Advances in Biochemical Engineering/Biotechnology 141
Series Editor: T. Scheper

Axel Schippers
Franz Glombitza
Wolfgang Sand *Editors*

Geobiotechnology I

Metal-related Issues

Springer

141
Advances in Biochemical Engineering/Biotechnology

Aims and Scope

This book series reviews current trends in modern biotechnology and biochemical engineering. Its aim is to cover all aspects of these interdisciplinary disciplines, where knowledge, methods and expertise are required from chemistry, biochemistry, microbiology, molecular biology, chemical engineering and computer science.

Volumes are organized topically and provide a comprehensive discussion of developments in the field over the past 3–5 years. The series also discusses new discoveries and applications. Special volumes are dedicated to selected topics which focus on new biotechnological products and new processes for their synthesis and purification.

In general, volumes are edited by well-known guest editors. The series editor and publisher will, however, always be pleased to receive suggestions and supplementary information. Manuscripts are accepted in English.

In references, Advances in Biochemical Engineering/Biotechnology is abbreviated as *Adv. Biochem. Engin./Biotechnol.* and cited as a journal.

More information about this series at http://www.springer.com/series/10

Axel Schippers · Franz Glombitza
Wolfgang Sand
Editors

Geobiotechnology I

Metal-related Issues

With contributions by

Malte Drobe · Franz Glombitza · Sabrina Hedrich
Robert Klein · Erika Kothe · Martin Mühling
René Phieler · Susan Reichel · Wolfgang Sand
Axel Schippers · Michael Schlömann
Judith S. Tischler · Marios Tsezos · Jürgen Vasters
Annekatrin Voit · Sabine Willscher

Springer

Editors
Axel Schippers
BGR—Fachbereich Geochemie d. Rohstoffe
Hannover
Germany

Wolfgang Sand
Fakultät für Chemie, Biofilm Centre
Universität Duisburg-Essen
Essen
Germany

Franz Glombitza
G.E.O.S. Ingenieurgesellschaft mbH
Halsbrücke
Germany

ISSN 0724-6145 ISSN 1616-8542 (electronic)
ISBN 978-3-642-54709-6 ISBN 978-3-642-54710-2 (eBook)
DOI 10.1007/978-3-642-54710-2

Library of Congress Control Number: 2014943414

Springer Heidelberg New York Dordrecht London

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Preface

Mineral and energy resources are increasingly being exploited to meet the demands of a worldwide growing population and economy. Despite technological developments, these raw materials cannot, or can only partly, be substituted by renewable resources within the next few decades. Thus, the efficient recovery and processing of mineral and energy resources, as well as recycling, are nowadays of significant importance in many countries.

Geobiotechnology can significantly contribute to new developments in this field and can be described as biotechnology in the geological context. This technology mainly takes advantage of the biological activity relevant for geochemical processes. Microorganisms control natural biogeochemical cycles and by doing so they contribute to the formation and alteration of metal, oil, coal, and phosphor deposits. Geobiotechnology comprises microbial processes in these deposits as well as in mining and environment. The interactions of microorganisms with raw materials enable an efficient geobiotechnological recovery of metals, oil and gas.

The ten chapters of this volume describe and summarize the scientific background and recent developments in metal bioleaching, bioextraction, biomineralization and bioremediation as well as in microbial enhanced oil and gas recovery (MEOR). Microbial processes in the underground and deposits, potentially used for the storage of raw materials or residues, or use of geothermal energy are also covered, including a chapter about basic mining legal principles.

The idea for this volume originated from the temporary working group Geobiotechnology of the German organisation DECHEMA e.V. Since many authors of this volume are active in this working group, geobiotechnological processes and applications are often described using examples from Germany and Europe.

The chapter on coal biotechnology is authored by the late Giovanni Rossi. He died in summer 2013 and could not live to see the publication. Giovanni Rossi was a dear friend, esteemed colleague, consummate engineer and researcher, and a pioneer in the field of biohydrometallurgy. We feel honored that he was able to finalize his contribution to this book. We dedicate this book in memory of Giovanni Rossi.

Axel Schippers
Franz Glombitza
Wolfgang Sand

Contents

Adv Biochem Eng Biotechnol (2014) 141: 1–47
DOI: 10.1007/10_2013_216

Published Online: 23 June 2013

Biomining: Metal Recovery from Ores with Microorganisms

Axel Schippers, Sabrina Hedrich, Jürgen Vasters, Malte Drobe, Wolfgang Sand and Sabine Willscher

Abstract Biomining is an increasingly applied biotechnological procedure for processing of ores in the mining industry (biohydrometallurgy). Nowadays the production of copper from low-grade ores is the most important industrial application and a significant part of world copper production already originates from heap or dump/stockpile bioleaching. Conceptual differences exist between the industrial processes of bioleaching and biooxidation. Bioleaching is a conversion of an insoluble valuable metal into a soluble form by means of microorganisms. In biooxidation, on the other hand, gold is predominantly unlocked from refractory ores in large-scale stirred-tank biooxidation arrangements for further processing steps. In addition to copper and gold production, biomining is also used to produce cobalt, nickel, zinc, and uranium. Up to now, biomining has merely been used as a procedure in the processing of sulfide ores and uranium ore, but laboratory and pilot procedures already exist for the processing of silicate and oxide ores (e.g., laterites), for leaching of processing residues or mine waste dumps (mine tailings), as well as for the extraction of metals from industrial residues and waste (recycling). This chapter estimates the world production of copper, gold, and other metals by means of biomining and chemical leaching (bio-/hydrometallurgy) compared with metal production by pyrometallurgical procedures, and describes new developments in biomining. In addition, an overview is given about metal sulfide oxidizing microorganisms, fundamentals of biomining including bioleaching mechanisms and interface processes, as well as anaerobic bioleaching and bioleaching with heterotrophic microorganisms.

A. Schippers (✉) · S. Hedrich · J. Vasters · M. Drobe
Federal Institute for Geosciences and Natural Resources (BGR), Hannover, Germany
e-mail: Axel.Schippers@bgr.de

S. Hedrich
School of Biological Sciences, Bangor University, Bangor, UK

W. Sand
Biofilm Centre, University of Duisburg-Essen, Essen, Germany

S. Willscher
Institute of Waste Management and Contaminated Site Treatment,
TU Dresden, Pirna, Germany

Keywords Biohydrometallurgy · Bioleaching · Biomining · Biooxidation · Cobalt · Copper · Gold · Nickel · Uranium · Zinc

Abbreviations

A	Autotroph
AFM	Atomic force microscopy
BGR	Bundesanstalt für Geowissenschaften und Rohstoffe
BRGM	Bureau de Recherches Géologiques et Minières
DNA	Deoxyribonucleic acid
EPS	Extracellular polymeric substances
F	Facultative autotroph and/or mixotroph
G+C	Mole% guanine+cytosine content of genomic DNA
H	Heterotroph
KPM	Kelvin probe microscopy
na	Data not available

Contents

1 Introduction

The naturally occurring ores of copper, zinc, and nickel exist largely in the form of metal sulfides. These sulfides are insoluble under normal environmental conditions as well as in weak acids, in contrast to oxidic minerals of these metals. Therefore, sulfide ores are concentrated by flotation. From the concentrates unrefined metals are produced by pyrometallurgical smelting. In the case of copper the smelting process begins with the production of copper stone from dried concentrates, nowadays mostly in fluid bed arrangements, or flash smelters, in which the roasting, smelting, and slag formation take place in a single working step. The intermediate product of this step is copper matte with approximately 70 % copper, primarily as copper sulfide as well as iron sulfide. In the conversion process sulfur is removed at high temperature as sulfur dioxide by blowing air or oxygen through the molten matte. The resulting product of the converter treatment is blister copper that contains about 98 % copper. For the refining process, the molten blister copper has to be treated in an anode furnace where excess oxygen is removed by reduction with natural gas. The deoxygenated copper is poured into moulds to produce anodes, which are placed in the electro refining bath. The final product of the copper extraction process is copper cathode with a quality of 99.99 % copper.

The roasting or oxidation of the metal sulfides leads to emission of sulfur dioxide gas. This does not cause environmental problems in modern plants, because sulfur dioxide is currently converted to more than 99 % sulfuric acid using the double contact process. Sulfuric acid is an important side product of copper production and sales comprise about 20 % of the revenues of the Chilean copper smelters.

An ecologically acceptable and yet economic alternative for processing of sulfidic low-grade ores, where metal sulfides cannot be concentrated economically by flotation, is the extraction of metals by means of microorganisms. This procedure is called biomining [18, 20–22, 38, 40, 124–127, 144, 150, 158, 179]. Nowadays biomining is an established biotechnology and is applied worldwide. Progress in the construction of leaching plants, in the construction and management of heap bioleaching operations, as well as in the process design resulted in a worldwide spreading of the technology. This may include the defined application and monitoring of the abundance and activity of the metal sulfide oxidizing microorganisms. All these developments enable biomining to compete successfully with other hydrometallurgical or chemical procedures. In addition, bioleaching appears more and more industrially proven as a portfolio of flexible techniques to provide a way of recovering base metals. Bioleaching is envisaged for processing low-grade ores, particularly when they contain deleterious elements that result in heavy penalties in pyrometallurgical treatments.

Bioleaching is the biological change of an insoluble metal compound into a water-soluble form. In the case of bioleaching of metal sulfides these are oxidized to metal ions and sulfate plus intermediate sulfur compounds in acidic solution by aerobic, acidophilic Fe(II)–, and/or sulfur compound oxidizing bacteria or archaea.

Important leaching bacteria are, for instance, *Acidithiobacillus ferrooxidans* (formerly *Thiobacillus ferrooxidans*) and *Leptospirillum ferriphilum* [125, 143]. The oxidative compound Fe(III) for the metal sulfide oxidation is provided by the microbial Fe(II)-oxidation. The sulfur compounds occurring in the course of metal sulfide oxidation, such as elemental sulfur, are oxidized by the microorganisms to sulfuric acid, creating an acidic environment.

