatic repulsive force between mineral particles and slime coatings is so strong at this pH level that desliming is no longer necessary.

It is noteworthy to point out that in Anshan, China, magnetic separation is often used in combination with reverse anionic/cationic flotation to upgrade low-grade iron ore from ~29% Fe to ~50% Fe before flotation. Such a configuration has the advantage of removing some of the slimes in the magnetic separation stage. For the reverse cationic flotation circuits once popular in this area, the desliming effect of magnetic separation was not adequate and typically produced concentrate of 65.22% Fe grade at a recovery of 78.42% from a feed of 29.16% Fe. The iron ore in USA and Anshan are both of Lake Superior type. The desliming effect of magnetic separation prior to reverse cationic flotation was also found to be insufficient at Groveland Mine, Michigan, United States. The superior performance of reverse anionic flotation circuits in Anshan demonstrated their better tolerance toward slimes.

Column Flotation

The application of column flotation cells in the mineral processing industry has gone from virtually zero in 1983 to wide acceptance in 1990. The major operating benefits of column flotation cells comparing to mechanical flotation cells is the lack of agitation in column flotation which results in energy and maintenance cost savings. The practice of froth washing in direct flotation increases concentrate grades without significant recovery losses. In the reverse flotation of iron ores, froth washing was found effective in reducing the loss of fine iron oxide particles to froth. It was reported that the cost of installing a column flotation circuit is approximately 25–40% less than an equivalent flotation circuit of mechanical flotation cells.

Industrial application of column flotation cells in iron ore processing has been reported in several Brazilian operations and Sydvaranger mine in Norway. Samarco is the first Brazilian producer to use column cells to increase its flotation capacity as part of a plant expansion program. Other companies using column cells at present

include CVRD (Companhia Vale do Rio Doce), CSN (Companhia Siderúrgica Nacional), and MBR (Minerações Brasileiras Reunidas).

However, negative reports on the use of column cells were also found in the literature. According to Dobby, there were several failures in the application of column cells in the iron ore industry. At CVRD's Samitri (Alegria) concentrator in Brazil, after three-column flotation stages, i.e., rougher, cleaner, and recleaner, a secondary circuit of mechanical cells was still installed to produce the final concentrate. Despite the advantages of column flotation reported in the literature, the use of column cells in iron ore flotation has mainly been reported in South America. In a typical mill in North America, the iron ore flotation section consists of mechanical flotation cells arranged in rows.

4.3 Magnetic Separation

Magnetic separation is a simple, yet effective method of iron ore beneficiation that exploit the difference in magnetic properties between the ore minerals in which magnetically susceptible materials are separated from a mixture using a magnetic force. Magnetic separation methods are mainly used to separate magnetic mineral such as magnetite, in some application hematite, from nonmagnetic gangue such as quartz. Magnetic separators can be classified into dry-feed and wet-feed separators. When the mineral is magnetite, low-intensity (500–1200 gauss) separation is normally practiced because it is low cost and effective. If the particles are of comparatively large size, greater than 6 mm, dry magnetic separations are used. When the particles are less than 100 micron, wet magnetic separation is used. If the size of the ore is intermediate, it is possible to use either method. High-intensity (1200–22,000 gauss) separators can be used to separate weakly magnetic materials, such as hematite and hydrated hematite, from gangue materials. This process is suitable for use with dry ores. Wet high-intensity magnetic separation method has been widely used in the beneficiation of low-grade iron ores containing hematite, which frequently replaces flotation methods.

Depending on their operation principles and intensity of magnetic field, common magnetic separators are wet high-intensity magnetic separators (WHIMS), wet/dry low-intensity magnetic drums (LIMS), induced roll magnetic separators (IRM), lift roll magnetic separators, cross belt magnetic separators, disk magnetic separators, rare-earth magnetic drums (RED), and rare-earth magnetic rolls (RER).

Wet High-Intensity Magnetic Separators (WHIMS)

Wet high-intensity magnetic separators are commonly used to recover/reject ilmenite from HMC ahead of dry separation. The ilmenite generally needs to be of a low TiO_2 content (around 50%), so the magnetic susceptibility is high. Typically, the unit consists of a rotating carousel, which has vertically inclined salient plates
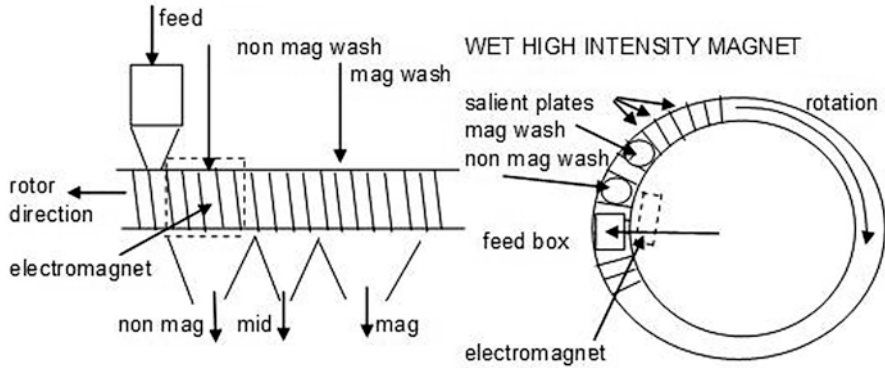
Fig. 10 Principle of wet intensity magnetic separator

through which feed slurry is passed. As the carousel rotates, it passes through fields of magnetic influence generated by surrounding electromagnets, followed by fields of no magnetic influence. The magnetic grains are initially held up in the plates, while the nonmagnetic grains are washed through into a launder below. When the plates are in the nonmagnetic field, the magnetic grains are then wash off into a separate launder below (Fig. 10).

Other type of WHIMS works on similar principles but uses a matrix that can consist of wire wool, steel balls, and expanded metal.

Wet/Dry Low-Intensity Magnetic Drums (LIMS)

Low-intensity magnetic drums are commonly used to recover/reject magnetite from HMC ahead of high-intensity wet or dry magnetic separation.

For the wet LIMS, there are two models, counter-current and cocurrent flow. All LIMS consists of a rotating drum within which a fixed permanent magnet is located. Feed in the form of a slurry flowing through a bath is presented to the submerged part of the drum and depending on the magnetic susceptibility of the grains that is either attracted by the magnetic field or held to the drum surface or is unaffected and discharges from the bath with the slurry. As the drum rotates, the magnetic grains leave the magnet field and are discharged separately usually with the aid of a scraper/brush (and spray water for the wet LIMS).

The level of the slurry bath and magnet position is the variables used in the wet LIMS. As the names suggest, the counter and cocurrent wet LIMS remove the products at different points in relation to the direction of the feed flow. The latter is more suited to cleaning duties (Fig. 11).

For the dry LIMS, feed is distributed across the drum at the top, and as the drum rotates enters the magnetic field of a permanent magnet, with the nonmagnetic grains being unaffected and thrown off by centrifugal force, while the magnetic grains are held to the drum surface until they leave the magnetic field and either fall or are scraped off separately (Fig. 12).
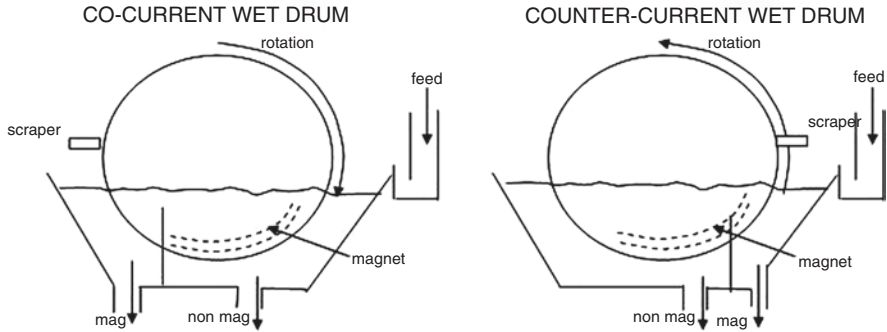
Fig. 11 Principle of wet low-intensity magnetic drum separator
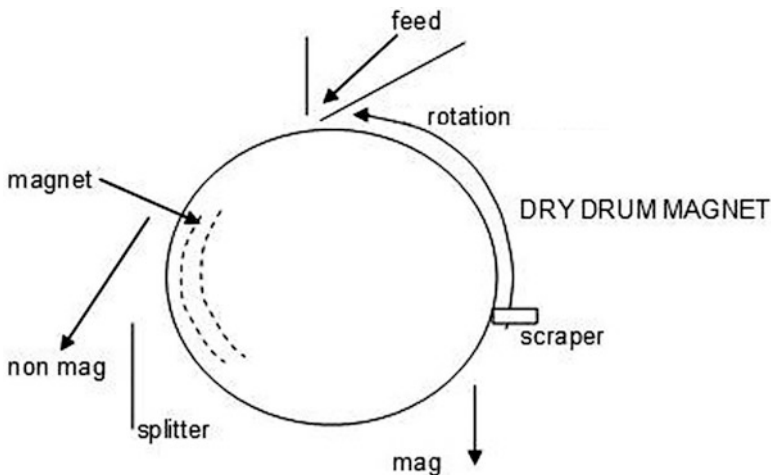
Fig. 12 Principle of dry magnetic drum separator

Induced Roll Magnetic Separators (IRM)

The induced roll magnetic separators are commonly used to separate ilmenite from less magnetic and nonmagnetic in dry mills. The IRM consists of an electromagnet which induces a magnetic field onto a serrated rotating roll via a cast iron pole located a short distance away. Dry feed is distributed across and onto the surface of the roll at the top, where depending on the magnetic susceptibility of the grains is either held to the roll or discharges off due to the centrifugal motion of the roll. The machine has two parallel feed points, each with two rolls which are mounted vertically above each other, with the lower roll operating as a nonmagnetic cleaner (Fig. 13).

INDUCED ROLL MAGNET
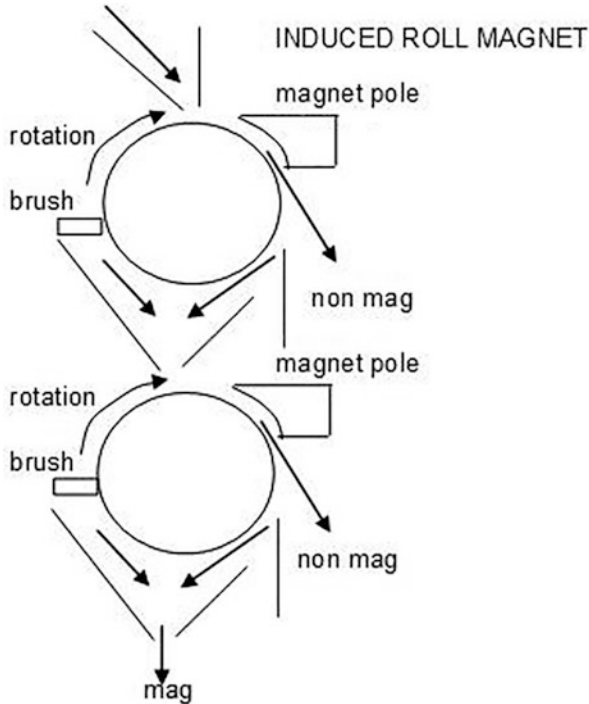
Fig. 13 Principle of induced roll magnetic separator

Lift Roll Magnetic Separators

The life roll magnetic separators reverse the effect of grain size (coarse magnetic grains with nonmagnetic grains) due to the centrifugal action of the IRM, as in this case the magnetic grains are lifted onto a rotating roll from a moving feed prior to separate discharge from the nonmagnetic grains which are unaffected (Fig. 14).

Cross Belt Magnetic Separators

The cross belt magnetic separator consists of a single belt upon which the feed is distributed across and is transported slowly underneath a series of five electromagnets, the magnetic fields of which lift the magnetic grains off the belt depending on their susceptibility. Underneath each magnet is a smaller faster moving belt across the main belt at right angles which picks up the magnetic grains and transports them away from the magnetic field to discharge into launders. The gap between the main belt and the magnets becomes progressively smaller until only the nonmagnetic grains unaffected by the magnetic fields remain on the main belt and discharge off the end (Fig. 15).
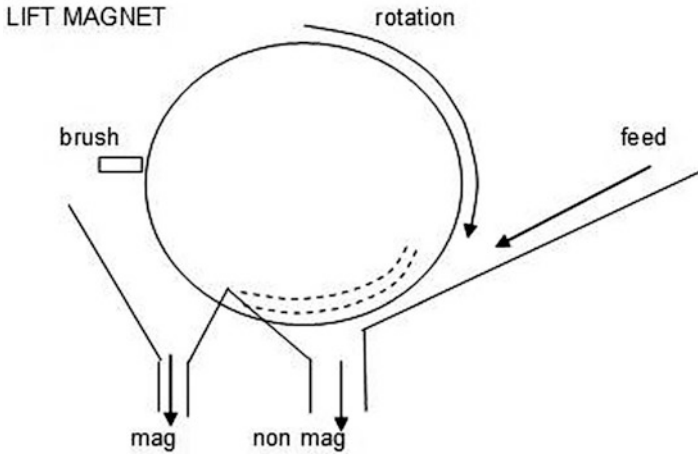
Fig. 14 Principle of lift roll magnetic separator
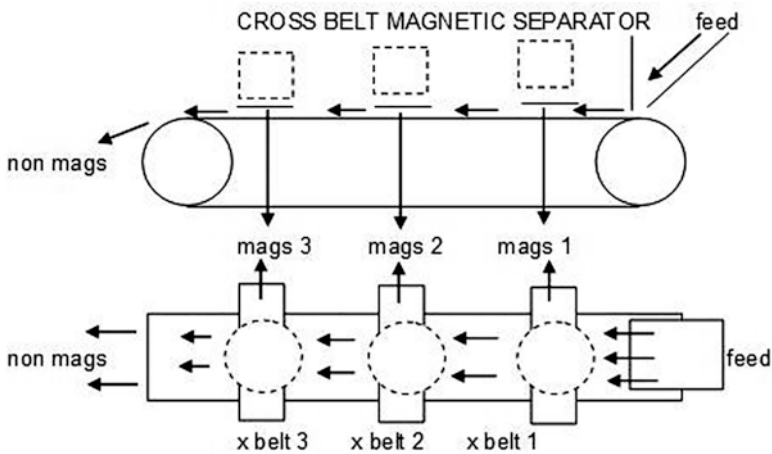
Fig. 15 Principle of cross belt magnetic separator

Disk Magnetic Separators

These dry separators are similar to cross belts, except that instead of using belts to transport magnetic material they employ rotating disks. Operating variables are similar. The disk magnetic separator is particularly suitable for final cleaning.

Rare-Earth Magnetic Drums (RED)

Examples of these separators are the Eriez RED manufactured by Eriez. They are similar to the dry LIMS except that they employ an exotic permanent magnet capable of producing much higher-intensity magnetic field. This magnet is positioned inside a large diameter drum (Fig. 16).
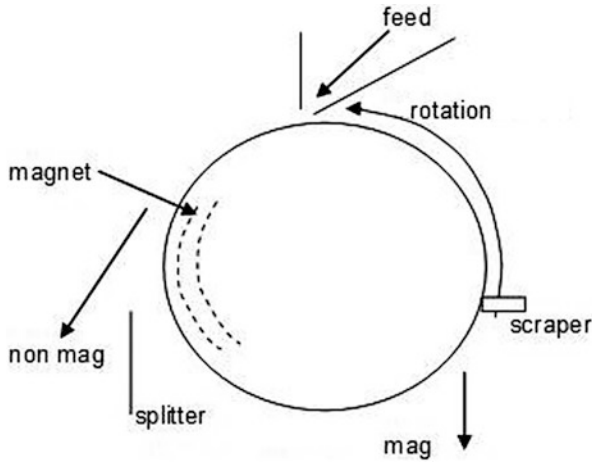
Fig. 16 Principle of rare-earth drum magnetic separator
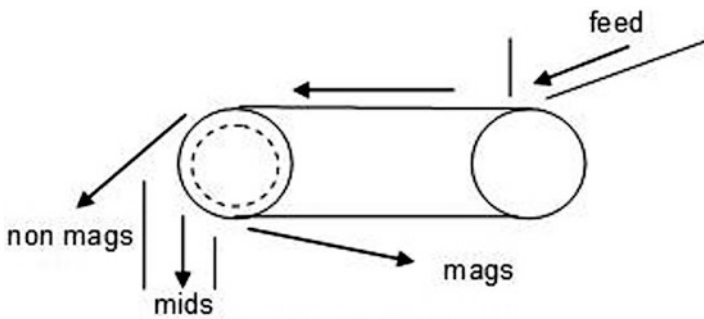
Fig. 17 Principle of raw earth magnetic rolls

Rare-Earth Magnetic Rolls (RER)

Rare-earth magnetic roll separator differs from REDs in that they have two smaller diameter rolls between and around which a belt tracks. Feed material is distributed onto the belt which transports it to the end roll which contains an exotic permanent magnet similar to the RED. The rolls and belt move at high speed, and the trajectory of the grains discharging from the belt depends on their magnetic susceptibility and operating variables such as belt speed, belt thickness, and splitter position (Fig. 17).

5   Agglomeration of Iron Ores

Iron ore agglomerations, i.e., briquettes, sinter, or pellets, are not the final products. They are formed from such fine-grained iron ores which, in this physical shape, cannot be utilized and served as an intermediate product on the way from the ore mine

to the blast furnace or direct reduction plant. The sole purpose of agglomerate production is to keep the cost price of pig iron or steel at the lowest level. For many years until about the turn of the century, the iron ores charged to blast furnaces had been crushed and partly classified either at the mine or at the iron and steel works. In this case, lump ores were preferred although small portions of fine ores could be tolerated.

As a result, the fines which were not utilized formed continuously growing dumps with no economic use. They could only be employed to a limited extent in the blast furnace since they decreased the gas permeability of the blast furnace operation.

Moreover, a great part of these fines was blown out of the blast furnace and had to be recovered as flue dust. These dust quantities represented a considerable iron value which, like the unused fine ore dumps, was lost; this was of lesser importance in countries with great iron reserves than in those with small iron reserves. The amount of the accumulating dust depends largely on the ore type treated. In the case of Minette or other ores with a high loss of ignition, it is substantially greater than in the case of high-grade, dense ores with a small loss on ignition.

Possibilities were examined, and tests to agglomerate the flue dust by sintering or briquetting and to recycle it to the blast furnace were started at approximately the turn of the century in various industrialized countries although with differing intensities. Countries with important iron reserves were less interested in this agglomeration. They considered sintering as a "necessary evil." The situation was quite different in countries with small ore reserves. Here, the development of the sinter process continued intensively, and not only flue dust but also other iron-bearing secondary raw materials such as mill scale or red mud were of great interest. The sintering of fines, obtained during the crushing and screening of unclassified lump ores, was also gaining significance.

At about the same time, some researchers were looking for an alternative process to sintering, especially in areas in which very fine ores or concentrates were available. This was the beginning of the pelletizing process.

5.1   Sintering

Iron ore sintering plants are associated with the manufacture of iron and steel, often in integrated steel mills. The sintering process is a pretreatment step in the production of iron, where fine particles of iron ores and in some plants, also secondary iron oxide wastes (collected dusts, mill scale), are agglomerated by combustion. Agglomeration of the fines is necessary to enable the passage of hot gases during the subsequent blast furnace operation.

Sintering involves the heating of fine iron ore with flux and coke fines or coal to produce a semi-molten mass that solidifies into porous pieces of sinter with the size and strength characteristics necessary for feeding into the blast furnace. Moistened feed is delivered as a layer onto a continuously moving grate or "strand." The

surface is ignited with gas burners at the start of the strand, and air is drawn through the moving bed causing the fuel to burn. Strand velocity and gas flow are controlled to ensure that "burn through" (i.e., the point at which the burning fuel layer reaches the base of the strand) occurs just prior to the sinter being discharged. The solidified sinter is then broken into pieces in a crusher and is air-cooled. Product outside the required size range is screened out, oversize material is recrushed, and undersize material is recycled back to the process. Sinter plants that are located in a steel plant recycle iron ore fines from the raw material storage and handling operations and from waste iron oxides from steel plant operations and environmental control systems. Iron ore may also be processed in on-site sinter plants.

The flexibility of the sintering process permits conversion of a variety of materials, including iron ore fines, captured dusts, ore concentrates, and other iron-bearing materials of small particle size (e.g., mill scale) into a clinker-like agglomerate.

Waste gases are usually treated for dust removal in a cyclone, electrostatic precipitator, wet scrubber, or fabric filter. Figures 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, and 18 provides a schematic for a typical iron ore sintering plant which uses an electrostatic precipitator for dust control.

Generally, every sinter plant comprises the raw materials storage bins with metering devices to proportion the components of the sinter mix; a mixer and/or a micropelletizer; a sintering strand; including a feeding arrangement and discharge through a breaker or crusher; sinter cooler; screens to size the sinter for blast furnace feed; hearth layer and return fines; facilities to clean the exhaust gaseous prior to exhausting them to atmosphere; and numerous conveyors to move materials.
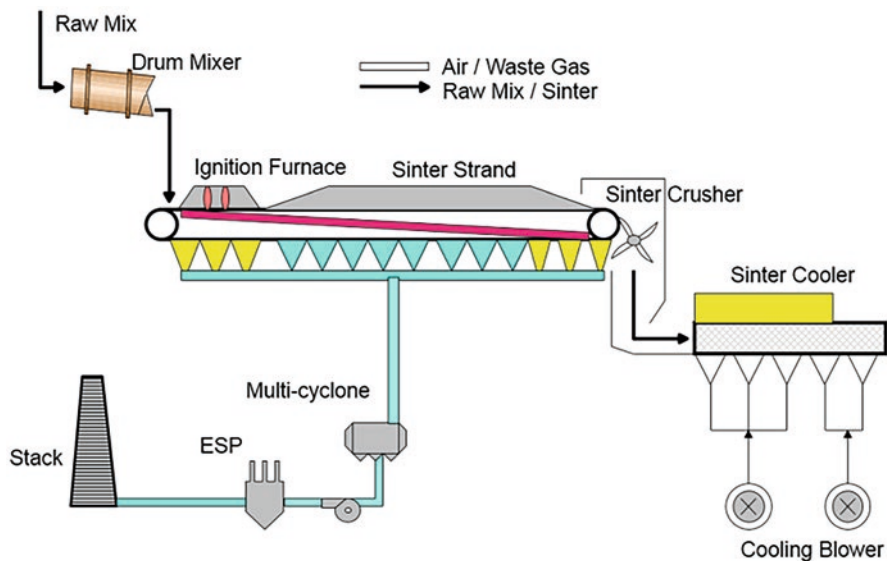
Fig. 18 Typical process flow sheet of an iron ore sinter plant

Reclaimed ores from the ore storage yard are conveyed to the sinter ore bins. Flux and solid fuels (coke breeze and/or flue dust) as well as hot and cold return fines are also transferred to the appropriate bins. To ensure a continuous flow of materials from the bins to the table feeder or belt weigh feeders, the bins are sometimes equipped with a vibrating bin bottom. The bin discharge feeders proportion the ores, flux, and coke breeze onto the main collecting conveyor at desired, controlled rates.

The moisture of the coke breeze is monitored continuously, and coke is added on a dry basis as a ratio of the raw feed. Return fines are metered to the mix as a fixed ratio to the weight of the raw feed. The ratio of return fines to raw mix is normally varied to maintain a desired level in the return fines bin; i.e., they are consumed at the same rate at which they are produced.

The total sinter mix is conveyed to the mixing device such as a mixing drum for conditioning. Usually, water is added into the drum, where lifters are installed for thorough blending of the raw materials and water. The final moisture addition is made in the second half of the drum or in the granulation drum, where the mix is rolled to promote micropelletizing of the fines to develop optimum mix permeability. A downstream moisture gauge is usually installed to provide control of the final mix moisture.

Approximately 1 inch of hearth layer, screened from product sinter and sized to between 10 and 40 mm, is laid down on the grate of the sintering machine. The hearth layer protects the grate bars from excessive heat, reduces the amount of fines drawn through the grate bars, and helps to prevent "stickers," i.e., molten sinter adhering to the grate bars. The hearth layer bin is usually mounted on lead cells to assure an adequate supply of hearth layer in the bin.

Sinter mix is conveyed to a roll feeder hopper from where it is deposited on top of the hearth layer by the roll feeder. The roll feeder hopper is mounted on load cells to control the volume of mix in the hopper. The roll feeder speed is ratioed to machine speed to maintain a desired amount of mix behind the cutoff plate.

As sinter mix passes beneath the cutoff plate, the surface of the mix is prepared (slightly compressed and leveled) prior to ignition. Mix level upstream of the cutoff plate is critical, since bed compaction and loss of mix permeability can result from too much mix being piled behind the plate. Conversely, insufficient mix will result in an uneven bed for ignition, and, consequently, the top of the sinter bed will be of poor quality.

The sinter mix on the traveling grate passes under the ignition hood. Temperatures in the ignition hood are controlled to prevent the sinter surface from slagging which restricts airflow. In some installations, the latter section of the ignition furnace is used as an annealing zone. That is, firing temperatures are moderated to anneal the sinter surface to prevent weakening of the sinter when it is contacted by cold air after exiting the ignition hood.

Sintering continues as the strand moves from the ignition zone over the windboxes. Completion of sintering, determined by peak windbox temperature, is desired to occur in the first section of the last windbox. Burn-through control (control of strand speed) prevents unsintered material from being discharged and maximizes sinter production.

Sinter is then discharged from the grate to a crashing deck and then is reduced in size by a revolving toothed breaker to a manageable size range (6–8″ top size). At many sinter plants, the hot sinter goes to a hot sinter screen for scalping at certain size (1/4″ or 5 mm, typically). The undersized fines generated during crushing and screening are discharged to a pan conveyor for transfer to the return fine bin. The oversized sinter is fed to the sinter cooler, the speed of which is controlled to maintain a full bed of sinter. Cooled sinter is discharged into a surge hopper for transfer to the cold screening station.

Most modern sinter plants also set a top size for their product sinter. In this case, a scalping screen separates the oversize product for crushing. The crushed sinter rejoins the undersize product from oversize scalping screen for screening into one or two sizes of sinter product and to extract hearth layer sinter as required for the process. Minus ¼″ or 5 mm fines are sent to the return fine bin by conveyor. The final sinter product is conveyed to the blast furnace stockhouse. An automatic sampler cuts representative samples for physical testing and chemical analysis.

5.2   Pelletizing

Originally, the pelletizing process was developed in the United States to treat the ultra-fine mineral dressing products obtained from the upgrading of Mesabi ore. In fact, depletion of higher-grade iron ore reserves in North America promoted the development of low-grade "taconite" ores, which require very fine grinding for beneficiation. These fine concentrates were not amenable to conventional sintering and led to development of the pelletizing process. The decline in hot metal production in the 1980s, along with excess modern pellet plant capacity and environmental issues, caused a reduction in sintering capacity. The subsequent development of fluxed pellets and improvement of acid pellet properties have made pellets the prime feed material in North America and parts of Europe and a valuable supplementary feed material elsewhere.

The pelletizing process is essentially based on the formation of green balls by rolling a finely ground ore or concentrate to which bentonite is usually added together with a critical amount of water. These balls are then dried, preheated and fired, all under oxidizing conditions, to a temperature of 2280–2460 °F (1250–1350 °C). As a result, oxide bridging, grain growth, and some slag bonding occur, and pellet strength are developed. The pellets are then cooled in air and the sensible heat recovered in the form of hot air used as process air in the previous heating operations. The process thus produces pellets in a highly oxidized state, as opposed to the sintering process in which solid fuel is added to the raw mix and results in a ferrous iron content from 5 to 15 weight percent in the product sinter.

Figure 19 illustrates a typical traveling grate pelletizing plant. Basic steps involved in the pelletizing process are,

1. Feed preparation
2. Green ball production
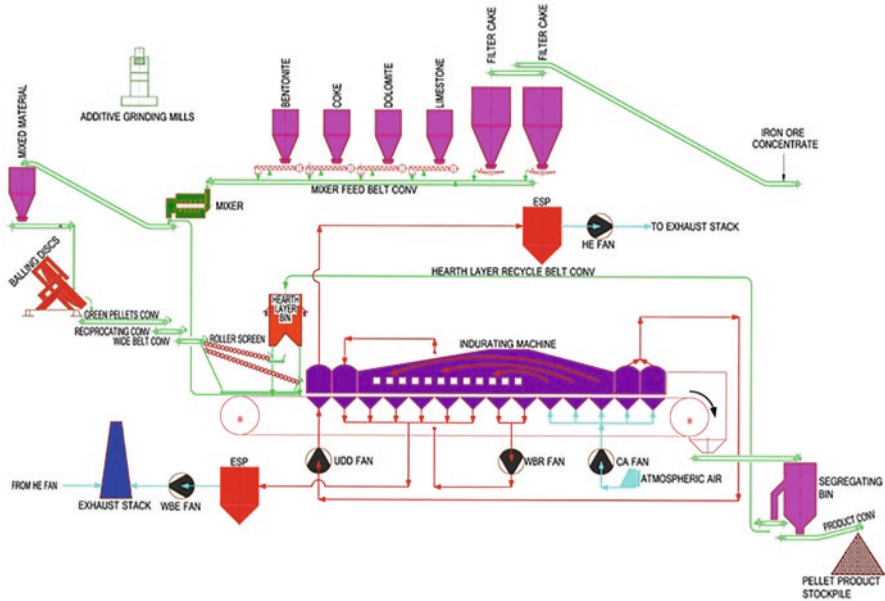
Fig. 19 Typical iron ore pelletizing plant with traveling grate

3. Green ball induration (drying stage, preheating stage, and firing stage)
4. Pellet cooling

East of these steps can be modified to suit both the equipment employed and the ore being processed.

Successful pellet production needs an optimum efficiency and harmony among all four steps with the preceding stage highly influencing the subsequent one. An error made in the preceding stage can only be corrected to a limited extent in the subsequent process stages. Even during induration, no first-class pellet can be produced from a defective green ball. The purpose of the green ball formation is to obtain balls of the desired size range and have a mechanical strength which enables them to be safely transported from the balling equipment to the induration furnaces.

Feed Preparation

The location of a pelletizing plant affects the method of receiving raw materials such as iron ore, additives, and binders. Many pelletizing plants are located near ore mines. This is because these plants were developed to pelletize the raw materials that are beneficiated at these mines. Such plants receive the raw materials via railways and/or slurry pipelines. Other pelletizing plants exist at a distance from and independent of ore mines. In such cases, the receiving method involves the

transportation of the ore in a dedicated ship, unloading the ore at a quay and stock-piling it in a yard. Iron ore must be shipped in bulk for maximum economy.

In the preparation of pelletizing process, the iron ore is first ground into fines having qualities required for the subsequent balling process. The pretreatment includes concentrating, dewatering, grinding, drying, and prewetting.

In general, low-grade iron ore is ground into fines to upgrade the quality of the iron ore, remove gangues containing sulfur and phosphorus, and control the size of the grains. In the case of magnetite, a magnetic separator is employed for upgrading and gangue removal. With hematite, on the other hand, these operations are accomplished by gravity beneficiation, flotation, and/or a wet-type, high-intensity magnetic separator.

The grinding methods are roughly categorized as to the following three aspects:

1. Wet grinding–dry grinding
2. Open-circuit grinding–closed-circuit grinding
3. Single-stage grinding–multiple-stage grinding

These methods are used in combination depending on the types and characteristics of the ore and the mixing ratio, taking into account the economic feasibility. A wet grinding system accompanies a dewatering unit with a thickener and filter, while a dry grinding system requires a prewetting unit. Drying is usually provided in association with dry grinding. Prewetting includes adding an adequate amount of water homogeneously into the dry ground material to prepare prewetted material suitable for balling. This is a process for adjusting the characteristics of the material that significantly affect pellet quality. Occasionally, the chemical composition of the product pellets is also adjusted in this process to produce high-quality pellets. A typical binder is bentonite or organic binder. Adding lime and/or dolomite to the ore adjusts the pellets so as to have the target chemical composition.

Green Ball Production

In this process, balling equipment produces green balls from the prewetted material prepared in the previous process. The green balls are produced either by a balling drum (Fig. 20) or by a balling disk (Fig. 21). Both of the units utilize centrifugal force to form the fine materials into spheroids. The green balls produced by a drum are not uniform in diameter. A significant portion of the discharge (about 70%) is smaller than target size and must be returned to the drum after screening. It is difficult to adjust the drum operation for varying raw material conditions. The operation, however, is stable for uniform raw material conditions (chemical composition, particle size, moisture, etc.). A balling disk, on the other hand, classifies green balls by itself, reducing the amount of pellets returned. The disk operation can easily be adjusted for varying raw material conditions by changing the revolution, inclined angle and depth of the pan.
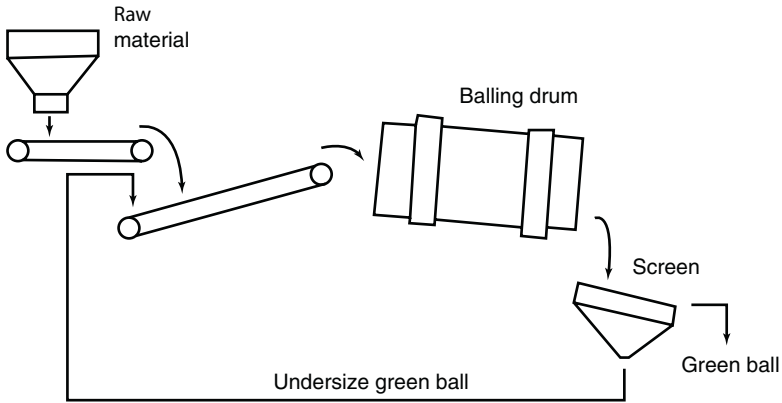
Fig. 20 Balling drum process for green ball production
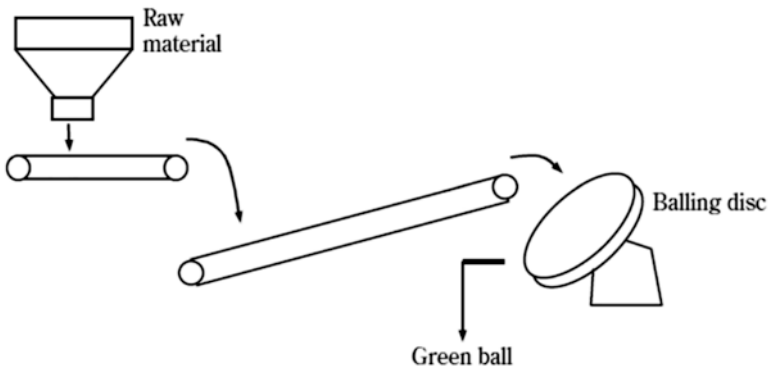
Fig. 21 Balling disk process for green ball production

Induration of Green Balls

Green balls leaving the balling plant are transported to the induration plant where the balls are subjected to drying, preheating, firing, and cooling. The firing of pellets establishes the binding of hematite particles at an elevated temperature ranging from 1250 to 1350 °C in oxidizing condition. Slag with a low melting point may form in the pellets during this firing step, if the raw material contains fluxed gangue, or if limestone is added to it. In these cases, the product may have an intermediate structure with both hematite binding and slag binding. The firing process is characterized by process temperatures lower than those required by sintering which requires partially melting and sintering fine ore mixed with coke breeze, a fuel which generates combustion heat.