2 Applied Biomining

2.1 Copper

2.1.1 Industrial Production

Sulfide copper ores are predominantly recovered by means of the flotation process and pyrometallurgical smelting of the flotation concentrate. A strong increase in the number of copper leaching operations (chemical and biological leaching) in the 1990s and at the beginning of the twenty-first century increased the share of leaching operations from 10 to 20 % of total copper production (Fig. 1). Since then the proportion of copper produced by leaching has remained stable at about 21 %. Nevertheless, in absolute figures, copper production from leaching processes has grown because of a strong increase in global mine production. In 1981, production from leaching processes amounted to about 0.6 million t and has increased to about 3.3 million t today. In the same period the total production of copper has doubled from approximately 8–16 million t (BGR databank).

The leaching processes have changed in the copper mining industry in this period. The conventional leaching of oxide ores with dilute sulfuric acid is increasingly being substituted by a growing share of bioleaching of sulfide ores. An important reason for this change is the decreasing resource of oxide copper ore in comparison to low-grade sulfide ore. In 2010 about 38 % of the leached copper or 8 % of the total primary mine production of copper (15.7 million t in 2010) originated, according to the BGR databank and literature research [48], from the bioleaching of sulfide copper minerals (Table 1). This includes dump/stockpile bioleaching operations where low-grade sulfide run-of-mine ores or primary crushed ores are used in leaching operations without further grain size reduction (e.g., in the United States). As there are no specific production figures for this type of leaching operation, it can be assumed that the share of bioleached copper can be estimated to be considerably higher than the above-mentioned production for copper bioleaching. Some authors estimate the share of bioleaching in primary copper production as more than 20 % [20, 21, 125]. There are some additional unlisted smaller leaching operations, for example, in Europe (Bulgaria: Asarel–Medet, Elshitza; Macedonia: Bucim, Kadiitza).

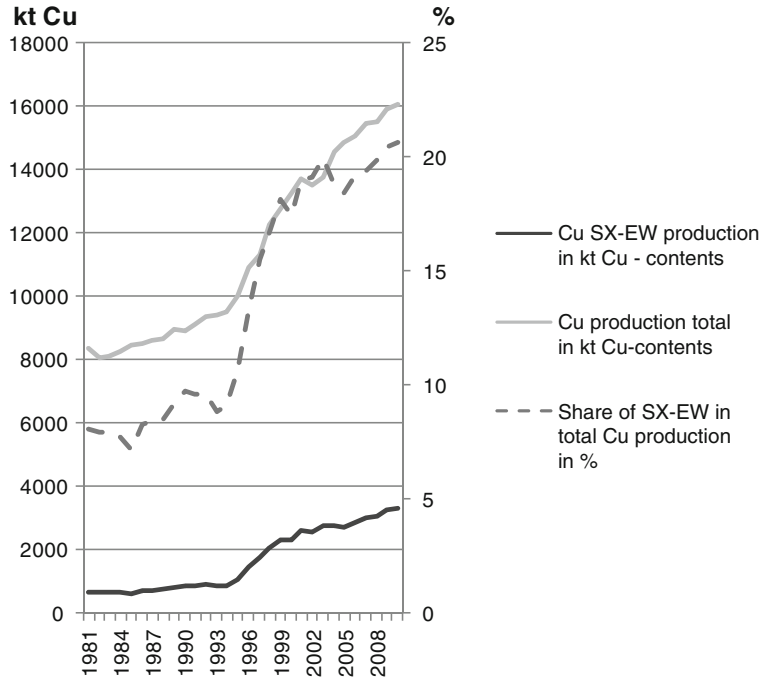

Fig. 1 Development of the total mined copper production as well as share of leaching (chemical and biological leaching), according to BGR databank research. SX-EW (solvent extraction-electro winning) is a standard procedure for the production of cathode copper in the mining industry

According to BGR databank research it is expected that copper production from bioleaching will increase by 40 % until 2014 compared with 2010. This shall be achieved by the production start-up of the large-scale bioleaching operation La Granja of Rio Tinto in Peru with a planned annual production of about 300,000 t of copper. Additionally, several smaller bioleaching projects with a total capacity of 200,000 t of copper will start production.

Three different processes for bioleaching of sulfide ores can be distinguished:

- Heap or dump/stockpile bioleaching with secondary, mostly low-grade sulfide ores, which contain minerals such as chalcocite (Cu_2S) and covellite (CuS).
- Heap bioleaching of low-grade primary copper sulfides such as chalcopyrite ($CuFeS_2$; pilot or demonstration scale).
- Stirred-tank bioleaching with copper concentrates (pilot or demonstration scale).

Most important for copper bioleaching to date is heap or dump/stockpile bioleaching with secondary copper ores. About 80 % of the bioleached copper originates from projects with secondary copper ores. In terms of the remaining 20 %, low-grade primary ore is increasingly bioleached via dump/stockpile leaching. Heap leaching of any copper ore has a recognized potential of expansion.

Table 1 2010 status of annual copper production from projects employing bioleaching, according to databank and literature research. The list of dump/stockpile leaching operations is incomplete

Copper production from bioleaching projects				
Country	Operation	Operator	Process	Cu in t
Australia	Whim Creek	Venturex		4.000
Australia	Lady Annie	CST Mining	Heap leach	20.000
Chile	Tres Valles	Vale SA	Heap leach	18.500
Chile	Andina Division	Codelco	Stockpile/Heap leach	25.000
Chile	Escondida Sulfide Lea	BHP Billiton Group	Stockpile/Heap leach	180.000
Chile	Ivan Zar	Cia Minera Milpo SA	Heap leach	10.000
Chile	Los Bronces	Anglo American plc		50.000
Chile	Quebrada Blanca	Teck Resources Ltd	Heap leach	85.000
Chile	Spence	BHP Billiton Group		120.000
Chile	Radomiro Tomic	Codelco	Heap leach	100.000
Chile	Collahuasi	Xstrata Plc		60.000
Chile	Cerro Colorado	BHP Billiton Group	Heap leach	100.000
Chile	Andacollo	Teck Resources Ltd	Dump leach	10.000
Chile	Zaldivar	Barrick Gold Corp	Heap leach	140.000
Chile	Chuquicamata	Codelco		20.000
China	Zijinshan Copper	Zijin Mining Group Co Ltd	Heap leach	12.840
China	Huogeqi	Local Government	Heap leach	20.000
Myanmar	Monywa	Rio Tinto, State of Myanmar	Heap leach	9.000
Peru	Cerro Verde	Freeport-McMoran	Heap leach	66.000
USA	Morenci	Freeport-McMoran	Heap/Dump leach	230.000
			Total	1,280.340

It has been subject to the most significant investment to control the bioleaching process at the level of growth of the microorganisms by understanding the microbial ecology in order to improve the overall efficiency of the process. These measures, as well as modeling of these operations, should be able to tackle the challenge of making heap leaching applicable to primary copper ores as it is to secondary ones. Among the forerunners in this field are BHP-Billiton, Codelco, and Mintek [37]. Chile and Peru together have a stake of 42 % of global copper mining and of 38 % of global copper reserves (BGR databank based on ICSG Data, 2010). The ratio of the copper content of oxide to sulfide ores is about 1:4 in Peru and 1:3 in Chile [154]. Further information on the geology of porphyry copper ore deposits and its implications for metal recovery via biomining can be found elsewhere [36, 179].

Assuming that oxide copper ore is usually processed by conventional chemical acid leaching, the long-term stake of conventional leaching in copper production in these two countries is about 20 % in Peru and 28 % in Chile. Today the proportion

of conventional leaching in Chilean copper production already amounts to about 23 %. A further increase can therefore only be achieved with difficulty. The proportion of bioleaching in the total leached copper production in Chile amounts to date to at least 42 % (918,500 t of Cu of a total of 2,160,000 t of Cu). An increase of leached copper in the total copper production of Chile is only possible in the long run if bioleaching of sulfide copper ores is further developed. Nowadays in Peru about 15 % of the produced copper originates from leaching processes. The proportion of bioleached copper in the total production of leached copper in Peru to date is about one third. It can be expected that the share of leached copper will further rise in Peru because of the reinforced leaching of oxide copper ores, as well as the bioleaching of sulfide ores.

2.1.2 New Developments

In view of the generally decreasing metal content of ore deposits or, respectively, the increasing depth of economically feasible copper ore deposits, classic processing technologies (grinding, flotation, roasting, and smelting) will lose their importance. However, this trend may be compensated by discoveries of new high-grade/-volume ore deposits and/or the introduction of alternative mining methods that allow large-scale production, for example, the caving method in deep underground mining operations (see below). With classic processing technologies, the power demand increases in inverse proportion to the metal content in the ore; that is, with very low metal content these conventional processes become increasingly uneconomical. Hence, it can be anticipated that with the expected dramatic increasing demand for raw materials, and considering the depletion of oxide ores, the biohydrometallurgical processing of sulfide ores will play a more important role in the future. In the case of copper, the biggest reserves exist as primary copper sulfides, as, for example, chalcopyrite ($CuFeS_2$). To date, the processing of this type of copper ore by leaching is limited in operations that usually apply heap bioleaching with mesophilic bacteria in the temperate range. However, heap bioleaching is under optimization by improved monitoring and modeling of the relevant parameters and processes including the microbial communities in the heap ecosystem [22, 88, 120, 126, 128, 152]. Further research is nowadays focused on the development of new biomining procedures for primary copper sulfides. A high copper extraction rate could be achieved with bioleaching using moderate thermophilic bacteria at approximately 50 °C or thermophilic archaea (genera *Acidianus, Metallosphaera, Sulfolobus*) up to 80 °C [3, 34, 57, 114]. In pilot and demonstration scale, tank bioleaching as well as heap bioleaching applications at high temperatures with high requirements to the material of process equipment and to process control already exist [10, 28, 45, 179].

2.1.3 Perspectives

The caving mining method, for example, the block caving method in the case of underground production from porphyry copper deposits is comparable in cost and production capacity to open pit mining and can be used, hence, also for low-grade copper ores, in particular when the technical and economic depth limit for an open pit mining operation is reached. A detailed description of this mining method can be found elsewhere [85]. The application of the block caving mining method is also planned for the transition from the open pit mining operation of Chuquicamata (Fig. 2) to an underground mining operation in Chile.