In contrast to sintering, for which the downdraft sintering method is employed, pellets are today indurated according to three methods: shaft furnace system,
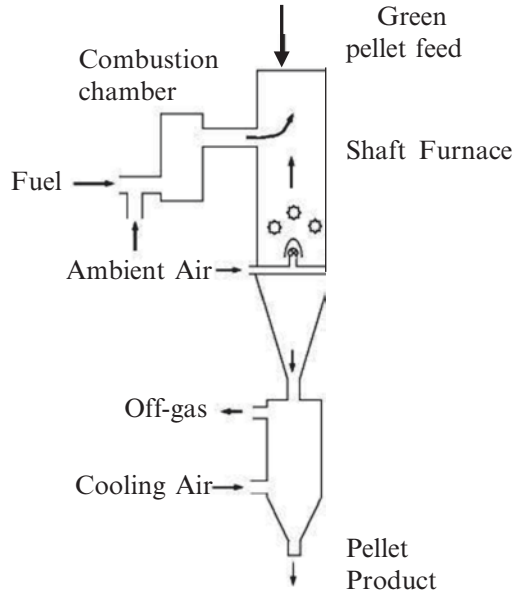
traveling grate system, and grate-kiln-cooler system. Shaft furnaces are the most
traditional facilities as shown in Fig. 22; however, few plants use this system these
days because of their limited scale. A traveling grate system emerged in the industry
soon after the shaft furnaces. It consists of a single unit which moves a static layer
of pellets. The system has a simple structure for drying, preheating, firing, and cool-
ing pellets as shown in Fig. 23. Due to its relative ease of operation and ease of
scaling-up, the traveling grate system is the most common pellet induration process.
A grate-kiln-cooler system consists mainly of a grate, a kiln, and a cooler, respec-
tively, designed for drying/preheating, firing, and cooling the pellets as shown in
Fig. 24. The system is easy to control, and the product pellets have a consistent
quality. It can also be scaled up to a fairly large degree, and these systems are used
by many plants along with straight grate systems.

Table 4 summarizes taconite pellet induration processes and production in United
States. The data show that grate-kiln and traveling grate are the main pellet indura-
tion processes, although each plant has varied ore chemical composition and has
individual operational practices.

Extensive comparisons of the shaft furnace, grate-kiln, and traveling grate indu-
ration have been made by Dor et al. (1970), Ilmoni et al. (1970), and Stjernberg
et al. (2015). Comparison of the time–temperature curves of the three processes
shows that indurating time for the shaft furnace is very long, whereas that for the
traveling grate is very short, and the grate-kiln is intermediate. The curves for the
shaft furnace and the traveling grate refer to the optimum treatment the pellets
receive. However, because of uneven gas flow, some pellets receive an inferior indu-
rating cycle during the shaft furnace and traveling grate processes. This is not the
case with the grate-kiln since within the rotary kiln all pellets receive the same
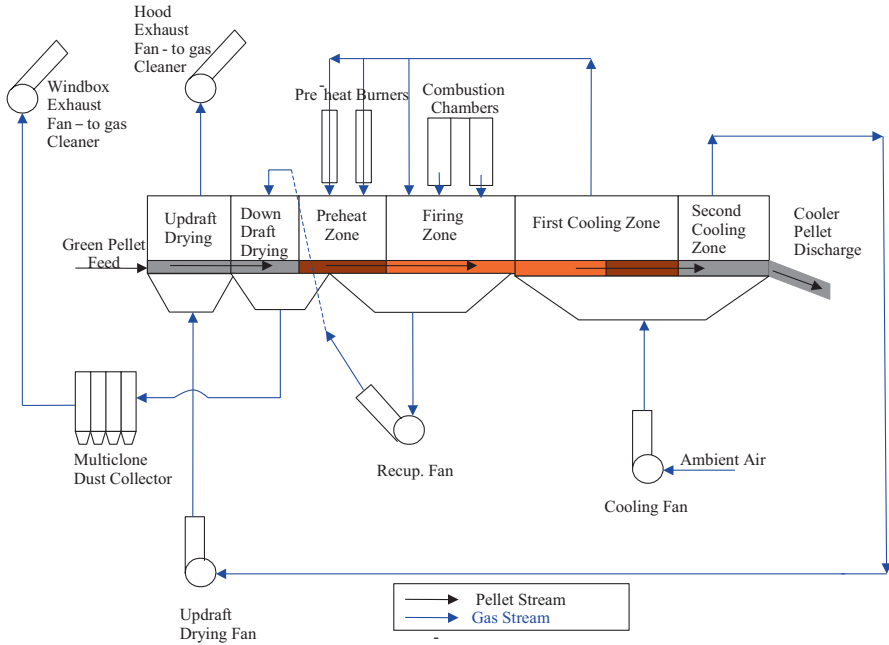
Fig. 23 Typical traveling grate system for pellet induration
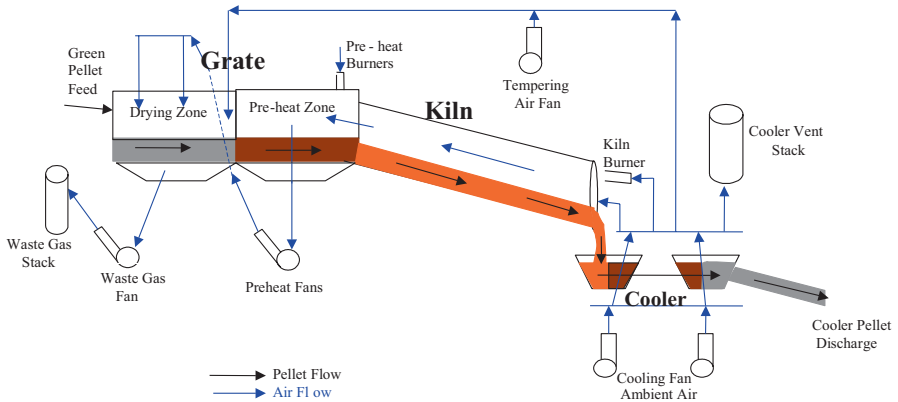
Fig. 24 Typical grate-kiln system for pellet induration

treatment. This enables all pellets to be uniformly and adequately heat-hardened by tumbling action and be held at the peak temperature for longer period than that of shaft furnace and traveling grate.

The fuel consumption for the three processes was also compared by Ilmoni et al. (1970) and Yamaguchi et al. (2010). The shaft furnace has the lowest fuel consumption among all three processes because of its greater efficiency of counter-current

Table 4 Taconite processing facilities on Minnesota's iron range

Plant	Lines	Furnace type	Pellet production
US Steel, Keewatin Taconite	1	Grate-kiln	Standard
Hibbing Taconite	3	Traveling grate	Standard
US Steel, Minntac	5	Grate-kiln	Standard/fluxed
United Taconite	2	Grate-kiln	Standard
Mittal Steel	1	Traveling grate	Fluxed
Northshore Mining Co.	3	Traveling grate	Standard

heat exchange. It would be even lower if the pellets were cooled further in the shaft furnace. Grate-kiln process consumes less fuel and power than traveling grate process mainly because of two reasons, i.e., more efficient heat transfer, and lower pressure drop across the pellet bed due to the fact that it has lower height of pellet bed and it does not require hearth layer and side layer.

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Microorganisms and Bioprocessing, General

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1 Introduction

Microorganisms play an important role on nutritional chains that are an important part of the earth's biological balance. Adapting several abilities, microorganisms have become an important influence on the ecological systems, making them necessary for superior organisms and their life in this planet. Microorganisms were among the first tools used for the discovery of biologically active compounds. Their utility reached a zenith during the era of antibiotic development in the 1950s and 1960s and then declined. Subsequently, a substantial role for microorganisms in the mining industry developed with the realization of bioleaching of nonferrous metals (Dresher, 2004; Manchee, 1977). Nowadays, bioprocessing is an essential part of many foods and chemical, pharmaceutical, and mining industries.

Ability of microorganisms to transform many types of natural resources such as iron sulfides and iron oxides has been widely recognized during the last several decades. Microorganisms survive in extreme environments because they are metabolically capable of utilizing its resources and can occupy a suitable niche. Metal elements are often potential nutrients or energy sources for microorganisms. Due to their ability to attach to mineral surfaces and interact with iron ore minerals, potential application of microbial-assisted iron ore beneficiation methods has been reported. Currently, microorganisms are widely applied in the leaching of mineral sulfides and the remediation of mineral processing waste and industry-contaminated environments (Johnson & Hallberg, 2005). With the increasing demand for minerals and the depletion of high-grade mineral deposits, mineral research is increasingly focusing on the beneficiation of low-grade ores to produce material suitable for a global market. Due to resource and economic considerations, the industry's focus on reducing the environmental impact of mining and mineral processing has led to significant advances in the application of biotechnology in mineral processing. Emerging from this is the relatively recent technology of bioflotation and bioflocculation, in which bacteria may be used as flotation reagents, collectors, or

modifiers enabling the selective separation of minerals (Deo & Natarajan, 1998; Dwyer et al., 2012; Misra et al., 1993; Sarvamangala et al., 2012; Sharma & Hanumantha, 1999). Essentially, the role of the bacteria is to effect changes in the surface chemistry of the minerals to achieve efficient separation.

This may be of particular importance to the iron ore mining and steelmaking industries, where an increasing global demand for steel and depleting resources of high-grade iron ores mean that lower-grade deposits will soon need to be mined to meet the market demand. Several conventional beneficiation processes, including flotation and flocculation, have been successfully integrated into a number of iron ore processing operations in the USA and Brazil (Houot, 1983). Often, the efficiency of these conventional treatment processes is highly dependent on the ore composition and nature, meaning that one particular process may not necessarily be universally applied to all mineral deposits (Groudev, 1987). In the future, as high-grade reserves are depleted, the iron ore industry will need to embrace cost-effective, environmentally benign treatment processes for the beneficiation of these lower-grade ores. A biological approach may be one such option.

2   Classification of Microorganisms

All living species are classified according to a hierarchical system which groups like organisms into different levels (called ranks or taxa). The highest taxonomic rank is domain. Organisms can be divided into three main domains: archaea, bacteria, and eukaryotes based on a biological classification introduced by Woese & Fox in 1977. The first two domains consist of microbes and are all prokaryotes that lack a cell nucleus. The third domain, eukaryotes, consists of both microbes and larger multicellular organisms, such as plants and animals. Eukaryotes have a cell nucleus, a membrane that envelopes the cell's genetic material. Initially, scientists used physical features to group similar organisms. Today, species of organisms are arranged according to evolutionary relationships. While this is based partially upon physical features, scientists also use fossil and genetic evidence. Microbes fall into five categories: Archaea, bacteria, fungi, protists, viruses, and prions. The last two types are not actually living cells. They are included with the microbes because they can only be viewed with a microscope and have the ability to replicate themselves (Fig. 1).

The second highest rank and the largest group are kingdom. There are two commonly known classification systems, six-kingdom system (mainly used in the USA) and five-kingdom system (mainly used in Great Britain, India, Australia, Latin America, and other countries). It should be noted that some recent classifications based on modern cladistics have explicitly abandoned the term "kingdom" mainly because the traditional kingdoms are not monophyletic.

The six-kingdom system consists of Protista (the single-celled eukaryotes), Fungi (fungus and related organisms), Plantae (the plants), Animalia (the animals), Archaea/Archaebacteria, and Bacteria/Eubacteria (prokaryotic, unicellular). Each kingdom is further divided into smaller groups called phyla, based on a few features

Bacteria
- Mitochondrion
- Cyanobacteria
- Gram-negative bacteria
- Gram-positive bacteria
- Chloroplast
- Thermotoga

Archaea
- Euryarchaeota
- Methanogens
- Methanosarcina
- Halophiles
- Methanobacterium
- T. celer

- Crenarchaeota
- Thermoproteus
- Pyrodictium

Eukarya
- Archaezoa
- Microspora
- Plasmodial Slime molds
- Chromista
- Euglenozoa
- Amoeba
- Ciliates
- Plants
- Oomycotes
- Cellular Slime molds
- Fungi
- Animals

Fig. 1 Three-domain classification of microorganisms. (Adopted from Woese & Fox, 1977, Woese et al., 1990)
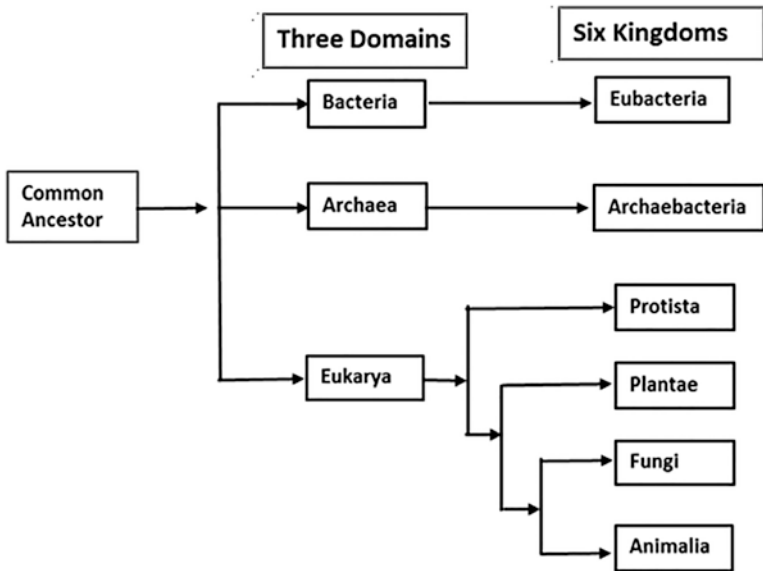
Fig. 2 Classification of domains and kingdoms

that are shared by some organism. A phylum is then subdivided into classes, orders, families, genera, and finally species (Fig. 2).

Microorganisms are a heterogeneous group of several distinct classes of living beings. Based on the difference in cellular organization and biochemistry, the kingdom Protista has been divided into two groups, namely prokaryotes and eukaryotes.

Bacteria and blue-green algae are prokaryotes, while fungi, other algae, slime molds, and protozoa are eukaryotes. Bacteria are prokaryotic microorganisms that do not contain chlorophyll. They are unicellular and do not show true branching, except in higher bacteria like *actinomycetales*.

The most industrially important microorganisms are traditionally bacteria and fungi. However, there is an increasing interest on the potential of archaea for industrial bioprocesses. The ability of archaea to perform in adverse conditions such as high temperatures (with advantageously high reaction rates) has generated increasing interest. Some archaea live optimally in environments considered the extreme limits of life. In fact, this has prompted many scientists to suggest that should life exist on other planets, these life forms would be similar to the extremophilic archaea. Existing examples of the use of archaea include *Sulfolobus*, acidothermophilic archaea which grow at an optimal temperature around 80 °C and a pH below 3, used for bioleaching of copper from sulfide ores (Lindstrom et al., 1993), and *Methanopyrus*, a methanogen which can survive at a temperature of 120 °C and has potential for both energy generation and CO_2 sequestration. Further, enzymes extracted from extremophilic archaea are particularly sought after and scientists travel to extreme environments to collect and harness these thermophilic, halophilic, and cryophilic treasures.

3   Microorganisms for Ferrous Metal Extraction

Iron is an essential nutrient for all known life forms, with the seeming exception of *Lactobacillus spp.* (Archibald, 1983). It is usually required only in trace amounts (i.e., it is a micronutrient), although in some exceptional cases such as the magnetotactic bacteria, cellular iron contents are up to 11.5-fold greater than in more "typical" bacteria (Chavadar & Bajekal, 2008).

Many types of microorganisms including autotrophic and heterotrophic archaea, bacteria, eukarya, fungi, and yeasts can act as reagents, collectors, or modifiers, to bring about beneficiation of iron ores. Table 1 summarizes the reported microorganism used in biobeneficiation; most of these microorganisms are present ubiquitously in many iron ore deposits. Generally, these microorganisms that catalyze the dissimilatory oxidation or reduction of iron ore can be subdivided into four main physiological groups: (i). acidophilic, aerobic; (ii). neutrophilic, aerobic; (iii). neutrophilic, anaerobic (nitrate-dependent); and (iv). anaerobic photosynthetic. Among these microorganisms, *Gallionella spp* and *leptothrix spp* are invariably associated with biogenic iron oxides at neutral pH in oxygen-rich zones. In acidic environments, *acidithiobacillus spp.* brings about ferrous iron oxidation. Iron-oxidizing archaea such as *Thermoplasmatales* could be identified in extreme acidic environments. Bioreagents such as exopolysaccharides and proteins are secreted by iron bacteria during the process of iron biogenesis and conversion. Microorganisms capable of producing polyphosphate granules, sulfur granules, and other intracellular and intercellular inorganic polymers have also been located in mining

Table 1 Summary of potential microorganisms for bioprocessing iron ore studied by various investigators

Domain	Organism	Nutrition type	Main Fe minerals	Main application	PH range	Reference
Archaea	*Sulfolobus acidocaldarius*	Chemolithoautotrophic	Pyrite	Bioleaching of iron ore	0.9–5.8	Acuna et al. (1992)
	Ferroplasma acidarmanus	Chemolithoautotrophic	Pyrite	Bioleaching of iron ore	0.35–2.20	Dopson et al. (2004)
	Thermoplasma acidophilum	Chemolithoautotrophic	Pyrite	Bioleaching of iron ore		Johnson (1998)
Bacteria	*Leptospirillum ferrooxidans*	Chemolithoautotrophic	Goethite and magnetite	Dephosphorization of iron ore	2.5–3.0	Chime (2013)
	Shewanella putrefaciens	Chemolithoautotrophic	Magnetite	Bioleaching of iron ore	1.4–6.0	Roberts et al. (2006)
	Bacillus subtilis	Neutrophilic	Hematite	Selective flotation of hematite, corundum, calcite, and quartz		Poorni and Natarajan (2013, 2014)
	Desulfovibrio desulfuricans	Chemolithoautotrophic	Hematite	Hematite and quartz separation		Sabari Prakasan & Natarajan, 2010
Eukarya	*Actinomucor sp.*	Heterotrophic	N/A	N/A		Muller (1964)
Fungi	*Aspergillus terreus*	Heterotrophic	Goethite and magnetite	Dephosphorization of iron ore		Anyakwo and Obot (2010)
	Aspergillus niger	Heterotrophic	Hematite and goethite	Dephosphorization of iron ore		Delvasto et al. (2005, 2007, 2008)
	Penicillium sp.	Heterotrophic	Hematite	Removal of potassium and phosphorus from iron ore		Adeleke and Damase (2010)
Yeasts	*Saccharomyces cerevisiae*	Heterotrophic	Hematite	Separation of quartz from hematite and calcite		Natarajan and Padukone (2012)
Algae	Not identified					
Protozoa	Not identified					
Amoebae	Not identified					

environments. Many iron bacteria exhibit magnetotaxis and are implicated in the biosynthesis of magnetite (Liu et al., 2006). An iron-reducing bacterium, such as *Shewanella putrefaciens,* is capable of production of intracellular particles of iron minerals (Roberts et al., 2006). With reference to banded iron formations, it is possible for iron-oxidizing bacteria to produce and precipitate iron-rich sediments on a large scale.

A list of microorganisms that have been reported to date to be dissimilatory iron reducers and iron oxidizers is given in Table 2. It should be noted that the majority of these microorganisms can utilize other electron acceptors than iron if they are available. In order to promote iron reduction or oxidation, it is therefore important to restrict the availability of alternative electron acceptors. It is important to note that the bacteria in the wild do not grow in isolation, but as part of a community of diverse organisms, some of which it may not be possible to culture in the laboratory.

Table 2 Typical iron-reducing and iron-oxidizing microorganisms

Iron-reducing	Iron-oxidizing	Iron-oxidizing/−reducing
Bacteria		
Acidiphilium (A.)	*Leptospirillum (L.)*	*Acidithiobacillus (At.)*
A. cryptum	*L. ferrooxidans*	*At. ferrooxidans*
A. acidophilum	*L. ferriphilum*	*At. ferrivorans*
A. angustum/rubrum	*L. ferrodiazotrophum*	*Acidiferrobacter*
A. organovorum	*Ferrovum myxofaciens*	*Thiooxydans*
A. multivorum	*Thiobacillus prosperous*	*Ferrimicrobium*
Acidocella (Ac.)		*acidiphilum*
Ac. Facilis		*Acidimicrobium*
Ac. Aromatic		*Ferrooxidans*
Acidobacterium		*Ferrithirix thermotolerans*
Acb. capsulatum		*Sulfobacillus (Sb.)*
Acidobacterium spp.		*Sb. acidophilus*
		Sb. thermosulfidooxidans
		Sb. benetaciens
		Alicyclobacillus (Alb.)
		Alb. tolerans
		Alb. terrooxydans
		Alb. aeris
		Alb. Pohliae
		Alicyclobacillus sp. GSM
Archaea		
	Sulfolobus (S.)	*Ferroplasma (Fp.) spp.*
	Metallosphaera	*Fp. acidiphilum*
	Sedula	*Fp. acidarmanus*
	S. metallicus	*Acidiplasma (Ap.)*
	S. tokodali	*Ap. Cupricumulans*
		Ap. aeolicum

The use of pure cultures of single organisms does not appear to be the most effective approach to solubilization of iron, as more rapid dissolution is generally observed by mixed cultures of sometimes unidentified organisms (Lovley, 1993; Coates et al., 1996). In the majority of cases, the bacteria community that promotes iron dissolution attaches to the mineral surfaces as a biofilm (Gadd, 2010) although the strength and the speed of attachment have been shown to vary greatly (Johnson & Clegg, 2010). Such biofilms are very different from cultures grown in the laboratory and require special procedures for manipulation and use in an industrial application.

In such communities, different microorganisms can each have different functions that, together, promote iron dissolution. For example, fermentative organisms can convert complex organic compounds to simpler short-chain organics that can be used as food or vitamins by the iron reducers, while other organisms can produce complexing agents for the reduced iron, and still others can liberate vital nutrients such as phosphorus from minerals. The organisms will even organize themselves spatially, with the iron reducers moving to sources of oxidized iron (Childers et al., 2002), while other organisms would move toward their respective nutrient sources. It would in any case be difficult to maintain pure cultures during leaching operations on an industrial scale, as it would be impractical to sterilize all incoming solutions. This is not necessarily an impediment to industrial use, as the existing biohydrometallurgical operations not only use natural bacterial mixtures, but also the microbial community changes markedly over time as the ore is leached (Brierley & Brierley, 2001).

3.1   Iron-Oxidizing Microorganism

Iron-oxidizing microorganisms can be divided into four main physiological groups according to their metabolism conditions: (1) acidophilic, aerobic iron oxidizers; (2) neutrophilic, aerobic iron oxidizers; (3) anaerobic, photosynthetic iron oxidizers; and (4) neutrophilic, anaerobic (nitrate-dependent) iron oxidizers. Except the nitrate-dependent iron oxidizers, most species in first three of these groups identified so far fall into one class within the phylum *Proteobacteria*, one of the major group of Gram-negative bacteria.

Acidophilic, Aerobic Iron-Oxidizing Microorganism

The most well-known acidophilic iron oxidizers are the acidophile *At. ferrooxidans*, whose isolates have been identified as strains on the basis of iron and sulfur oxidation at very low pH in the late 1940s (Colmer et al., 1950). Since then, the acidophilic iron-oxidizing microorganism has been extensively studied mainly because of their successful application in biomining (bioleaching of copper and other metals) and in environmental pollution (their role in generating acidic and metal-enriched mine drainage). *Acidophilic prokaryotes* have the capability of keeping

their intracellular pH at values close to neutrality and maintaining a proton gradient over their cytoplasmic membranes of up to five orders of magnitude. This pH differential enables them to produce ATP, though the influx of protons that drives this needs to be balanced with electrons derived from the oxidation of ferrous iron. While most acidophiles can obtain energy from the oxidation of ferrous iron alone when this is coupled to the reduction of molecular oxygen, most reported species are in fact facultative anaerobes that can also couple the oxidation of reduced sulfur compounds or hydrogen in some cases to the reduction of ferric iron in anoxic environments.

Hallberg et al. (2010) reported a novel iron-oxidizing species, *Acidithiobacillus ferrivorans* (*At. ferrivorans*), based on a phenotypic and genotypic analysis on four iron- and sulfur-oxidizing acidophilic bacteria (the "NO-37 group") isolated from different parts of the world. The isolates he obtained from the NO-37 group exhibited different physiological traits (most notably in being psychrotolerant) from the typical strain of *At. ferrooxidans* and clearly belong to a species that is different to those already recognized in the genus *Acidithiobacillus*. It also appears that *At. ferrivorans* have a different biochemical mechanism for oxidizing ferrous iron than *At. ferrooxidans* (Hallberg et al., 2010). Amouric et al. (2011) have proposed that iron-oxidizing *acidithiobacilli* comprises at least four distinct species, though currently only two species are recognized (i.e., *At. ferrooxidans* and *At. ferrivorans*). These divisions were supported by data obtained from a multilocus sequence analysis of 21 strains of iron-oxidizing *acidithiobacilli*.

Acidithiobacillus spp. were among the Gram-negative bacteria first identified as species of *Thiobacillus* according to their rod-shaped morphology and their capability of oxidizing reduced forms of sulfur. Most acidophilic species were reclassified as *Acidithiobacillus spp.* following phylogenetic analysis made by Kelly and Wood (2000), but two "species" of iron-oxidizing *acidithiobacilli* could not be affiliated with the new genus. One of these, "*Thiobacillus ferrooxidans*" strain m-1, had previously been highlighted by Harrison Jr (1982) as a probable distinct species. Later, it was fully described as the type strain of the novel genus and species *Acidiferrobacter thiooxydans* (*Af. thiooxydans*) (Hallberg et al., 2011). *Af. thiooxydans* shares many physiological characteristics with the type strain of *At. ferrooxidans*, including being a facultative anaerobe that oxidizes iron and reduced sulfur. Differences include a requirement for reduced sulfur by *Af. thiooxydans* and the fact that the latter is more tolerant of extreme acidity and moderately high temperatures (up to 47 Celsius degree) than *At. ferrooxidans*. The main distinguishing feature of this acidophile is its tolerance to salt, being able to grow in up to 3.5% (w/v) sodium chloride solution, whereas most iron-oxidizing *acidithiobacilli* are inhibited by 1% (w/v) sodium chloride salt (Nicolle et al., 2009). Both the original strain and a novel strain have been shown to grow optimally in the presence of 1–2% (w/v) sodium chloride and also, like *Af. thiooxydans*, require reduced sulfur for rapid oxidation of ferrous iron in liquid media (Nicolle et al., 2009).

It should be noted that Kimura et al. (2011) reported one *betaproteobacterium* "*Ferrovum myxofaciens*" ('*Fv. myxofaciens*'), which does not follow the pattern that all acidophilic iron-oxidizing proteobacteria are *Gammaproteobacteria*. Like all the

other iron oxidizers, "*Fv. myxofaciens*" is an extreme acidophile, though with a pH optimum of 3.0 and a pH minimum of 2, it is less acidophilic than *Acidithiobacillus spp.*, *Af. Thiooxydans,* and "*T. prosperus*." Uniquely among these bacteria, it appears to oxidize ferrous iron only and is an obligate aerobe. "*Fv. myxofaciens*" is widely distributed in acidic, iron-rich streams and rivers, where it is frequently observed as macroscopic streamer growths (Hallberg et al., 2006). It has also been identified as the major iron-oxidizing bacterium colonizing a pilot-scale mine water treatment plant designed to oxidize and precipitate iron from contaminated groundwater (Heinzel et al., 2009). Other bacteria, classified as "moderate acidophiles" (optimum pH for growth: 3–5), have also been occasionally reported to be able to catalyze the dissimilatory oxidation of iron under aerobic conditions. These include some *Thiomonas spp.* (mixotrophic sulfur-oxidizing *Betaproteobacteria*) that have been reported to precipitate ferric iron when grown in liquid and on solid media (Battaglia-Brunet et al., 2006). However, great care has to be taken to differentiate biological and abiotic iron oxidation, as noted above, particularly as small changes in the culture pH of moderate acidophiles can induce rapid chemical oxidation of iron. Slyemi et al. (2011) reported that *Thiomonas* strains that deposited ferric iron in shake flasks and on solid media did not oxidize iron in pH-controlled bioreactor, leading to the conclusion that *Thiomonas spp.* probably does not directly catalyze ferrous iron oxidation.

Neutrophilic, Aerobic Iron-Oxidizing Microorganism

Most neutrophilic aerobic iron-oxidizing bacteria have been isolated and characterized only relatively recently. So far, all known neutrophilic, oxygen-dependent lithotrophic iron oxidizers are proteobacteria (Emerson et al., 2010). Because of the potential for fast abiotic oxidation of ferrous iron in oxygen-rich, pH-neutral waters, aerobic, neutrophilic iron oxidizers often colonize the interface between aerobic and anoxic zones in sediments and groundwaters and have often been described as "gradient" microorganisms. Methods used to isolate these bacteria have usually attempted to mimic these environmental conditions in vitro, such as incubating in microaerobic atmospheres and using "gradient tubes" (Druschel et al., 2008; Emerson & Floyd, 2005; Hallbeck et al., 1993). In contrast to their acidophilic counterparts, neutrophilic iron oxidizers do not have preexisting pH gradients across their membranes that can facilitate ATP synthesis, and although the redox potentials of the ferrous/ferric couple(s) are lower at neutral pH than at acidic pH, so is that of the oxygen/water couple. Considering these reasons, lithotrophic, iron-oxidizing microorganisms have been described by Neubauer et al. (2002) as living on the "thermodynamic edge" (Fig. 3).

Gallionella ferruginea was one of the most known neutrophilic iron oxidizer that was first described by Ehrenberg in 1838. *G. ferruginea* can grow autotrophically or mixotrophically using ferrous iron as electron donor (Hallbeck & Pedersen, 1991). It forms bean-shaped cells with characteristic long, twisted stalks of ferrihydrite-like precipitates (Hanert, 1981). *G. ferruginea* remains as the only classified species

Fig. 3 Example of
gradient tubes (from left to
right: uninoculated tube,
tube inoculated with sterile
water and tube inoculated
with iron ore slurry
sample. Dye is added to
the tube inoculated with
sterile water to show that.
Note that all three tubes
have a brown iron band
near the bottom; only the
tube with slurry shows a
higher iron band, indicative
of growth)
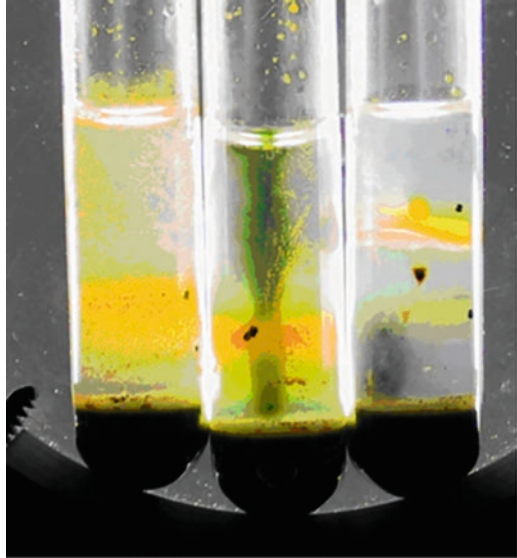
of this genus. Recently, bacteria that appear to be distinct species of *Gallionella*
have been found to be matching in clone libraries of DNA extracted from acidic
(pH 2.5–3.0) metal-rich water bodies (Hallberg et al., 2006; Heinzel et al., 2009;
Kimura et al., 2011), raising the possibility that acidophilic *Gallionella spp.* may
exist and still need to be isolated and characterized.

The dissimilatory iron oxidation by sheath-forming *Leptothrix spp.* and
Sphaerotilus natans is much less understood comparing to that of *Gallionella spp.*
Leptothrix currently comprises four recognized species, three of which (*Leptothrix
discophora*, *Leptothrix cholodnii,* and *Leptothrix mobilis*) are obligate heterotrophs,
while the other (*Leptothrix ochracea*) has not been cultivated and studied in the
laboratory in pure culture or subjected to thorough phylogenetic analysis. While all
four species (and *S. natans*, which is also a heterotroph) can accumulate ferric iron
and/or manganese (IV) on their sheaths, the only species for which there is circum-
stantial evidence for autotrophic growth using energy derived from iron oxidation is
L. ochracea. It is conceivable that the accumulation of ferric iron deposits by het-
erotrophic, sheath-forming betaproteobacteria is serendipitous and derives from the
breakdown of organic iron complexes by these bacteria, with the sheath acting as a
focal point for the hydrolysis and precipitation of the ferric iron released.

Two novel genera of aerobic, neutrophilic iron-oxidizing *Betaproteobacteria*
have been proposed more recently. The proposed genus "*Sideroxydans*" currently
includes two species, "*Sideroxydans*" sp. ES-1 (Emerson & Moyer, 1997) and
"*Sideroxydans paludicola*," while "*Ferritrophicum radicicola*" ("*Ft. radicicola*") is
currently the only known species of the genus "*Ferritrophicum*" (Weiss et al., 2007).
While the genus "*Sideroxydans*" belongs to the same bacterial order as *G. ferru-
ginea* (the *Gallionellales*), "*Ft. radicicola*" is currently the sole representative of a
new *Betaproteobacterial* order, the "*Ferritrophicales*." "*Sideroxydans*" *spp.* and

"*Ft. radicicola*" share the physiological traits of being unicellular rods that do not form sheaths or stalks, and all three species are obligate aerobes (microaerophiles) that appear to use ferrous iron as sole energy source and are autotrophic (Emerson et al., 2010). Interestingly, "*Sideroxydans" sp.* ES-1 was also the dominant phylotype detected in gene libraries obtained from a ferrous iron-oxidizing/nitrate-reducing enrichment culture by Blöthe and Roden (2009).

Sobolev and Roden (2004) reported another neutrophilic and autotrophic iron oxidizer (strain TW2) isolated from freshwater sediments. This bacterium, though as yet unclassified, was initially identified to be a novel genus and actually belongs to the *Betaproteobacteria*. In contrast with most other aerobic and neutrophilic iron oxidizers, strain TW2 can grow both as an autotroph and as a mixotroph.

Marine waters are typically pH 8.3–8.4, and the half-life of ferrous iron in seawater (pH 8.0) is about 2 min (Millero et al., 1987). As with freshwaters, microaerophilic iron-oxidizing proteobacteria have recently been isolated from iron mats in submarine geothermal areas and characterized in vitro (Emerson et al., 2007). *M. ferrooxydans* is a marine, mesophilic, autotrophic iron oxidizer that has close morphological similarity (bean-shaped cells and stalk formation) to *G. ferruginea*, though phylogenetically it is very distant from the freshwater bacterium and is affiliated with the *Candidatus* class "*Zetaproteobacteria*" (Emerson et al., 2010). Another strain of *M. ferrooxydans* has recently been isolated from a near-shore marine environment (McBeth et al., 2011). In addition to *M. ferrooxydans*, other (as yet unclassified) marine strains of iron-oxidizing *proteobacteria* were described by Edwards et al. (2003). These were psychrophilic facultative anaerobes that were related to *Alphaproteobacteria* and *Gammaproteobacteria*. More recently, Sudek et al. (2009) reported that heterotrophic Pseudomonas/Pseudoalteromonas-like *Gammaproteobacteria* isolated from a volcanic seamount could also catalyze ferrous iron oxidation under microaerobic conditions and therefore contribute to the formation of iron mats in the deep oceans. The status of other neutrophilic, aerobic iron oxidizers (e.g., *Siderocapsa*, *Metallogenium,* and *Crenothrix*) has long been brought into question, given the conflicting and sometimes sparse information on some of these bacteria. This issue has been eloquently addressed by Emerson et al. (2010).

Neutrophilic Iron-Oxidizing Microorganism Utilizing Nitrate/Nitrite

The fact that some bacteria are able to catalyze the dissimilatory oxidation of ferrous iron in anaerobic as well as in aerobic conditions has been recognized only since the early 1990s (Straub et al., 1996; Widdel et al., 1993). Two distinct metabolisms are known: one in which iron oxidation is used as a source of electrons by some photosynthetic bacteria and one that is a variant of anaerobic respiration, in which ferrous iron is used as electron donor and nitrate as electron acceptor. The feasibility of the latter being an energy-yielding reaction depends on the redox potential of the ferrous/ferric couple being more negative than that of the nitrate/nitrite couple (+0.43 V), which restricts this metabolic lifestyle to environments that

have circumneutral (and higher) pH values, and where the redox potential of the ferrous/ferric couple(s) is much lower (about +0.20 V) than in acidic liquors (+0.77 V). Iron-oxidizing/nitrate-reducing bacteria have been found in marine, brackish and freshwaters, and in anaerobic sediments (Benz et al., 1998; Kappler & Straub, 2005; Straub & Buchholz-Cleven, 1998). In contrast to other groups of iron-oxidizing *proteobacteria*, the anaerobic nitrate-reducing iron oxidizers are not found exclusively or predominantly in one class of the *Proteobacteria*, and the (relatively few) bacteria described are randomly affiliated with the classes *Alpha-*, *Beta-*, *Gamma-,* and *Deltaproteobacteria*.

Bacteria that couple iron oxidation and nitrate reduction in anaerobic environments can be divided into those that are autotrophic and those that use organic materials as carbon sources and can also grow as heterotrophs. In the first report of this form of metabolism, Straub et al. (1996) isolated three Gram-negative bacteria from an active enrichment culture containing ferrous iron and nitrate, all of which could grow on organic acids using either nitrate or oxygen as electron acceptor. While all three isolates could also oxidize ferrous iron in anaerobic, nitrate-containing media, rates of iron oxidation were relatively slow when no organic acid (acetate or fumarate) was provided, and even then iron oxidation by pure cultures of the isolates was never as rapid as observed with the enrichment culture. The end product of nitrate reduction in all three cases was predominantly nitrogen gas, and small amounts of nitrous oxide (N_2O) were also detected. Ferric iron was deposited as the mineral ferrihydrite, and the overall reaction that occurred is depicted in equation:

$$10FeCO_3 + 2NO_3^- + 24H_2O \rightarrow 10Fe(OH)_3 + N_2 + 10HCO_3^- + 8H^+ \qquad (1)$$
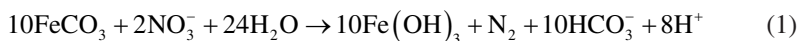
Later, Straub et al. (2004) identified one of these isolates as a strain of *Acidovorax* and another as a strain of *Aquabacterium* (both *Betaproteobacteria*). A third anaerobic iron-oxidizing isolate was most closely related to the *Gammaproteobacterial* genus *Thermomonas*. Kappler et al. (2005) also isolated a strain of *Acidovorax* (strain BoFeN1) from a freshwater lake sediment, and this strain could couple iron oxidation to nitrate reduction. Like the isolate of Straub and coworkers, this Acidovorax strain was a mixotroph and could only oxidize ferrous iron effectively in the presence of an organic acid, such as acetate (Muehe et al., 2009). *Acidovorax sp.* strain BoFeN1 could also reduce nitrite, nitrous oxide, and oxygen. Another heterotrophic *Betaproteobacterium*, isolated from a swine-waste lagoon (*Dechlorosoma suillum* strain PS, subsequently renamed as *A. oryzae* strain PS), was found to oxidize ferrous iron using either nitrate or chlorate as electron acceptor, with acetate as cosubstrate (Chaudhuri et al., 2001).

In circumneutral pH environments, ferrous iron oxidation can also, in theory, be coupled to the reduction of nitrate to ammonium. Weber et al. (2006) reported that oxidation of ferrous iron correlated with the appearance of ammonium in a nitrate-containing anaerobic enrichment culture containing *Geobacter* and *Dechloromonas spp.*, though no iron-oxidizing bacterium that could reduce nitrate to ammonium in pure culture was identified.

Anaerobic, nitrate-dependent oxidation of iron by autotrophic bacteria is also known. One of the first indications of this was an observation by Straub et al. (1996) that *Thiobacillus denitrificans* oxidized ferrous sulfide (FeS) in the presence of nitrate. *T. denitrificans* is a strictly autotrophic *Betaproteobacterium* that is best known for coupling the oxidation of various reduced inorganic sulfur compounds (or elemental sulfur) to the reduction of nitrate. *T. denitrificans* has also been implicated in the anaerobic oxidation of pyrite in anoxic sediments (Jørgensen et al., 2009). Whether the sulfide or ferrous iron moiety (or both) is the primary electron donor in this context is unclear, though in the absence of molecular oxygen pyrite oxidation is mediated by ferric iron, implying that *T. denitrificans* does indeed oxidize ferrous iron.

Weber et al. (2006) obtained an isolate (*Pseudogulbenkiania* strain 2002) from a freshwater lake sediment that could oxidize ferrous iron and reduce nitrate while growing as an autotroph, though it was also reported to grow heterotrophically on a variety of organic compounds (Weber et al., 2009). Analysis of its 16S rRNA gene sequence showed that this isolate (a facultative anaerobe) was very closely related (99.3% sequence similarity) to the *Betaproteobacterium Pseudogulbenkiania subflava* (Weber et al., 2009). A different approach to enrich for nitrate-dependent iron oxidizers was used by Kumaraswamy et al. (2006), who included EDTA-complexed ferrous iron as the electron donor.

These authors obtained an isolate that was related to species of the *Alphaproteobacterial* genus *Paracoccus*. A new species designation ("*Paracoccus ferrooxidans*") was proposed for the isolate, which was described as a facultative autotroph capable of growth in both aerobic and anoxic conditions. Intriguingly, there has been at least one report describing nitrate-dependent ferrous iron oxidation by the strict anaerobe *Geobacter metallireducens* (Finneran et al., 2002). Whether this deltaproteobacterium could use the energy from this reaction to support its growth was not ascertained, but given the widespread abundance of *Geobacter spp.* in anaerobic sediments (Lovley, 1991), the possibility exists that these anaerobic iron-reducing bacteria can also oxidize iron when nitrate is available.

Anaerobic Phototrophic Iron-Oxidizing Microorganisms

The fact that the photosynthetic iron-oxidizing microorganisms can promote iron oxidation in the absence of oxygen and without alternative electron donor (such as nitrate) has major implications for the occurrence of vast deposits of oxidized banded iron formation (BIF) on the planet earth. Large-scale oxidation of ferrous iron, originating from the weathering of ferro magnesium and other reduced minerals associated with the extensive volcanism, is thought to predate the development of an oxygen-enriched atmosphere (Ehrenreich & Widdel, 1994; Koehler et al., 2010).

The existence of oxygen-independent biological oxidation of ferrous iron was recognized first in cultures of anoxygenic phototrophic bacteria. Phototrophic purple bacteria are one of the representative microorganisms that can oxidize ferrous

iron in anaerobic environments according to the pioneering work by Ehrenreich and Widdel (Ehrenreich & Widdel, 1994; Widdel et al., 1993). Later, iron-oxidizing phototrophs have been isolated from a variety of freshwater and marine environments (Croal et al., 2004b; Heising & Schink, 1998; Jiao et al., 2005; Straub et al., 1999). Most of the iron-oxidizing phototrophs that have been reported are affiliated with the class Alphaproteobacteria, with the notable exception of *Thiodictyon* strain L7, which is a *Gammaproteobacterium*. Ferrous iron is utilized by this group of bacteria as a source of reductant for carbon dioxide as shown in Eq. 2,

$$4Fe^{2+} + CO_2 + 11H_2O \rightarrow 4Fe(OH)_3 + (CH_2O) + 8H^+ \tag{2}$$
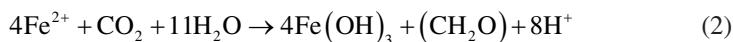
Following this reaction, carbon dioxide is transformed into fixed biomass carbon in the form of CH_2O. In addition to carbon assimilation, most photosynthetic bacteria can also use this ferrous iron oxidation process as a detoxification mechanism. During the process, the ferric iron precipitates (insoluble ferric hydroxide) are generated as waste products. This iron precipitates could be potential hazard to iron-oxidizing phototrophs, as the bacteria risk being enshrouded by these ferrihydrite-like minerals, which would restrict their access to light (Heising & Schink, 1998). However, this phenomenon has only, so far, been noted for cultures of *Rhodomicrobium vannielii* (*Rm. vannielii*), in which encrustation of cells has been reported to result in incomplete oxidation of ferrous iron due to restricted light access (Heising & Schink, 1998).

Ferrous iron has been sometimes regarded as a high potential electron donor at any pH, and the midpoint potential of the purple bacteria with one photosystem at pH 7 is about +0.45 V. Therefore, the redox potential of ferrous iron is more positive than that of the ferrous carbonate/ferric hydroxide couple ($Fe(OH)_3 + HCO_3^-$/$FeCO_3$, +0.20 V). Also, ferrous iron is a less favorable electron donor in energetic terms than sulfide, which is more widely used by anaerobic photosynthetic bacteria (the redox potential of the sulfide/sulfur couple is −0.18 V). Table 3 summarizes the alternative electron donors that have been reported to be used by phototrophic iron-oxidizing bacteria. It has been reported that phototrophic iron oxidizers can use

Table 3 Alternative electron donors of phototrophic iron-oxidizing microorganisms

Microorganisms	Electron donors
Rhodovulum iodosum, Rhodovulum robiginosum	$S_2O_3^{2-}$, HS^-, elemental S
Rm. Vannielii	H_2, HS^-, fatty acids, amino acids, sugars, and aromatic compounds
Rhodobacter sp. SW2	H_2, fatty acids, amino acids, sugars, and aromatic compounds
Rp. Palustris TIE-1	H_2, $S_2O_3^{2-}$
Thiodictyon strain L7	H_2, fatty acids, amino acids, sugars, and aromatic compounds

Duchow & Douglas, 1949; Imhoff, 2005; Jiao et al., 2005; Straub et al., 1999

soluble ferrous iron and minerals such as iron sulfide (FeS) or carbonate (FeCO$_3$) as sources of reductant, but are not able to access ferrous iron in more crystalline minerals such as pyrite (FeS$_2$) and magnetite (Fe$_3$O$_4$). (Kappler & Newman, 2004).

Almost all currently known phototrophic iron oxidizers can be attributed to the genus of the same family, *Rhodobacteraceae*, which is a highly diverse family within the class *Alphaproteobacteria*. *Rhodobacter* has been well defined by Imhoff (Imhoff, 2005, 2006), among all the phototrophic alphaproteobacteria, by a set of characters that include cellular morphology and division mode, intracytoplasmic membrane type, pigment composition, optimal phototrophic conditions, polar lipid content, saline preferences, and major products of sulfide oxidation. *Rhodobacter* cells are ovoid or short rods, with polar flagella (when motile) that divide by binary fission (sometimes forming chains) and exhibit vesicular intracytoplasmic membrane systems when grown phototrophically (an exception is *R. blasticus*, formerly *Rhodopseudomonas blastica*, which forms peripheral lamellae and divide by budding).

Ehrenreich and Widdel (1994) characterized the first iron-oxidizing phototroph, *Rhodobacter sp.* strain SW2, which oxidizes ferrous iron only when organic carbon source is available and also utilizes hydrogen and organic compounds. More recently, Poulain and Newman (2009) described and tested the effects of light, Fe (II) speciation, pH, and salinity on the Fe (II) oxidation rate by *Rhodobacter sp.* (*Rhodobacter capsulatus*), formerly classified as a species of *Rhodopseudomonas*. This phototroph is highly sensitive to low concentrations of Fe (II), and its growth is inhibited in the presence of concentrations as low as 5 μM. However, this toxicity can be relieved once the ferrous iron is oxidized to the highly insoluble ferric forms.

Other iron-oxidizing phototrophic bacteria include *Rhodobacteraceae* family species of *Rhodovulum* (*Rhodovulum robiginosum* and *Rhodovulum iodosum*) and the green phototrophic sulfur bacterium *Chlorobium ferrooxidans* strain KoFox. All species oxidize ferrous iron and sulfide when organic cosubstrates, such as acetate, are available (Straub et al., 1999). A phototrophic isolate, identified as a strain (BS-1) of *Rm. vannielii*, a heterotrophic nonsulfur purple bacterium of the family *Hyphomicrobiaceae*, was shown by Heising and Schink (1998) to oxidize ferrous iron; this trait was subsequently confirmed in the type strain of this species. Interestingly, *Rm. vannielii* had been tentatively identified by Widdel et al. (1993) as one of the iron-oxidizing phototrophic isolates that they obtained from freshwaters. Growth of strain BS-1 in the presence of ferrous iron was stimulated by adding acetate or succinate as cosubstrates. Heising and Schink (1998) concluded that the oxidation of ferrous iron is only a peripheral activity for *Rm. Vannielii* strain BS-1. Another member of the *Hyphomicrobiaceae*, *Rhodopseudomonas palustris* (*Rp. palustris*) strain TIE-1, was isolated from an iron-rich mat by Jiao et al. (2005) and used subsequently as a model organism for genetic studies.

In summary, seven cultures of phototrophic iron oxidizers have been well described: the freshwater strains *Rhodobacter ferrooxidans* strain SW2 (Ehrenreich & Widdel, 1994), *Rhodopseudomonas palustris* strain TIE-1 (Jiao et al., 2005), *Chlorobium ferrooxidans* strain KoFox (Heising et al., 1999), *Thiodictyon sp.* strain F4 (Croal et al. 2004, b), and *Rhodomicrobium vannielii* strain BS-1 (Heising &

Schink, 1998) as well as the marine strains *Rhodovulum iodosum* and *Rhodovulum robiginosum* (Straub et al., 1999). Currently, only two photosynthetic iron-oxidizing *Gammaproteobacteria* have been reported, and both are strains of *Thiodictyon*. One of these, strain L7, was isolated from the same source as *Rhodobacter sp.* SW2 (Ehrenreich & Widdel, 1994), while *Thiodictyon sp.* strain f4 was isolated from a marsh by Croal et al. (2004, b). *Thiodictyon sp.* strain f4 displays the fastest rates of iron oxidation of all phototrophic iron-oxidizing bacteria that have been isolated (Hegler et al., 2008).

3.2   Iron-Reducing Microorganism

Under natural environment, iron (III) minerals can be microbiologically reduced by strictly anaerobic or facultative iron-reducing bacteria using a wide range of organic compounds as electron donors or by using H_2 (Ehrlich & Newman, 2009). The ability to use iron (III) as a terminal electron acceptor is widespread across the domains Bacteria and Archaea.

Bromfield first reported reduction of iron by microbial activity in 1954. Since then, it was long believed that certain microorganisms can produce organic compounds or chemicals that are capable of reducing iron. Later, the discovery of *Geobacter metallireducens* confirmed that some organisms could use iron directly and specifically in their respiration (Lovley et al., 1987; Lovley and Phillips, 1988; Lovley, 1991). This iron-reducing ability has been discovered in a wide range of microorganisms (Coupland & Johnson, 2008; Fredrickson & Gorby, 1996). Most of them are obligate anaerobes, but it has also been found that some normally aerobic iron-oxidizing bacteria such as *Acidithiobacillus ferrooxidans* (*A. ferrooxidans*) can change their ways of existing as iron reducers in anaerobic environments (Johnson & Clegg, 2010).

Geobacter metallireducens (strain GS-15), *Geobacter sulfurreducens* (strains KN400 and PCA), *Shewanella oneidensis* (strain MR-1), and *Shewanella putrefaciens* (strains 200 and ATCC 8071) are the most studied iron (III)-reducing bacteria. Results indicate that for dissimilatory growth on iron (III) minerals, *Geobacter* species require direct contact with insoluble iron (III) oxide surfaces (Nevin & Lovley, 2000). In *Geobacter spp.*, motility enables contact with Iron (III) oxides (Childers et al., 2002), which cells may reduce via conductive "nanowires" that provide conduits for electron transfer (Reguera et al., 2005a, 2005b). Diverse periplasmic and outer surface c-type cytochromes in *Geobacter* species also play critical roles in extracellular electron transfer to iron (III) (Ding et al., 2006; Leang et al., 2010; Lloyd et al., 1999; Qian et al., 2011). In contrast to other iron (III)-reducing species, such as *Shewanella*, *Geobacter* cannot produce soluble electron shuttles to reduce iron (III) minerals (Nevin & Lovley, 2000).

Geobacter metallireducens predominantly uses acetate, a fermentation product, as electron donor, while it can also oxidize other organic compounds like alcohol and fatty acid. Theoretically, fermentation would contribute more electron to iron

(III) during the metabolism than oxygen-based respiration calculation based on the efficiency of iron reduction coupled to fermentation. The iron reduction reaction for *Geobacter metallireducens* is as follows:

$$CH_3CO_2^- \left(acetate\right) + 8Fe^{3+} + 4H_2O \rightarrow 8Fe^{2+} + 2HCO_3^- + 9H^+ \tag{3}$$
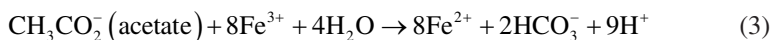
Among the *Geobacter* family, the TCA cycle leads to complete oxidation of acetates or other electron donors and ATP is generated primarily through oxidative phosphorylation. To transfer electrons to iron (III), *Geobacter metallireducens* possesses a membrane-bound iron (III) reductase. It also produces soluble form and membrane-bound c-type cytochromes. Another method to transfer electron to iron (III) is extracellular transport of electrons to iron (III) through microbial nanowires. According to a knockout mutation experiment of *Geobacter sulfurreducens*, the results indicate that mutants that lack conductive pili are not able to grow because they are not able to reduce iron (III) oxides yet pili is not necessary for iron (III) oxide attachment to cell.

Myers and Nealson (1988a) isolated and identified as *Alteromonas putrefaciens* (later renamed as *Shewanella putrefaciens*), a microorganism capable of anaerobic growth using either manganese (IV) or iron (III) as electron acceptors. This bacterium (MR-1) is a facultative anaerobe, capable of reduction of many other electron acceptors. It was considered as one of the most versatile respiratory microorganism ever reported. However, MR-1 was isolated by using an enrichment culture that utilizes nonfermentable carbon compounds as a source of energy. Using this approach, it is a routine matter to isolate iron reducers, and they are found to span a wide range of bacterial group, including both Gram-positive and Gram-negative and facultative anaerobes to strict anaerobes. It is speculated that in the rich carbon sources the metal reducers were simply outcompeted by fermentative bacteria that altered the pH conditions or outgrew them.

S. putrefaciens is another bacterium that is capable of iron (III) reduction. Unlike *Geobacter* family, *Shewanella*, respiring anaerobically, utilizes substrate-level phosphorylation as a primary energy conservation mechanism to sustain growth. Major organic electron donors for this particular bacterium are formate (HCO_2^-), lactate ($CH_3CH\left(OH\right)CO_2^-$), and pyruvate ($CH_3COCO_2^-$).

$$HCO_2^- + 2Fe^{3+} + H_2O \rightarrow HCO_3^- + 2Fe^{2+} + 2H^+ \tag{4}$$

$$CH_3CH\left(OH\right)CO_2^- + 4Fe^{3+} + 2H_2O \rightarrow CH_3CO_2^- + HCO_3^- + 4Fe^{2+} + 5H^+ \tag{5}$$

$$CH_3COCO_2^- + 2Fe^{3+} + 2H_2O \rightarrow CH_3CO_2^- + HCO_3^- + 2Fe^{2+} + 3H^+ \tag{6}$$
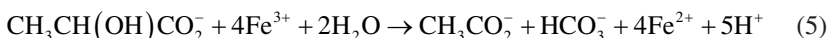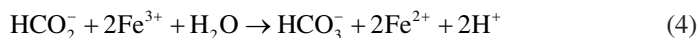
As indicated in the above reactions, lactate and pyruvate are not completely oxidized. Thus, it is expected that lactate and pyruvate only contribute a little to electron and carbon flow in iron (III)-reducing environment. Under anaerobic conditions, the production of c-type cytochromes is stimulated in the outer membrane; If c-type

cytochromes serve as a mediator for electron transfer to reduce insoluble Iron (III) oxides, this hypothesizes a possible mechanism for iron (III) reduction in the absence of iron (III) reductase identification. Also, there is an association between proton translocation and electron transfer to iron (III) because of the experimental finding of depressed pH value in the medium while incubating *S. putrefaciens* anaerobically. In another study of *S. oneidensis* MR-1, the experimental finding suggests that the presence of different oxidation states of Fe on both the cell surface and in extracellular environment offers an insight on the iron (III)-reducing mechanism; the researchers also conclude that surface contact-mediated electron transfer is significant.

4   Industrial Microbiology

Microorganisms have been used to produce certain food products and beverages since ancient times, but today they are grown on industrial scale to produce valuable commercial products or to assist to carry out chemical reactions. In this chapter, a range of practical topics will be investigated to study microbial growth, growth cycles, and the factors that affect microorganism population growth. Examples will also be given to help understand how growth conditions can be manipulated to influence the end product and the role of genetic engineering in industrial microbiology. In addition, common physical and chemical methods will be discussed for microbial contamination control and quality control.

4.1   Industrial Microorganisms

Numerous microorganisms are used within industrial microbiology; these include naturally occurring organisms, laboratory-selected mutants, or even genetically modified organisms (GMOs). Currently, the debate in the use of genetically modified organisms (GMOs) in food sources is gaining both momentum, with more and more supporters on both sides. However, the use of microorganisms at an industrial level is deeply rooted into today's society. The following is a brief overview of the various microorganisms that have industrial uses and of the roles they play.

Archaea are specific types of prokaryotic microbes that exhibit the ability to sustain populations in unusual and typically harsh environments. Those surviving in the most hostile and extreme settings are known as extremophile archaea. The isolation and identification of various types of Archaea, particularly the extremophile archaea, have allowed for analysis of their metabolic processes, which have then been manipulated and utilized for industrial purposes.

Extremophile archaea species are of particular interest due to the enzymes and molecules they produce that allow them to sustain life in extreme climates, including very high or low temperatures, extremely acid or base solutions, or when

exposed to other harmful factors, including radiation. Specific enzymes which have been isolated and used for industrial purposes include thermostable DNA polymerases from the *Pyrococcus furiosus*. This type of polymerase is a common tool in molecular biology; it is capable of withstanding the high temperatures that are necessary to complete polymerase chain reactions. Additional enzymes isolated from Pyrococcus species include specific types of amylases and galactosidases which allow food processing to occur at high temperatures as well.

Corynebacteria are characterized by their diverse origins. They are found in numerous ecological niches and are most often used in industry for the mass production of amino acids and nutritional factors. In particular, the amino acids produced by *Corynebacterium glutamicum* include the amino acid glutamic acid. Glutamic acid is used as a common additive in food production, where it is known as monosodium glutamate (MSG). *Corynebacterium* can also be used in steroid conversion and in the degradation of hydrocarbons. Steroid conversion is an important process in the development of pharmaceuticals. Degradation of hydrocarbons is key in the breakdown and elimination of environmental toxins. Items such as plastics and oils are hydrocarbons; the use of microorganisms which exhibit the ability to break down these compounds is critical for environmental protection.

Xanthomonas, a type of *Proteobacteria*, is known for its ability to cause disease in plants. The bacterial species which are classified under *Xanthomonas* exhibit the ability to produce the acidic exopolysaccharide commonly marketed as xanthan gum, used as a thickening and stabilizing agent in foods and in cosmetic ingredients to prevent separation.

Another type of microorganism utilized by industry includes various species of Aspergillus. This genus includes several hundred types of mold. Aspergillus has become a key component in industrial microbiology, where it is used in the production of alcoholic beverages and pharmaceutical development. *Aspergillus niger* is most commonly used to produce citric acid, which is used in numerous products ranging from household cleaners, pharmaceuticals, foods, cosmetics, photography, and construction. Aspergillus is also commonly used in large-scale fermentation in the production of alcoholic beverages such as Japanese sake.

4.2 Microbial Nutrition and Growth Kinetics

The biosynthesis of cellular components necessary for growth, reproduction, and maintenance requires a supply of basic nutrients and an energy source. Microorganisms have evolved a wide range of mechanisms to gain energy, but are essentially divided into two categories. Chemotrophs obtain energy by the oxidation of organic or inorganic compounds, whereas phototrophs use energy derived from light. Both have two possible sources of hydrogen atoms or electrons. Organotrophs oxidize preformed organic molecules, such as sugars, to obtain electrons or hydrogen, whereas lithotrophs acquire electrons from reduced inorganic molecules, including hydrogen sulfide and ammonia. Carbon nutrition is divided into two

classes. Autotrophs utilize CO_2 as their sole or primary source of carbon, whereas various heterotrophs use a wide range of reduced organic molecules, including hydrocarbons, lipids, organic acids, simple sugars, and polysaccharides.

Microbial cells must obtain a range of chemical elements to fulfill their nutritional requirements. Four of these, the macronutrients, carbon, hydrogen, oxygen and nitrogen, must be available in gram quantities per liter of growth medium. These elements, along with phosphorus and sulfur, are the principal components of major cellular polymers: lipids, nucleic acids, polysaccharides, and proteins. Other minor elements, including calcium, iron, potassium, and magnesium, are required at levels of a few milligrams per liter; the trace elements, primarily cobalt, copper, manganese, molybdenum, nickel, selenium, and zinc, are needed in only microgram quantities.

Microbial Nutrition, Growth, and Cultivation

Microbial growth can be defined as an orderly increase in cellular components, resulting in cell enlargement and eventually leading to cell division. This definition is not strictly accurate as it implies that a consequence of growth is always an increase in cell numbers. However, under certain conditions, growth can occur without cell division, for example, when cells are synthesizing storage compounds, e.g., glycogen or poly b-hydroxybutyrate. In this situation, the cell numbers remain constant, but the concentration of biomass continues to increase. This is also true for coenocytic organisms, such as some fungi, that are not divided into separate cells. Their growth results only in increased size.

Microbial growth in liquid media can be carried out under different operating conditions, i.e., batch growth, fed-batch growth, or continuous growth. Batch growth involves a closed system where all nutrients are present at the start of the growth within a fixed volume. The only further additions may be acids or bases for pH control or gases (e.g., aeration, if required). In fed-batch systems, fresh medium or medium components are fed continuously, intermittently, or are added as a single supplement, and the volume of the batch increases with time. Continuous growth is open systems where fresh medium is continuously fed into the vessel, but the volume remains constant as spent medium and cells are removed at the same rate.

Growth in closed culture systems, such as a batch culture in LB broth, where no additional nutrients are added and waste products are not removed, the bacterial growth will follow a predicted growth curve and can be modeled as shown in Fig. 4. Generally, there are four distinct growth phases, lag, exponential, stagnant, and death phases. During lag phase, bacteria adapt themselves to growth conditions. It is the period where the individual bacteria are maturing and not yet able to divide. During the lag phase of the bacterial growth cycle, synthesis of RNA, enzymes, and other molecules occurs. Exponential phase (sometimes called the log or logarithmic phase) is a period characterized by cell doubling. The number of new bacteria appearing per unit time is proportional to the present population. Under controlled conditions, cyanobacteria can double their population four times a day. Exponential
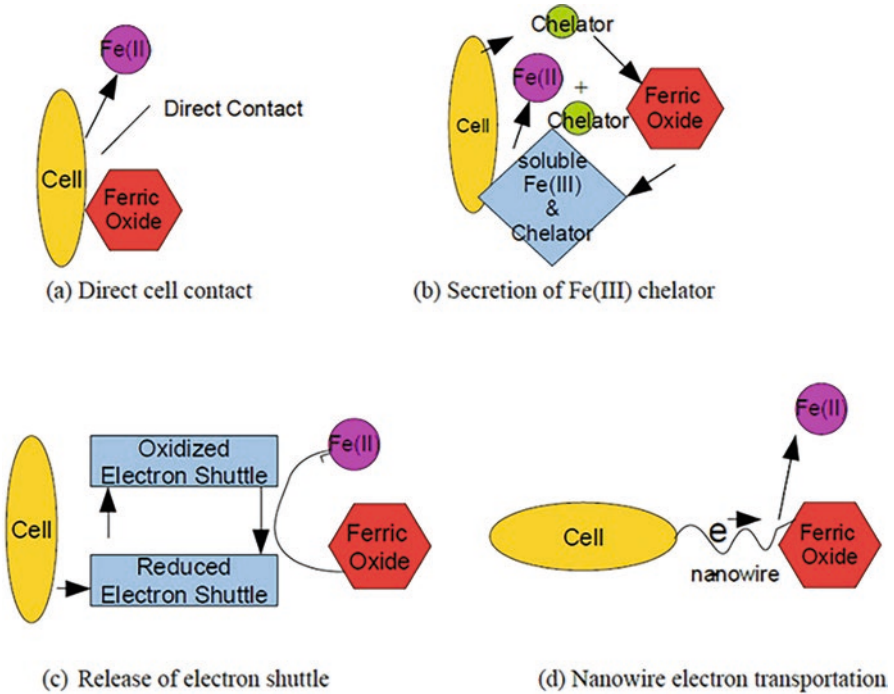
Fig. 4 Mechanism of iron reduction by microorganisms (**a**) Direct cell contact (**b**) Secretion of Fe(III) chelator (**c**) Release of electron shuttle (**d**) Nanowire electron transportation

growth cannot continue indefinitely, however, because the medium is soon depleted of nutrients and enriched with wastes. The stationary phase is due to a growth-limiting factor, this is, mostly depletion of a nutrient and/or the formation of inhibitory products such as organic acids. At death phase, bacteria run out of nutrients and die (Table 4).

In reality, the four phases are not well defined even in batch cultures. The cells do not reproduce in synchrony without explicit and continual prompting, and their logarithmic phase growth is often not over a constant rate, but instead a slowly decaying rate, a constant stochastic response to pressures both to reproduce and to go dormant in the face of declining nutrient concentrations and increasing waste concentrations.

Batch culture is the most common laboratory growth method in which bacterial growth is studied, but it is only one of many. The bacterial culture is incubated in a closed vessel with a single batch of medium.

In some experimental regimes, some of the bacterial culture is periodically removed and added to fresh sterile medium. In the extreme case, this leads to the continual renewal of the nutrients. This is a chemostat, also known as an open or continuous culture: a steady state defined by the rates of nutrient supply and bacterial growth. In comparison to batch culture, bacteria are maintained in exponential

Table 4 Microbial growth phases and growth characteristics

Phase	Description	Specific growth rate
Lag	A period before growth when microorganisms adapt to their new environment. Genes are switched on, and the required enzymes are synthesized. The transfer to a new medium may cause a change in pH, increase in available nutrients, and reduction of growth inhibitors. Length of lag phase can vary—If the inoculum is taken from a culture during its log phase, then there may be no lag phase, but if it is taken from a culture in its stationary phase, then the lag phase may be quite long.	$r_X = \mu x$ $\mu \approx 0$ Where r_X is the volumetric rate of biomass production with units of, for example, $kg\ m^{-3}s^{-1}$; x is viable cell concentration with units of, for example, $kg\ m^{-3}$, and μ is the specific growth rate.
Exponential	Cells have adapted to the new conditions and can now double their number (filamentous organisms such as fungi double their biomass) per unit time, giving an exponential growth rate. Growth curves are plotted on logarithmic graph paper giving a straight line. The specific growth rate depends on concentration of substrate (S), maximum growth rate (μm) and a substrate-specific constant (Ks). Ks is equivalent to the Michaelis constant (km) in enzyme kinetics and is the substrate concentration where half the maximum specific growth rate occurs.	Growth starts: $\mu < \mu_{max}$ Growth achieves its maximum rate: $\mu \approx \mu_{max}$
Stagnant	Growth slows due to the substrate having been completely metabolized or to the accumulation of toxic by-products. The biomass remains constant during this phase as number of cells produced = number of cells dying. However, as dead cells lyse (split), carbohydrates, lipids or proteins are released, which are new substrates and energy sources for the remaining surviving cells. Metabolites produced during the stationary phase are called secondary metabolites. The length of the stagnant phase depends on the microorganism and on the process being used for its culture.	Growth slows: $\mu < \mu_{max}$ Growth stops: $\mu = 0$
Death	Cells die at an exponential rate, giving a straight line on logarithmic graph paper. They die because they have exhausted all their energy reserves. In commercial and industrial processes, the fermentation is usually interrupted before the end of the log phase or before the death phase begins.	Cell lyses: $\mu < 0$

growth phase, and the growth rate of the bacteria is known. Related devices include turbidostats and auxostats.

Bacterial growth can be suppressed with bacteriostats, without necessarily killing the bacteria. In a synecological culture, a true-to-nature situation in which more than one bacterial species is present, the growth of microbes is more dynamic and continual. Liquid is not the only laboratory environment for bacterial growth. Spatially structured environments such as biofilms or agar surfaces have been used widely to form complex growth models.

The continuous culture of microorganisms is a technique of increasing importance in microbiology. The essential feature of this technique is that microbial growth in a continuous culture takes place under steady-state conditions; that is, growth occurs at a constant rate and in a constant environment. Such factors as pH value, concentrations of nutrients, metabolic products, and oxygen, which inevitably change during the "growth cycle" of a batch culture, are all maintained constant in a continuous culture; moreover, they may be independently controlled by the experimenter. These features of the continuous culture technique make it a valuable research tool, while it offers many advantages, in the form of more economical production techniques, to the industrial microbiologist.

Continuous culture of microorganisms has many applications for both laboratory research and industrial-scale processes. Studies can be performed on all aspects of cell growth, physiology, and biochemistry. They are useful for ecological studies and as a genetics tool for the examination of mutation rates, mutagenic effects, etc. Application in industrial bioprocesses overcomes many limitations of batch processes.

Continuous Growth Kinetics

Growth kinetics of homogeneous unicellular suspension cultures can be described using differential equations in a continuum model. However, filamentous growth and growth in heterogeneous cell aggregates and assemblages, particularly biofilms, colonies, flocs, mats, and pellicles, are much more complex. In fact, heterogeneous systems require a very different approach using cellular automaton and Swarm system models. The growth kinetics of filamentous organisms and heterogeneous systems will not be discussed here.