Because of the increasing dilution with waste rock in the case of an advanced block caving operation, up to 25–30 % of the ore has to be left in situ [178]. After the extraction of the ore from the block, which is carried out until a pre-calculated cut-off grade is reached; ore intermingled with waste rock remains in the mining block. Ore and waste materials in such a block are size reduced by natural fracturing and caving processes and resemble, with respect to structure and mineral content, low-grade ore or waste dump/stockpile. Hence, it can be supposed that the goaf area or, respectively, the abandoned working filled with caved-in rock and ore, is accessible for in situ (or in-place) leaching.

To the best of our knowledge nobody has yet considered in pre-mine planning stages the application of bioleaching for the ore losses originating in block caving. The effectiveness of the mining operation could be increased and resource efficiency be improved by taking into consideration in situ leaching utilizing caving mining methods in the planning phase of large-scale mining projects.

Fig. 2 Open pit mining operation of Chuquicamata, Chile

The new underground mine at Chuquicamata, which will be commissioned in 2018, will use the block caving mining method to produce about 350,000 t of copper content annually. Consequently with an assumed ore loss of 30 % due to the caving mining method, about 105,000 t of prepared copper content would annually remain in the abandoned blocks. Assuming a typical copper extraction rate of 50 % for dump/stockpile bioleaching, additionally more than 50,000 t of copper could be recovered by in situ post-leaching per year. This corresponds to the annual production of a medium-sized copper mine. In addition to this middle- or longer-term perspective within copper mining, bioleaching is already now of interest for the treatment of concentrates those are not accepted in regular copper smelters due to their high amounts of pollutant compounds (e.g., arsenic and bismuth), their unfavorable polymetallic composition, or high transportation costs.

Newer activities have opened the door for metal extraction from mine waste or processing residues (mine tailings, e.g., [29, 105, 151]). Furthermore, in the case of mine tailings, biomining could additionally be an option for bioremediation of mine waste that produces acid mine drainage, inasmuch as the removal of metals from tailings for metal recovery also significantly reduces the source for acid mine drainage formation. The bioleaching of base metal sulfide concentrates in stirred tanks is today far from being a standard process. The feasibility of bioleaching was shown not only for the production of copper, but also for a row of other metals including nickel, gold, silver, and uranium. The production of metals relevant for electronics such as indium, gallium, and germanium by means of bioleaching could also be shown in the laboratory [169].

2.2 Gold

Gold is not leached biologically because oxidation of the gold, which exists in the metallic state, does not take place. However, sulfidic iron and, perhaps, the arsenic matrix, in which the gold is either bound in the crystal lattice or enclosed as a particle, is biologically oxidized (biooxidation). The liberation of the originally refractory gold is facilitated by extracting solubilized oxidized mineral components. Afterwards the gold can be attacked by cyanide leaching [124].

The number of biooxidation projects in gold mining has strongly increased since its beginnings in the 1980s and 1990s. At least 14 active gold projects with biooxidation could be identified in the commercial project databank of the Minerals Economic Group (www.metalseconomics.com) and by other sources (BGR databanks). These projects produced at least 84 t of gold and 161 t of silver in 2010 (Table 2). The share of biooxidized gold corresponds to about 3.3 % of the total global production which amounted to about 2,450 t in 2010. For comparison, there are 444 gold projects that produce gold by hydrometallurgical processes either as main- or by-products. Their total gold production was about 1,950 t in 2010. Hence, the share of production derived from projects that include a

Table 2 2010 status of annual gold production from projects employing biooxidation processes, according to databank and literature research

Country	Operation	Project operator	Au in oz
Australia	Fosterville	NuEnergy Capital Ltd	112.000
Australia	Tasmania	BCD Resources NL	80.000
Australia	Wiluna	Franco-Nevada Corp	120.000
China	Jiaojia		55.000
China	Jinfeng	Eldorado Gold	150.000
China	Laizhou	Shandong Tiancheng Biotechn.	65.000
Ghana	Bogoso-Prestea	Golden Star Resources	280.000
Ghana	Obuasi	Anglogold Ashanti	400.000
Kazakhstan	Suzdal	Nord Gold (Severstal)	72.000
New Zealand	Reefton	Royalco Resources Ltd	87.300
Peru	Coricancha (Previously Tamboraque)	Nyrstar	15.000
Russia	Olimpiada	Polyus Gold Mining	839.000
South Africa	Fairview	Pan African Resources PLC	98.000
Uzbekistan	Kokpatas	Navoi Mining	353.000
		Total	2,726.300

biooxidation step in the processing route amounts to about 4.5 % of the total hydrometallurgically produced gold.

Because the leachable gold deposits that occurred close to surfaces were preferentially exploited in the past and are now going to be depleted [180], it can be assumed that the future production of gold coming from refractory or low-grade sulfide ores will increase significantly. Many of the newly developed, more deeply situated gold deposits have to be assumed as refractory in terms of mineralogy, because the gold is encased in sulfide minerals. In order to prepare these refractory ores for cyanide leaching, a pre-treatment that includes oxidation of the sulfides is required.

For many years the roasting of the sulfide ore was the only economical process for a pre-treatment of sulfide ores prior to cyanide leaching. Another process that can be used economically for the pre-treatment of rich sulfide ores and concentrates is the pressure oxidation in autoclaves. In the 1990s biooxidation as an alternative pre-treatment process, particularly for low-grade sulfide gold ores, was introduced. Biooxidation takes place in large-scale tank reactors set up in series in which several process parameters such as temperature, pH, O_2, and CO_2 are controlled. Optimum conditions for the metal sulfide-oxidizing bacteria are regulated. Because of the relatively slow process kinetics the residence time in the bioreactor is up to a few days. In contrast to pressure oxidation, the general advantages of biooxidation projects are the relatively low capital expenditures and the ease of operability [93, 177].

There are 13 new projects that include biooxidation processes currently in the project pipeline. These projects could additionally produce 26 t of gold in the next several years. There are three different technical processes distinguished:

- Biooxidation in heaps for low-grade, refractory gold ores (e.g., applied by Newmont Mining Corporation).
- Biooxidation in stirred tanks for refractory gold ores with higher content of gold (e.g., applied by Gold Fields, BIOX® process).
- Coating of inert tailings material with sulfidic gold concentrates and its biooxidation in heaps (e.g., applied by GeoBiotics).

Biooxidation in heaps: The reaction rate is relatively slow (months up to years for a heap), but the operating expenses and capital costs are very low. Diminished gold extraction in comparison to the other processes—between 50 and 75 % gold recovery—is observed. Likewise the long operation time, because of the long-term capital commitment, is considered a drawback in the case of the subsequently applied heap cyanide leach process. The process can be profitable especially for low-grade sulfide gold ores.

Biooxidation with stirred tanks: This process is characterized by a rapid reaction rate. The capital costs and operating expenses are substantially higher in comparison to the other processes, so that the application can only be used for high-grade gold ores and gold concentrates. This is considered at the moment to be the only biooxidation technology that is widely applied at industrial scale.

Biooxidation with thin layer technology (coating): This process lies with respect to performance indicators and the technical-economic parameters between the stirred-tank and heap leaching process.

2.3 Nickel, Cobalt, and Zinc

In comparison to copper leaching and the biooxidation of refractory gold ores and concentrates, bioleaching of other metals, for example, nickel, cobalt, and zinc, is still an exception. Bioleaching for the recovery of the above-mentioned metals is only used when the framework conditions (low-grade or refractory character of the ore, remoteness of the production plant) exclude conventional processing of the ore. An example for heap bioleaching of polymetallic ore is the Talvivaara mine in Finland, where nickel, zinc, copper, cobalt, and uranium are produced [123, 135]. According to the size of the planned production, in the future this project could deliver about 3 % of the total primary nickel production.

In a stirred-tank bioleaching plant at Kasese, Uganda, 240 t of pyrite concentrate are oxidized daily for the production of cobalt, copper, nickel, and zinc (Fig. 3 [105]). At Kasese about 1,100 t of cobalt per year are produced, corresponding to 1.25 % of the world production of cobalt which amounted to about 88,000 t in 2010.

Fig. 3 Stirred-tank bioleaching plant at Kasese, Uganda (picture from BRGM/Kasese Cobalt Company Ltd.)

2.4 Uranium

For chemical in situ leaching of uranium, an acid or alkaline digestion is applied depending on the acid-consuming characteristics of the rocks in the deposit. For this purpose, an oxidizing solution with complexing agents is introduced in the ore deposit via bore holes and the uranium-enriched solution is pumped to the surface for further processing.

In situ bioleaching of uranium ores is a procedure in which insoluble UO_2 is oxidized to water-soluble uranyl ions $(UO_2)^{2+}$ by means of microorganisms such as *At. ferrooxidans*. In the process, U(IV) is oxidized to U(VI) and in a coupled redox-reaction Fe(III) is reduced to Fe(II). The oxidizing agent Fe(III) for UO_2 is provided by the microbial Fe(II) oxidation as in copper biooxidation.

In 1984 Denison Mines, Ontario, Canada, commercially applied bioleaching of uranium and produced 10–15 % of that mine's total uranium production [99].

The current worldwide capacity of about 30 active uranium in situ leaching projects is about 34,000 t of uranium contents. This is a third of the worldwide production capacity for uranium. In situ leaching of uranium is considered to be

very efficient. The extraction efficiency is estimated to be within the range of 70–80 %. Environmental problems with respect to in situ leaching can originate from uncontrolled seeping of the solution.

In Germany until 1990 uranium was extracted by means of dump leaching (Ronneburg, Thuringia) as well as leaching mainly in blocks underground (Königsstein, Saxony). In Königsstein, 5,755 t of uranium were recovered via leaching from 1969 until 1990 (Franz Glombitza, personal communication).