The continuous growth model that will be discussed here is bacterial binary fission in homogeneous suspension cultures, where cell division produces identical daughter cells. Each time a cell divides is called a generation, and the time taken for the cell to divide is referred to as the generation time. Therefore, the generation time or doubling time is the time required for a microbial population to double. Theoretically, after one generation, both the microbial cell population and biomass concentration have doubled. However, as previously stated, under certain conditions, growth can be associated with an increase in biomass and not cell numbers. Also, the generation time recorded during microbial growth is in reality an average value, as the cells will not be dividing at exactly the same rate. At any one time, there are cells at different stages of their cell cycle. This is termed asynchronous growth. However, under certain conditions, synchronous growth can be induced so that all cells divide simultaneously, which is a useful research tool in the study of microbial physiology.

Since all continuous cultures start initially as batch cultures, the growth kinetics of continuous culture can be described by extending exponential growth indefinitely, in theory, through the continuous addition of fresh medium. The reactor is continuously stirred, and a constant volume is maintained by incorporating an
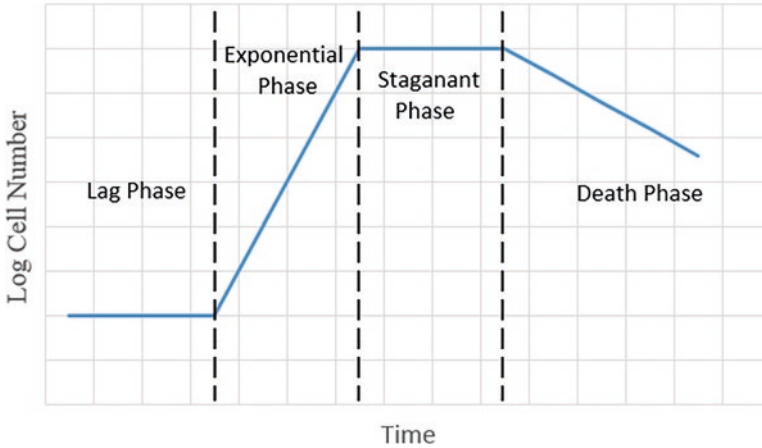
Fig. 5 Typical microbial growth curve

Table 5 Assumptions in the operation of continuous cultivation

Conditions	Assumption
Continuous feed	No batch or drop feeding
All biomass in suspension	No wall growth
Biomass in reactor and effluent in steady state	Equal, no biomass retention at outlet
Ideal mixed	Conversion rates not too high

overflow weir or other leveling device (Fig. 5). Fresh medium is continuously added and displaces an equal volume of spent medium and cells at the same rate as fresh medium is introduced. Steady-state and other ideal conditions are assumed throughout the process, where the rate of microbial cell growth equals the rate at which the cells are displaced from the vessel as shown in Table 5.

As with batch culturing, the specific rate at which the microorganism grows in continuous culture is controlled by the availability of the rate-limiting nutrient. Therefore, the rate of addition of fresh medium controls the rate at which the microorganisms grow. However, the actual rate of growth depends not only on the volumetric flow rate of the medium into the reactor, but also on the dilution rate (D). This equals the number of reactor volumes passing through the reactor per unit time and is expressed in units of reciprocal time, per hour.

$$D = \frac{F}{V} \tag{7}$$

where D is the dilution rate; F is the flow rate, and V is the volume of the reactor.

Addition of fresh medium into the reactor can be controlled at a fixed value; therefore, the rate of addition of the rate-limiting nutrient is constant. Within certain

limits, the growth rate and the rate of loss of cells from the fermenter will be determined by the rate of medium input. Therefore, under steady-state conditions, the net rate of increase of biomass can be described as follows:

$$\frac{dx}{dt} = \left(\text{growth rate in reactor}\right) - \left(\text{loss rate or output}\right) \tag{8}$$

or

$$\frac{dx}{dt} = \mu x - Dx \tag{9}$$

Under steady-state conditions, the growth rate = rate of loss; i.e., $dx/dt = 0$; therefore,

$$\mu x = Dx \text{ or } \mu = D \tag{10}$$

Consequently, at fixed flow rates and dilution rates under constant physical and chemical operating conditions, i.e., under steady-state conditions, the specific growth rate of the microorganism is dependent on the operating dilution rate, up to a maximum value equal to μ_{max} (shown in Fig. 6, maximum specific growth rate). If the dilution rate is increased above μ_{max}, complete washout of the cells occurs, as the cells have insufficient time to "double" before being washed out of the reactor via the overflow. The point at which this is just avoided is referred to as the critical dilution rate (D_{Crit}).

For any given dilution rate, under steady-state conditions, the residual substrate concentration in the reactor can be predicted by substituting D for μ in the Monod equation as shown below:

$$\mu = \frac{\mu_{max} S}{K_s + S} \tag{11}$$

$$D = \frac{\mu_{max} S_r}{K_s + S_r} \tag{12}$$

where S is the concentration of limiting substrate; K_s is the saturation constant; and S_r is the steady-state residual substrate concentration in the reactor at a fixed dilution rate. Rearranging the above equation gives the following:

$$D\left(K_s + S_r\right) = \mu_{max} S_r \tag{13}$$

Dividing by S_r, then gives the following:

$$\frac{DK_s}{S_r} + D = \mu_{max} \tag{14}$$
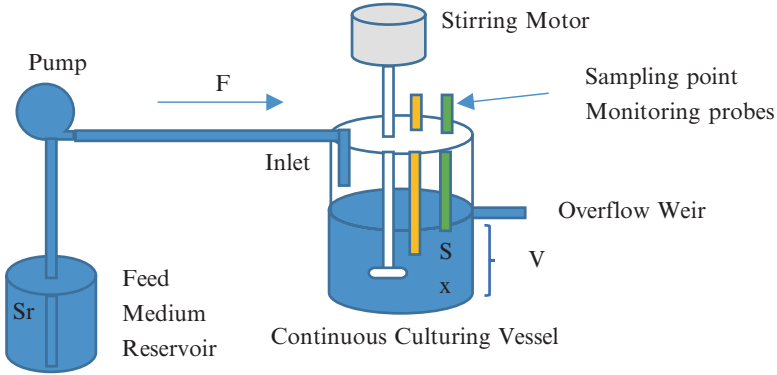
Fig. 6 Schematic of a continuous chemostat for culturing microorganism

Therefore,

$$S_r = \frac{DK_s}{\mu_{max} - D} \tag{15}$$

Consequently, the residual substrate concentration in the reactor is controlled by the dilution rate. Any alteration to this dilution rate results in a change in the growth rate of the cells that will be dependent on substrate availability at the new dilution rate. Thus, growth is controlled by the availability of a rate-limiting nutrient. This system, where the concentration of the rate-limiting nutrient entering the system is fixed, is often described as a chemostat, as opposed to operation as a turbidostat, where nutrients in the medium are not limiting. In this case, turbidity or absorbance of the culture is monitored and maintained at a constant value by regulating the dilution rate; i.e., cell concentration is held constant.

At low dilution rates with fixed substrate concentrations, the residual substrate concentration will be low (Fig. 6). However, as D approaches μ_{max}, the residual substrate concentration increases along with the growth rate of the microorganism. Beyond D_{Crit}, input substrate concentration will equal output concentration, as all the cells have been lost from the system. Thus, this continuous reactor can be described as a self-regulating nutrient-limited chemostat.

The concentration of biomass or microbial metabolite in a continuous fermenter under steady-state conditions can be related to the yield coefficient, as described in the batch fermentation section. Inserting the equation for residual substrate (Eq. 15) into the biomass or metabolic product yield coefficient equation (Eq. 16) gives, in this case for steady-state biomass ($\bar{x}$),

$$\bar{x} = Y_{x/S} \left(S_R - S_r \right) \tag{16}$$

where S_R is the substrate concentration of inflowing medium or

$$\bar{x} = Y_{x/S}\left(S_R - \frac{DK_s}{\mu_{max} - D}\right)$$

(17)

Therefore, the biomass concentration under steady-state conditions is controlled by the substrate feed concentration and the operating dilution rate. Under noninhibitory conditions, where there is no substrate or product inhibition, the higher the feed concentration, the greater the biomass concentration and residual substrate concentration remains constant. However, the higher the dilution rate, the faster the cells grow, which results in a simultaneous increase in the residual substrate concentration and a consequent reduction in the steady-state biomass concentration. As D approaches μ_{max}, the biomass concentration becomes even lower, yet the cells grow faster, and there is a concurrent increase in the residual substrate concentration (Figs. 6 and 7).

4.3 Industrial Bioprocess

The birth of industrial bioprocess largely began with the fermentation processes, such as those involved in the production of fermented dairy products and alcoholic beverages in 1800s. The development of pure culture techniques by Hansen at the Carlsberg Brewery in Denmark greatly advanced the progress of industrial fermentation processes in 1883. In the early 1920s, an industrial fermentation process was introduced for the manufacture of citric acid, employing a filamentous fungus
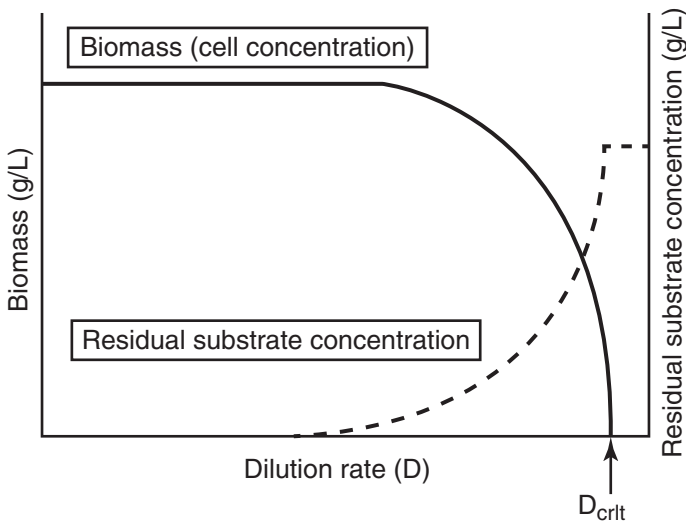
Fig. 7 Growth of a microorganism in continuous culture

(mold), *Aspergillus niger*. Further innovations in fermentation technology were greatly accelerated in the 1940s through efforts to produce the penicillin, stimulated by the vital need for this antibiotic during World War II. Not only did production rapidly move from small-scale surface culture to large-scale submerged fermentations, but it led to great advances in both media and microbial strain development.

Later at the turn of the twentieth century, there had been major advancements in the large-scale treatment of wastewater and sewage. The increasing global trend of industrialization, coupled with continual effort in improving process efficiency and waste minimization, has led to the development of bioprocessing of industrial wastewater. Noticeably developed even before or along with wastewater bioprocessing is the application of microbial-based "clean technology" in mineral biomining and the desulphurization of fuels, along with composting of solid organic wastes and ensiling of plant biomass. This technology also reduces our reliance on synthetic chemical pesticides by allowing the implementation of biological control measures using bioinsecticides, biofungicides, etc.

Many of the greatest advances in industrial bioprocess have followed the massive developments in genetic engineering (recombinant DNA technology) over the last 20 years. This technology has had, and will continue to have, a tremendous influence on traditional, established and novel bioprocesses and products. It allows genes to be transferred from one organism to another and allows new approaches to strain improvement. The basis of gene transfer is the insertion of a specific gene sequence from a donor organism, via an expression vector, into a suitable host. Hosts for expression vectors can be prokaryotes such as the bacterium *Escherichia coli*; alternatively, where posttranslational processing is required, as with some human proteins, a eukaryotic host is usually required, e.g., a yeast.

A vast range of important products, many of which were formerly manufactured by chemical processes, are now most economically produced by microbial-assisted or biotransformation processes. Microorganisms also provide valuable services. They have proved to be particularly useful because of the ease of their mass cultivation, speed of growth, use of cheap substrates that in many cases are wastes, and the diversity of potential products. In addition, their ability to readily undergo genetic manipulation has opened up almost limitless possibilities for new applications.

Successful development of an industrial bioprocess requires major contributions from a wide range of other disciplines, particularly biochemistry, genetics and molecular biology, chemistry, chemical and process engineering, and mathematics and computer technology. All industrial bioprocesses can be divided into two step operations which involve both upstream processing (USP) and downstream processing (DSP) stages.

Upstream Processing

The upstream part of a bioprocess refers to the first step in which biomolecules are grown, usually by bacterial or mammalian cell lines in bioreactors. When they reach the desired density (for batch and fed-batch cultures), they are harvested and moved

to the downstream section of the bioprocess. Key factors relating to this process are as follows: the strategy for initially obtaining a suitable industrial microorganism, strain improvement to enhance productivity and yield, maintenance of strain purity, preparation of a reliable inoculum, and the continuing development of selected strains to improve the economic efficiency of the process.

Downstream Processing

Downstream processing encompasses all operational steps following the preparation of industrial microorganisms. It has the primary aim of efficiently, reproducibly, and safely recovering the target product to the required specifications (biological activity, purity, etc.), while maximizing recovery yield and minimizing costs. The target product may be recovered by processing the cells or the spent medium depending upon whether it is an intracellular or extracellular product. The level of purity that must be achieved is usually determined by the specific use of the product. Often, a product's purity will be defined by what is not present rather than what is. Purity of an enzyme, for example, is expressed as units of enzyme activity per unit of total protein. Not only is it important to reduce losses of product mass, but in many cases retention of the product's biological activity is vitally important (Fig. 8).
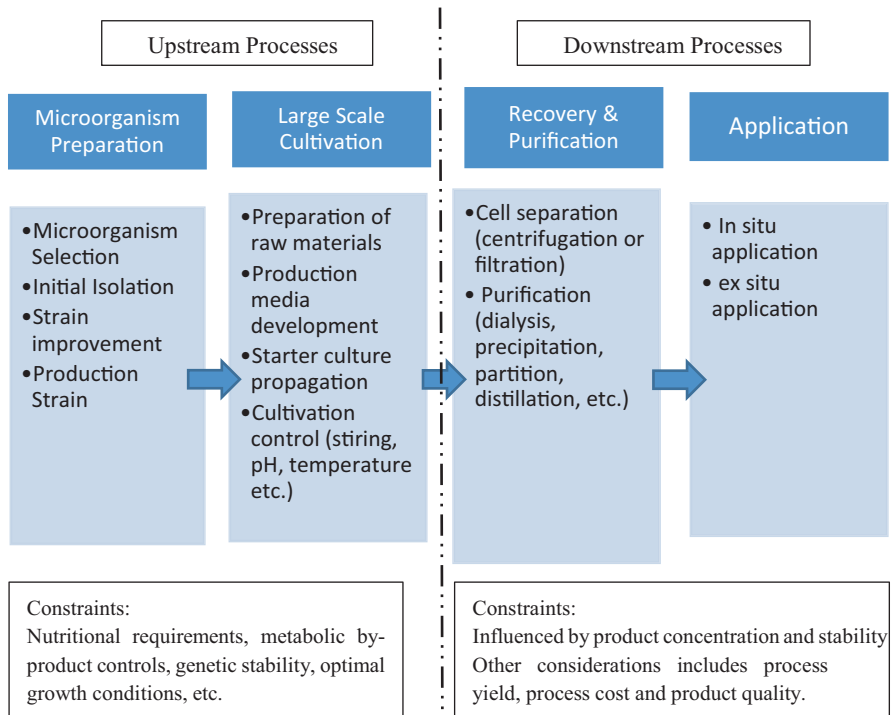

Fig. 8   Outline of an industrial bioprocess—upstream and downstream processing operations

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Physicochemical and Biological Processes in Iron Ore Bioprocessing

1 Introduction

The role of microorganisms is of great importance in the biologically assisted iron ore processing systems. They can form or secrete various kinds of complex-forming organic species/compounds (chelators) which react with alumina/silicates or silicates to solubilize them. Microbes also play key roles in the areas of element biotransformations and biogeochemical cycling, metal and mineral transformations, decomposition, bioweathering, and soil and sediment formation. All kinds of microbes, including prokaryotes and eukaryotes and their symbiotic associations with each other and "higher organisms," can contribute actively to geological phenomena, and central to many such geomicrobial processes are transformations of metals and minerals. Microbes have a variety of properties that can effect changes in metal speciation, toxicity, and mobility, as well as mineral formation or mineral dissolution or deterioration. Such mechanisms are important components of natural biogeochemical cycles for metals as well as associated elements in biomass, soil, rocks, and minerals, e.g., sulfur and phosphorus, and metalloids, actinides, and metal radionuclides. Apart from being important in natural biosphere processes, metal and mineral transformations can have beneficial or detrimental consequences in a human context.

2 Principles of Microbial Interaction with Iron Ores

Mineralytic effects of microorganisms on minerals are based mainly on three principles, namely acidolysis, complexolysis, and redoxolysis. Microorganisms are able to mobilize metals by (1) the formation of organic or inorganic acids (protons); (2) oxidation and reduction reactions; and (3) the excretion of complexing agents. Sulfuric acid is the main inorganic acid found in leaching environments. It is formed

by sulfur-oxidizing microorganisms such as thiobacilli. A series of organic acids are formed by bacterial (as well as fungal) metabolism resulting in organic acidolysis, complex, and chelate formation. A kinetic model of the coordination chemistry of mineral solubilization has been developed which describes the dissolution of oxides by the protonation of the mineral surface as well as the surface concentration of suitable complex-forming ligands such as oxalate, malonate, citrate, and succinate. Proton-induced and ligand-induced mineral solubilization occurs simultaneously in the presence of ligands under acidic conditions (Fig. 1).

Chemical weathering of minerals during pedogenesis can be enhanced by microbial activity by a factor as high as 10^6 (Kurek, 2002). Microorganisms can dissolve minerals by direct and indirect actions under aerobic and anaerobic conditions (Ehrlich, 2002, Kurek, 2002). In some cases of attack, the microorganism may be dispersed in the soil solution; in others, they may grow in biofilms on the surface of susceptible minerals.

Oxidation by chemolithotrophic microorganisms of the sulfur entities of metal sulfides to obtain energy is an example of direct dissolving action under aerobic condition (Kurek, 2002). Compounds of many other oxidized metals and metalloids such as Fe (III), Mn (IV), and As (V) can act as electron acceptors in anaerobic environments. Under such conditions, anaerobic respiration becomes an example of direct dissolving action of oxidized metal and metalloid compounds.

Fungi can adsorb potassium from solution and thus shift potassium equilibrium in suspension of trioctahedral and dioctahedral micas and transfer them to vermiculites (Weed et al., 1969). Similar processes can also occur for many major and trace elements (Robert & Berthelin, 1986). Because their small size, microorganisms
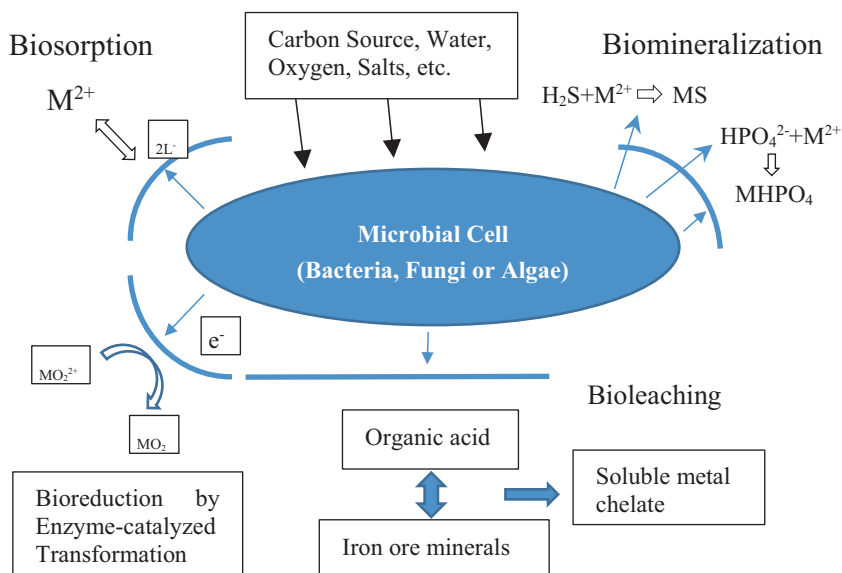
Fig. 1 Mechanism of microorganism interactions with minerals

provide a large contact area that interacts with metals in the environment. Microbial metal accumulation has recently received much attention particularly due to the potential use of microorganisms for cleaning metal-polluted water. The different accumulation processes that microorganisms perform have been analyzed and their potential significance in soil system has been addressed. Different mechanisms can be involved in the accumulation of metals by microorganisms, e.g., adsorption, precipitation, complexation, and active transport into the cell. Physicochemical parameters such as pH, ionic strength, ionic composition, as well as biochemical and biological factors are of importance in influencing the magnitude of accumulation. Several recent studies have applied surface complexation theory to model metal adsorption behavior onto microorganisms (Burnett et al., 2006). Surface complexation models (incorporating the Dorman electrostatic model) have been developed to determine stability constants corresponding to specific adsorption reactions. Adsorption reactions and stoichiometries have been constrained using spectroscopies such as attenuated total reflectance FTIR (ATRFTIR), X-ray absorption near edge structure (XANES), and extended X-ray absorption fine structure (EXAFS). Molecular simulations of metal adsorption to microbial surfaces have recently been reported; force field-based simulation techniques can adequately describe the interactions of Cd with the cell wall, defining both metal ion coordination and binding distances (Johnson et al., 2006). These research findings further indicate that microorganisms should also accumulate metals in soils and the amounts accumulated may be considerable. Therefore, much work remains to be done, with focus on mechanisms of microbial accumulation of metals in soils. Considerable less attention has been paid to the role of microorganisms in metal mobility. It is, thus, important to determine the overall influence of soil microorganisms on metal accumulation and mobility and to quantify these processes.

Metal diagenesis, which is the transformation of one mineral into another by some microorganisms, can be an indirect effect of aerobic and anaerobic microbial metabolism (Ehrlich, 2002; Kurek, 2002). The formation of a new mineral can be resulted from a chemical reaction between products of microbial dissolution of a mineral in the environment.

The physical and chemical characteristics of bacteria, such as their large surface area-to-volume ratio, serve to increase the metal-binding capacity of their charged surfaces leading to precipitation and formation of mineral phases on their cell walls or other surface polymers (McLean et al., 2002). Therefore, geochemical modeling of metal speciation and transport is beginning to include bacteria as geochemically active surfaces (Huang and Bollag, 1999; Burnett et al., 2006). The mechanisms by which bacteria initiate the formation of minerals in bulk solution very widely between species. There may be a combination of biochemical and surface-mediated reactions during the process.

2.1 Metal Mobilization

Originally, a model with two types of mechanisms which are involved in the microbial mobilization of metals has been proposed (Silverman and Ehrlich, 1964): (1) Microorganisms can oxidize metal sulfides by a "direct" mechanism obtaining electrons directly from the reduced minerals. Cells have to be attached to the mineral surface, and a close contact is needed. The adsorption of cells to suspended mineral particles takes place within minutes or hours. This has been demonstrated using either radioactively labeled Thiobacillus ferrooxidans cells grown on $NaH_{14}CO_3$ or the oxidative capacity of bacteria attached to the mineral surface (Escobar et al., 1996). Cells adhere selectively to mineral surfaces occupying preferentially irregularities of the surface structure (Edwards et al., 1999). In addition, a chemotactic behavior to copper, iron, or nickel ions has been demonstrated for Leptospirillum ferrooxidans (Acuna et al., 1992). Genes involved in the chemotaxis were also detected in *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans* (Acuna et al., 1992). (2) The oxidation of reduced metals through the "indirect" mechanism is mediated by ferric iron (Fe^{3+}) originating from the microbial oxidation of ferrous iron (Fe^{2+}) compounds present in the minerals. Ferric iron is an oxidizing agent and can oxidize, e.g., metal sulfides, and is (chemically) reduced to ferrous iron which, in turn, can be microbially oxidized again. In this case, iron has a role as electron carrier. It was proposed that no direct physical contact is needed for the oxidation of iron.

In many cases, it was concluded that the "direct" mechanism dominates over the "indirect" mostly due to the fact that "direct" was equated with "direct physical contact." This domination has been observed for the oxidation of covellite or pyrite in studies employing mesophilic T. ferrooxidans and thermophilic Acidianus brierleyi in bioreactors which consisted of chambers separated with dialysis membranes to avoid physical contact (Larsson et al., 1993; Pogliani et al., 1990). However, the attachment of microorganisms on surfaces is not an indication per se for the existence of a direct mechanism (Edwards et al., 1999). The term "contact leaching" has been introduced to indicate the importance of bacterial attachment to mineral surfaces.

The following equations describe the "direct" and "indirect" mechanisms for the oxidation of pyrite:

Direct:

$$2FeS_2 + 7O_2 + 2H_2O \overset{\text{Thiobacilli}}{\Rightarrow} 2FeSO_4 + 2H_2SO_4 \tag{1}$$

Indirect:

$$4FeSO_4 + O_2 + 2H_2SO_4 \overset{\substack{\text{T.ferrooxidans} \\ \text{L.ferrooxidans}}}{\Rightarrow} 2Fe_2(SO_4)_3 + 2H_2O \tag{2}$$

$$FeS_2 + Fe_2(SO_4)_3 \overset{\text{Chemical oxidation}}{\Rightarrow} 2FeSO_4 + 2S \tag{3}$$

$$2S + 3O_2 + H_2O \overset{\text{T.thiooxidans}}{\Rightarrow} 2H_2SO_4 \tag{4}$$
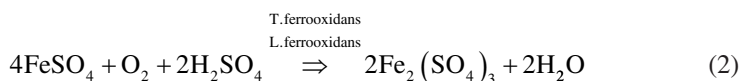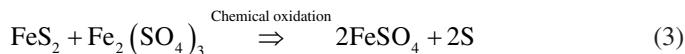
However, the model of "direct" and "indirect" metal leaching is still under discussion. Recently, this model has been revised and replaced by another one which is not dependent on the differentiation between a "direct" and an "indirect" leaching mechanisms (Sand et al., 1995, 1999). All facts have been combined, and a mechanism has been developed which is characterized by the following features: (1) Cells have to be attached to the minerals and in physical contact with the surface; (2) cells form and excrete exopolymers; (3) these exopolymeric cell envelopes contain ferric iron compounds which are complexed to glucuronic acid residues. These are part of the primary attack mechanism; (4) thiosulfate is formed as intermediate during the oxidation of sulfur compounds; and (5) sulfur or polythionate granules are formed in the periplasmic space or in the cell envelope.

Thiosulfate and traces of sulfite have been found as intermediates during the oxidation of sulfur. Sulfur granules (colloidal sulfur) have been identified as energy reserves in the exopolymeric capsule around cells of T. ferrooxidans during growth on synthetic pyrite films (Rojas et al., 1995). "Footprints" of organic films containing colloidal sulfur granules are left on the mineral surface upon detachment of the bacteria. From the existing data, two "indirect" leaching mechanisms have been proposed, whereas no evidence for a "direct" enzymatically mediated process has been found (Sand et al., 1999). The mineral structure is the determining factor for the prevailing type of leaching mechanism. In the "thiosulfate mechanism," thiosulfate is the main intermediate resulting from the oxidation of pyrite, molybdenite, or tungstenite. Polysulfide and elemental sulfur are the main intermediates in the "polysulfide mechanism" during the oxidation of galena, sphalerite, chalcopyrite, hauerite, orpiment, or realgar. The presence of iron (III) at the beginning of mineral degradation is an important prerequisite (Sand et al., 1999).

The following equations summarize the oxidation mechanisms (Sand et al., 1999):
Thiosulfate mechanism (found for FeS_2, MoS_2, WS_2):

$$FeS_2 + 6Fe^{3+} + 3H_2O \Rightarrow S_2O_3^{2+} + 7Fe^{2+} + 6H^+ \tag{5}$$

$$S_2O_3^{2-} + 8Fe^{3+} + 5H_2O \Rightarrow 2SO_4^{2-} + 8Fe^{2+} + 10H^+ \tag{6}$$
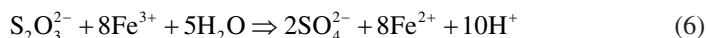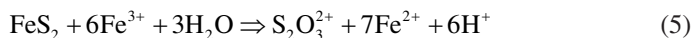
Polysulfide mechanism (found for PbS, $CuFeS_2$, ZnS, MnS_2, As_2S_3, As_3S_4):

$$2MS + 2Fe^{3+} + 2H^+ \Rightarrow 2M^{2+} + H_2S_n + 2Fe^{2+} \tag{7}$$

$$H_2S_n + 2Fe^{3+} \Rightarrow 1/4S_8 + 2Fe^{2+} + 2H^+ \tag{8}$$

$$1/4S_8 + 3O_2 + 2H_2O \Rightarrow 2SO_4^{2-} + 4H^+ \tag{9}$$
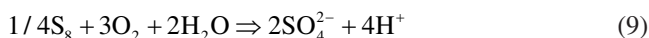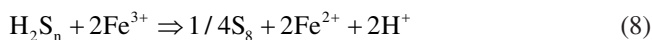
Several biomolecules are involved in the aerobic respiration on reduced sulfur and iron compounds. It has been found that up to 5% of soluble proteins of *T. ferrooxidans* is made of an acid-stable blue copper protein, called rusticyanin (Blake et al., 1993). Additionally, the iron (II) respiratory system contains a (putative) green copper protein, two types of cytochrome c, one or more types of cytochrome a, a porin, and an iron (II) sulfate chelate (Blake et al., 1993). The acid stability of rusticyanin suggests that it is located in the periplasmic space. Figure 2 shows a scheme of the model which combines the electron transport sequence proposed earlier with concepts stemming from the debate on "direct"/"indirect" leaching mechanisms (Blake and Shute, 1994; Blake et al., 1993; Hazra et al., 1992; Sand et al., 1995).

Microbes can also mobilize metals and attack mineral surfaces by redox processes: Fe(III) and Mn(IV) solubility is increased by reduction to Fe(II) and Mn(II), respectively (Figs. 2 and 3). Microbial reduction of Fe(III) and Mn(IV) may also be a means for releasing contaminant metals adsorbed to Fe(III) and Mn(IV) oxides, and this process may be enhanced by humic materials or related compounds (Lovley & Coates, 1997; Lloyd et al., 2003). Ferric iron, Fe(III), can be enzymatically
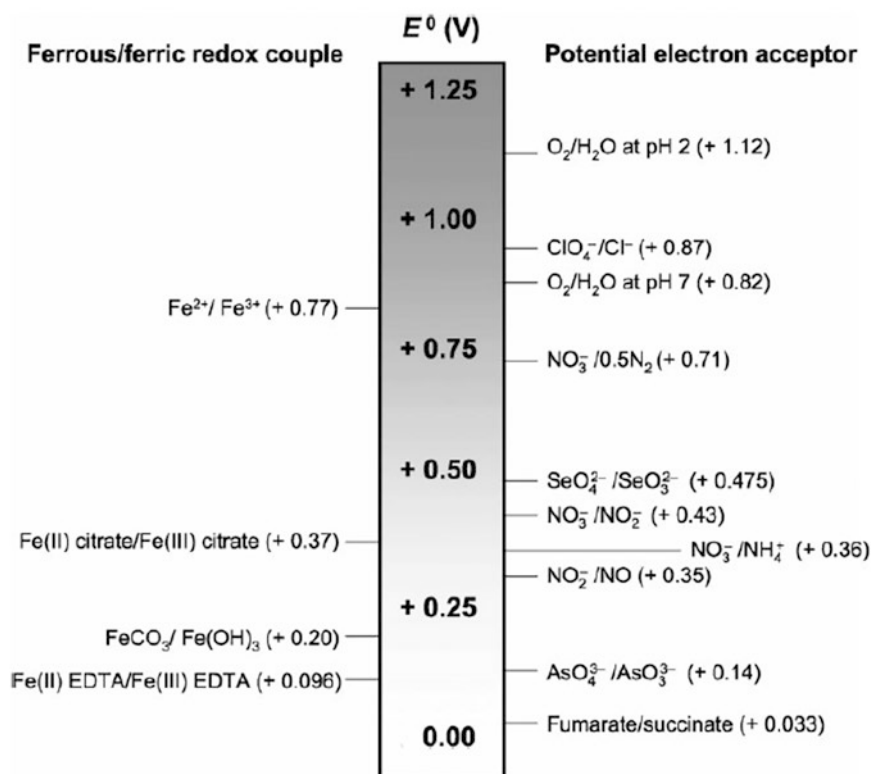
Fig. 2 Different redox potentials of soluble and insoluble ferrous/ferric couples, as well as those of other couples that serve as electron acceptors for iron-oxidizing microorganism.

reduced to ferrous iron, Fe(II), with a suitable electron donor (Schro¨der et al., 2003). Many Fe(III) reducers are heterotrophs, and in some anaerobic environments such Fe(III) respiration may be a more important mechanism of carbon source decomposition than sulfate reduction (Ehrlich & Newman, 2009). Some Fe(III) reduction can be the result of metabolic products such as H2S or formate or other secondary metabolites. Naturally occurring microbially produced metal chelators that may solubilize Fe(III) include oxalate, citrate, humic acids, and tannins.

Some species of microorganisms that oxidize ferrous iron are also able to catalyze the dissimilatory reduction of ferric iron, where the latter acts as the sole or major electron acceptor in anaerobic respiration (Lovley, 1997; Lovley et al., 2004). Both organic and inorganic materials can be used as electron donors for iron reduction. While in most cases proteobacteria that reduce or oxidize iron are distinct species, some, described below, can both oxidize and reduce iron, depending on the prevailing environmental conditions. Cycling of iron mediated by microbiological oxidation–reduction of iron is an important process in the environment, both on the microscale and the global scale (Fig. 3). Iron-oxidizing proteobacteria have an important role in facilitating ferric iron reduction in the environment, as their solid-phase end products (e.g., schwertmannite and ferrihydrite) are much more reactive than other ferric iron minerals such as goethite and hematite, which are often more abundant in the environment. In the case of extreme acidophiles, the end product of iron oxidation is often soluble ferric iron, which is much more readily reduced than amorphous or crystalline forms, and ferric iron respiration appears to be widespread among acidophilic proteobacteria. Since the redox potential of the soluble ferric/ferrous couple is not much less positive than that of the oxygen/water couple,
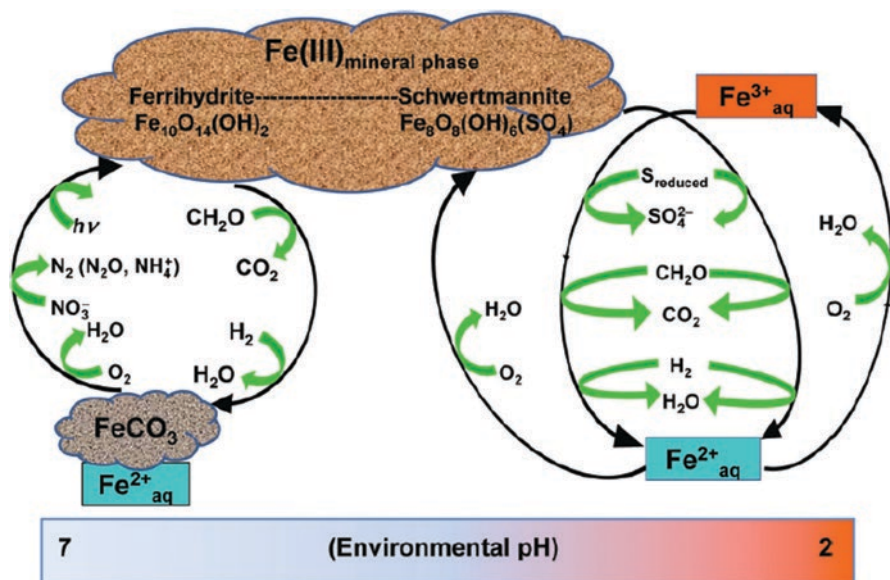
Fig. 3 Microbially mediated cycling of iron in neutral and acidic environments

facultative anaerobic acidophiles that can use ferric iron as an electron acceptor do not suffer such a thermodynamic "penalty" for switching electron acceptors as their neutrophilic counterparts.

2.2   Metal Immobilization

Microbial biomass provides a metal sink, by biosorption to cell walls, pigments and extracellular polysaccharides, intracellular accumulation, or precipitation of metal compounds in and/or around cells, hyphae, or other structures. All microbial materials can be effective biosorbents for metals except for mobile alkali metal cations like Na + and K+, and this can be an important passive process in living and dead organisms (Wang & Chen, 2009).

In natural systems, metal bioavailability is determined by interactions with environmental components, such components including clay and other minerals, humic substances, soil colloidal materials, biogenic debris and exudates, and living organisms. Sorption is one of the most important reactions that influence bioavailability, and metal sorption to cells is likely to play an important role in all microbe–metal–mineral interactions (Burford et al., 2003a), taking place over a massive range of timescales from milliseconds to years (Borda & Sparks, 2008; Theng & Yuan, 2008). Metal interactions with specific cell surface groups may also enhance or inhibit metal transport, metal transformations, and biomineralization processes (Barkay & Schaefer, 2001). The major biosphere compartments, such as soil and the oceans, contain a vast amount of metal-sorbing material with high surface area-to-volume ratios: bacteria have the highest surface area-to-volume ratios of any living organisms. Microbes are major components of the soil, while biogenic particles dominate oceanic detrital phases (Stumm & Morgan, 1996). Many studies have shown that microbial cells, on a specific unit area basis, can exhibit higher sorption values for metals than even clay minerals (Garnham et al., 1993; Morley & Gadd, 1995). It is possible that biosorption phenomena have a more significant role in metal/radionuclide speciation, bioavailability, and mobility in the biosphere than has previously been supposed (Krantz-Rulcker et al., 1993, 1996; Ledin et al., 1996; McLean et al., 2002). This may also accompany or precede nucleation, precipitation, and biomineral formation (Burford et al., 2003a; Gadd, 2007). Where microbial reduction of a metal to a lower redox state occurs, mobility and toxicity may be decreased for several elements (Lovley, 2001; Lloyd & Lovley, 2001; Finneran et al., 2002a, b; Lloyd et al., 2003; Holden & Adams, 2003; Wall & Krumholz, 2006), e.g., U(VI) to U(IV) and Cr(VI) to Cr(III) (Phillips et al., 1995; Smith & Gadd, 2000). U(VI) reduction to U(IV) can be the basis of U removal from contaminated waters and leachates as well as the formation of uraninite (UO2) (Lovley & Coates, 1997; Lovley, 2001; Finneran et al., 2002a, b; Lloyd, 2003; Lloyd & Renshaw, 2005; Landa, 2005). Anaerobically, hexavalent U(VI) can be reduced to tetravalent U(IV) by a number of bacteria using either H2 or one of a variety of organic electron donors (Lovley & Coates, 1997;

Landa, 2005; Wall & Krumholz, 2006). Aerobic or anaerobic microbial reduction of Cr(VI) to Cr(III) is widespread (Smith & Gadd, 2000; McLean & Beveridge, 2001). Sulfur- and sulfate-reducing bacteria are particularly important in reductive precipitation of U(VI), Cr(VI), Tc(VII), and Pd(II) (Aubert et al., 1998; Lloyd et al., 1999a, b; Lloyd & Macaskie, 1998; Lloyd, 2003; Lloyd & Renshaw, 2005). Some sulfate-reducing bacteria such as Desulfotomaculum reducens share physiological properties of both sulfate- and metal-reducing groups of bacteria and can use Cr(VI), Mn(IV), Fe(III), and U(IV) as sole electron acceptors (Tebo & Obraztsova, 1998). Such direct processes may accompany indirect mechanisms of reductive metal precipitation, e.g., in sulfate-reducing bacterial systems, where reduction of Cr(VI) can be a result of indirect reduction by Fe2+ and the produced sulfide. Elemental silver (Ag0) and gold (Au0) species result during microbial reduction of ionic silver and gold species (Kierans et al., 1991; Holden & Adams, 2003; Southam et al., 2009). Other redox transformations of metals such as Mo, V, and Sb are also known, which must play a role in their speciation, although rather less is known about such rarer elements. Microbes can also mediate formation of several inorganic and organic biominerals, e.g., oxalates, phosphates, sulfides, oxides, and carbonates, which lead to metal immobilization (Gadd, 2007).

Weathering of iron-containing minerals in rocks, soils, and sediments is promoted partly by bacterial and fungal action and partly by chemical activity (Lovley, 2000). Mobilized ferrous iron, Fe(II), may be biologically or abiotically oxidized to Fe(III) at pH.5 under anaerobic or partially or fully aerobic conditions. Some bacteria can oxidize ferrous iron enzymatically with the generation of energy, e.g., acidophiles such as Acidithiobacillus ferrooxidans, Leptospirillum ferrooxidans, Sulfolobus spp., Acidianus brierleyi, and Sulfobacillus thermosulfidooxidans. Fe(II) is least susceptible to autoxidation below pH 5. Some bacteria growing at circumneutral pH can also oxidize Fe(II) enzymatically under partially reduced conditions, e.g., the stalked bacterium Gallionella ferruginea and sheathed bacteria such as Leptothrix spp. (Ehrlich & Newman, 2009). Fe(II) can also be oxidized nonenzymatically by microbes when their metabolic activities alter the microenvironment to favor autoxidation. Some Fe(III) precipitation may also occur as a result of the destruction of ferric iron chelates. Fe(III) may also be locally concentrated by adsorption to microbial surfaces and metal oxides. Microbial formation of hydrous iron oxides in aqueous environments may cause accumulation of other metal ions by coprecipitation or adsorption: Such adsorbed metals may be remobilized by reduction of the iron oxides or acidification (Ehrlich & Newman, 2009).

2.3 Organic Matter Decomposition

Organic matter decomposition is one of the most important microbial activities in the biosphere, and the ability of microbes, mainly bacteria and fungi, to utilize a wide spectrum of organic compounds is well known. These range from simple compounds such as sugars, organic acids, and amino acids to more complex molecules which

may be broken down by extracellular enzymes before cellular uptake and metabolism. These latter compounds include cellulose, pectin, lignin, lignocellulose, chitin and starch, and also hydrocarbons, pesticides, and other xenobiotics that may be anthropogenically produced. Degradation of such substances results in redistribution of component elements between organisms and environmental compartments. The vast majority of elements in plant, animal, and microbial biomass comprise carbon, hydrogen, oxygen, nitrogen, phosphorus, and sulfur. Several other elements are typically found in living organisms, most with essential biochemical and structural functions, e.g., K, Ca, Mg, B, Cl, Fe, Mn, Zn, Cu, Mo, Ni, Co, Se, Na, and Si. However, all 90 or so naturally occurring elements may be found in plants, animals, and microbes, including Au, As, Hg, Pb, Cd, and U. Some of these elements will be taken up as contaminants in food and from the environment. Therefore, it should be stressed that all decomposition, degradative, and pathogenic microbial activities are linked to the cycling of these constituent elements, most of which are metals and some of which may be radionuclides accumulated from anthropogenic sources. This simple perspective on organic matter decomposition illustrates the global involvement of microbes in almost all elemental cycles. Biodegradation of organometallic (and organometalloid) compounds, still widely used in agriculture and industry, can result from direct enzymatic action or by microbial facilitation of abiotic degradation, e.g., by alteration of pH and excretion of metabolites (Gadd, 1993b, 2000b). Organotins, such as tributyltin oxide and tributyltin naphthenate, may be degraded to monobutyltin and dibutyltin, inorganic Sn(II) being the ultimate product (Gadd, 2000b). Organomercury compounds may be detoxified by organomercury lyase, the resultant Hg2+ being subsequently reduced to the less toxic, diffusible, and volatile Hg0 by mercuric reductase (Gadd, 1993b).

2.4   Common Biological Transformations of Minerals by Microbes

Silicates

Silica, silicates, and aluminosilicates in rocks are weathered by biological, chemical, and physical processes (Brehm et al., 2005). Silicon liberated as soluble silicate can be assimilated by several microbial groups in order to fabricate cell support structures. These groups include diatoms, some chrysophytes, silicoflagellates, some xanthophytes, radiolaria, and actinopods. Silicon-assimilating microbes such as diatoms and radiolaria are important in the formation of oceanic siliceous oozes, while diatoms are important in forming such oozes in lakes. Such "cellular" silicon may later be returned to solution by weathering processes. Biosilicification of natural microbial mats has been shown to be a microbially mediated geochemical process, with a requirement for colloidal silica, an acidic pH and exposed organic surfaces, both of which favor colloidal silica sorption (Amores & Warren, 2007). Silicon dioxide, when combined with oxides of magnesium, aluminum, calcium,

and iron, forms the silicate minerals in rocks and soil (Bergna, 1994). Silicates are the largest class of minerals, comprising 30% of all minerals and making up 90% of the Earth's crust (Ehrlich, 1998; Ehrlich & Newman, 2009). Silicate minerals are unstable in the biosphere and break down readily to form clays (Adamo et al., 2002; Tazaki, 2006). Many kinds of bacteria, fungi, and lichens play an important role in the dissolution of silicates, and therefore in the genesis of clay minerals, and in soil and sediment formation (Barker & Banfield, 1996, 1998; Rodriguez Navarro et al., 1997; Banfield et al., 1999; Adamo & Violante, 2000; Arocena et al., 1999, 2003; Tazaki, 2006; Theng & Yuan, 2008; Cockell et al., 2009; Ehrlich & Newman, 2009). Even silicates of great physical and chemical resistance can be attacked, e.g., quartz sand, crystalline quartz, and commercial glass (Brehm et al., 2005). Microbial action is mainly indirect, through either the production of chelates or the production of acids (mineral or organic) or other metabolites, together with biomechanical effects (Cromack et al., 1979; De La Torre et al., 1992; Mandal et al., 2002). In bioweathering of rock silicates and aluminosilicates, cleavage of Si–O–Si (siloxane) or Al–O bonds or removal of cations from the silicate crystal lattice may cause collapse of the silicate lattice structure. The mechanisms of attack may include microbially produced (1) ligands of cations; (2) organic or inorganic acids (a source of protons); (3) alkali (ammonia or amines); or (4) extracellular polysaccharides that act at acidic pH. Such agents may be excreted into the bulk phase but may also involve attached biofilm microbes on surfaces of silica or silicates resulting in etching (Bennett et al., 1996, 2001). Such mechanisms of silicate dissolution may be instrumental in releasing limiting nutrients such as bound phosphorus and iron (Rogers & Bennett, 2004). For several bacteria, dissolution of silicates results from complexation of cationic components by 2-ketogluconate. Quartz (SiO_2) can be subject to slow dissolution by organic acids such as citric and oxalic acid (Bennett et al., 1988), the mechanism of action being chelation rather than protonation. Hydration of respiratory or fermentative CO_2 to give the weak carbonic acid H_2CO_3 can also result in solubilization of silicates. Alkaline conditions can help mobilize silicon from silicates, and facilitate ammonia production from urea hydrolysis with bacterial mechanism (Ehrlich & Newman, 2009). In lichen, weathering of silicate minerals, calcium, potassium, iron clay minerals, and nanocrystalline aluminous iron oxyhydroxides becomes mixed with fungal organic polymers (Barker & Banfield, 1998), while biotite was interpenetrated by fungal hyphae along cleavages, partially converting it to vermiculite (Barker & Banfield, 1996). The fungal partner has also been reported to be involved in the formation of secondary silicates, such as opal and forsterite, in lichen thalli (Gorbushina et al., 2001). The transformation rate of mica and chlorite to 2:1 expandable clays was pronounced in ectomycorrhizosphere soil and was probably a result of the high production of organic acids and direct extraction of K+ and Mg^{2+} by fungal hyphae (Arocena et al., 1999). Silicon compounds in the form of clays (aluminosilicates) can exert many effects on microbes in soil and stimulate or inhibit metabolism (Marshall, 1971; Marshman & Marshall, 1981a, b; Weaver & Dugan, 1972; Theng & Yuan, 2008). Effects of clays are mostly indirect and arise from physicochemical effects of clays on the microenvironment, e.g., action as buffers, and as sorptive agents for cells, metabolites, ions,

and enzymes (Tazaki, 2006; Ehrlich & Newman, 2009). Clay minerals (bentonite, palygorskite, and kaolinite) can also influence the size, shape, and structure of fungal mycelial pellets in liquid culture.

Bauxite

Aluminum is the third most abundant element in the Earth's crust after silicon and oxygen. Various microbes are involved in the formation of some aluminum-containing minerals through bioweathering. The formation of bauxite (bauxitization) involves two stages where microbes are involved. The major constituents of bauxite are Al_2O_3, Fe_2O_3, and SiO_2 or aluminosilicate in various forms, and the source material for bauxitization may be volcanic and other aluminosilicate rocks, limestone, and alluvium. Weathering of source rock (formation of protobauxite) is promoted by those activities of bacteria and fungi that mobilize aluminum, iron, and silicon, which are then subsequently precipitated as oxides, silica, and silicate minerals. Maturation of protobauxite to bauxite is promoted by iron-reducing and fermentative bacteria under anaerobic conditions, which selectively mobilize iron oxides and silica or silicate, and enrich the bauxite in aluminum (Ehrlich & Newman, 2009).

Carbonates

Certain bacteria, cyanobacteria, and fungi can deposit calcium carbonate extracellularly (Verrecchia et al., 1990; Chafetz & Buczynski, 1992). Mineralized carbonate precipitates are also found in association with bacterial biofilms. Some algae, including certain green, brown and red algae, and chrysophytes, such as coccolithophores, deposit calcium carbonate as cell surface structures, while some protozoa (foraminifera) use it for tests or shells. Calcium, as well as some magnesium ions, can combine with carbonate ions of biogenic origin sometimes to massive scales; e.g., the White Cliffs of Dover, UK, is a biogenic carbonate deposit in the form of chalk. Carbonate precipitation is possibly the most important process that influences global carbon cycling (Dupraz et al., 2009). Calcium carbonate associated with stromatolites, originating from types of cyanobacterial mats, may be a result of calcium carbonate entrapment or deposition as well as cyanobacterial photosynthesis and bacterial activities.

Calcium carbonate associated with travertine (a porous limestone) and lacustrine carbonate crusts and nodules can result from cyanobacterial photosynthesis in freshwater environments. Calcareous nodules are formed around rounded rocks, stones, pebbles, shells, etc., to which calcium carbonate-depositing cyanobacteria are attached. Most bacteria, including cyanobacteria, and some algae cause precipitation of $CaCO_3$ close to or at their cell surface. In contrast, some algae and protozoa form $CaCO_3$ intracellularly and then export it to the cell surface to become support structures. These include the coccolithophores (green algae) and foraminifera (Protozoa,

Sarcodina), the mineral form of calcium carbonate deposited being calcite or aragonite. Apart from calcium and magnesium carbonates, others that may have a microbial involvement in their formation include hydromagnesite [$Mg_5(CO_3)_4(OH)_2.4 H_2O$], $SrCO_3$, siderite ($FeCO_3$), rhodochrosite ($MnCO_3$), and sodium carbonate (natron, $Na_2CO_3.10H_2O$) (Ehrlich & Newman, 2009). The range of composition and crystallography of carbonate precipitates produced by microbial communities is influenced by environmental conditions and community species composition (Dupraz & Visscher, 2005). Key components of carbonate biomineralization in microbial mat systems are the "alkalinity" engine (microbial metabolism and environmental conditions which affect the calcium carbonate saturation index) and microbially produced extracellular polymeric substances which provide a template for carbonate nucleation (Dupraz et al., 2009). Alkalinity produced by sulfate-reducing bacteria has a prominent role in such carbonate deposition (Dupraz et al., 2009).

Insoluble carbonates may be broken down by microbial attack. This is usually the result of organic and inorganic acid formation but may also involve physical processes (Lian et al., 2008a). Various bacteria, fungi, lichens, cyanobacteria, and even algae have been implicated (Schneider & Le Campion-Alsumard, 1999; Adamo & Violante, 2000; Hoppert et al., 2004; Cockell & Herrera, 2008; Lian et al., 2008a). Such activity is evident on limestones and marble used in building construction, and also in natural limestone formations such as coral reefs, where limestone-boring cyanobacteria, algae, and fungi are active in the breakdown process (Golubic et al., 2005; Cockell & Herrera, 2008). Bacteria and fungi contribute to the discoloration and destruction of structural limestone and marble and are also involved in patina formation. Fungal attack on carbonate substrates (dolomites and limestones) can result in significant microbial diagenesis of these substrates to neo-dolomite, glushinskite, weddellite, whewellite, and possibly struvite, as well as intense substrate "de-micritization" and "micritization" with oxalates, grain bridging and cementation, open-space filling, formation of intergranular and intragranular porosity, and permeability enhancement. Advanced stages of diagenesis were characterized by dissolution and replacement of the original minerals by the new substrates produced by fungal biomineralization processes (Kolo et al., 2007).

Oxalates

Calcium oxalate is the most common form of oxalate encountered in the environment, mostly occurring as the dihydrate (weddellite) or the more stable monohydrate (whewellite) (Gadd, 1999). Calcium oxalate crystals are commonly associated with free-living, pathogenic and plant symbiotic fungi, and lichens, and are formed by the precipitation of solubilized calcium as the oxalate (Gadd, 1999; Gharieb et al., 1998; Adamo & Violante, 2000; Adamo et al., 2002). Biotic fungal calcium oxalate can exhibit a variety of crystalline forms (tetragonal, bipyramidal, plate-like, rhombohedral, or needles) (Arnott, 1995). Calcium oxalate precipitation has an important influence on biogeochemical processes in soils, acting as a reservoir for

calcium, and also influencing phosphate availability. Fungi can also produce other metal oxalates on interacting with a variety of different metals and metal-bearing minerals, e.g., Ca, Cd, Co, Cu, Mg, Mn, Sr, Zn, Ni, and Pb (White et al., 1997; Gadd, 1999; Sayer et al., 1999; Sayer & Gadd, 1997; Gadd, 2007). The formation of toxic metal oxalates may also provide a mechanism enabling fungi to tolerate high concentrations of toxic metals (Gadd, 1993a; Jarosz-Wilkołazka & Gadd, 2003). In many arid and semi-arid regions, calcareous soils and near-surface lime-stones (calcretes) are secondarily cemented with calcite ($CaCO_3$) and whewellite (calcium oxalate monohydrate, $CaC_2O_4.H_2O$). The presence of fungal filaments mineralized with calcite, together with whewellite, has been reported in limestone and calcareous soils from a range of localities (Verrecchia, 2000). Calcium oxalate can also be degraded to calcium carbonate, and this may again cement preexisting limestones (Verrecchia et al., 2006). During the decomposition of fungal hyphae, calcite crystals can act as sites of further secondary calcite precipitation. Calcite will also readily nucleate on chitin, an important component of fungal cell walls. Other experimental work has demonstrated fungal precipitation of secondary calcite, whewellite, and glushkinskite ($MgC_2O_4.2H_2O$) (Burford et al., 2003a, b, 2006; Gadd, 2007). Fungal attack on dolomitic and seawater substrates resulted in the formation of calcium oxalates (weddellite, whewellite) and glushinskite (Kolo & Claeys, 2005).

Oxides

A good example of microbial oxide formation is provided by manganese. Many bacterial species can oxidize this metal, which is then deposited on cells, sheaths, or spores as oxides (Tebo et al., 2005). Some species promote oxidation nonenzymatically, others enzymatically, with possible involvement of a multicopper oxidase system; they include spore-forming and nonspore-forming rods, sheathed, and appendaged bacteria as well as the usual morphological forms of Gram-positive and Gram-negative bacteria from a diverse range of freshwater, marine, and terrestrial ecosystems (Tebo et al., 2005). Several fungi can also promote Mn(II) oxidation to Mn(IV)O_2, including Acremonium spp. (Miyata et al., 2004, 2006, 2007; Saratovsky et al., 2009). In many cases, fungal oxidation is probably nonenzymatic and due to interaction with a metabolic product (e.g., a hydroxy acid) or a cellular component (Ehrlich & Newman, 2009), although the involvement of laccase and/or multicopper oxidases has been shown in ascomycetes, which are ubiquitous in natural environments (Miyata et al., 2004, 2007; Tebo et al., 2005).

For some basidiomycete white-rot fungi such as Phanerochaete chrysosporium, redox transformations of manganese occur during lignin degradation, but this oxidation is thought to be of minor significance regarding manganese redistribution in the environment (Ehrlich & Newman, 2009). The MnOx material produced by Acremonium KR21-2 is manifested as small crystalline particles which adopt a

todorokite-like tunnel structure; this is in striking contrast to previously reported microbial MnOx materials, which adopt layered birnessite-type structures (Saratovsky et al., 2009). Nonenzymatic microbial Mn(II) oxidation may be effected through production of metabolites, e.g., hydroxycarboxylic acids such as citrate, lactate, malate, gluconate, or tartrate. Some microbes can oxidize Mn(II) and Fe(II) in metal-bearing minerals such as siderite ($FeCO_3$) and rhodochrosite ($MnCO_3$) and precipitate them as oxides (Grote & Krumbein, 1992). Manganese and iron oxides are major components (20–30%) along with clay (~60%) and various trace elements in the brown-to-black veneers known as desert varnish or rock varnish (Grote & Krumbein, 1992; Gorbushina, 2007). The prevalence of manganese oxidizers in desert varnish implies a role for these bacteria in its formation (Ehrlich & Newman, 2009). Manganese-oxidizing and manganese-reducing bacteria also play an important role in the manganese cycle in freshwater and marine environments through the accumulation of manganese oxides in sediments. The oxides they form may be deposited as concretions formed around sediment grains, pebbles, mollusc shells, coral fragments, or other debris. Manganese oxide phases have high sorption capacities for numerous metal cations (e.g., Ni, Zn, Cu, Co, Mn, Pb, and Cd) and also serve as strong oxidants for inorganic [e.g., As(III) to As(V); Cr(III) to Cr(IV)] and organic compounds such as humic substances (Tebo et al., 2004; Miyata et al., 2007). Furthermore, in anoxic environments, manganese oxides as well as Fe(III) (hydr)oxides become terminal electron acceptors for microbial metal respiration, potentially controlling the fates of a wide variety of organic compounds such as organic acids, fatty acids, and aromatics in the environment (Lovley, 2000).

Conversely, manganese-reducing microbes may mobilize oxidized or fixed manganese, releasing it into the aqueous phase. A number of different, taxonomically unrelated bacteria can reduce manganese enzymatically or nonenzymatically. The bacteria that reduce manganese enzymatically often do so as a form of respiration where oxidized manganese serves as a terminal electron acceptor and is reduced to Mn(II) (Lovley, 2000). Some bacteria can reduce the oxidized manganese aerobically or anaerobically, whereas others can reduce it only anaerobically. Microbial reduction of oxidized manganese can also be enzymatic or nonenzymatic. Some bacteria and most of those fungi that reduce Mn(IV) oxides such as MnO_2 reduce them indirectly (nonenzymatically), with the likely mechanism being the production of metabolic products that can act as reductants for Mn(IV) oxides such as formic acid, pyruvate, H2S, sulfite, Fe(II) (bacteria), and oxalate (fungi) (Ehrlich & Newman, 2009). Microbial reduction of manganese oxides may also lead to the formation of manganous carbonate. Ferromanganese nodules on parts of the ocean floor are inhabited by manganese-oxidizing and manganese-reducing bacteria, and these are likely to contribute to nodule formation (Ehrlich & Newman, 2009). Many bacteria can precipitate and deposit Fe(III) oxides and hydroxides (e.g., FeOOH, Fe_3O_4) around their cells by enzymatic, e.g., Gallionella sp., and nonenzymatic processes, e.g., Leptothrix sp. (Ehrlich & Newman, 2009).

Phosphates

Phosphorus occurs primarily as organic phosphate esters and as inorganic forms, e.g., calcium, aluminum, and iron phosphates. Organic phosphates are hydrolyzed by phosphatases, which liberate orthophosphate during microbial decomposition of organic material. Microbes also liberate free orthophosphate from insoluble inorganic phosphates by producing organic or mineral acids or chelators, e.g., gluconate and 2-keto-gluconate, citrate, oxalate, and lactate, which complex the metal resulting in dissociation or, for iron phosphates, by producing H_2S. Phosphate-solubilizing activity is very important in the plant rhizosphere (Whitelaw et al., 1999). Microbes can also play a role in the formation of phosphate minerals such as vivianite [$Fe_3(PO_4)_2.8H_2O$], strengite ($FePO_4.2H_2O$) and variscite ($AlPO_4.2H_2O$). Here, the orthophosphate may arise from organic phosphate degradation, while Fe or Al may arise from microbial solubilization of other minerals. Such formation of phosphate minerals is probably most common in soil (Ehrlich & Newman, 2009). Secondary mycogenic uranium mineral precipitates on fungal mycelia growing in the presence of uranium oxides or depleted uranium were found to be uranyl phosphate minerals of the meta-autunite group, uramphite, and/or chernikovite (Fomina et al., 2007a, 2008).

Sulfides

Most nonferrous sulfides are formed abiotically, but some sedimentary deposits are of biogenic origin. Sulfate-reducing bacteria play an important role in some sedimentary environments in mediating the formation of certain sulfide minerals, especially pyrite (FeS_2). Microbial roles in the biogenesis of sulfide deposits arise from the generation of H_2S, usually from bacterial reduction of sulfate (Ehrlich & Newman, 2009), and reaction of metal ions with the biogenic sulfide, although some sulfide may also result from decomposition of sulfur-containing organic compounds. Metal sulfides are subject to oxidation by bacteria such as *Acidithiobacillus ferrooxidans*, *Leptospirillum ferrooxidans*, *Sulfolobus spp.,* and *Acidianus brierleyi*. The bacterial action may involve direct oxidative attack of the crystal lattice of a metal sulfide or indirect oxidative attack by generation of acid ferric sulfate, which oxidizes the metal sulfide chemically. The indirect mechanism is of primary importance in the solubilization of uraninite (UO_2). Microbial oxidation of metal sulfides is exploited industrially in extracting metals from low-grade metal sulfide ores and uraninite. In bituminous coal seams that are exposed as a result of mining activity, pyrite oxidation by these bacteria is an environmentally deleterious process and the source of acid mine drainage (Rawlings et al., 2003; Ehrlich & Newman, 2009; Jerez, 2009).

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Engineering Design and Process Requirements

1 Introduction

The use of microorganisms to recover precious metals from ores and concentrates has been adopted into industrial operations, and the bioprocessing has developed into a promising and expanding area of biotechnology. Bioprocessing is gaining importance as a unit process which involves biological organisms in mineral extraction industries worldwide. With the decreasing high-grade ore reserves and increased concern regarding the effect of mining on the environment, iron ore bioprocessing or biomining technology, which was nevertheless age-old deserted technique, is now being developed as a potential main process in the mining industry to meet the demand. Another important aspect is the feasibility of iron ore bioprocessing technologies to treat ores deposits with complex mineralogy, which could be difficult to treat by conventional methods. Besides, the most attractive feature of iron ore bioprocessing is economic feasibility compared to other competitive techniques. It has been comparatively analyzed how gold and copper bioprocessing operations played a role with the increase or decrease in metal pricing over time. The analysis results indicated that most biohydrometallurgical innovations have been commercially implemented during leaner times. Economic factors such as eliminations of high-temperature smelting and refining and possibility of the use bioleaching for on-site ore pretreatment to reduce operational costs are the reasons behind this observation.

Engineering options for bioprocessing have evolved from relatively inexpensive, partly controlled, irrigated dump, or heap reactors to sophisticated, highly controlled and expensive stirred tank reactors. Dump or heap reactors range from randomly packed, low-efficiency dumps to carefully designed heaps that are stacked, aerated, irrigated, and sometimes thermally insulated for higher levels of mineral-leaching efficiency. Dump and heap reactors are typically used for leaching low-grade, run-of-mine rock that would otherwise be discarded (used widely for copper ores), or for low-value mineral ores that do not allow for the use of expensive reactors. Stirred tank reactors consist of a series of aerated continuous-flow tanks that

are used mostly in a pretreatment process for the recovery of high-value metals, such as gold, from mineral concentrates. These reactors are more expensive to construct and operate than heap reactors but allow for the precise control of parameters such as temperature, pH, and aeration, all of which have a major impact on the microbial populations and metal recovery efficiency. Details of these different engineering configurations and variants on them, such as the coating of inert rock particles with high-value sulfide concentrates in heap reactors, can be found in various reviews (e.g., Rawlings et al., 2003; Harvey & Bath, 2007). Mineral heaps and stirred tanks provide very different environments and challenges for mineral-leaching microorganisms, and different "optimal" populations might be expected to emerge with similar target minerals depending on the reactor used.

2   Process Flowsheet Development

Process flowsheet development (PFD) is the conceptual work done prior to building, expanding, or retrofitting any process plant. It consists of two main activities, process synthesis and process analysis. Process synthesis is the selection and arrangement of a set of unit operations (process steps) capable of producing the desired product at an acceptable cost and quality. Process analysis is the evaluation and comparison of different process synthesis solutions. In general, a synthesis step is usually followed by an analysis step and the results of analysis determine the subsequent synthesis step. Process design and project economic evaluation require integration of knowledge from many different scientific and engineering disciplines and are carried out at various levels of detail.

All design starts with a perceived need. In the process design of an iron ore bioprocessing process, the ultimate goal is to meet customer's need for the product, creating a commercial opportunity, as foreseen by the sales and marketing organization. Within this overall objective, the designer will recognize subobjectives, the requirements of the various units that make up the overall process. Before starting work, the designer should obtain as complete, and as unambiguous, a statement of the requirements as possible. If the requirement (need) arises from outside the design group, from a customer or from another department, then the designer will have to elucidate the real requirements through discussion. It is important to distinguish between the needs that are "must-haves" and those that are "should-haves." The "should-haves" are those parts of the initial specification that may be thought desirable, but that can be relaxed if required as the design develops. For example, a particular product specification may be considered desirable by the sales department, but may be difficult and costly to obtain, and some relaxation of the specification may be possible, producing a saleable but cheaper product. Whenever possible, the designer should always question the design requirements (the project and equipment specifications) and keep them under review as the design progresses. It is important for the design engineer to work closely with the sales or marketing department or with the customer directly to have as clear as possible an understanding of
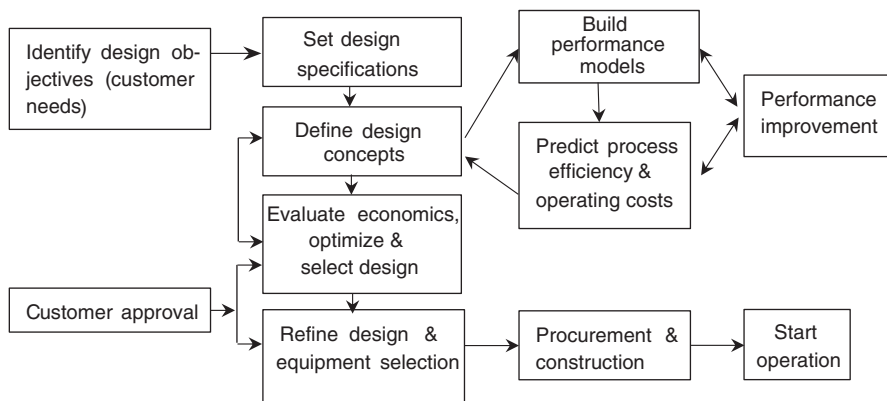
Fig. 1 General design process

the customer's needs. When writing specifications for others, such as for the mechanical design or purchase of a piece of equipment, the design engineer should be aware of the restrictions (constraints) that are being placed on other designers. A well-thought-out, comprehensive specification of the requirements for a piece of equipment defines the external constraints within which the other designers must work as shown in Figure. 1.

Once the design team has assembled information on the alternative commercial processes, they will usually need to carry out substantial customization and optimization of the designs before a selection can be made. The first step is usually a full process flowsheet development, and mass and energy balance of the process. These can be used for preliminary sizing and costing of the main process equipment to obtain an estimate of the required capital investment. The feed and product flow rates and energy consumption can be used to estimate the costs of production. The economic analysis methods can then be applied to determine the overall project economics and choose which design gives the best overall economic performance according to the criteria established by the company.

If one process flowsheet has a particular cost advantage, this will usually become clear in the economic analysis. Factors such as feedstock or fixed cost advantages can be very important in selecting between flowsheets. The selection is usually influenced more by process yields, energy consumption, and capital requirements and hence is sensitive to catalyst, organism, or enzyme performance and process design and optimization.

In an industrial context, technology vendors or engineering, procurement, and construction contractors will often supply detailed PFDs and materials and energy balances to a client.

Typical mineral engineering projects involving iron ores can be divided into three types, depending on the novelty involved: (i) modifications, and additions, to existing plant; usually carried out by the plant design group; (ii) new production capacity to meet growing sales demand and the sale of established processes by

contractors. Repetition of existing designs, with only minor design changes, including designs of vendors' or competitors' processes carried out to understand whether they have a compellingly better cost of production; (iii) new processes, developed from laboratory research, through pilot plant, to a commercial process. Even here, most of the unit operations and process equipment will use established designs.

2.1   *Revamping of Existing Plants*

Flowsheet development for plant revamps requires access to an operating plant and the data it produces. Revamps generally fall into two categories. Debottlenecking projects are carried out to increase the production rate of a plant while making the same product. Retrofit projects are carried out to change the design of a plant to handle different feeds; make different products; exploit better reactor, catalyst, or separation technology; or improve plant environmental impact in response to new regulatory requirements.

One of the critical requirements of a revamp project is always to minimize project cost by maximizing reuse of existing equipment. The revamped flowsheet therefore always requires compromises between desired objectives and what can be obtained with the equipment available.

Many features of a revamped flowsheet will be different from the flowsheet of a corresponding new plant. The development of a revamped flowsheet thus requires a lot of information on the performance of existing equipment so that the equipment can be related or modified for a role in the new flowsheet. When the existing equipment cannot be upgraded, the designer must find the cheapest method to add new capacity or augment the existing capacity.

Once the revamped flowsheet has been completed, the designers can assess the costs of the new components that must be added. The cost of revamping a plant should always be compared to the cost of building a new plant from scratch. The revamp will usually be a cheaper method for adding small increments of capacity, but for larger capacity increases a new unit will become more attractive.