2.5 Silicate, Carbonate, and Oxide Ores

The biotechnological processing of silicate, carbonate, and oxide ores on an industrial scale does not exist yet. Application potential exists, for example, for the production of lithium from spodumene, cobalt and nickel from laterites, or cobalt, nickel, copper, and manganese from polymetallic deep-sea nodules (manganese nodules [46, 53, 76]). Successful bioleaching of such ores by heterotrophic bacteria and fungi could be shown in the laboratory [18, 26, 77]. However, these microorganisms require (as opposed to the autotrophic metal sulfide-oxidizing bacteria and archaea) the addition of organic carbon (e.g., processed waste from the agricultural or food industries). This makes control of heterotrophic bioleaching processes more costly, and undesirable microorganisms may disturb these processes under real industrial operation conditions. A new perspective for the processing of silicate, carbonate, and oxide ores is offered by anaerobic bioleaching, for example, by the recently developed Ferredox process [46]. In that case, *At. ferrooxidans* is used, which solubilizes the ore, such as laterites, via Fe(III)-reduction, coupled with the oxidation of added elemental sulfur under the exclusion of aerial oxygen (anaerobic).

2.6 Metal Recovery from Waste

Metal recovery from waste is the topic of chap. 2 of this book, however, a brief overview is given here. Mine tailings from former ore processing activities often contain significant amounts of valuable metals. Thus, reprocessing of tailings including bioleaching or biooxidation steps has been demonstrated as feasible in laboratory to industrial scales [29, 105, 151]. Industrial residues including fly ash from waste or coal combustion, slag, sludge, electronic scrap, and the like, can be processed either with autotrophic microorganisms (*Acidithiobacillus*) and/or heterotrophic microorganisms, as already shown in laboratory or pilot scale [18, 26, 77, 91]. In addition, the extraction of metals from mine and industrial wastewater by means of biotechnological processes (bioremediation/biosorption) has already been applied. Also, some microorganisms have demonstrated the ability to form nanoparticles, consisting of pure metals or metal compounds (biomineralization [33, 78, 107]).

3 Metal Sulfide Oxidizing Microorganisms

Bioleaching and biooxidation require acidophilic metal sulfide oxidizing microorganisms that in fact oxidize Fe(II) and/or sulfur compounds. Most described acidophilic metal sulfide oxidizing microorganisms belong to the mesophilic and moderately thermophilic *Bacteria*. The *Archaea* are usually extremely thermophilic (in addition to the genus *Ferroplasma*). Most industrial heap and tank bioleaching operations run below 40 °C but operations at higher temperatures promise higher reaction rates [3, 10, 118]. Most of these microorganisms fix CO_2 and grow chemolithoautotrophically. A list of the metal sulfide oxidizing *Bacteria* or *Archaea*, their phylogeny, and some of their physiological properties is given in Tables 3, 4, and 5 (amended from [143]). The organisms can be separated into three groups according to their optimum temperature for growth: mesophiles up to ~40 °C, moderate themophiles between ~40 and ~55 °C, and extreme thermophiles between ~55 and ~80 °C. Further information on these microorganisms can be found elsewhere [41, 68–70, 73, 75, 83, 84, 115, 121, 143, 190].

The role of microorganisms in the bioleaching process is to oxidize metal sulfide oxidation [Fe(II) ions and sulfur compounds] products in order to provide Fe(III) and protons, the metal sulfide attacking agents. In addition, proton production keeps the pH low and thus, the Fe ions in solution. Aerobic, acidophilic Fe(II) oxidizing *Bacteria* or *Archaea* provide Fe(III) by the equation:

$$2\ Fe^{2+} + 0.5\ O_2 + 2\ H^+ \rightarrow 2\ Fe^{3+} + H_2O. \quad (1)$$

Aerobic, acidophilic, sulfur-compound oxidizing *Bacteria* or *Archaea* oxidize intermediate sulfur compounds to sulfate and protons (sulfuric acid). Most relevant is the oxidation of elemental sulfur to sulfuric acid inasmuch as elemental sulfur can only be biologically oxidized under bioleaching conditions:

$$0.125\ S_8 + 1.5\ O_2 + H_2O \rightarrow SO_4^{2-} + 2\ H^+. \quad (2)$$

The sulfur-compound oxidizing *Bacteria* or *Archaea* produce protons that dissolve metal sulfides in addition to pyrite which is not acid-soluble. Pyrite is only attacked by Fe(III) ions (not by protons) and therefore only dissolved by Fe(II) oxidizing *Bacteria* or *Archaea*.

4 Bioleaching Mechanisms

The mechanisms of bioleaching have been intensively discussed in the recent past. In the older literature "direct" versus "indirect" bioleaching is described [18, 54, 133]. Direct leaching means a direct electron transfer from the metal sulfide to the cell attached to the mineral surface. Indirect leaching proceeds via the metal sulfide oxidizing agent Fe(III) which is generated by Fe(II) oxidizing bacteria

Table 3 Phylogeny of acidophilic, metal sulfide oxidizing microorganisms (amended from [143])

Species[a]	Phylum	G + C (mol %)
Mesophilic and moderately thermophilic bacteria		
Acidiferrobacter thiooxydans	Proteobacteria	63
Acidimicrobium ferrooxidans	Actinobacteria	67–69
Acidithiobacillus albertensis	Proteobacteria	61.5
Acidithiobacillus caldus	Proteobacteria	63–64
Acidithiobacillus ferrooxidans	Proteobacteria	58–59
Acidithiobacillus ferrivorans	Proteobacteria	55.5
Acidithiobacillus thiooxidans	Proteobacteria	52
"*Acidithiomicrobium* P1/P2"	Actinobacteria	55/51
Alicyclobacillus disulfidooxidans	Firmicutes	53
Alicyclobacillus tolerans	Firmicutes	49
Alicyclobacillus GSM	Firmicutes	50.5
Ferrimicrobium acidiphilum	Actinobacteria	55
Ferrithrix thermotolerans	Actinobacteria	50
Leptospirillum ferriphilum	Nitrospira	55–58
"*Leptospirillum ferrodiazotrophum*"	Nitrospira	na
Leptospirillum ferrooxidans	Nitrospira	52
Sulfobacillus acidophilus	Firmicutes	55–57
Sulfobacillus benefaciens	Firmicutes	50
"*Sulfobacillus montserratensis*"	Firmicutes	52
Sulfobacillus sibiricus	Firmicutes	48
Sulfobacillus thermosulfidooxidans	Firmicutes	48–50
Sulfobacillus thermotolerans	Firmicutes	48
"*Thiobacillus plumbophilus*"	Proteobacteria	66
"*Thiobacillus prosperus*"	Proteobacteria	64
Thiomonas cuprina	Proteobacteria	66–69
Mesophilic and moderately thermophilic archaea		
Acidiplasma cupricumulans	Euryarchaeota	34
"*Ferroplasma acidarmanus*"	Euryarchaeota	37
Ferroplasma acidiphilum	Euryarchaeota	36.5
Extremely thermophilic archaea		
Acidianus brierleyi	Crenarchaeota	31
Acidianus infernus	Crenarchaeota	31
Acidianus sulfidivorans	Crenarchaeota	31
Metallosphaera hakonensis	Crenarchaeota	46
Metallosphaera prunae	Crenarchaeota	46
Metallosphaera sedula	Crenarchaeota	45
Sulfolobus metallicus	Crenarchaeota	38
Sulfolobus yangmingensis	Crenarchaeota	42
Sulfurococcus mirabilis	Crenarchaeota	~44
Sulfurococcus yellowstonensis	Crenarchaeota	45

[a] Listed in alphabetical order; G+C = mole% guanine+cytosine content of genomic DNA; na = data not available; species without standing in nomenclature (http://www.bacterio.cict.fr/) are given in quotation marks

Table 4 Optimum and range of growth for pH and temperature of acidophilic, metal sulfide oxidizing, microorganisms (amended from [143])

Species[a]	pH optimum	pH Minimum–Maximum	Optimum temperature (°C)	Minimum–Maximum temperature (°C)
Mesophilic and moderately thermophilic bacteria				
Acidiferrobacter thiooxydans	2	>1.2	38	>5–47
Acidimicrobium ferrooxidans	~2	na	45–50	<30–55
Acidithiobacillus albertensis	3.5–4.0	2.0-4.5	25–30	na
Acidithiobacillus caldus	2.0–2.5	1.0-3.5	45	32–52
Acidithiobacillus ferrooxidans	2.5	1.3–4.5	30–35	10–37
Acidithiobacillus ferrivorans	2.5	1.9–3.4	27–32	4–37
Acidithiobacillus thiooxidans	2.0–3.0	0.5–5.5	28–30	10–37
"Acidithiomicrobium P1/P2"	2.5	na	50	na
Alicyclobacillus disulfidooxidans	1.5–2.5	0.5–6.0	35	4–40
Alicyclobacillus tolerans	2.5–2.7	1.5–5	37–42	<20–55
Alicyclobacillus GSM	1.8	1.3– > 2	47	<30–60
Ferrimicrobium acidiphilum	2	>1.4	35	<37
Ferrithrix thermotolerans	1.8	>1.6	43	<50
Leptospirillum ferriphilum	1.3-–1.8	na	30–37	na–45
"*Leptospirillum ferrodiazotrophum*"	na	<1.2<	na	<37<
Leptospirillum ferrooxidans	1.5–3.0	1.3–4.0	28–30	na
Sulfobacillus acidophilus	~ 2	na	45–50	<30–55
Sulfobacillus benefaciens	1.5	>0.8	38.5	<47
"Sulfobacillus montserratensis"	1.6	0.7– > 2	37	<30–43
Sulfobacillus sibiricus	2.2–2.5	1.1–3.5	55	17–60
Sulfobacillus thermosulfidooxidans	~2	1.5–5.5	45–48	20–60
Sulfobacillus thermotolerans	2–2.5	1.2–5	40	20–60
"*Thiobacillus plumbophilus*"	na	4.0–6.5	27	9–41
"*Thiobacillus prosperus*"	~2	1.0–4.5	33–37	23–41

(continued)

Table 4 (continued)

Species[a]	pH optimum	pH Minimum–Maximum	Optimum temperature (°C)	Minimum–Maximum temperature (°C)
Thiomonas cuprina	3.5–4	1.5–7.2	30–36	20–45
Mesophilic and moderately thermophilic archaea				
Acidiplasma cupricumulans	1–1.2	0.4–1.8	54	22–63
"*Ferroplasma acidarmanus*"	1.2	<0–1.5	42	23–46
Ferroplasma acidiphilum	1.7	1.3–2.2	35	15–45
Extremely thermophilic archaea				
Acidianus brierleyi	1.5–2.0	1-6	~70	45–75
Acidianus infernus	~2	1–5.5	~90	65–96
Acidianus sulfidivorans	0.35–3	0.8–1.4	74	45–83
Metallosphaera hakonensis	3	1-4	70	50–80
Metallosphaera prunae	2–3	1–4.5	~75	55–80
Metallosphaera sedula	2–3	1–4.5	75	50–80
Sulfolobus metallicus	2–3	1–4.5	65	50–75
Sulfolobus yangmingensis	4	2–6	80	65–95
Sulfurococcus mirabilis	2–2.6	1–5.8	70–75	50–86
Sulfurococcus yellowstonensis	2–2.6	1–5.5	60	40–80

[a] Listed in alphabetical order; na = data not available; species without standing in nomenclature (http://www.bacterio.cict.fr/) are given in quotation marks

either planktonic or attached to the mineral surface. Because a direct electron transfer via enzymes, nanowires, and the like between the metal sulfide and the attached cell has not been demonstrated, a direct mechanism does not seem to exist. Instead, attached cells provide an efficient EPS-filled reaction compartment for indirect leaching with Fe(III) [138, 140]. Thus, to avoid the terms "direct leaching" and "indirect leaching", the new terms "contact leaching" and "non-contact leaching" have been proposed for bioleaching by attached and planktonic cells, respectively. A third term "cooperative leaching" describes the dissolution of sulfur colloids, sulfur intermediates, and mineral fragments by planktonic cells [124, 170]. These new terms may be useful for a description of the physical status of cells involved in bioleaching, but they do not tell us anything about the underlying chemical mechanisms of biological metal sulfide dissolution. Metal sulfide oxidation can be described by two different pathways, namely the thiosulfate mechanism and the polysulfide mechanism (Fig. 4 [140, 145, 147, 148]). The formation of the intermediate sulfur compounds in the two reaction pathways depends on the mineralogy of the metal sulfide and the geochemical conditions in the environment, mainly the pH and the presence of different oxidants [142]. Microorganisms play a crucial role in the oxidation of intermediate sulfur

Table 5 Physiological properties of acidophilic, metal sulfide oxidizing microorganisms (amended from [143])

Species[a]	Oxidation of				Growth
	Pyrite	Other MS[b]	Fe(II) Ions	Sulfur	
Mesophilic and moderately thermophilic bacteria					
Acidiferrobacter thiooxydans	+	na	+	+	A
Acidimicrobium ferrooxidans	+	na	+	–	F
Acidithiobacillus albertensis	–	+	–	+	A
Acidithiobacillus caldus	–	+	–	+	F
Acidithiobacillus ferrooxidans	+	+	+	+	A
Acidithiobacillus ferrivorans	+	+	+	+	A
Acidithiobacillus thiooxidans	–	+	–	+	A
"*Acidithiomicrobium* P1/P2"	na	+	+	+	F
Alicyclobacillus disulfidooxidans	+	na	+	+	F
Alicyclobacillus tolerans	+	+	+	+	F
Alicyclobacillus GSM	+	na	+	+	F
Ferrimicrobium acidiphilum	+	na	+	–	H
Ferrithrix thermotolerans	+	na	+	–	H
Leptospirillum ferriphilum	+	+	+	–	A
"*Leptospirillum ferrodiazotrophum*"	na	na	+	na	A
Leptospirillum ferrooxidans	+	+	+	–	A
Sulfobacillus acidophilus	+	+	+	+	F
Sulfobacillus benefaciens	+	na	+	+	F
"*Sulfobacillus montserratensis*"	+	na	+	+	F
Sulfobacillus sibiricus	+	+	+	+	F
Sulfobacillus thermosulfidooxidans	+	+	+	+	F
Sulfobacillus thermotolerans	+	+	+	+	F
"*Thiobacillus plumbophilus*"	–	+	–	+	A
"*Thiobacillus prosperus*"	+	+	+	+	A
Thiomonas cuprina	–	+	–	+	F
Mesophilic and moderately thermophilic archaea					
Acidiplasma cupricumulans	na	+	+	+	F
"*Ferroplasma acidarmanus*"	+	na	+	–	F
Ferroplasma acidiphilum	+	na	+	–	F
Extremely thermophilic archaea					
Acidianus brierleyi	+	+	+	+	F
Acidianus infernus	+	+	+	+	A
Acidianus sulfidivorans	+	+	+	+	A
Metallosphaera hakonensis	na	+	na	+	F
Metallosphaera prunae	+	+	+	+	F
Metallosphaera sedula	+	+	+	+	F
Sulfolobus metallicus	+	+	+	+	A
Sulfolobus yangmingensis	na	+	na	+	F
Sulfurococcus mirabilis	+	+	+	+	F
Sulfurococcus yellowstonensis	+	+	+	+	F

[a] Listed in alphabetical order; [b] *MS* Metal sulfides other than pyrite; *A* Autotroph; *F* Facultative autotroph and/or mixotroph; *H* Heterotroph; na = data not available; species without standing in nomenclature (http://www.bacterio.cict.fr/) are given in quotation marks

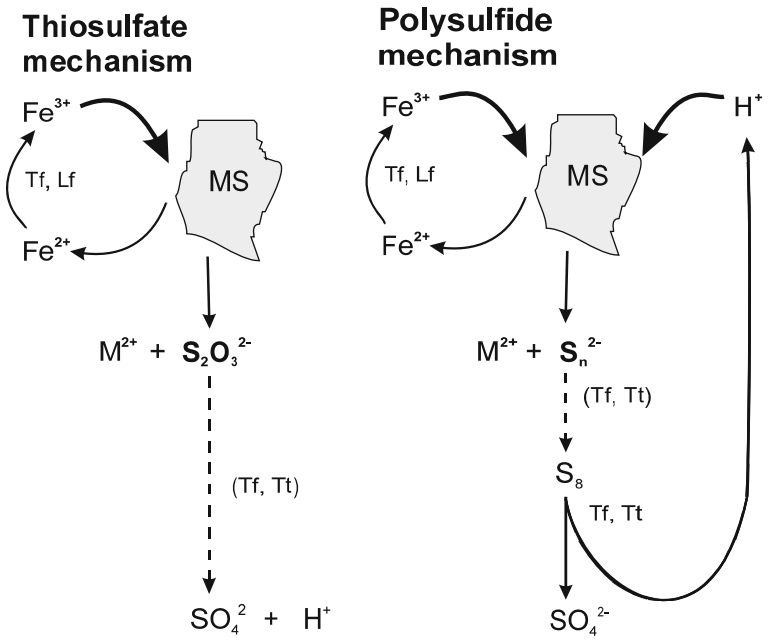

Fig. 4 Scheme of the two metal sulfide oxidation pathways (mechanisms) via thiosulfate or via polysulfides and sulfur based on the properties of metal sulfides (MS). In the case of bioleaching, the reactions are catalyzed by acidophilic Fe(II)- and sulfur compound-oxidizing bacteria or archaea (Tf, Tt, and Lf stand for *Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans,* and *Leptospirillum ferrooxidans*, respectively). *Dashed lines* indicate occurrence of intermediate sulfur compounds [147]

compounds that are formed by the chemical dissolution of the metal sulfides. Under oxic and acidic conditions relevant for bioleaching, microorganisms oxidize Fe(II) to Fe(III), which serves as oxidant for the metal sufides and for most of the intermediate sulfur compounds. Additionally microorganisms may catalyze the oxidation of intermediate sulfur compounds to sulfate and protons (sulfuric acid).

Metal sulfides are conductors, semiconductors, or insulators and their metal and sulfur atoms are bound in the crystal lattice [174, 189]. According to molecular orbital and valence band theory, the orbitals of single atoms or molecules form electron bands with different energy levels. The metal sulfides FeS_2 (pyrite), MoS_2 (molybdenite), and WS_2 (tungstenite) consist of pairs of sulfur atoms [174] that form nonbonding orbitals. Consequently, the valence bands of these metal sulfides are only derived from orbitals of metal atoms, whereas the valence bands of all other metal sulfides are derived from both metal and sulfur orbitals [15]. Thus, the valence bands of FeS_2, MoS_2, and WS_2 do not contribute to the bonding between the metal and the sulfur moiety of the metal sulfide which explains the resistance of these metal sulfides against a proton attack. The bonds can only be broken via multistep electron transfers with an oxidant such as Fe(III). For the other metal sulfides, in addition to an oxidant such as Fe(III), protons can remove electrons

from the valence band, causing a cleavage of the bonds between the metal and the sulfur moiety of the metal sulfide. Consequently, these metal sulfides are more or less soluble in acid, whereas FeS_2, MoS_2, and WS_2 are insoluble [31, 134, 140, 156, 171, 172].

Because two different groups of metal sulfides exist, two different metal sulfide oxidation mechanisms have been proposed [140, 145, 147, 148]. These mechanisms are able to explain the occurrence of all inorganic sulfur compounds that have been documented for bioleaching environments.

4.1 Pyrite and Other Non-acid-Soluble Metal Sulfides: Thiosulfate Pathway

Because FeS_2 oxidation is also the most studied among metal sulfides (for reviews see, e.g., [42, 44, 55, 96, 112, 129, 130, 142]), FeS_2 is used as an example for the three metal sulfides FeS_2, MoS_2, and WS_2 here. After the initial attack of the oxidant Fe(III), the sulfur moiety of FeS_2 is oxidized to soluble sulfur intermediates. Moses et al. [106] and Luther [97] presented a detailed reaction mechanism for FeS_2 dissolution by Fe(III) in which thiosulfate is the first soluble sulfur intermediate. According to this mechanism, hydrated Fe(III) ions oxidize the S_2 of FeS_2 to a sulfonic acid group by several electron transfers. Due to this transformation, the bonds between Fe and the two sulfur atoms are cleaved and hydrated Fe(II) ions and thiosulfate are formed. Thiosulfate as the first soluble sulfur compound intermediate is then almost quantitatively oxidized to tetrathionate [147, 181]. Tetrathionate is further degraded to various sulfur compounds, that is, trithionate, pentathionate, elemental sulfur, and sulfite [42, 142, 147]. These sulfur compounds are finally oxidized to sulfate in chemical and/or biological reactions. Overall, the thiosulfate pathway can be summarized by the following equations.

$$FeS_2 + 6\ Fe^{3+} + 3\ H_2O \rightarrow S_2O_3^{2-} + 7\ Fe^{2+} + 6\ H^+ \quad (3)$$

$$S_2O_3^{2-} + 8\ Fe^{3+} + 5\ H_2O \rightarrow 2\ SO_4^{2-} + 8\ Fe^{2+} + 10\ H^+. \quad (4)$$

The stoichiometry of the thiosulfate pathway has been confirmed in bioleaching experiments with *At. ferrooxidans*, in which the stable isotopes of oxygen and sulfur were determined in the pyrite oxidation reaction products [8].