3   Equipment Selection, Specifications, and Design

Iron ore mining has long been considered as capital and energy-intensive and is associated with negative impacts on the environmental. Conventional iron ore crushing, milling, and concentrating processes consume large amount of energy. Uncontrolled release of gases, solids, and wastewater has been a long-standing problem in many mining operations. Another major problem is the depletion of high-grade mineral deposits and the consequent need to mine at greater depths with higher costs.
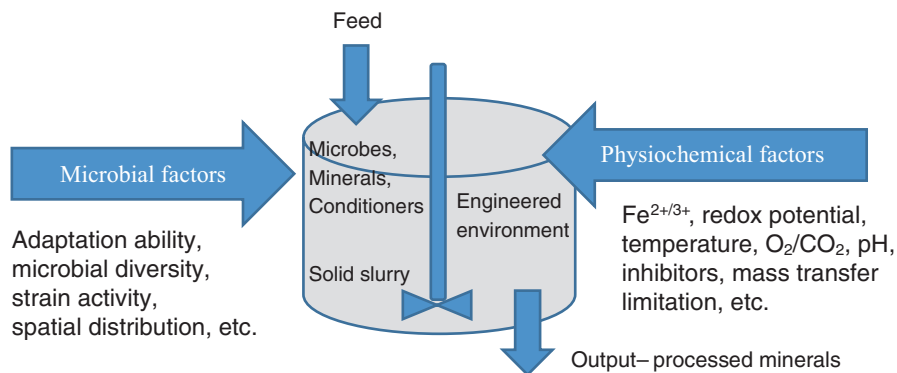
Fig. 2 Factors involved in application of bioreactors in iron ore bioprocessing

Iron ore bioprocessing or biomining in general is the exploitation of chemolithotrophic microorganisms to assist the extraction of metals from iron oxide or sulfide ores or concentrates. It is a combination of two major operations bioleaching and biooxidation. The metal solubilization process is a blend of microbiology and chemistry. Knowledge in microbiology is required as specific microorganisms are responsible for producing the ferric iron and acid leading biooxidation, while strong base on chemistry is equally necessary since the solubilization of the metal is a result of the action of ferric iron and/or acid on the mineral further known as bioleaching. Conventional biomining is usually performed in heaps of ground ore or in the dumps of waste or spent material. Though this process offers several advantages such as simple equipment and operation, low investment, operational costs, and acceptable yields, it is beset with severe operational limitations, such as high heterogeneity of piled material and practically no close process control. Moreover, the low oxygen and carbon dioxide transfer rate and extended periods of operation to achieve sufficient conversions are very challenging. From the process engineering point of view, bioreactors are the best choice for regulating the complex network of biochemical reactions comprehended in biomining as they allow for a close control of the variables involved, rendering significantly better performances. The reactors as shown in Fig. 2 are usually arranged in series, with a continuous flow of material into the first, which overflows to the next, and so on for reduction of reaction volume required. Therefore, process designing approach along with the defined application and monitoring of the abundance and activity of the metal oxidizing microorganisms will make the biomining process more industrially popular and as a portfolio of flexible techniques to provide a way of recovering metal.

Current commercial bacterial leaching of copper is usually performed in heaps of ground ore or in dumps of waste or spent material. Heaps and dumps are irrigated in closed circuit with an acidic liquor that contains a fraction of the bacterial population, the rest being attached to mineral. When the desired metal concentration is attained, the rich liquor is pumped to the solvent extraction (SE) section and then sent to electrowinning (EW), where the fine metal is recovered. The raffinate from

the SE section is recycled to the heap or dump, and the spent liquor of the EW section is recycled to the SE operation (Montealegre et al., 1993; Avendaño & Domic, 1994; Readett, 1999).

Heaps and dumps present a number of advantages such as simple equipment and operation, low investment and operation costs and acceptable yields. On the other hand, it must be realized that the operation suffers from some severe limitations: The piled material is very heterogeneous and practically no close process control can be exerted, except for intermittent pH adjustment and the addition of some nutrients. Moreover, the rates of oxygen and carbon dioxide transfer that can be obtained are low, and extended periods of operation are required in order to achieve sufficient conversions (Acevedo & Gentina, 1989).

From a process engineering standpoint, the complex network of biochemical reactions encompassed in bioleaching would best be performed in reactors. The use of reactors would allow a good control of the pertinent variables, resulting in a better performance. Parameters such as volumetric productivity and degree of extraction can be significantly increased (Pinches et al., 1988; Acevedo & Gentina, 1989; Gormely & Brannion, 1989; Adamov et al., 1990). The main limitation in the use of reactors in biomining is the very large amounts of run-of-mine ore that in most cases is to be treated. The Chuquicamata copper mine in Chile produced 630,000 tons of fine copper in 1999. The production of that amount of metal implied the treatment of around six million tons of run of mine. If such amount would to be treated in bioreactors, the required equipment volume would be of the order of 30 million m^3, an unthinkable figure. This limits their application to the treatment of mineral concentrates or when moderate volumes of ore are to be processed. For instance, over 11,000 tons of gold concentrates are biooxidized in reactors every year.

The discussion that follows will center on the use of bioreactors in bioprocessing of minerals, with emphasis on oxygen and carbon dioxide transfer, the maintenance of an adequate solids suspensions, and the application of bioreactors to commercial iron ore mining operations.

3.1   Reactors in Bioleaching

The selection of a suitable reactor for a biomining process and its design should be based on the physical, chemical, and biological characteristics of the system. Adequate attention should be paid to the complex nature of the reacting sludge, composed by an aqueous liquid, suspended, and attached cells, suspended solids, and air bubbles (Gormely & Brannion, 1989). Because of the very large volumes of material to be processed, bioleaching and biooxidation are best performed in a continuous mode of operation in which volumetric productivity is high and reactor volumes can be kept low. Considering the kinetic characteristics of microbial growth, a continuous stirred tank reactor (CSTR) appears as the first choice.

An important consideration in selecting a suitable reactor refers to the autocatalytic nature of microbial growth. This fact is common to all fermentation operations,

but in bioleaching there is an important difference. In industrial fermentations, the nutrients are chosen by their high affinity with the microbial population, while in biomining the mineral species involved are usually recalcitrant to microbial action, implying that the affinity is quite low. The substrate microorganism affinity is related to Monod's saturation constant, KS (Monod, 1949). High affinities are reflected in low KS values, of the order of a few milligrams per liter, as in the case of most sugars. Some minerals have saturation constants as high as 3–6 g/L, that is, thousands of orders of magnitude higher. This situation affects the selection of the reactor. If a high degree of conversion is desired, a single agitated tank will require a very large volume, so an arrangement of reactors will be more suitable (Dew, 1995). It can be shown that a CSTR followed by a tubular plug flow reactor, PFR, gives the minimum reaction volume to attain a certain conversion (Levenspiel, 1972). Because the need of aeration and the presence of solid particles make PFRs unpractical, their performance can be approximated by a series of CSTRs (González et al., 1999).

Other types of reactors that have been studied for their application in biomining are the percolation column, the Pachuca tank, the air-lift column, and some special designs such as rotary reactors (Atkins & Pooley, 1983; Atkins et al., 1986; Nikolov et al., 1986; Acevedo et al., 1988; Barrette & Couillard, 1993; Loi et al., 1995; Herrera et al., 1997; Acevedo et al., 1999; Canales et al., 1999; Nedeltchev et al., 1999; Rossi, 1999).

3.2 Gas Mass Transfer

Several mass transfer operations occur in a biomining operation. Nutrients have to reach the attached and suspended cells, metabolic products have to migrate from the cells to the liquid, and solubilized species must be transported from the surface of the mineral particles to the liquid. In addition, two other important transport processes are to be considered: the supply of oxygen and carbon dioxide from the air to the cells. Carbon dioxide is demanded by the cell population as carbon source, while oxygen is needed as the final electron acceptor of the overall oxidation process. In reactors, these gases are usually supplied by bubbling air into the liquid. In order to be used by the cells, oxygen and carbon dioxide must dissolve in the liquid, a mass transfer operation that presents a high resistance and can become limiting for the overall process rate.

A gas mass balance around the bioreactor gives (Wang & Humphrey, 1968):

$$\frac{dC_{Li}}{dt} = k_L a_i \left(C_i^* - C_{Li} \right) - N_i \tag{1}$$

where i stands for oxygen or carbon dioxide, N is the gas demand (g gas/l·h), $k_L a_i$ is the volumetric gas transfer coefficient (h^{-1}), C_i^* is the gas equilibrium concentration (g/L), C_{Li} is the dissolved gas concentration, and t is time (h).

The gas supply, $k_L a_i \left(C_i^* - C_{Li} \right)$ must equal the gas demand in order to avoid growth limitation, so

$$N_i = k_L a_i \left(C_i^* - C_{Li} \right) \tag{2}$$

The gas demands can be calculated as follows:

$$N_i = \frac{\mu X}{Y_i} \tag{3}$$

where μ is the specific growth rate of the cells (h^{-1}), X is cell mass concentration (g/L), and Y_i is the gas cell yield (g cells/g gas).

The bioleaching process can be represented by a stoichiometric equation (Acevedo, 1987; Acevedo & Gentina, 1989). In the case of a leaching organism such as *Thiobacillus ferrooxidans* growing in a simple defined culture media with ferrous iron as the energy source, the following equation can be written as follows:

$$l\,CO_2 + m\,NH_3 + n\,FeSO_4 + o\,H_2SO_4 + p\,O_2 \rightarrow$$
$$C_5H_7O_2N + r\,Fe_2\left(SO\right)_3 + s\,H_2O \tag{4}$$
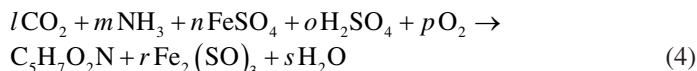
$C_5H_7O_2N$ represents the biomass with an elemental composition of 53.1% C, 6.2% H, 28.3% O, and 12.4% N (Jensen & Webb, 1995). Elemental mass balances on C, H, O, and N, together with the experimental value of the ferrous ion cell yield of 0.0086 g cells/g Fe^{2+} allow for the calculation of the stoichiometric coefficients:

$$5.0\,CO_2 + NH_3 + 241.4\,FeSO_4 + 120.7\,H_2SO_4 + 55.3\,O_2 \rightarrow$$
$$C_5H_7O_2N + 120.7\,Fe_2\left(SO\right)_3 + 118.7\,H_2O \tag{5}$$
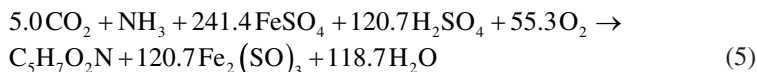
The oxygen and carbon dioxide cell yields can be calculated from Eq. (5) as follows:

$$Y_{O_2} = \frac{M_c}{55.3\,M_{O_2}} \tag{6}$$

$$Y_{CO_2} = \frac{M_c}{5.0\,M_{CO_2}} \tag{7}$$

where M_c, M_{O_2}, and M_{CO_2} are the molecular masses of the cells, oxygen, and carbon dioxide, respectively. In an actual bioleaching operation, a similar stoichiometric representation can be made. For instance, for the biooxidation of enargite (Cu_3AsS_4) from a refractory gold concentrate the following equation applies, considering an experimental value of 0.6 g cell/g Cu (García, 1997):

$$5.0CO_2 + NH_3 + Cu_3AsS_4 + 4.5H_2O + 3.75O_2 \rightarrow$$
$$C_5H_7O_2N + 3.0CuSO_4 + H_3AsO_4 + H_2SO_4 \tag{8}$$
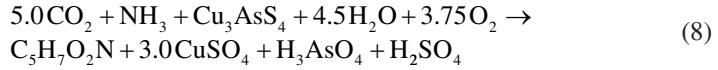
In this case, the oxygen and carbon dioxide cell yields are as follows:

$$Y_{O_2} = \frac{113}{3.75 \times 32} = 0.94 \, g/g \tag{9}$$

$$Y_{CO_2} = \frac{113}{5.44} = 0.51 \, g/g \tag{10}$$

As could be expected, the carbon dioxide yield, related only with cell growth, is the same in the defined soluble medium and in the bioleaching of a mineral. Equations (2), (3), (6), (7), (9), and (10) can be used to estimate the required mass transfer coefficients, as shown in Table 1. When not enough experimental data are available, the required coefficient for oxygen can be estimated from the stoichiometry equation of the main oxidation reaction. Oxygen transfer coefficients estimated by such method are included for chalcocite (Cu_2S), covellite (CuS), and chalcopyrite (CuFeS). The required kLas for oxygen are of the same order of magnitude or less than those that have been obtained experimentally in bioreactors (Acevedo et al., 1988; Liu et al., 1988; Boon & Heijen, 1998; Harvey et al., 1999; Rossi, 1999; Veljkovic et al., 1999). This implies that at the usual experimental conditions of 5–18% w/v pulp density, the process is not limited by oxygen supply. This situation may change at higher pulp densities (Bailey & Hansford, 1993; Hansford & Bailey, 1993; Loi et al., 1995).

In fermentation technology, it is usual to correlate kLa with agitation power per unit volume and gas superficial velocity (Wang & Humphrey, 1968; Boon & Heijen, 1998; Harvey et al., 1999):

$$k_L a_O = K \left(\frac{P_g}{V} \right)^{\alpha} \cdot v_s^{\beta} \tag{11}$$

Table 1 Required kLa values for the biooxidation of ferrous iron and an enargite gold concentrate (García, 1997; Acevedo et al., 1988)

	kLa (h^{-1})	
	O_2	CO_2
Ferrous iron	30	65
Enargite	3	84
Chalcocite	12	–
Covellite	20	–
Chalcopyrite	42	–

In leaching bioreactors, the transfer coefficient may be influenced by the presence of solids (Mills et al., 1987), so the equations derived specifically for bioleaching are required. Table 2 shows some correlations of this type. Because of its very low concentration in air, 0.03% v/v, the equilibrium concentration of carbon dioxide is also very low, 0.00039 g/L at 30 °C. The magnitude of the transfer potential, $C^* - C_L$, is severely limited, leading to CO_2-limited growth, as air flow rate is commonly determined based on the oxygen demand. Carbon dioxide limitation has been demonstrated by several authors (Torma et al., 1972; Norris, 1989; Boogard et al., 1990; Haddadin et al., 1993; Nagpal et al., 1993; Jensen & Webb, 1995; Jaworska & Urbanek, 1997; Acevedo et al., 1998; Boon & Heijen, 1998), but more work is required on this topic.

The CO_2 transfer coefficient can be experimentally determined by a dynamic method on the exit gas (André et al., 1981) or estimated from de oxygen transfer coefficient (Liu et al., 1983; Nagpal et al., 1993):

$$k_{LC} = K_{Lo} \left(\frac{D_O}{D_C} \right)^{-2/3} \tag{12}$$

3.3   Suspension of Solids

The CSTR is an ideal conception that implies a completely mixed content that presents no gradients, so the value of each variable is the same at every point within the liquid. That being the case, the exit stream has the same composition as the fluid

Table 2   Correlations for the volumetric oxygen transfer coefficient in bioleaching processes

Correlation	Comments	Reference
$k_L a_O = K \left(\frac{P_g}{V} \right)^{0.93} \cdot v_s^{0.8}$	Stirred tank with ferrous iron as energy source	Gormely and Brannion (1989)
$k_L a_O = K \rho^{-2.8} N^{2.65} v_s^{0.57}$	20-L stirred tank with up to 20% w/v glass beads	Liu et al. (1988)
$k_L a_O = K \left(\frac{P_g}{V} \right)^{0.09} \cdot v_s^{1.13}$	7-L stirred tank with 15% w/v chalcopyrite concentrate and air enriched with 1% CO_2	Acevedo et al. (1988)
$k_L a_O = K \left(\frac{P_g}{V} \right)^{0.33}$	7-L Pachuca tank with 15% chalcopyrite concentrate and air enriched with 1% CO_2	Acevedo et al. (1988)
$k_L a_O = (a - b\varnothing) \left(\frac{P_g}{V} \right)^{0.67} \cdot v_s^{0.31}$	18-L stirred tank, model system with up to 40% w/v solids	Mills et al. (1987)
$k_L a_O = K \cdot v_s^{(0.72 - 0.01\varnothing)}$	4-L air-lift column with up to 24% w/v gold concentrate	Canales et al. (1999)
$k_L a_O = K \varnothing^{-0.0015} F^{2.8} N^{1.49}$	15-L rotating drum reactor with 50% w/w gold concentrate	Herrera et al. (1997)

within the reactor. As stated previously, tank bioleaching is a three-phase system composed by the incoming air and the outlet gas, the acidic aqueous liquor, and the microbial cells and mineral particles. The complex nature of this slurry makes the attainment of homogeneity especially difficult (Brucato & Brucato, 1998). Agitation has a double purpose: to increase the rate of transfer operations, such as oxygen and carbon dioxide transfer and heat transfer, and to mix the reactor content. Under conditions of insufficient agitation, the transfer operations may become limiting and the overall reaction performance will decline because of the appearance of zones of the fluid with insufficient nutrients or inadequate temperature or pH (Namdev et al., 1994). For several decades, the use of disk turbines (or Rushton turbines) has been common in industrial fermentors. Back in the 1950s, investigators were mainly looking for impellers that specifically enhanced oxygen transfer, but they neglected other important factors such as mixing, impeller gas flooding, and power consumption, which are all negative assets for the disk turbine (Humphrey, 1998). The high shear stress exerted by the disk turbine on the fluid may also produce metabolic stress and cell growth inhibition (Toma et al., 1991). When mixing is especially important, axial flow impellers such as the hydrofoils become an advantageous alternative (Nienow, 1997; Junker et al., 1998; Myers & Bakker, 1998).

This is the case of bioleaching, where the oxygen demands are modest but the presence of fine solid particles imposes an additional difficulty in obtaining homogeneous slurries. Table 3 lists the most commonly used type of impellers. It can be seen that the power required by disk turbines is very high compared with the requirements of other impellers. NP, the power number, is a dimensionless number defined as $P/\rho \cdot N3 \cdot D5$.

Some of the hydrofoil designs developed in the mid-1980's (Lally, 1987) present convenient characteristics for their use in bioleaching reactors. Their power requirement is low, the mixing and solids suspension capabilities are good, and oxygen and carbon dioxide transfer coefficients are comparable with those of the disk turbine (Kubera & Oldshue, 1992; Kaufman et al., 1997).

The problem of solids suspension in agitated vessels has been addressed by several investigators. An important early work was that of Zwietering (1958), who studied the minimum required stirrer speed (referred afterward as critical speed)

Table 3 Impeller types used in bioreactors (Dickey & Fenic, 1976; Kubera & Oldshue, 1992)

Impeller	Flow	Np
Disk turbine	Radial	6.0
Flat blade turbine	Radial	2.8
Pitched blade turbine	Axial	1.2
Curved blade turbine	Radial	2.8
Hydrofoil	Axial	0.3
Gas dispersing hydrofoil	Axial	0.8
Marine helix	Axial	0.3
Bar turbine	Radial	0.7

and the stirrer dimensions for the complete suspension of solids. In this work, the main objective was to avoid solids deposition on the bottom of the tank, but the homogeneity of the slurry was not of special concern. Different expressions for the critical speed have been proposed since then (Oldshue, 1983; Tatterson, 1996).

The concept of critical speed was developed for solid/liquid systems and does not consider the effect of bubble aeration. In this respect, some early work by Oldshue (1969, 1983) can be cited. This author points out that air bubbling has a negative effect on solids suspension and homogeneity, because the bubbles tend to disturb the flow pattern established by each type of impeller. In the specific field of tank bioleaching, Acevedo and Aroca (1986) present a comparative study of the effect of pulp density and aeration rate on the critical agitation speed when using three types of turbines: flat blade, curved blade, and pitched blade. The critical speed increased with aeration, and the pulp density showed a minor effect. The pitched blade turbine gave the lowest critical speeds, pointing to the fact that that the presence of an axial component in the flow is positive for solids suspension.

3.4 Future Development and Applications

In the last several decades, quite a few large-scale commercial copper and gold bioprocessing units have been established. So far, most of them use heap leaching or stirred tank reactors to process flotation concentrates, although a few use heaps for low-grade ores and tailings.

The future of bioreactors in mining appears promising. Previous metal biooxidation operations have been increased in number and size in several countries. The use of reactors will most likely extend to the bioleaching of other base metals, such as copper and iron. Currently, studies are being carried out on for the development of bioprocesses for the bioleaching of iron concentrates. The experience gained in the heap and dump leaching of copper and in the biooxidation of gold concentrates is being used in these studies. The bioleaching of chalcopyrite copper concentrates in the next few years will constitute a big breakthrough in biomining. The application of these technologies to the processing of nickel, zinc, and other metals may also become a reality in the near future.

As discussed above, several mathematical models of key genetic circuits are currently available for a variety of bioprocesses with the level of fidelity that would be required for industrial applications. However, mechanistic models are still not yet substantially applied for industrial bioprocess development. Nevertheless, interest in them has grown considerably as mechanistic models may provide an outstanding summary of process knowledge. The improvement of experimental techniques and computational power provides the opportunity to develop increasingly complex models. Given these advances, mechanistic models are expected to be gradually used to a greater extent in the future and may eventually become routine for industrial application. Consequently, the construction of complex models that capture the dynamics of various interacting genes, proteins, and metabolites will be used to

simulate conditions that are too expensive or time-consuming to be tested experimentally intervening beneficially in bioprocess development.

Many of the potential microorganisms for application at large scale are capable of producing very small quantities of the desired product and under conditions other than those usually applied in the industry. The improvement of those microorganisms remains one of the greatest challenges in biotechnology and requires the combination of engineering and molecular biology disciplines for the analysis and modification of strains and bioprocesses. The development of next-generation bioprocesses, with high selectivity for the production of pharmaceuticals and for production of chemicals from renewable sources, is an effort where process systems engineers are expected to have an important role. In line with the above, the modeling framework discussed in the present work provides a methodology that organizes experimental information systematically, omitting unnecessary experiments and developing models with a priori established aims. Future studies may also utilize the current progress in molecular biology to construct detailed mechanistic models of key regulatory processes facilitating the development of high-fidelity bioprocess models. Maybe then, we can upgrade the simplified models that describe microbial growth kinetics based on enzyme kinetics with more detailed mechanistic models that capture the dynamics of gene regulation controlling upstream the production of catabolic enzymes.

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Economics of Bioprocessing Technologies

1 Introduction

Iron ore is primarily found as the oxides of iron, notably hematite and magnetite and as hydroxides like goethite and limonite. Small amounts are found as the carbonates in siderite, as sulfides in pyrites and as silicates in chamosite and greenalite. Broadly iron ores may be grouped as follows: direct shipping ore generally contains higher than 60% iron (Fe), which is mined and used in blast furnaces requiring only simple preparation; and beneficiable ore which contains as little as 25% Fe and can be upgraded to around 60% Fe by magnetic or heavy media separation. Lump ores are naturally mined ores that are crushed and screened to a certain grain size before their use. However, as a result of preparation and enrichment processes in the iron ore mines to increase the Fe content, very fine-grained ores increasingly accumulate which have to undergo agglomeration. This is done by means of sintering and pelletizing. The physical and metallurgical characteristics of the ores are as important as chemical properties. The steel industry requires iron ore which is high in iron and low in impurities particularly SiO_2, Al_2O_3, sulfur, and phosphorous. It is a prerequisite that the lumps should have high strength. Therefore, beneficiation of iron ore after mining is an important stage to prepare ore to meet both physical and chemical properties suitable for the various metallurgical processes. The important aspect of ore preparation is to increase iron content, lower impurities such as Al_2O_3, SiO_2, etc., and generate lumps with closer sizes and utilization of fines and slimes, so as to improve the economics of mining operations.

Nevertheless, even in modern iron ore processing techniques, there still remain a number of problems of considerable economic importance which have not been solved. Thus, for example, iron ores containing a substantial quantity of iron silicates cannot be adequately beneficiated by existing mechanical means, and no economically viable solution has yet been found for the beneficiation of comparatively low-grade iron ores in basic rock. No economically advantageous techniques for the efficient beneficiation of brown hematite and siderites have yet been devised, and

only a beginning has been made with the utilization of complex iron ores and more particularly polymetallic ores. In the present state of iron ore beneficiation technology, with the progressive decline of ore process brought about by the discovery and operation of several large deposits of extremely rich iron ores in recent years, and with the decreasing cost of ore transport by sea, a number of countries with deposits of low-grade iron ores which are difficult to beneficiate, or which cannot be beneficiated by existing methods, are finding that the imported high-grade ores are becoming increasingly competitive with the domestic low-grade iron ones.

2 Economic Aspects of Iron Ore Bioprocessing

The preliminary economic evaluation of an iron ore bioprocessing project usually involves the estimation of capital investment, estimation of operating costs, and analysis of profitability. For any new process with application of new bioreagents, significant costs could be related to the new bioreagent development including research and development (R&D) spending for all unsuccessful products. In other words, the average cost per successful project may be 20–50% higher than actual costs because more than 90% of new projects never reach commercialization. This reinforces the need for effective process design tools and methodologies that assist engineers and scientists to efficiently evaluate and eliminate nonpromising project ideas at the very early stages of product and process development.

2.1 Capital Cost Estimation

The capital investment for a new plant includes three main items: (1) direct fixed capital (DFC), (2) working capital, and (3) startup and validation cost. The DFC for small biotechnology facilities is usually in the range of $30–60 million, whereas for large facilities it is in the range of $100–250 million. For preliminary design purposes, the various items of DFC are estimated based on the total equipment purchase cost (PC) using several multipliers. Table 1 provides ranges and average values for the multipliers and a skeleton for the calculations. Detailed definitions of the various cost items and additional information can be found in traditional process design textbooks and the technical literature (Douglas, 1988; Garrett, 1989; Peters & Timmerhaus, 1991; Seider et al., 1999; Ulrich, 1984; Valle-Riestra, 1983).

Notice the wide range of multiplier factors for estimating the cost of buildings. Plants for commonly used biochemicals, such as ethanol and citric acid, fall on the low end of the range, whereas small biotech facilities that manufacture small amounts of high-value products fall on the high end. The average value of 0.45 corresponds to relatively large plants that produce medium to high-value products. For more accurate estimation of building costs, it is necessary to estimate the process area required based on the footprint of the equipment and the space required around

Table 1 Fixed capital cost estimation

Cost item	Multiply factors	Range of values
Total plant direct cost (TPDC)		
1. Equipment purchase cost		
2. Installation	0.50 × PC	0.20–1.50
3. Process piping	0.40 × PC	030–0.60
4. Instrumentation	0.35 × PC	0.20–0.60
5. Insulation	0.03 × PC	0.01–0.05
6. Electrical	0.15 × PC	0.10–0.20
7. Building	0.45 × PC	0.10–2.00
8. Raw material yard	0.15 × PC	0.05–0.30
9. Auxiliary facility	0.50 × PC	0.20–1.50
Total plant indirect cost (TPIC)		
10. Engineering	0.25 × TPDC	0.20–0.30
11. Construction	0.35 × TPDC	0.30–0.50
Total plant cost (TPC)		
12. Contractors	0.05 × TPC	0.03–0.08
13. Contingency	0.10 × TPC	0.07–0.15
Direct fixed capital (DFC)	TPC + 12 + 13	

Table 2 Operating cost items

Cost item	Type of cost	Percentage of total cost, %
A. Raw materials	Direct	10–80
B. Labor	Direct	20–50
C. Consumables	Direct	1–50
D. Lab/QC/QA	Direct	2–50
E. Waste disposal	Direct	1–20
F. Utilities	Direct	1–30
G. Equipment-dependent	Indirect	10–70
H. Miscellaneous	Indirect	0–20

the equipment for safe and efficient operation and maintenance. Then, the building cost is estimated by multiplying the area of the various sections (e.g., process, laboratory, and office) of a plant by an appropriate unit cost.

As indicated in Table 2, the equipment installation cost has one of the highest multipliers among all costs. For better accuracy, one should use multipliers that are specific to individual equipment items. In general, preassembled equipment delivered mounted on skids has a lower installation cost.

For preliminary cost estimates, Table 2 clearly shows that the fixed capital investment of a plant is a multiple (usually 5–8 times) of its equipment purchase cost. The equipment purchase cost can be estimated from vendor quotations, published data, company data compiled from previous projects, and by using process simulators and other computer aids. Vendor quotations are time-consuming to obtain and are

therefore usually avoided for preliminary cost estimates. Instead, engineers tend to rely on the other three sources. The data represent average values from several vendors. Oftentimes, cost data for one or two discrete equipment sizes are available, but the cost for a different size piece of equipment must be estimated. In such cases, the scaling law (expressed by the equation below) can be used as follows:

$$\text{Cost}_2 = \text{Cost}_1 \left(\frac{\text{Size}_2}{\text{Size}_1} \right)^a \tag{1}$$

The mathematical form of the scaling law explains why cost versus size data graphed on logarithmic coordinates tend to fall on a straight line. The value of the exponent (a) in the equation above ranges between 0.5 and 1.0 with an average value for vessels of around 0.6 (this explains why the scaling law is also known as the "0.6 rule," which is just under 2/3, the ratio of surface to volume for vessels). According to this rule, when the size of a vessel doubles, its cost will increase by a factor of (2/1)0.6 or approximately 52%. This is often referred to as the economy of scale. When using the scaling law, it is important to make sure that the piece of equipment whose cost is being estimated has a size that does not exceed the maximum available size for that type of equipment. The price of equipment changes with time due to inflation and other market conditions. That change in price is captured by the Chemical Engineering Plant Cost Index (CE INDEX) that is published monthly by chemical engineering magazine. The index I is used to update equipment cost data according to the following equation.

Another factor that affects equipment purchase cost is the material of construction. A stainless steel chromatography column is more expensive than a plastic one of the same size. Similarly, a stainless steel tank costs 2.5–3 times as much as a carbon steel tank of the same size. Fortunately, in bioprocessing, most of the equipment is made of stainless steel for GMP (good manufacturing practice) reasons, and selection of materials is less of a problem. Other factors that affect equipment cost include the finishing of the metal surface and the instrumentation that is provided with the equipment. This is the major cause for the wide range in prices for bioreactors. Additional cost data for chemical processing equipment can be found in the literature (Garrett, 1989; Peters & Timmerhaus, 1991 and Ulrich, 1984). The choices are rather limited when it comes to cost data for bioprocessing equipment (Kalk & Langlykke, 1986; Reisman, 1988).

In addition to direct fixed capital costs, money must also be available to pay for the following items: (1) raw materials for 1–2 months, (2) labor for 2–3 months, (3) utilities for a month, (4) waste treatment/disposal for a month, and (5) other miscellaneous expenses. "Working capital" accounts for these investments in temporary expenses and consumable materials. The required amount of working capital for a process is usually 10–20% of the DFC. Startup and validation costs can also represent a significant capital investment for a biopharmaceutical plant. A value of 5–10% of DFC is quite common.

2.2   Operating Cost Estimation

The operating cost to run a bioprocessing plant is the sum of all expenses associated with raw materials, labor, utilities, waste disposal, overhead, etc. Dividing the annual operating cost by the annual production rate yields the unit production cost (in \$/ton). Biotechnology is a unique industry when it comes to the range in unit production cost. There are products that cost less than \$1.0/kg and others that cost more than \$10,000,000/kg to make. The citric acid and therapeutic monoclonal antibody processes that are described in the examples section of this chapter lie close to these two extremes. If one also considers biological wastewater treatment with a unit cost of \$0.1–0.5/m³ (or \$0.0001/kg), then the range in order of magnitude in the unit processing cost is 1011. Table 2 displays the various types of operating costs, their direct or indirect nature, and ranges for their values relative to the total operating cost. Sometimes cost items are categorized as either fixed or variable. Fixed costs are those that are incurred regardless of volume of product output. The clearest case of a fixed cost is depreciation, which is part of the equipment-dependent cost. The clearest case of a variable cost would be the cost of raw materials. Most other costs have a fixed and a variable component.

It is obvious from the wide range of values in Table 2 that one cannot estimate the operating cost of a product based on average values. A certain level of detailed calculations is required.

Raw Materials   This accounts for the cost of all fermentation media, recovery chemicals, and cleaning materials. For commodity biochemicals, such as ethanol, it is mainly the cost of fermentation media. For high-value products, the buffers used for product recovery and equipment cleaning can be a major part of the materials cost. Table 3 provides a list of commonly used raw materials in the biochemical industries. Note that the price of a raw material can vary widely depending on its required purity. This can be clearly seen in the case of water. Water for injection (WFI), for instance, costs 100–500 times as much as city water. Prices of various raw materials can be found in the chemical marketing reporter. More recently, a number of websites have come online where a buyer can find pricing information and request bids from suppliers.

Labor   This is estimated based on the total number of operators, which in turn is calculated by summing up the operator requirements of the various operations as a function of time. As will become clear in the examples later in this chapter, the labor requirement in a batch manufacturing facility varies with time. In a single product facility, the number of operators in each shift must be based on maximum demand during that shift. In multiproduct facilities, each product line can employ a certain number of dedicated operators and utilize floating operators during periods of peak demand. In general, smaller facilities tend to utilize a larger number of operators per processing step because they are less automated. For instance, a small biotech company may utilize 2–3 operators to set up a fermentor, whereas in a large, highly automated fermentation facility a single operator may remotely handle the setup of

Table 3 Common bioprocessing raw materials (2010 prices)

Raw material	Grades	Price ($/kg)
Carbon sources		
Glucose	Solution 70% w/v	0.25–0.35
Corn syrup	95% dextrose equivalent	0.35–0.45
Molasses	50% fermentable sugars	0.08–0.12
Soybean oil	Refined	0.80–0.90
Corn oil	Refined	0.85–0.95
Ethanol	USP tax free	0.50–0.60
Methanol	Gulf Coast	0.20–0.25
n-alkanes		0.35–0.50
Nitrogen sources		
Ammonia	Anhydrous	0.20–0.25
Soybean flour	44% protein	0.25–0.30
Cottonseed flour	62% protein	0.45–0.55
Casein	13.5% w/w total N	2.40–3.00
Ammonium sulfate	Technical	0.15–0.25
Ammonium nitrate	Fertilizer grade 33.5% N	0.15–0.20
Urea	Agricultural grade 46% N	0.20–0.25
Yeast	Brewers	2.60–3.20
Whey	Dried, 4.5% w/w N	0.45–0.60
Salts		
KH2PO4	USP, granular	1.65–1.85
K2SO4	Granular, purified	2.20–2.50
Na2HPO4		1.30–1.50
MgSO4.7H2O		0.25–0.35
ZnSO4.7H2O	Agricultural grade	0.50–0.60
Other		
City water		0.0005
Distilled water		0.01–0.05
Water for injection		0.05–0.20

six different fermentors from the control room. In general, a typical biotech company that deals with high-value products will allocate at least one operator to each processing step, such as centrifugation, membrane filtration, and chromatography. During its operation. The setup of a step may require multiple operators for a short period.

Consumables This includes the cost of periodically replacing items that may be used up, fouled, or otherwise damaged during processing, such as membranes, chromatography resins, and activated carbon. As the examples later in this chapter will illustrate, the high unit cost of chromatography resins and their frequent replacement can make this item a major component of the operating cost.

Laboratory/QC/QA This accounts for the cost of off-line analysis, quality control (QC), and quality assurance (QA) costs. Chemical and biochemical analysis and physical property characterization, from raw materials to final product, are a vital part of biochemical operations. This cost is usually 10–20% of the operating labor cost. However, for certain biopharmaceuticals that require a large number of very expensive assays, this cost can be as high as the operating labor. For such cases, it is important to account for the number and frequency of the various assays in detail. Changes in lot size that can reduce the frequency of analysis can have a major impact on the bottom line.