4.2 Acid-Soluble Metal Sulfides: Polysulfide Pathway

In contrast to FeS_2 oxidation, the metal–sulfur bonds in the acid-soluble metal sulfides can be cleaved before the sulfidic sulfur is oxidized. These metal sulfides such as As_2S_3 (orpiment), As_4S_4 (realgar), $CuFeS_2$ (chalcopyrite), FeS (troilite),

Fe_7S_8 (pyrrhotite), MnS_2 (hauerite), PbS (galena), and ZnS (sphalerite) can thus be dissolved by protons. At low pH, the sulfur moiety of these metal sulfides is oxidized mainly to elemental sulfur [44, 101, 145]. A series of reactions for acid-soluble metal sulfides inherently explains the formation of elemental sulfur via polysulfides [145], which have been detected during dissolution of, for example, Fe_7S_8 [166, 167], PbS [157], and $CuFeS_2$ [66]. Consequently, the oxidation mechanism for acid-soluble metal sulfides has been named the polysulfide mechanism [145]. Although elemental sulfur is chemically inert in natural environments, it can be biologically oxidized to sulfuric acid. Overall, the polysulfide mechanism can be described by the following equations [145].

$$MS + Fe^{3+} + H^+ \rightarrow M^{2+} + 0.5\ H_2S_n + Fe^{2+} (n \geq 2) \quad (5)$$

$$0.5\ H_2S_n + Fe^{3+} \rightarrow 0.125\ S_8 + Fe^{2+} + H^+ \quad (6)$$

$$0.125\ S_8 + 1.5\ O_2 + H_2O \rightarrow SO_4^{2-} + 2\ H^+. \quad (7)$$

The polysulfide pathway is in agreement with results of bioleaching experiments with *At. ferrooxidans*, in which the stable isotopes of oxygen and sulfur were determined in the products of chalcopyrite and sphalerite oxidation [9, 169].

5 Interfacial Processes in Bioleaching

Bioleaching is a process that takes place at the interface of (sulfide) mineral, bacterium, and air/solution [87, 132, 137]. The majority of the leaching bacteria grow attached on the surfaces of mineral sulfides. After inoculation of experiments an important finding has been made: more than 80 % of inoculated cells can disappear from the solution within 24 h in the case of a nonlimiting surface space of the mineral [2, 7, 35, 58, 72, 111]. If the inoculum exceeds the available surface area, some cells may remain in the planktonic state. Interestingly this occurs even though the surface area is only less than 5 % covered by cells (unpublished data) and, thus, the space on the surface of a metal sulfide must be nonlimiting for attachment [139]. The reasons for this finding seem to be connected with electrochemical phenomena and still are not fully elucidated. However, some assumptions with a high probability are discussed.

The attachment process of bacteria to a mineral surface is predominantly mediated by the extracellular polymeric substances (EPS) that surround the cells. The EPS are the "contact substances" and consist chemically of sugars, lipids, proteins, and nucleic acids and combinations thereof. Attachment/surface contact stimulates the EPS production considerably up to 100-fold [111,173]. This effect is based on the induction of plentiful genes and their products [11, 12, 175]. Recently we were able to show that upon surface contact more than 75 proteins/genes were induced in cells of *At. ferrooxidans*. This is equivalent to about 3 % of all genes

and indicates the serious change in metabolism upon contact with a substrate/substratum surface, respectively, surface growth. Furthermore, N-acylhomoserine lactones via quorum sensing are involved in the process of surface attachment and biofilm formation on mineral sulfides [59]. In the case of *At. ferrooxidans* strain R1 growing on pyrite, it was demonstrated unequivocally that these EPS consist of the sugars glucose, rhamnose, fucose, xylose, mannose, C12–C20 saturated fatty acids, glucuronic acid, and Fe(III) ions [57, 58]. The primary attachment occurs mainly by electrostatic interactions between positively charged cells (actually the EPS surrounding the cells, where 2 mol negatively charged glucuronic acid residues complex 1 mol positively charged Fe(III) ions resulting in a net positive charge) with the negatively charged pyrite surface (at pH 2 in sulfuric acid solution [13, 65, 159, 176]. Also hydrophobic interactions contribute somewhat to the attachment to metal sulfide surfaces [58, 136], although this applies especially to very hydrophobic surfaces, for example, those of elemental sulfur. Here, the hydrophobic interactions may be the dominant ones for attachment. Hydrophobic interactions as well as covalent bonds seem to mediate the secondary (tight) surface attachment. In addition, attachment is dependent on the composition of the EPS, the latter being a result of the growth history of the bacteria. Cells grown on elemental sulfur do not attach to pyrite due to a considerably different EPS composition and, thus, exhibit a lag-phase of several days after the inoculation to a sulfur substrate. These EPS contain considerably fewer sugars and uronic acids, but many more fatty acids than EPS of cells grown on pyrite. The most important difference, however, is the total lack of complexed Fe(III) ions or other positively charged ions. Consequently, exclusively hydrophobic interactions are relevant for attachment of cells of *At. ferrooxidans* to sulfur [57]. This means as a consequence that the bacteria are able to adapt the composition and also the amount of their EPS to the growth substrate [planktonic cells grown with soluble substrates, e.g., Fe(II) sulfate, produce almost no EPS]. Attachment to mineral surfaces is quite strong: a simple treatment by whirling or shaking is insufficient for a quantitative removal of the cells from the mineral surface. Even with the addition of detergents such as Tween 20 only less than 10 % of the sessile cells can be removed and won for analyses (unpublished data). The adhesion force for cells of leaching bacteria fall in the range of 0.6 up to 1.1 nN between a single cell and the surface of the mineral chalcopyrite [191]. Recent findings indicate that not all leaching bacteria are able to attach to metal sulfide surfaces; for example, *At. caldus* needs a preformed biofilm for attachment, preferentially by *Leptospirillum ferrooxidans* [56]. This has serious consequences: in order to enhance bioleaching (as would be necessary in technical applications such as heap leaching or with bioreactors) or to inhibit this process (as intended in acid mine/rock drainage cases) it is necessary to discover which member of a leaching consortium is the primary colonizer of metal sulfides and which is only using a preformed biofilm. Furthermore, one needs to discover whether all bioleaching bacteria attach specifically to metal sulfide minerals or also unspecifically to gangue minerals to increase the efficiency of the processes.

The site for attachment on a mineral (sulfide or gangue material) and the detection/sensing of this site by the cells are still open questions. There are indications from the literature [6, 47, 49–51, 58, 59, 116, 141, 153] that attachment to metal sulfides does not occur randomly but is a deliberate cell process. For example, atomic force microscopy (AFM) images demonstrate that cells of *At. ferrooxidans* preferentially (> 80 %) attach to sites with visible surface imperfections (scratches, etc.; Fig. 5). If one looks at the surface in the nanoscale, the crystal units become visible. The crystal units of pyrite have a size in the range of 20–50 nm (Fig. 6). This means that one attached cell of, for example, *At. ferrooxidans,* measuring 0.5 by 0.3 μm on average, covers several crystal units. If one of these crystals has a defect, it is practically impossible to localize this same crystal under the bacterium with the currently available instrumentation. Such imperfections can be defects in the crystal lattice such as nonstoichiometric iron-to-sulfur ratios in pyrite as an example (1 to 2, but there may be deficiencies in sulfur such as FeS_{2-x}). Furthermore, attachment to areas with a low degree of crystallization (= amorphous) is favored and the attached cells seem to orient

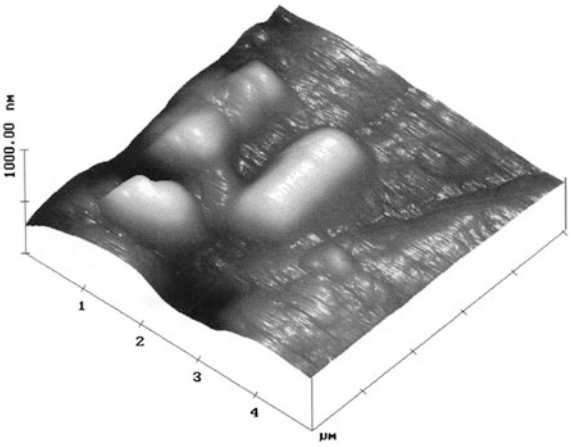

Fig. 5 High-resolution AFM image of cells of *Acidithiobacillus ferrooxidans* on a pyrite surface with surface defects (contact in air; Gehrke et al. 1998)

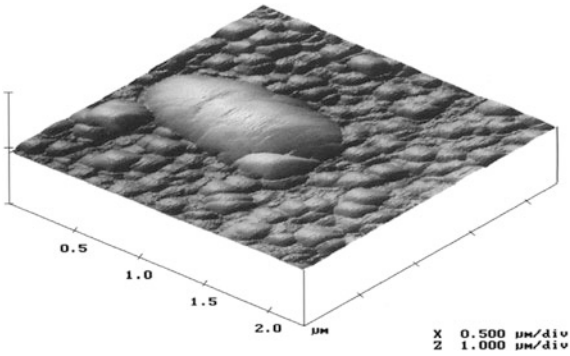

Fig. 6 High-resolution AFM image of a cell of *Acidithiobacillus ferrooxidans* on a pyrite surface showing the crystal units of the pyrite with a diameter of 20–50 nm (contact in air, Telegdi and Sand, unpublished)

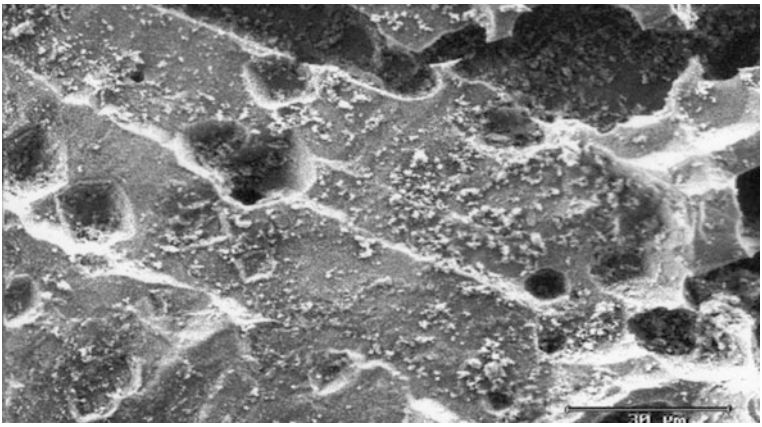

Fig. 7 Scanning electron micrograph of a pyrite particle after 5 months bioleaching (Telegdi and Sand unpublished) exhibiting preferential attack on the pyrite lattice along planes

themselves along crystallographic axes/planes (e.g., < 100 > or < 110 > plane orientation), in whose direction oxidation fronts may propagate (Fig. 7). Whereas adhesion to scratches might be explained by mere contact area enhancement, areas with low crystallization and crystallographic axis are often not related to changes in surface topography. Therefore, attachment to specific sites on the mineral surface may be principally related to different attractants, most likely caused by charge imbalances on the surface as discussed above or as a result of the orientation of the crystal planes, which cause the bacteria to face either an atomic iron, sulfur, or a mixed iron–sulfur surface. Different attractants may also be released by previous chemical and/or biological oxidation processes. Both *At. ferrooxidans* and *L. ferrooxidans* have clearly been shown to possess a chemosensory system–chemotaxis reacting positively to gradients of Fe(II)/(III) ions, thiosulfate, and the like [1, 103]. These compounds occur compulsorily in the course of metal sulfide dissolution (Fig. 4). Dissolution occurs in an electrochemical sense at local anodes bringing Fe(II) ions and thiosulfate in solution in the case of pyrite. A review on the anodic and cathodic reactions is given by Rimstidt and Vaughan [129]. It may be speculated that these local anodes are the sites towards which the cells are chemotactically attracted. These anodes and cathodes may result from the above-mentioned imperfections in the crystal lattice (with iron-to-sulfur ratios not exactly 1 to 2), an inclusion of other metal atoms (heteroatoms, the concentration may reach several percent of the whole crystal matrix) during the process of crystallization (from saturated solutions), and/or from variations of temperature during crystallization (causing amorphous up to highly crystalline structures).

Experiments with a Kelvin probe to measure surface potential on minerals were used to detect local anodes and cathodes on a pyrite surface. These experiments remained unsuccessful due to a lateral resolution of the instrument of 10 μm [58]. Obviously, an increased resolution is necessary given that a bacterial cell is about 0.5 up to 1 μm and a unit crystal of pyrite is in the range of 20–50 nm.

The limitations of the Kelvin probe can be overcome by the recently developed combination of an atomic force microscope (AFM) with a Kelvin probe. Present work using an AFM equipped for Kelvin probe force mapping (Kuklinski and Sand, unpublished data) indicates that the cells of *L. ferriphilum* attached to a pyrite surface are more negatively charged (about 100–200 mV) than the surrounding surface. This can be interpreted as the cells being able to extract electrons from the pyrite (Fig. 8). In another case of a biological dissolution of materials, here biocorrosion of steel by sulfate-reducing prokaryotes, Little and White with coworkers [92] were looking for the attachment sites of sulfate-reducing bacteria on steel surfaces. They detected that the bacteria were attached in the immediate vicinity (nanometer range) of the anode. Consequently, the cathode must be either in the vicinity of the anode (because sulfate-reducing bacteria preferentially attach to negatively charged sites) or the transiently negatively charged anodes attracted the bacteria and caused them to attach at these sites. As a consequence of bacterial attachment, the anode (and most likely the cathode) become permanent (manifest), and steel dissolution commences. This mechanism also seems to be fully applicable to the bioleaching of metal sulfides.

To summarize, cells are attracted to transiently (electrically) charged dissolution sites by their chemotactic sensory system and cause the anodes and cathodes on the metal sulfide or the metal surface to become permanent. The dissolution process occurs in the EPS layer (Fig. 9). This layer fills the void volume between the outer membrane (of the cells) and the material's surface (of the metal sulfide) and can thus be considered as a reaction space. The pioneering work of Tributsch

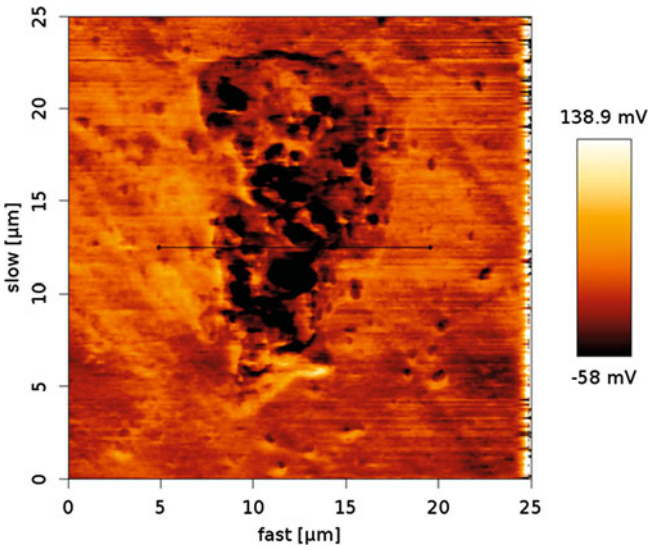

Fig. 8 Electrochemical analysis by Kelvin probe microscopy (KPM): Surface potential mapping of a colony of *Leptospirillum ferriphilum* versus pyrite surface (contact in air, Biomaterials workstation, Kuklinski, Noel and Sand, unpublished)

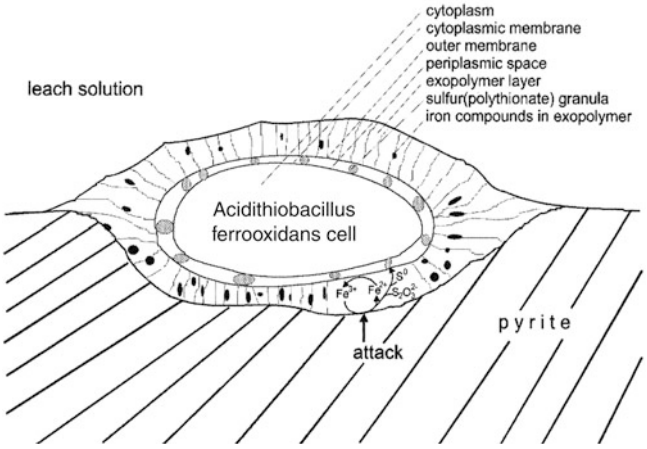

Fig. 9 Scheme of a cell of *Acidithiobacillus ferrooxidans* on the surface of the substrate pyrite with the surrounding EPS layer indicating the dissolution process (bioleaching [138])

and coworkers [131] demonstrated that between the cells and the material's surface there exists a distance of several nm width. More precise measurements do not exist. In the case of metal sulfides such as pyrite, which need an oxidizing attack by Fe(III) ions for dissolution, the EPS-complexed Fe(III) ions must fulfill this function. However, this very process is not at all understood. Currently, the most likely explanation is based on two plausible assumptions. In order for these ions to become reduced (to take up an electron from the metal surface, which in turn becomes oxidized and thus starts to dissolve liberating Fe(II) ions, thiosulfate, etc.) the first assumption considers the electron tunneling effect. It is known that electrons can bridge distances of up to 2 nm (20 Å) by tunneling from one electron hole to another [102]. All abundantly commercially available enzyme electrodes are based on this effect. Consequently, the Fe(III) ions have to be exposed to the pyrite surface within this distance (to be reducible by tunneling electrons). Considering the 2 nm distance between the cell membrane and the substrate surface, this hypothesis seems to be reasonably sound and would serve as an explanation for the reduction of the Fe(III) ions. The second assumption is that Fe(II) ion–glucuronic acid complexes are less stable than the corresponding Fe(III) ion complexes. This has been demonstrated for various iron–carbonic acid complexes (NIST [110]). Consequently, Fe(II) ions produced by the cathodic electron transfer may be released from their EPS chelators, the two glucuronic acid residues. The remaining uronic acid residues will recruit a new Fe(III) ion at the pyrite surface out of solution as it stands in equilibrium with the dissolved ones, as well as other complexed Fe(III) ions. If the now mobile Fe(II) ions diffuse towards the outer membrane, they will be (re)oxidized by the enzymatic system of the cells and can enter the cycle by (re)complexation by two glucuronic acid residues. Consequently, in such a system Fe(II)/Fe(III) ions serve as electron shuttle compounds.

These two assumptions currently underpin the most likely explanation of the complex electrochemical mechanism of (bio)leaching of metal sulfides. Furthermore, the biocorrosion of metals seems to be a comparable process using similar reactions.

The chemical reactions occur outside the cells, in fact outside the outer membrane, but still within the EPS-generated microenvironment (Fig. 9). Similar observations have been recorded for *Geobacter* by Lovley et al. [95] explaining the use of this bacterium for bioelectricity.

6 Anaerobic Bioprocesses in Mineral Leaching and Metal Recovery

6.1 Anaerobic Bioleaching of Oxidized Ores

Biomining is currently known as a technology that focuses on the oxidative dissolution of reduced sulfidic ores by microorganisms. Large amounts of valuable metals are, however, hosted in oxidized nonsulfidic (mostly ferric iron-based) ores, which are not yet subject to aerobic industrial bioleaching processes. With the decline of sulfidic ores and increasing demand for valuable metals (e.g., nickel), novel technologies focusing on the dissolution of reduced ores under anaerobic conditions become more and more attractive [52, 53, 79].