Waste Treatment/Disposal This accounts for the treatment of wastewater and the disposal of solid and hazardous materials. The amount and composition of the various waste streams are derived from the material balances. Multiplying the amount by the appropriate unit cost yields the cost of treatment and disposal. Treatment of low biological oxygen demand (BOD) wastewater (less than 1000 mg/L) by a municipal wastewater treatment facility usually costs $0.2–0.5/m^3. This is not a major expense for most biotech facilities that deal with high-value products. Disposal, however, of contaminated solvents (generated by chromatography steps) and other regulated compounds can become a major expense because their unit disposal cost is in the range of $2–20/kg (usually higher that the purchase price of the same chemical). Waste disposal may also become a problem if an unwanted by-product is generated as part of the recovery chemistry of a process.

Utilities This accounts for heating and cooling utilities as well as electricity. The amounts are calculated as part of the material and energy balances. Aerobic fermentors are major consumers of electricity, but downstream processing equipment generally does not consume much electricity. In terms of unit cost, electricity costs around $0.1/kWh, heating steam is around $4–8/1000 kg, clean steam (generated utilizing purified water) is around $10–50/1000 kg (depending on the scale of production and level of water purity), and refrigerants around $0.05–0.1 per 1000 kcal of heat removed. In downstream processing, clean steam is mainly used for sterilizing equipment as part of equipment cleaning. Another common use is for sterilizing fermentation media. Note that purified water used for buffer preparation and equipment cleaning is often classified as a utility and not as a raw material, thus increasing the cost contribution of utilities.

Equipment-Dependent This cost accounts for the depreciation of the fixed capital investment, maintenance of equipment, insurance, local (property) taxes, and possibly other overhead-type expenses. For preliminary cost estimates, the entire fixed capital investment is usually depreciated linearly over a 10-year period. In the real world, the government allows corporations to depreciate equipment in 5–7 years and buildings in 25–30 years. Land is never depreciated. The annual equipment maintenance cost can be estimated as a percentage of the equipment's purchase cost (usually 10%). Insurance rates depend to a considerable extent upon the maintenance of a safe plant in good repair condition. A value for insurance in the range of

0.5–1% of DFC is appropriate for most bioprocessing facilities. The processing of flammable, explosive, or dangerously toxic materials usually results in higher insurance rates. The local (property) tax is usually 2–5% of DFC. The factory expense represents overhead cost incurred by the operation of nonprocess-oriented facilities and organizations, such as accounting, payroll, fire protection, security, and cafeteria. A value of 5–10% of DFC is appropriate for these costs.

Miscellaneous This accounts for ongoing R&D, process validation, and other overhead-type expenses. Expenses of this type can be ignored in preliminary cost estimates. Other general expenses of a corporation include royalties, advertising, and selling. If any part of the process or any equipment used in the process is covered by a patent not assigned to the corporation undertaking the new project, permission to use the teachings of the patent must be negotiated, and some form of royalties is usually required. Advertising and selling cover expenses associated with the activities of the sales department.

2.3 Profitability Analysis

With estimates of capital investment, operating cost, and revenues of a project, one can proceed to assess its profitability and attractiveness from an investment point of view. There are various measures for assessing profitability. The simplest ones include gross margin, return on investment (ROI), and payback time, and they are calculated using the following equations:

$$\text{Gross Margin} = \text{Gross Profit / Revenues} \tag{2}$$

$$\text{Return on Investment}\left(\text{ROI}\right) = \left(\text{Net Profit / Total Investment}\right) \times 100\% \tag{3}$$

$$\text{Payback Time}\left(\text{in years}\right) = \text{Total Investment / Net Profit} \tag{4}$$

where gross profit is equal to annual revenues minus the annual operating cost and net profit is equal to gross profit minus income taxes plus depreciation. All variables are averaged over the lifetime of a project.

Other measures that are more involved, such as the net present value (NPV) and internal rate of return (IRR), consider the cash flows of a project over its evaluation life and the value of money as a function of time. Detailed definitions for NPV and IRR can be found in the literature (Peters & Timmerhaus, 1991). The examples that are presented later in this chapter demonstrate how these measures facilitate the decision-making process.

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Environmental Considerations

1 Introduction

Over the past few decades, the preoccupation with environmental factors has deterred conventional iron ore mining and smelters expansions in most developed countries. For a long period, there was a general excess of iron ore production capacity, operating on fully depreciated plant, willing to offer attractive contracts to maintain adequate supplies. The situation has now changed; however, the environmental restrictions have stimulated the development of iron ore bioprocessing with much less environmental impacts and also obliged conventional mining and smelters to either reduce their throughput rates or add considerable capital expenditure to overcome emission problems.

In the short term, because of the general downturn in economic activity, the environmental restrictions placed on conventional iron ore mining and smelting operations have not caused a general shortage of capacity. As market demands and consumer requirements are reestablished, new iron ore mining and smelting facilities must be developed with much less environmental footprint. It can be assumed that in the industrialized countries the new environmental requirements will have increased the cost, in real terms, of conventional iron ore smelting and refining.

Bioprocessing of low-grade ores and concentrates has been well established as a commercial-scale technology for extracting value from various nonferrous and precious metal minerals. Microorganisms are also increasingly being used for recovering value from mine wastes, such as tailings, slags, and ashes, as well as urban mining of end-of-life consumer products such as batteries and electronic wastes. The capability of microbes to catalyze oxidative and reductive bioprocesses as well as degrade organic compounds has been utilized for the removal of various contaminants from hydrometallurgical process waters and the treatment of effluents prior to release into the environment. Biological iron oxidation, bioreduction of nitrate, selenate and sulfate, neutralization of acidity with biogenic alkalinity, and bioprecipitation of metals offer alternatives for chemical water treatment. Emerging technologies,

such as bioelectrochemical systems and synthetic biology, are also opening new avenues to mining companies for monitoring and mitigating environmental impacts. Several recent developments in the environmental applications of biotechnology in mining have been reported (Lawrence et al., 1998; Ehrlich & Brierly, 1990).

The environmental impacts of iron ore mining, as the very first step in steel production, are important information for understanding the sustainability profile of steelmaking process. Steel consumers are keenly aware of this need and are looking to better understand the contribution of iron ore processing to the overall footprint of steel products. More and more steel consumers including carmakers, for example, are setting up schemes to evaluate the maturity of their suppliers when it comes to sustainability, and major certification programs in the construction sector are rewarding products with visible data on their raw material extraction stage.

But iron ore environmental knowledge has been limited by the availability of reliable data. Until now, any lifecycle analysis (LCA) studies—key to understanding the potential environmental impact of a product throughout its entire lifecycle—have used general data from the mining sector. These data are not readily available on either conventional iron ore mining or bioprocessing. This chapter will try to bring insights into the lifecycle assessment of iron ore bioprocessing operations and particularly its environmental impacts. The assessment covered in this chapter is intended to provide environmental indicators beyond CO_2 emissions—which are already well reported—including primary energy demand, global warming potential, acidification (mainly caused by combustion processes, in particular coal power plants, and on-site diesel engines), eutrophication (caused by phosphates and nitrates emissions), and photochemical ozone creation.

2   Environmental Regulations

Bioprocessing is no stranger to environmental regulatory concerns. Any kind of industrial bioprocessing must at least go through the motions of basic environmental assessment to meet EPA and local state environmental requirements. But nowadays more and more emphasis has been placed on sustainability, due not only to public pressure, but also a world of decreasing resources. How the bioprocessing industry is beginning to incorporate related ideas into its processes and facilities? What degree of sustainability is realistic to strive for? What hidden costs of not modernizing do companies tend to miss in their evaluations, and what are the real economic advantages of going green? Where are the tradeoffs specific to various methods of disposal, and how are they to be evaluated? And what lessons can the iron ore bioprocessing industry learn from attention paid to this topic by many nonferrous companies and regulatory agencies?

For any kind of industrial bioprocesses, the evaluation of its environmental impact might potentially use the same structure but is likely to require some different tools and business processes. These differences stem from differing bioprocess characteristics and the associated alternative regulatory framework as shown in Table 1.

Table 1 Aspects of bioprocessing and their corresponding US regulatory agencies

Aspects	Regulatory agency
Biosafety	National Institutes of Health (NIH)
Recombinant DNA	National Institutes of Health (NIH)
Environmental (plant) pathogenicity/recombinant organism release	US Department of Agriculture (USDA)
Air emissions (fermentor off-gas and other volatiles from solvent feeds or solvent usage in isolation)	State Department of Environmental Protection (DEP)
Wastewater discharges (e.g., trace metals, high/low pH, biological demand)	Environmental Protection Agency (EPA), state DEP, municipal wastewater facility
Solid discharges (e.g., disposables, cell mass)	EPA, state DEP
Water usage and consumption	Voluntary (incentive-based)
Power use (e.g., steam generation, equipment electricity)	Voluntary (incentive-based)
Commuters and deliveries	Voluntary (incentive-based)

3   Environmental Impact Assessment

Environmental impact assessments (EIA) qualitatively and quantitatively determine, assess, and mitigate the biological, physical, chemical, economic, and social consequences of a proposed project on the environment. The key steps are to identify, predict, and evaluate impacts. An important first step is to identify major inputs and outputs for an iron ore bioprocessing process as shown in Fig. 1. The E factor (also known as the process mass index) is defined either as the mass of waste divided by the mass of products (direct calculation) or the difference between the mass of inputs and the mass of outputs divided by the mass of products (indirect calculation with product mass also including recyclable mass). The latter definition incorporates the benefit of potential water recycling for bioprocessing processes. The engineering quotient, EQ, multiplies the E factor by the unfriendliness quotient, Q, where Q ranges from 1 for innocuous salts to 100–1000 for metal salts based on their toxicity. This parameter considers the lower environmental impact of most bioprocess wastes.

Environmental risk impact assessments that characterize waste and air emissions identify opportunities to minimize their generation. They also identify opportunities for potential recovery of materials for recycling, although for biopharmaceutical processes these opportunities need to be evaluated carefully against GMP considerations. Environmental wastes in production—solid waste, wastewater, and air emissions—have no value to a customer. Furthermore, because of the lack of a consistent evaluation framework, environmental waste reduction opportunities remain largely untapped. The remainder of this article offers an initial framework for assessing microbial and animal cell biopharmaceutical processes, specifically focusing on opportunities in early-stage process development. An additional goal is that this environmental evaluation framework also can be transferred to internal or contract manufacturing organizations.
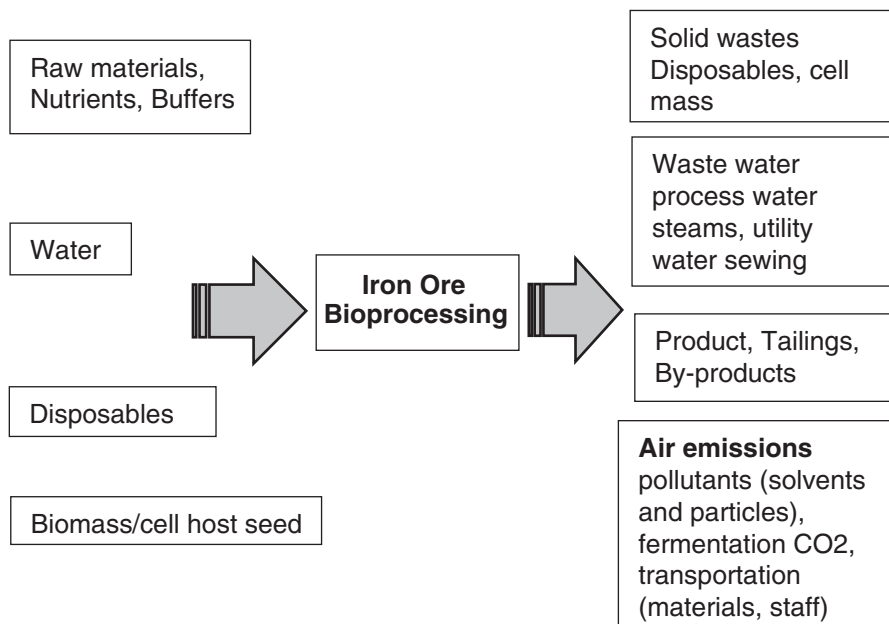
Raw materials, Nutrients, Buffers		Solid wastes Disposables, cell mass

| Water | → | **Iron Ore Bioprocessing** | → | Waste water process water steams, utility water sewing |

| Disposables |

| Biomass/cell host seed |

| Product, Tailings, By-products |

| **Air emissions** pollutants (solvents and particles), fermentation CO2, transportation (materials, staff) |

Fig. 1 Input/output elements for assessing bioprocess environmental footprint

3.1 Environmental Impacts of Iron Ore Mining

The environmental problems associated with the iron ore mining are diverse. The removal of vegetation, topsoil, overburden/waste, and ore brings about the inevitable natural consequences, which manifest in many ways, deforestation, climatic change, erosion, air and water pollution, and health hazards. A discussion of the potential environmental effects associated with iron ore mining is presented in the following sections.

Water Pollution

Exposed ore, overburden piles, waste rock and ore piles, tailings impoundments, and other disturbed areas can contribute sediment and increase the total solids load to surface water bodies. Other potential sources of surface and groundwater contamination include fuel spills, flotation reagents, cleaning solutions, and other chemicals used or stored at the site. For iron recovered from sulfide-bearing ores, acid generation due to the oxidation of sulfides (e.g., pyrite and pyrrhotite) in the ore body, host rock, and waste material may be of concern. Trace elements and minerals often associated with iron deposits include aluminum, antimony, arsenic, beryllium, cadmium, chromium, copper, lead, manganese, nickel, selenium, silver, sulfur,

titanium, and zinc. Lowering pH increases the solubility of these constituents and may make them available for transport in both surface water and groundwater. After a mine is abandoned, pumping is usually stopped, allowing the pit or underground workings to fill with water. Over time, this may lead to uncontrolled releases of mine water.

Air Pollution Due to Dust and Noxious Fumes

The primary sources of air contamination at mine sites are fugitive dust from dry surfaces of dry tailings impoundments, as well as overburden, waste rock, and ore piles. Often, tailings impoundments are not completely covered by pooled water; thus, dry tailings may be available for windblown transport. Deposition of windblown tailings provides exposure routes for contamination of groundwater, surface water, and soil. Air pollution is also a major problem in the mines area, with concentration of suspended particulate matter (SPM) in ambient air much above the permissible limit in many places, particularly at crusher loading and transfer points.

Soil Erosion and Contamination

Environmental impacts to soils as a result of mining activities are most commonly associated with erosion and contamination. Erosion may be caused by land disturbances and removal of vegetation related to mining activities. Under these conditions, precipitation events, such as snowmelt, may lead to erosion of soils. Contamination of soils may result from water discharge, runoff, seepage from tailings impoundments, pits and mine workings, as well as from the overburden, waste rock, and ore piles directly to soils. In addition, deposition of windblown particulates from piles and dry tailings impoundments may also be a source of soil contamination. Other sources of soils contamination include spills of fuels, flotation reagents, cleaning solutions, as well as other chemicals used or stored at the site.

Waste Management in Mining Industries

Disposal of mine wastes historically involved either returning the materials to the mining site; dumping into the ocean, a stream, or lake; or placing them into a receiving pond. Today, surface containment of tailings within embankments remains a commonly used approach. In 1995, it was estimated that on an annual basis over 700 million kg of metals in mine tailings were disposed on land (Warhurst, 2000). Alternatively, tailings may be returned to the mine (in-pit storage or backfilling) or mixed with coarse mine waste (codisposal). However, they remain unstable and subject to eolian dispersion and water erosion with the potential to contaminate nearby communities and environmentally sensitive areas.

3.2   Remediation Processes for Iron Ore Mining

Conventional technologies for remediation of mine tailings have focused on physical and chemical stabilization. Physical stabilization entails covering mine waste with an innocuous material, generally waste rock from mining operations, gravel, topsoil from an adjacent site, or a clay capping, to reduce wind and water erosion. These solutions are often temporary in nature because of the impermanence of the capping process. Engineering techniques such as soil washing, burning, excavation, and removal are used to remediate heavy metal-contaminated soils, but the cost of these procedures is very high (Pilon-Smits, 2005). For this reason, the development of low-cost, effective, and sustainable technologies to remediate heavy metal-contaminated soils is very important and long overdue (LeDuc and Terry, 2005), and it should receive considerably more attention. At the same time, phytoremediation is a cost-effective and eco-friendly "green" remediation technology for environmental cleanup. (Mohanty et al., 2010; Mohanty and Patra, 2011).

The conventional techniques used for remediation have been to dig up contaminated soil and remove it to a landfill or to cap and contain the contaminated areas of a site. The methods have some drawbacks. The first method simply moves the contamination elsewhere and may create significant risks in the excavation, handling, and transport of hazardous material. Additionally, it is very difficult and increasingly expensive to find new landfill sites for the final disposal of the material. The cap and contain method is only an interim solution since the contamination remains on-site, requiring monitoring and maintenance of the isolation barriers long into the future, with all the associated costs and potential liability.

A better approach than these traditional methods is to destroy the pollutants if possible or at least to transform them to innocuous substances. Some technologies that have been used are high-temperature incineration and various types of chemical decomposition (e.g., base-catalyzed dechlorination, UV oxidation). They can be very effective at reducing levels of a range of contaminants, but have several drawbacks, principally their technological complexity, the cost for small-scale application, and the lack of public acceptance, especially for incineration that may increase the exposure to contaminants for both the workers at the site and nearby residents.

Bioremediation is an option that offers the possibility to destroy or render harmless various contaminants using natural biological activity. As such, it uses relatively low-cost, low-technology techniques, which generally have a high public acceptance and can often be carried out on-site. It will not always be suitable, however, as the range of contaminants on which it is effective is limited, the time scales involved are relatively long, and the residual contaminant levels achievable may not always be appropriate. Although the methodologies employed are not technically complex, considerable experience and expertise may be required to design and implement a successful bioremediation program, due to the need to thoroughly assess a site for suitability and to optimize conditions to achieve a satisfactory result.

Because bioremediation seems to be a good alternative to conventional cleanup technologies research in this field, especially in the United States, rapidly

increasing. It has been used at a number of sites worldwide, including Europe, with varying degrees of success. Techniques are improving as greater knowledge and experience are gained, and there is no doubt that bioremediation has great potential for dealing with certain types of site contamination. Unfortunately, the principles, techniques, advantages, and disadvantages of bioremediation are not widely known or understood, especially among those who will have to deal directly with bioremediation proposals, such as site owners and regulators. Here, we intended to assist by providing a straightforward, pragmatic view of the processes involved in bioremediation, the pros and cons of the technique, and the issues to be considered when dealing with a proposal for bioremediation. Some tests make an exhaustive examination of the literature of bioremediation of organic (King et al., 1997; Norris et al., 1993) and inorganic pollutants (Hinchee et al., 1995), and another test takes a look at pertinent field application case histories (Flathman et al., 1993) as shown in Table 2.

Industrial bioprocessing is a combination of chemical and biological processes. The most traditional approach suggested supplementing strong mineral acids such as sulfuric acid to help destroy/dissolve carbonate minerals, stimulate oxidation of sulfide minerals and form favorable conditions for microbial organisms. However, addition of the acid brings negative environmental effects. For example, the acid application can lead to acidification of the industrial site and requires the following remediation of the environment that means increasing costs for pollution abatement.

Thus, it is possible to see fruitful ways and steps to create new biotechnology that shall be both profitable and more eco-friendly not only in comparison with pyrometallurgy but also in competition with some modern biomining processes too. One of the mode evident tasks for the green technology is to minimize input of sulfuric acid as an industrial supplement. The same effect of leaching can be reached with a minimum of the technical acid via application of (i) moderate acidophilic or neutrophilic leaching bacteria, (ii) leaching bacteria which produce enough amounts of sulfuric acid themselves, or, as well, (iii) these two groups combined. Application of the moderate acidophilic bacteria decreased the local pH value in the treated pulp and changed it to threshold valuation that also permits the subsequent activity of the strong acidophilic bacteria without introducing chemical acidifying supplements. The suggested replacement of strong acidophilic bacteria for moderate acidophilic ones shall decrease cost of production and reduce the expenses for the site remediation.

Biofiltration   This is a pollution control technique employing the use of living material to capture and biologically degraded process pollutants. Common uses of biofiltration processes are for processing wastewater, capturing harmful chemicals or silt from surface runoff, and microbiotic oxidation of contaminants in air.

In multimedia-multiphase bioremediation, waste streams containing volatile organic compounds (VOCs) may be treated with combinations of phases, that is, solid media, gas, and liquid flow in complete biological systems. These systems are classified as three basic types: biofilters, biotrickling filters, and bioscrubbers (http://www.biofilter.com/). Biofilms of microorganisms (bacteria and fungi) are grown on

Table 2 Bioremediation processes for handling iron ore mining environmental impacts

Environmental conditions	Biosystems or microbes	Bioremediation benefits
Wastewater and industrial effluents	Sulfur-metabolizing bacteria	Microorganisms in sewage treatment plants remove common pollutants (heavy metals and sulfur compounds) from wastewater before it is discharged into rivers or sea. Useful biogas (methane, etc.) production from anaerobic wastewater treatment.
Process water	Organic degrading microbes (bacteria, fungi, and algae)	Reclamation and purification of iron ore mineral processing wastewaters for reuse Remove chemicals and wastes before discharging into ecosystem
Air and waste gases	Bacteria and fungi	Biofilter application of pollutant purifying bacteria. Application of bioscrubbers, immobilized microorganism in inert matrix and nutrient film trickling devices for better air and gas purification. For example, bioscrubber-based system for removal of nitrogen and sulfur oxides from flue gas of blast furnaces replacing limestone gypsum process
Soil and land treatment	Pseudomonas spp., Bacillus spp., Fungi, Rhodococcus, Acinetobacter, and Mycobacterium	Both in situ and ex situ are commercially implemented for the cleanup of soil and groundwater. Use of microorganisms (bioaugmentation, ventilation), and/or adding nutrient solution, i.e., biostimulation, for example, petroleum decontamination, can involve use of plants—phytoremediation. Bacteria in association with roots of plants (Rhizobacterium), and so forth. Use of bioreactors for ex situ treatment with introduction of suitable microbes and environmental factors.
Solid waste	Bacteria, fungi, and other microbes	Composting or anaerobic digestion of domestic and garden wastes helps in recovery of high-value biogas and useful organic compost without the toxic components. Free breakdown of solid waste by microbial biota for recyclable waste, an acceptable alternative to incineration.

porous media in biofilters and biotrickling systems. The application of this biotechnological tool includes the following.

Control of Air Pollution When applied to air filtration and purification, biofilters use microorganisms to remove air pollution. The air flows through a packed bed, and the pollutant transfers into a thin biofilm on the surface of the packing material. Microorganisms, including bacteria and fungi, are immobilized in the biofilm and degrade the pollutant. Trickling filters and bioscrubbers rely on a biofilm and the bacterial action in their recirculating waters. The air or other gas containing the VOCs is passed through the biologically active media, where the microbes break down the compounds to simpler compounds, eventually to carbon dioxide (if aerobic), methane (if anaerobic), and water. The major difference between biofiltra-
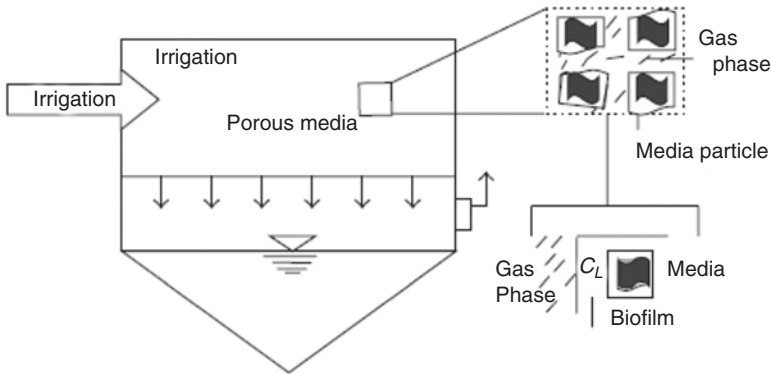
Fig. 2 Schematic of packed bed biological control system to treat volatile compounds. Air containing gas-phase pollutants (C_G) traverse porous media. The soluble fraction of the volatilized compounds in the air steam partition into the biofilm (C_L) according to Henry's law. $C_L = C_G/H$, where H is Henry's law constant

tion and trickling systems is how the liquid interfaces with the microbes. The liquid phase is stationary in a biofilter (Fig. 2), but liquids move through the porous media of a biotrickling system (i.e., the liquid "trickles").

A particular novel biotechnological method in biofiltration (Fig. 3) uses compost as the porous media. Compost contains numerous species of beneficial microbes that are already acclimated to organic wastes. Industrial compost biofilters have achieved removal rates at the 99% level (Vallero, 2010). Biofilters are also the most common method for removing VOCs and odorous compounds from air streams.

In addition to a wide assortment of volatile chain aromatic organic compounds, biological systems have successfully removed vapor-phase inorganics, such as ammonia, hydrogen sulfide, and other sulfides including carbon disulfide, as well as mercaptans. The operational key is the biofilm. The gas must interface with the film. Compost has been a particularly useful medium in providing this partitioning (Vallero, 2010). Industries employing the biofiltration technology include food and animal products, off-gas from wastewater treatment facilities, pharmaceuticals, wood products manufacturing, paints, and coatings application and manufacturing and resin manufacturing and application. Compounds treated are typically mixed VOCs and various sulfur compounds, including hydrogen sulfide. Maintaining proper moisture condition is an important factor in biofiltration. The air normally humidifies before it enters the bed with a watering (spray) system, humidified chamber, bioscrubber, or biotrickling filter. Properly maintained, a natural organic packing media peat, vegetable mulch, bark, or wood chips may last for several years. However, engineered combined natural organic and synthetic component packing materials will generally last much longer, up to 10 years. A number of companies offer these types or proprietary packing materials and multiyear guarantees, not usually provided with a conventional compost or wood chip bed biofilter. For large volumes of air, a biofilter may be the only cost-effective solution. There is no
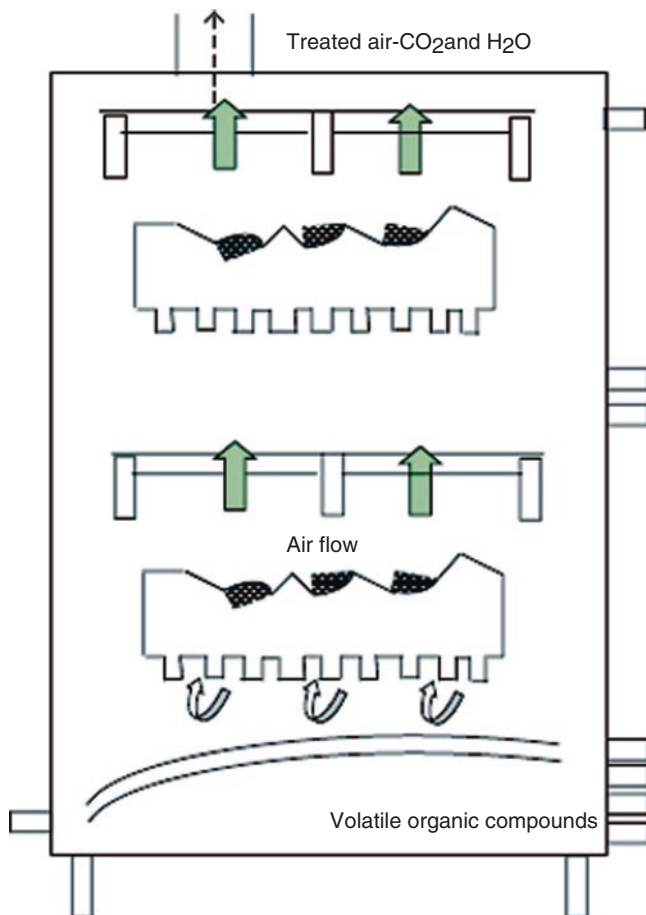
Fig. 3 Biofiltration without a liquid phase used to treat vapor-phase pollutants. Air carrying the volatilized contaminants upward through porous media (e.g., compost) containing microbes acclimated to break down the system can be heated to increase the partitioning to the gas phase. Microbes in the biofilm surrounding each individual compost particle metabolize the contaminants into simpler compounds, eventually converting them into carbon dioxide and water vapor

secondary pollution (unlike the case of incineration where additional CO_2, CO, and NO gases are produced from burning fuel(s) and degradation products form additional biomass, carbon dioxide, and water).

Water Treatment

Unlike any chemical treatment of iron ore mining wastewater, biological methods when used as membrane bioreactors or biofilters usually do not generate large amounts of sludge. Although biological treatment requires regular monitoring, the

fact that it is achieved inside biological media and being itself not a source of additional water pollution has ranked it as an economical and environmentally friendly one. The biological media could be either artificial or a natural one, the latter stimulated by addition of nutrient or energy sources like lactates, pyruvates, formates, malates, acetates, or even the organic pollutants themselves. Biological methods could be practiced as different systems (membrane bioreactors and biofilters, permeable reactive barriers (PRB) and constructed wetlands). They could be operated either in passive (static flow) or in active contact modes (Obreque-Contreras et al., 2015). Deo and Natarajan (1998a) provided evidence of biodegradation of flotation surfactants by the species Bacillus polymyxa used as biofilter. In a similar operation mode, biogenically generated sulfides, produced by some bacteria such as Desulfovibrio, Desulfotomaculum, etc., have been used as precipitating agents for the metallic ions found in acid mine drainage waters (Dobson and Burgess, 2007; Potvin, 2004; Obreque-Contreras et al., 2015). In some cases, by creation of conditions that are favorable to the growth for certain bacteria or plants like green algae blooms, the tailings pond could be transformed into a giant bioreactor (Obreque-Contreras et al., 2015; Shazia Iram et al., 2012; Sterritt and Lester, 1979). The same study indicated that the proliferation of green algae blooms inside the tailings pond reflecting in production of biogenic hydrogen sulfide from the dead algae has led to more than 90% removal of heavy metals via biosorption and precipitation as sulfides.

Concerning the biological treatment by means of constructed wetlands, it operates on the same principle as the passive sulfate-reducing biofilters but requires larger surface areas. Constructed wetlands provide an attractive means for long-term wastewaters management at abandoned mill and mine sites. The application of wetlands for industrial wastewater treatment is looked at by Awaleh and Soubaneh (2014) as a promising alternative apart from their significant merits of having low capital and operating costs compared to other conventional treatment systems.

When metallurgical wastewaters are to be treated, wetlands are usually placed as polishing step after the primary chemical neutralization when most heavy metals are already precipitated (Gaydardjiev et al., 1996). At the Doe Run Buick mine in Missouri, USA, the combination of settling in tailings pond and bio-treatment in series of artificially constructed meandering channels and polishing lagoon has led to removal of more than 95% zinc and manganese along with 50% and 60% copper and lead, respectively, from the flotation wastewaters (Erten-Unal and Wixson, 1999). It should be noted that although biological methods have found application both in the treatment as well as in the recycling of wastewaters from variety of industries, their industrial implementation for treatment and recycling of flotation wastewaters is still to be seen (Galil and Levinsky, 2007). Besides, the presence of bacteria in the treated water can perturb the process functioning particularly in the case of sulfide ores flotation (Levay et al., 2001).

4   Conclusions

Achieving LCA for the first time in iron ore bioprocessing will be a major accomplishment because it paves the way for building a complete environmental profile for green manufacturing of steel products and how conventional iron ore mining operations can factor into this—something that our customers will increasingly want to know.

The contamination of soil, air, and water resources with metals and bioreagents is one of the major environmental concern for iron ore bioprocessing processes. Metals and other inorganic contaminants are among the most prevalent forms of contamination found at waste sites, and their remediation in soils and sediments is technically most difficult due to their persistent nature. The high cost and ineffectiveness of existing cleanup technologies have led to the search for certain low-cost, low-impact, visually benign, and environmentally sound cleanup strategies. Under these circumstances, phytoremediation can be a better alternative to the environmentally destructive traditional remediation technologies. No doubt, phytoremediation technologies are still in research and development phase, but various field applications have shown potential for success. This, in turn, has helped to increase interest and research in both public and private sectors, to develop phytoremediation into a commercially viable industry. However, some key technical hurdles must be overcome for an industry to adopt this plant-based technology on commercial scale like identifying more species with remediation abilities, appropriate plant selection, and agronomic practices optimizing phytoremediation processes, understanding more about the mechanism of uptake, translocation, and metabolization of the contaminant concerned, to identify or genetically construct plants that are hardy enough to tolerate high shoot metal concentrations and still produce large amounts of biomass which in turn can reduce the required number of cropping cycles to a minimum, and identifying genes responsible for the tolerance of the concerned plant species with respect to a particular contaminant and extensive research under field conditions. Furthermore, the technique must be tailored to the physicochemical characteristics (pH, cation exchange capacity, electrical conductivity, and metal content) of individual mine tailings sites. Enough and honest advertisement of the successful ventures must be made to the scientific community as well as public to enhance its acceptability as global sustainable technology.

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Laboratory Research and Techniques

1 Introduction

For developing suitable bioprocessing processes and methods on a commercial scale, laboratory-based design and execution of metallurgical test programs are essential. Small-scale test facilities are necessary in this regard. Both batch and continuous bioprocessing reactor systems can be developed to suit a specific ore deposits to establish amenability and feasibility of a chose bio hydrometallurgical route.

The air-lift percolation techniques developed during the early period of biohydrometallurgy research are still used nowadays. Air-list percolators are the simplest and easy to construct in varying dimensions using pyre glass or PVC. It essentially consists of a column of suitable diameter and length with an open-up and a porous diaphragm at the bottom to support ore sample and solution drainage. Through a side parallel limb which runs from the top to bottom, drained solution can be recycled to the top through a compressed air or suction device. Percolation bioleach tests in air-lift percolators can yield valuable information on acid consumption, metal solubilization, and control of parameters such as Eh, pH, and temperature during biooxidation. However, these results cannot be directly scaled up. Larger column made of glass, fiberglass, PVC, stainless steel, or Perspex can be erected in different dimensions depending on the weight of the ore bed. Perforated bottom plate can be provided for the ore bed to rest on. Leach solution can be kept in a bottom vessel and circulated to the top through a peristaltic pump. Pump and heap leaching parameters can be simulated in column bioleaching tests. Several columns can be arranged in series. Column bioleaching tests can be performed on a long-term basis (as long as several years) to yield representative, commercially useful data. Scale-up studies using tons of ore samples can also be carried out in larger columns.

Specially for iron ore bioprocessing, microbial leaching is one of the most promising techniques. Depending on the working volume, the leaching techniques can be

divided into two domains: laboratory scale (up to 10 dm^3), and pilot plant scale (10 m^3) (Krebs et al., 1997).

Laboratory-scale leaching processes involve percolator leaching, column leaching, and submerged leaching (Bosecker, 1997). These processes may be scaled up to a pilot plant testing by considering certain scale-up factors, such as adequate oxygen and carbon dioxide supply, adequate agitation devices, and maintenance of specified pH and temperature for the growth of the microorganisms.

Percolator leaching is the first technique used in bacterial leaching. It consists of a vertical glass tube with sieve plate filled by solid particles at the bottom. The solid packing is flooded with nutrients inoculated with bacteria. The leaching liquor is pumped up by compressed air and is kept recirculated. This leaching technique generally suffers from inadequate oxygen supply, low efficiency, and fairly slow rates (Bosecker, 1997).

Column leaching is operated on the concept of percolator leaching and is often used for pilot plant-scale leaching. Column leaching is operated at a higher capacity (from few kilograms to tons) compared to percolator leaching (benchmark scale). Most column systems also apply online monitoring systems with the installation of monitoring instruments, such as a thermocouple and pH meter, compared to manual sampling from time to time in percolator leaching (Bosecker, 1997; Brombacher et al., 1997).

In submerged leaching, solid particles are agitated and kept in a suspension in the leaching medium, either in shake flasks or bioreactors. The ease in control and monitoring in this process allows the microorganisms to grow well and be active in bioleaching. Consequently, reaction times are shortened, and metal extraction yields increase (Bosecker, 1987). Bioleaching with fungi is commonly carried out using this technique, by means of either one-step or two-step bioleaching (Burgstaller & Schinner, 1993).

2   Isolation, Culture, and Adaptation of Microorganism

Isolation, culture, and adaptation of microorganism involve basic techniques in microbiology. The most common growth media for microorganisms are nutrient broths and agar plates; specialized media are required for some microorganisms. Most microorganisms related to iron ore processing require specialized environments due to complex nutritional requirements.

When growing in closed culture systems, such as a batch culture, where no additional nutrients are added and waste products are not removed, the bacterial growth will follow a predicted growth curve. The growth curve can be described in four different phases as shown in Fig. 1.

During lag phase, bacteria adapt themselves to growth conditions. It is the period where the individual bacteria are maturing and not yet able to divide. During the lag phase of the bacterial growth cycle, synthesis of RNA, enzymes, and other molecules occurs. Exponential phase (sometimes called the log or logarithmic phase) is a period characterized by cell doubling. The number of new bacteria appearing per

Fig. 1 Four different
phases of bacterial growth
curve in batch culture (lag
phase A, exponential or log
phase B, stationary phase
C, and death phase D)
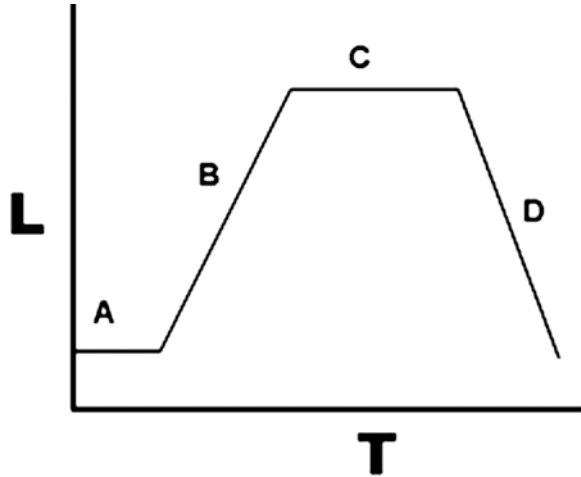
unit time is proportional to the present population. Under controlled conditions, cyanobacteria can double their population four times a day. Exponential growth cannot continue indefinitely, however, because the medium is soon depleted of nutrients and enriched with wastes. The stationary phase is due to a growth-limiting factor; this is mostly depletion of a nutrient and/or the formation of inhibitory products such as organic acids. In death phase, bacteria run out of nutrients and die.

Batch culture is the most common laboratory growth method in which bacterial growth is studied, but it is only one of many. The bacterial culture is incubated in a closed vessel with a single batch of medium.

In some experimental regimes, some of the bacterial culture is periodically removed and added to fresh sterile medium. In the extreme case, this leads to the continual renewal of the nutrients. This is a *chemostat*, also known as an open or continuous culture: a steady state defined by the rates of nutrient supply and bacterial growth. In comparison to batch culture, bacteria are maintained in exponential growth phase, and the growth rate of the bacteria is known. Related devices include *turbidostats* and *auxostats*. Bacterial growth can be suppressed with bacteriostats, without necessarily killing the bacteria.

In a synecological culture, a true-to-nature situation in which more than one bacterial species is present, the growth of microbes is more dynamic and continual (Fig. 2).

Much of the study of microbiology depends on the ability to grow and maintain microorganisms in the laboratory, and this is possible only if suitable culture media are available. In the natural habitats, microorganisms usually grow in complex mixed populations with many species. This presents problem for microbiologists because a single type of microorganism cannot be studied in a mixed culture; one needs a pure culture, a population of cells arising from a single cell, to characterize individual species. However, to isolate microorganisms from the natural environment, it is necessary to perform serial dilution to reduce or thin out the population of microorganisms in the sample sufficiently.

Serial Dilution   Typically, ten test tubes are filled with 9 ml of water and then steril-
ized (they are labeled −1 to −10). With a sterile pipette, 1 ml of the original sample is
taken and transferred into the first test tube (−1), vortexed, and 1 ml taken and trans-
ferred into the next test tube (−2). The process is repeated till the last test tube (−10).
Depending on the type of plating to be carried out, a known volume is taken from any
of the dilutions with a sterile pipette, and dropped on the agar in the petri dishes,
spreading is done with a sterile glass rod. The petri dishes are allowed to dry and then
incubated. Isolated cells grow into colonies and can be used to establish pure cultures.

Spread Plate   A small volume of dilute microbial mixture containing around
30–300 cells is transferred to the center of an agar plate and spread evenly over the
surface with sterile bent glass rod. The dispersed cells develop into isolated colo-
nies. Because the number of colonies should equal the number of viable organisms
in the sample, spread plate can be used to count the microbial population.

Streak Plate   The microbial mixture is transferred to the edge of an agar plate with
an inoculating loop or swab and then streaked out over the surface in one of several
patterns. After the first sector is streaked, the inoculating loop is sterilized and an
inoculum for the second sector is obtained from the first sector. A similar process is
followed for streaking the third sector, except that the inoculum is from the second
sector. Thus, this is essentially a dilution process. Eventually, very few cells will be
on the loop, and single cells will drop from it as it is rubbed along the agar surface.
These develop into separate colonies. In both spread plate and streak plate tech-
niques, successful isolation depends on spatial separation of single cells.

Pour Plate   The original sample is diluted several times to reduce the microbial
population sufficiently to obtain separate colonies when plating. Then, small vol-
umes of several diluted samples are mixed with liquid agar that has been cooled to
about 45 °C, and the mixtures are poured immediately into sterile culture dishes.
Most bacteria and fungi are not killed by a brief exposure to the warm agar after the
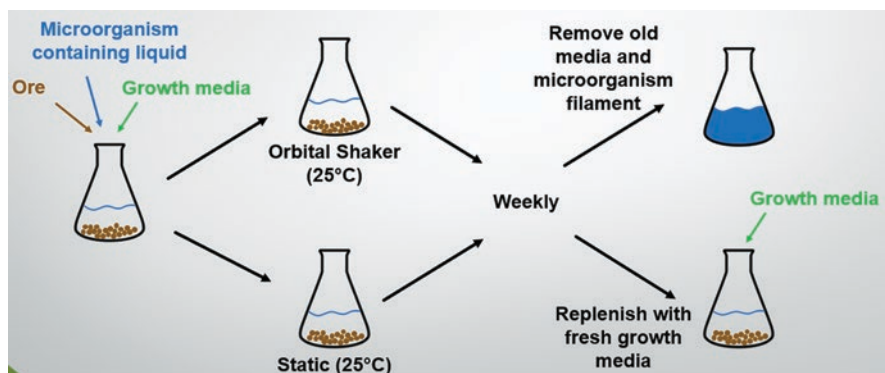

Fig. 2 Typical microorganism culturing method

agar has hardened; each cell is fixed in place and forms an individual colony. Like the spread plate, the pour plate can be used to determine the number of cells in a population. Plates containing between 30 and 300 colonies are counted. The total number of colonies equals the number of viable microorganisms in the sample that can grow in the medium as colonies growing on the surface also can be used to inoculate fresh medium and prepare pure cultures.

3   Laboratory Shaking Flask Test

Laboratory shake flask test basically consists of Erlenmeyer flasks and a shaker as test equipment. The shaking ensures mixing and homogenization as well as continuous changing of the liquid surface, thereby enhancing the dissolution of atmospheric oxygen and other gases which are utilized by microorganisms for their metabolism. The shake flask test is usually the initial experimental approach for most bioprocessing tests due to its simplicity, small sample size, quick response, and low cost. With continuously changing conditions, it offers little operational control, but is a convenient method of screening and replicating large numbers of cultures and ore samples.

All industrial processes began in the laboratory and, if successful, finish in the production plant. In transferring a laboratory-scale process to the production-scale process, various problems are likely to be encountered. For successful scale-up process, it is necessary to overcome all these problems. In typical fermentation process, the major scale-up problems fall into three major categories, namely development of inoculum, aeration–agitation, and media sterilization. In bioleaching process, sterilization is not very crucial scale-up problem as the process operates under specific extreme condition, so contamination problem is not very critical. In bioleaching process, presence of high pulp density, heavy metal ions, and autotrophic nature of the inoculum are responsible for additional scale-up problems.

There are two schools of thought concerning the ideal number of stages, which should be involved in scale-up from the flask to the production plant. Normally, four stages will be sufficient for majority of the fermentation processes, and only one size of fermenter should be selected from each of the mentioned scales (Table 1).

Table 1 Typical size of laboratory, pilot, and industrial-scale vessel for microorganism test

Type of vessel	Volume	Comments
Shake flask	50–1000 cm^3	Microorganism culturing and isolation
Laboratory fermenter/incubator	5–20 dm^3	Microorganism culturing for laboratory bioleaching tests
Pilot fermenter/incubator	50–5000 dm^3	Microorganism culturing for pilot-scale column or stirred tank leaching tests
Production fermenter/incubator	25–1000 m^3	Industrial-scale culturing

Normally, preliminary experimentation with a new project is always carried out at the shake flask scale. This study is very versatile. Results obtained at this level are transferred to the next stage of scale-up.

As bioleaching is a complex process, various physicochemical and biological factors affect it. Moreover, each level of scale-up has some specific factors influencing the process. Optimization of many of these factors can be done at shake flask level in less time with ease and economy.

Effectiveness of leaching depends largely on the efficiency of the microorganisms and on the chemical and mineralogical composition of the ore to be leached. The maximum yields of metal extraction can be achieved only when the leaching conditions correspond to the optimum growth conditions of the bacteria. Some important factors influencing the bioextraction of metals from minerals are illustrated.

4   Pilot Column and Tank Test

Laboratory column setups of different dimensions and designs are shown in Figs. 3, 4 and 5. The reactor can be operated both in fluidized bed and drain mode depending on particle size. Compressed air is passed through the top, and through controlled aeration, the ore particles in the slurry can be kept in suspension. The leach liquor can be drained off periodically and recirculated.

Fig. 3 Small-scale bioleaching column (2 kg capacity)

Fig. 4   Medium-size column bioleaching setup (50 kg capacity each)

Fig. 5   Large-size column
bioleaching setup (100 kg
capacity)

To study amenability for agitation bioleaching, shake flask tests using rotary incubator shakers on a laboratory scale can be performed. Though the kinetic data generated from share flask studies render plenty of useful information on the bioprocesses involved, they are yet insufficient for the development of models useful for design of commercial reactors. Continuous flow stirred of mineral concentrates. Pilot studies can be carried out using stirred tank system under controlled conditions. A pilot-scale stirred tank bioreactor is shown in Fig. 6.

In stirred tank bioreactors or in short stirred tank reactors (STRs), the air is added to the culture medium under pressure through a device called sparger. The sparger may be a ring with many holes or a tube with a single orifice. The sparger along with impellers (agitators) enables better gas distribution system throughout the vessel. The bubbles generated by sparger are broken down to smaller ones by impellers and dispersed throughout the medium. This enables the creation of a uniform and homogeneous environment throughout the bioreactor.

Fig. 6 Stirred tank bioreactor (5-liter capacity)

There are many advantages of STRs over other types. These include the efficient gas transfer to growing cells, good mixing of the contents, and flexible operating conditions, besides the commercial availability of the bioreactors.

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Pilot-Scale Studies, Scaling-Up, and Technology Transfer

1 Introduction

Expanding microbial processes for commercial production is a win-or-lose endeavor, entailing time and investment often exceeding that for laboratory process development. Mistakes, neglect, and carelessness can be expensive, even fatal to the overall project. A proper approach is required to scale up from laboratory to commercial. Three basic steps are needed, pilot-scale studies, scaling-up to commercial scales, and technology transfer. During each stage, the project executors must begin with the end in mind, be diligent in the details, and prepare for the unexpected. There is a particular emphasis on the fermentation step, which is usually the costliest and impacts downstream processing. It is advised that engineering resources skilled in integrated process development and scale-up be engaged from the very beginning of microbe and process development to guide ongoing R&D, thus ensuring a smooth and profitable path to the large-scale commercial end.

2 Pilot-Scale Studies

Pilot-scale studies are the first step of scaling up most bioprocesses and are often the limiting step for a process to a commercial scale. Moving from the laboratory and R&D scale to a pilot plant is the safest way to establish what aspects of production and process parameters need to be considered for the industrial-scale bioprocessing process.

A pilot plant aims to translate a laboratory process into a small-scale industrial process. Building a pilot facility is one of the most expensive parts of product development, so this phase must be completed on time and within budget. For this reason, pilot manufacturing is often outsourced, leading to issues with compatible facilities/equipment, process control, and contamination. Whichever route is taken,

the most crucial role of a pilot plant is to demonstrate proof of concept for a process. A pilot plant will provide material for downstream processing and quality assessment. It is also necessary to optimize the process at scale, often tweaking lessons learned at the benchtop to translate into full-scale manufacturing. Ultimately, the goal of the pilot plant is to develop and optimize the process for production manufacturing.

2.1   Key Considerations

To build a successful pilot plant, it is essential to know the objectives from the beginning. Understanding the production targets and downstream processing allows for the assessment of risk and helps to develop contingencies to mitigate issues that may arise.

A successful pilot plant can demonstrate a predictable, cost-effective, and robust process that produces iron ore products that meet steelmaking specifications. The process needs to meet the regulatory and environmental requirements set for the industry. This means that the process must often meet government and local laws authorizing and guiding mining activities, such as the Environmental Protection Agency (EPA), in regulating mining operations. Other acts, such as the Safe Drinking Water Act, can be relevant to specific mining operations. Establishing the blueprint for process validation at the pilot scale can ease the transition into full-scale production.

The most critical factor for any large-scale projects and tests is often the cost-efficiency. It is vital to perform an economic analysis to estimate capital costs (CAPEX) and the total operational costs (OPEX). Pilot plants are an excellent way to trial production and understand the costs associated with the production process. The expense of running a pilot-scale plant is often prohibitory, with fermentation iterations being the highest cost. Maximizing development speed and process fidelity is paramount. A return on investment must be demonstrated in a defined time-frame, or a project is doomed to failure.

2.2   Challenges for Scaling Up Bioprocesses

The challenges for pilot-scale studies are often different than those for laboratory-scale tests. Many of the parameters considered are the same (heat transfer, oxygen transfer rate, mixing, etc.), but the inherent dynamics of the system change when the volume is increased. Additionally, the equipment and controls available for industrial processes are fundamentally different from benchtop bioreactors. Thus, for various reasons, the same "recipe" developed in the laboratory does not necessarily translate well to a larger scale (Noorman & Heijnen, 2017; Yang, 2010).

A list of key parameters and considerations to consider when scaling up a bioprocess (Weiss, 2016) are as follows:

- Heat transfer.
- Mixing (impeller type, speed, shear).
- Oxygen transfer rate.
- Pressure.
- Solubility of gases (temperature, partial pressure).
- Bioreactor (tank/fermenter) aspect ratio.

    Other important considerations and challenges for scaling up a bioprocess:

- Sourcing and optimizing feedstocks/media components (variability of raw materials).
- Feed rates and switching feedstocks.
- Micronutrient vs. macronutrient (purity and availability).
- Contamination/sterility.
- Fermentation by-products.
- Utilities (appropriate for the application, interruption of services).
- Equipment failure.
- Compatibilities with the process, equipment, and regulation.

The fermentation is often the most challenging part of scaling-up (Culler, 2016; Yang, 2010). Pilot-scale fermentation requires a thorough understanding of not just the microbiology of the organism but the equipment necessary to make the process successful. It is essential to understand the iron ore bioprocessing system's physical and chemical limitations and how to address the challenges of scaling-up. Selecting the correct bioreactor for the iron ore bioprocessing process is key to optimizing product yield, quality, and efficiency.

3   Scale-Up and Technology Transfer

In a broad sense, scaling up any bioprocesses is a critical activity that enables a bioprocess achieved in research and development to operate a commercially viable manufacturing scale. A successful scale-up involves many aspects of successful preparation and planning beyond pure process scale-up technology. These include setting clear goals and expectations, timelines and milestones, resources and organization, facility fit considerations, and quality and specifications.

Technology scale-up and transfer are common and crucial biodevelopment activities. For any bioprocessing process, commercial success depends on increasing production volume quickly and effectively and maintaining production efficiency and cost-effectiveness with a certain variation of raw materials quality. A successful transfer is vital for product quality and efficiency, but failure's time and financial costs can be significant.

Regulators expect manufacturers and their contract partners to take a systematic approach and provide all required documentation to move a process to a new facility or convert it from demonstration to commercial scale. Technology transfer activities guide the transfer of product and process knowledge from development to manufacturing or between manufacturers.

3.1   Framework for Technology Transfer

Laying the groundwork is key in technology transfer. Ensuring that those who are working on the transfer have the required experience and skills will help ensure success and avoid pitfalls. Process parameters and process knowledge may need to be transferred from development to pilot study to commercial production. In all cases, final-scale and success parameters, such as critical quality attributes, must be defined clearly, in writing, before the transfer begins (Inzelt & Hilton, 1999).

Transfers between different companies demand extra care in planning and documentation. Given differences between facilities and equipment, standard operating procedures are unlikely to translate directly and will need to reinvent the target site.

Occasionally, the sending party has a minor stake in the project's success than the recipient, making a disciplined approach imperative. High-level due diligence regarding capacity, facilities, capability, and personnel will help teams assess the feasibility to prepare for transfer. Planning, for example, can allow teams to preorder equipment with long lead times, if necessary. Note that transfer does not end with completing qualification lots or approval but extends throughout the production process. Successful technology transfer follows an orderly progression to set expectations and ensure that all stakeholders work toward the same goals. Teams should follow all of the following steps, explained in subsequent sections.

Form technology transfer teams and governance structures and define a project charter with goals and timelines. Setting clear expectations and responsibilities between partners in the technology transfer is crucial to avoiding confusion and conflict down the road. The initial charter agreed upon by all parties must include the scope of the project, technology transfer timelines, and the team structure, specifying clearly defined roles and responsibilities. The charter should also establish clear communication paths and a government structure for addressing issues. Most importantly, success criteria must be clearly documented in the project charter.

Consolidate process knowledge into a technology transfer protocol. Communicating manufacturing challenges can be complex; the sending personnel may be so close to the process that they no longer see the difficulties. Nonetheless, both sending and receiving parties must collaborate to create a detailed flowsheet and description of the process.

The process description document overviews each step and must include critical process parameters. Everything from facility and equipment requirements to raw materials and consumables to vendors to analytical methods must be outlined, focusing on intrinsic, site-, and scale-independent process requirements. The

sending team should provide as much potentially helpful process information as possible, down to tacit knowledge of media color, etc.

Analyze gaps and risks to create a detailed project plan. Based on the process description document, the next step is a thorough process walk-through at the receiving site. This is a great learning exercise for the receiving team and identifies areas where changes will be necessary and what differences are acceptable. Information learned from this activity guides the project work plan by pinpointing needs for facility, equipment, training, procedure, or process modifications to address gaps.

Some process amendments are inevitable, based on significant differences in the facility, equipment, or operational practices, and risks are always inherent in technology transfers. To determine acceptability, changes may require specific new validation studies or be covered in the qualification validation process. Predefined success criteria are essential for promptly accepting or rejecting changes.

Execute the technology transfer as planned. Once the transfer protocol and project plans are in place, the teams can perform the actual transfer to be ready for process qualification. First, they must make the necessary equipment and facility modifications to mitigate identified risks. Next, they must execute the process at a small scale and qualify that model before progressing to a more extensive or full-scale process. Once the team has developed a successful operation, they must author and approve manufacturing instruction documents and train production and support staff.

Transferring a process between brands and sizes of equipment is always problematic. Computational fluid dynamic (CFD) simulation can help to navigate this step efficiently and successfully. A comprehensive methodology for modeling and adapting the equipment can mitigate risk by making the transfer much faster and easier. The method should be based on understanding the critical, systematic differences among brands and sizes of bioprocessing equipment. For example, mixing efficiency can vary from system to system because of impeller type, position or size, or size of the tank. Similarly, sparging efficiency can vary with sparger size, position, or bubble size differences. Temperature gradients are typically not comparable, and fluid dynamics can vary with baffle type, position, and interactions. These characteristics impact the growth of the cell line and may therefore affect titers or quality in the final batch an unacceptable result. However, mass transfer modeling, which models the behavior of the bioreactors in use, can help determine the right equipment settings to achieve consistent titers and quality of end products.

Technology transfer may be a matter of transferring a process and negotiating issues that come with it. In this case, during the planning and risk analysis stage, it became apparent that the sending laboratory had never actually run the process twice within the same template. Therefore, the robustness of the process may be in question. Experimentation showed that the process was not robust and resulted in inconsistent quality attributes. In the end, the receiving party may decide that the most likely way to achieve success would be to redevelop the process to meet the acceptance criteria set forth at the beginning of the transfer.

Demonstrate technical success: meet acceptance criteria (process qualification). Predefined success criteria are a must, whether they are part of a validation protocol

or not. Process qualification through a demonstration showing that the process is performing correctly at the receiving unit may be a formal validation exercise or a simple report following an early clinical campaign. Criteria should include key performance metrics such as step yields, impurities, growth rates, and titers. They may also delineate product quality ranges or require success in a formal validation. Advance agreement on success metrics can speed up decision making at crucial go/no-go points.

Finalize the transfer through documentation, support of regulatory activities, follow-up actions, and examining lessons learned. The last technology transfer tasks are geared toward process performance review and regulatory approval. If process issues still need correction, the receiving team must assign actions and complete the work. Recognized flaws in standard transfer procedures must be amended. The team must also prepare documents for regulatory submission, respond to questions, and prepare for inspections and implement systems for ongoing production technical support. Finally, the team must complete all required documentation.

4   Conclusions

A well-planned pilot-scale study, scale-up, and technology transfer plan are essential to minimize the risk of failure when scaling a bioprocess to commercial size. This requires a pilot-scale study program that focuses on the core technology and provides the necessary data for a larger-scale plant to handle separation steps, waste streams, and material handling operations. Additionally, in developing commercial bioprocesses to produce iron ore products, using pilot plant results to design and operate a continuous commercial plant helps address many challenges often overlooked in bench or batch laboratory tests. Material handling, solid–liquid separations, stream recycling, and waste stream handling need to be investigated in the pilot plant before the demonstration and commercial plants are built.

Following a successful pilot-scale study and scaling-up, a systematic approach for technology transfer must be followed to pass the documented knowledge and experience gained during development to an appropriate party for full-scale commercial production. First, it is paramount that success criteria be defined, ahead of time, in writing. Critical quality attributes must be identified, agreed upon, and recorded. All parties must agree on the measures of success. Second, regulations mandate that technology transfer be performed in a specific, organized way to avoid surprises. The best option is to follow the regulatory guidelines. Lastly, the culture and vocabulary of the sending and receiving facilities have to be on the same page. The sending facility may be unmotivated or unable to cooperate with the receiving facility, and standard operating procedures will likely not apply after changes in equipment and facilities. To ensure the best outcomes, receiving teams must plan and organize transfers down to the last detail and maintain a disciplined approach at all times. A partner experienced in technology transfer can help ensure success and minimize expenditures in time and cost.

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Outstanding Questions and Future Developments

1 Introduction

Bioprocess development for iron ore starts with a robust bioprocess capable of producing iron ore products with the desired yield and purity. It requires experiments to understand the interaction of parameters of the specific bioprocess. Usually, adequate laboratory tests are needed for screening the feasible bioprocesses, which would need lots of processing materials and time. Hence, the high throughput approach of implementing experiments at smaller scales is extremely important as it only requires a small amount of processing materials. With optimized parameters under laboratory scale, pilot studies and scaling-up are necessary before the commercialization of the bioprocesses. During each developing stage, the impact of multivariable on the bioprocess development needs to be studied at the same time, thus allowing a broader range of data to be collected. Due to the complexity of the biological system, experimental design with iterative selection methods that are based on the previous run data are vital when little is known about the process. How to define the design condition and integrate the manipulation of experiments and data analysis, as well as experiment design, will become outstanding questions of any developing bioprocess project. These outstanding questions must be addressed to bring the current practice, the experimental design, multivariable experiment execution, and assay analysis together and formulate a successful bioprocess solution.

2 Outstanding Questions

There are still many fundamental questions related to iron ore bioprocesses that need to be addressed before they can be implemented at commercial scales. First and foremost, the energy pathways and reaction rates related to iron ore

bioprocessing must be resolved. Direct use of tailor-made microorganisms might bypass some of the obstacles. The biological activities in a reservoir are also poorly understood. Growth control of reservoir microbes and the knowledge to achieve this control will be crucial. In any bioreactors, the main challenge will be how to achieve sufficient reaction rates that are required for a commercial process. This is not a challenge from the microbiological aspect only but also from a metallurgical engineering point of view.

The fast-evolving digitization and COVID-19 pandemic have compelled us to rearrange over routines. It also presented bioprocess development with new challenges to master, i.e., current working procedures state-of-the-art techniques must be reshaped to put bioprocessing into a new light. Over the last several years, many questions arose concerning how to accelerate the process from the early stage of laboratory tests to commercial production. The answers to these questions will be the key drivers to speed up bioprocess development timelines in the future (Neubauer et al., 2013):

- Consistent, scalable, sharable, and well-defined data with accessibility.
- Process modeling.
- Better process understanding.
- Process intensification.
- Improved knowledge transfer.
- Advanced process control.

Continuous integrated manufacturing, process intensification, and process modeling will be the enablers to speed up bioprocess development in the near future. Further improvement and progress in all fields are mandatory to advance bioprocess development to fulfill faster timelines. However, only the first steps have been set in this long-lasting journey. Although all the partners and infrastructure to speed up bioprocess development are available, the industry has to put these parts together to change the routine to speed up the process significantly.

2.1 How to Profit from Existing Data and Upcoming Technologies to Speed Up the Iron Ore Bioprocessing Development?

It is often not enough to collect as much process data as possible to build a good process model. These data has to be brought in context with the process's desired target parameters, often referred to as critical quality attributes (CQA). The design space is one of the crucial parameters to extract complete meaning information quickly and to prove the model boundaries for applications like soft sensors or in the future model predictive control (MPC). The experts believe that MPC implementations for CQAs might be seen in 5–10 years until fully implemented in bioprocess manufacturing (Nickel et al., 2017).

There are three main bottlenecks in using advanced process models for the industry, the limited knowledge of building meaningful process models, the current IT infrastructure, and the limited time to perform good experiments. The complexity of bioprocesses requires scientists to create new technologies and powerful process models. The process models migrating from the R&D stage to the manufacturing can shed light on the bioprocess definitions and design space characterizations and can use to understand the process better to decrease the development timelines. The applicability of predictive model control and knowledge transfer is usually not the main driving force. Each unit operation must be perfectly described with different modeling approaches before a holistic picture can be drawn. The proper design space description and robust hybrid modeling solutions are the key enablers to fulfill reduced timelines and increased understanding and ensure both a better knowledge transfer and to implement MPC in the future.

2.2 But Do We Need "New Technologies" for Speeding Up Iron Ore Bioprocess Development?

Another critical point to discuss was the application of "new technologies." Many approaches are, per se, not new and rather state of the art in other areas. For example, computational fluid dynamics (CFD) and hybrid models have been used in the chemical industry for years but only recently entered the area of bioprocessing (von Stosch et al., 2016). These approaches might not be assessed as new to change the timelines and knowledge within bioprocess development. One of the main reasons for not implementing more modeling approaches in the bioprocessing industries might be the limited modeling understanding, the limited timelines to focus on new technologies, and the relatively low budget for process development compared to other sectors such as the pharmaceutical industry.

However, it can be expected that the aim of increasing process understanding with the right partners while reducing development timelines can easily be achieved. The question remains where the project owner wants to invest. However, it is confident that if reduced timelines, such as seen in the COVID-19 vaccine development, would be transferred to the bioprocessing of iron ores, the pressure on bioprocess development will further increase. To cope with such timelines, alternative and better approaches are needed to have to be implemented in the industry as fast as possible.

To balance the beneficial and detrimental effects of microbial growth in the iron ore bioprocessing system, fundamental research has to be conducted to acquire new knowledge. Growth and possible excretion of products under different conditions are not well known. To date, various types of chemicals are used in iron ore mining and processing to upgrade iron grade, e.g., to counteract or minimize the influence of other undesirable constituents during concentrating. Some of these chemicals are known to serve as either energy sources or growth inhibitors for the microorganisms, i.e., nitrogen, phosphorus, and carbon sources. Mining conditions vary significantly,

and thus, the microbial communities will respond differently depending on this external influence. It is imperative to acquire an in-depth understanding of the growth and production of microbial products under the different iron ore mining and processing conditions. In this respect, modeling tools may be used to simulate how the changes will influence the indigenous microorganisms. Joint efforts from internal experts and external collaborators are vital to the success of this type of project. Much knowledge on microbial technologies already exists, but the molecular biology approach represents a bold and important step forward.

3   Future Development

Process development in biotechnology is still mainly driven by experienced personnel creating and evaluating experimental data. However, in recent times, automation, miniaturization, and data science are setting new paths for drastic changes in bioprocess development. Today, hundreds of automated experiments can be performed in parallel miniaturized cultivation and purification systems per day. Additionally, statistical experimental planning and evaluation are applied to utilize these facilities' experimental capacity efficiently. Still, innovative software concepts and workflows are needed to exploit the full potential of automated laboratories. Moreover, the conditions in which small-scale experiments are performed need to resemble the production scale as closely as possible.

Today, there are numerous possibilities to create, collect, store, and share data. This has significant potential in biotechnology where (i) the complexity of living microorganisms can be studied and analyzed by computer-aided tools, (ii) complex biochemical pathways including hundreds of thousands of reactions can be reconstructed, and (iii) proteins can be designed using in silico tools and gene synthesis. (Herold et al., 2017; Nickel et al., 2017).

Nevertheless, while today's development of processes and products in other areas of engineering can be performed firmly based on computer-based models with a minimum of practical experiments, the future bioprocess development is still coupled to extensive wet laboratory experimentation due to the nature and the complexity of bioprocesses. The most significant challenge is that it is challenging to create a reliable mathematical model of even the most straightforward biochemical reaction. Changes in protein expression, metabolic activity, mutations, etc., can neither be foreseen, described, nor deeply understood. In other words, model-based methods in biotechnology will always be coupled with experimental activities to validate and re-adjust models to the reality of living systems. From advanced plant-wide control and optimization strategies for bioprocesses to custom-made therapeutics, fully automated experimental facilities are required to run thousands of experiments in parallel to produce large amounts of data needed to validate the mathematical models constantly. These robotic laboratories must be intelligent when designing experiments and learning from the data.

Today's laboratory automation systems aim to increase experimental throughput instead of performing complex, intelligent operations. However, to make bioprocess development more efficient, these systems must be equipped with adaptive experimental design methods to plan and execute complex experiments and, e.g., decide when to take representative and informative samples and initiate the analysis. Errors should be detected automatically and promptly. The experimental strategies should be readjusted as data are being generated. The experiment results should deliver the maximum amount of information possible.

In the near future, scientists and engineers should be able to enter basic parameters in the computer models (e.g., operating conditions, available raw materials and substrates, desired process yield, and final product grade and recovery), which will be used to (i) find candidate microorganisms, (ii) design screening experiments, (iii) carry out the experiments, (iv) analyze, evaluate, and store the data, and (v) send the specification of the new process (including strain, required facilities, operating conditions, and control strategy) for its bioprocess application.

Methods for design of experiments (DoE) have been developed and widely applied to this end. Unfortunately, time-dependent responses are often the most important factors (e.g., industrial processes, slow kinetics associated with microorganisms). Therefore, the experimental design should consider the nonlinear dynamics of the process, in which case more advanced adaptive tools such as model-based design of experiments (MBDoE) are additionally required. These methods can be customized to a specific project and used to (i) design optimal dynamic experiments in a very short time, (ii) communicate with the testing facility that experiments, (iii) learn from the experimental data as it is being generated, (iv) readapt the parameters of the model to the data, and (v) redesign the experimental plan while keeping the biology of the system in focus.

There has been a significant advancement in bioprocess development, and several innovative approaches have been reported. Herold et al. (2017) reported the successful application of a progressive parameter-control workflow during a lactase production process. With this strategy, the time frame for process development was reduced to 3 months. Customized software solutions for the integration of model-based techniques are presented by Wellenbeck et al. (2017). One of the key elements of the efficient use of automation in biotechnology is the organized storage of all data in a database, as Schmid and Aschoff (2017) described. These data are immediately accessible for evaluation and replanning of the experiments. An example of how "intelligence" can be incorporated into such a complex system where the whole experiment is not only performed but also digitally controlled is described by Cruz Bournazou et al. (2017) and Nickel et al. (2017). At a somewhat lower level, automatic operations also can be used to identify important parameters for process development, such as the dependence of the specific product formation rate on the specific growth rate—important information for establishing a fed-batch process. When the amount of data is limited, hybrid models can be applied as an alternative to pure statistical analysis for better process understanding, as illustrated by von Stosch et al. (2016).

The broad range of topics illustrates the interdisciplinary nature of bioprocess development and highlights the necessity of combining these innovations into future process development platforms. If metallurgists, molecular and microbiologists, software programmers, process engineers, and business administrators work closely together, production efficiency, time to market, and costs of iron ore bioprocesses and iron ore products can be reduced significantly.

4   Conclusions

The biological characteristics of microorganisms and enzymes often impose constraints on bioprocessing; therefore, knowledge is an important prerequisite for rational engineering process design and development. For instance, bacterial thermostability properties must be considered when choosing the operating temperature for a bioleaching reactor. The susceptibility of a microorganism to substrate inhibition will determine whether the substrate is fed to the incubator or fermenter all at once or intermittently. It is equally valid, however, that biologists working in biotechnology must consider the engineering aspects of bioprocessing. Selection or manipulation of microorganisms should be carried out to achieve the best results in production-scale operations.

All areas of bioprocess development—the microorganism used, the culture conditions provided, the fermentation equipment, and the operations used for iron ore processing—are interdependent and also closed interconnected. Because improvement in one area can be advantageous or disadvantageous to another, the iron ore bioprocess development should proceed using an integrated approach. Combining the skills of metallurgical engineers and mathematical modelers with biologists with the strong experimental technique will facilitate the future development of iron ore bioprocess. Many outstanding questions still need to be answered in all areas of microbiological mechanism and kinetics, equipment design and process analysis, quantitative methods, physics, and engineering. To build a working iron ore bioprocessing plant, engineers must take a pragmatic approach with the help of specialists in all necessary fields to address the wonder, intricacy, and complexity of the iron ore bioprocessing process.

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