In 1983 Goodman et al. [61] predicted anaerobic leaching in a pyritic waste rock dump, where they discovered high cell numbers at 3-m depth (oxygen < 0.1 vol %, $CO_2 > 2$ vol %) and the highest copper concentrations in the dump. In their laboratory-scale experiments 63 % zinc was recovered from a zinc–iron sulfide under CO_2-rich, O_2-free atmosphere in a ferric-rich solution at pH 2.5. Donati et al. [39] confirmed the anaerobic leaching of covellite in the presence of ferric iron-reducing *At. ferrooxidans*. Sulfur formed during the chemical attack of covellite by ferric iron serves as an electron donor for iron reduction by *At. ferrooxidans* which is followed by an increase of copper leaching from covellite. Anaerobic processes find another useful application in copper leaching from chalcopyrite, which is inhibited by high concentrations of ferric iron but enhanced by ferrous iron [165]. Dissolution of chalcopyrite is therefore most efficient at low redox potentials in solution which is concurrent with high ferrous iron and low ferric iron concentration. The appropriate redox potential for chalcopyrite leaching can be achieved by controlling the oxygen supply to the system, which either allows the bacteria to oxidize ferrous iron (aerobic phase) or to reduce the ferric iron (anaerobic conditions; coupled to sulfur oxidation) in solution [165]. Hol et al. [76] confirmed an enhanced gold recovery from enargite by a combined milling and bioreduction process. The sulfur formed on the surface of the ore during milling, interfering with the gold recovery, is removed by sulfur-reducing bacteria

Table 6 Acidophilic bacteria able to grow by ferric iron reduction (anaerobic respiration)

Species	Atmosphere required for ferric iron reduction	Electron donor
Acidithiobacillus ferrooxidans	Anaerobic	Reduced S^0, H_2
At. ferrivorans	Anaerobic	Reduced S^0
Acidiferrobacter thiooxydans	Anaerobic	Reduced S^0
Ferrimicrobium acidiphilum	Anaerobic	Organic
Acidimicrobium ferrooxidans	Anaerobic[a]	Reduced S^0, organic
Ferrithrix thermotolerans	Anaerobic	Organic
Sulfobacillus acidophilus	Anaerobic[a]	Reduced S^0, organic
Sb. thermosulfidooxidans	Anaerobic[a]	Reduced S^0, organic
Sb. benefaciens	Anaerobic	Organic
Acidicaldus organivorans	Anaerobic	Organic
Acidiphilium cryptum	Microaerobic	Organic
A. acidophilum	Microaerobic	Organic
A. angustum/rubrum	Microaerobic	Organic
A. organovorum	Microaerobic	Organic
A. multivorum	Microaerobic	Organic
Acidocella facilis	Microaerobic	Organic
Acidobacterium capsulatum	Microaerobic	Organic

[a] Also capable of ferric iron reduction under microaerobic conditions

under mild acidic conditions (pH 5), forming hydrogen sulfide, allowing more efficient gold recovery.

Many acidophilic bacteria and archaea are able to reduce ferric iron under anaerobic (or microaerobic) conditions [30], and many of these (e.g., *At. ferrooxidans*) are also well known for their ability to oxidize ferrous iron and already used in (oxidative) biomining operations (see above). The majority of acidophilic bacteria are known to reduce ferric iron under anaerobic or microaerobic conditions coupled to the oxidation of either inorganic (e.g., sulfur, hydrogen) or low-weight organic compounds [25, 32, 122, 163]. Please see Table 6.

Acidophilic, iron-reducing bacteria are not only able to reduce soluble ferric iron but also have the ability to use ferric iron minerals as electron acceptors [23, 24]. When using ferric iron minerals as substrate, the mineral structure is destroyed by the bacteria and associated metals are released, a process also known as "reductive dissolution" and which has recently been applied to nickel laterites.

6.1.1 Reductive Dissolution of Laterites

Laterites contain ~72 % of the terrestrial nickel and cobalt resources in the world and are most abundant in the tropics. These oxidized nickel ores mostly occur as

limonite-types, where the target metal is associated with a host mineral, mainly ferric minerals (such as goethite), and saprolite-types, containing nickel-hosting magnesium silicates. Limonite typically consists of 40 % ferric iron, 11.3 % silicium, 1.4 % nickel, 1.3 % manganese, 1.3 % chromium, 1 % aluminum, and 0.2 % cobalt. Cobalt in the laterites is also associated with asbolane, a manganese oxyhydroxide, in which manganese, as does ferric iron, occurs in the oxidized form.

To release nickel from the goethite structure, laterites are conventionally hydrometallurgically processed under high pressure/temperature to break up the strong bond between ferric iron and oxygen [100]. This procedure is not only highly energy consuming, but also induces the co-dissolution of gangue minerals, causing the subsequent metal-recovering step to be more complex and cost-intensive.

Proposed biological processes for laterite leaching to date include the use of chelating agents, such as citric acid, produced by heterotrophic fungi [16]. However, in addition to the low leaching capacity of the chelating agents for the target metals, the approach causes high substrate costs. Experiments using sulfuric acid produced by acidophilic sulfur-oxidizing bacteria belonging to the genus *Acidithiobacillus* seemed a better approach for nickel extraction [29, 155], although slow dissolution kinetics limit the process.

A novel recently developed bioprocess allows leaching of nickel and cobalt under acidic conditions and at ambient temperature (30 °C) by iron-reducing bacteria (autotrophic or heterotrophic) that are able to dissolve the goethite structure [71]. The process utilizes a low-cost electron donor (sulfur) whereby electrons are shuffled from sulfur to ferric iron according to Eq. (8).

$$S^0 + 6\ FeO(OH) + 10\ H^+ \rightarrow SO_4^{2-} + 6\ Fe^{2+} + 8\ H_2O. \quad (8)$$

The iron-reducing bacteria involved in this process belong to species *At. ferrooxidans* and reduce ferric iron under anaerobic conditions coupled to the oxidation of elemental sulfur. The reductive dissolution process catalyzed by *At. ferrooxidans* at low pH (1.8) and ambient temperature (30 °C) achieved over 70 % extraction of nickel from the limonitic laterite within 14 days, which is up to seven times more effective then the same set-up under aerobic conditions. Reduction of manganese in the asbolane is also enhanced under the present conditions and either occurs indirectly via the ferrous iron produced from goethite (Eq. 9), in the case for cultures of *At. ferrooxidans*, or in the presence of manganese-reducing bacteria similar to ferric iron reduction (Eq. 10 [94]).

$$Mn_3O_3(OH)_6 + 6\ Fe^{2+} + 12H^+ \rightarrow 3\ Mn^{2+} + 6\ Fe^{3+} + 9\ H_2O \quad (9)$$

$$Mn_3O_3(OH)_6 + S^0 + 4\ H^+ \rightarrow 3\ Mn^{2+} + 5\ H_2O + SO_4^{2-} \quad (10)$$

Another advantage of the set-up is the low pH at which the system operates, which keeps the metals in solution and allows subsequent metal recovery.

Although a heterotrophic, moderate thermophilic organism, *Acidicaldus organivorans*, was tested positive for the reductive dissolution of goethite (coupled to

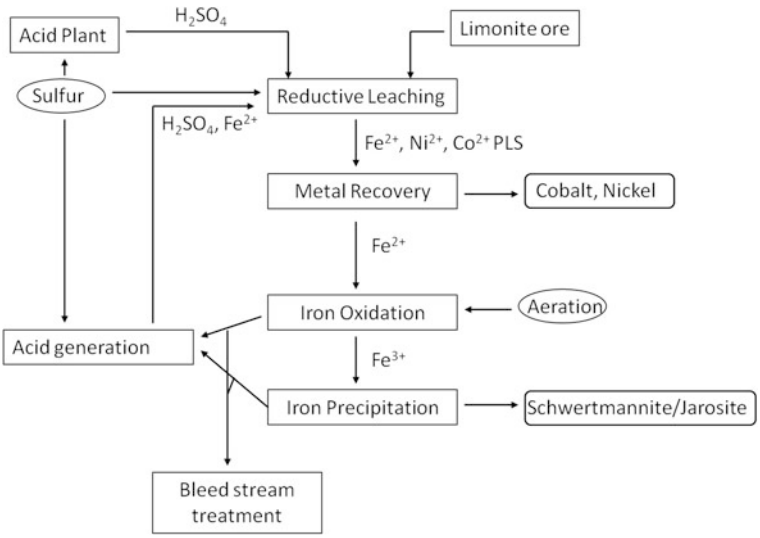

Fig. 10 Modified scheme of the Ferredox process [46] for reductive dissolution of limonitic laterite ore under anaerobic conditions and metal recovery

an organic electron donor), the disadvantages of using organic substances and the costs of achieving elevated temperatures (45 °C) would probably offset the process.

du Plessis et al. [46] proposed a flow sheet process, the Ferredox process, including the reductive dissolution of the limonitic ore and metal recovery as well as removal of impurities and regeneration of sulfuric acid required for the laterite dissolution (see Fig. 10). The core components of the Ferredox process are the reductive leaching coupled to sulfur oxidation by *At. ferrooxidans,* producing a ferrous-rich PLS, followed by the recovery of valuable metals (e.g., nickel, cobalt) from the solution. Ferrous iron is oxidized in the next step by aerobic, iron-oxidizing bacteria (see above) resulting in a ferric-rich liquor that can either be directly used for sulfuric acid regeneration or iron precipitated as iron-(oxy)hydroxide, which is again used for acid regeneration.

The ferrous iron contained in the laterite pregnant leach solution (PLS) is a major advantage to solutions containing the oxidized form, since ferric iron would interfere with conventional recovery methods for nickel and cobalt [182], and therefore needs to be removed from the solution prior to further treatment.

Recovery of nickel and cobalt (and other target metals) can be achieved without further pH adjustment by either direct sulfide precipitation or via solvent extraction or ion exchange [98]. Although sulfide precipitation is not selective for nickel and cobalt, because of their highly similar solubility products (see Table 7), separation from iron and further possible metals (e.g., copper and zinc) can be achieved by this method [91, 104, 108].

Table 7 Solubility product of some metal sulfides [161]

Metal sulfide	Log K_{SP}
CdS	28.9
CoS	22.1
CuS	35.9
FeS	18.8
MnS	13.3
NiS	21.0
ZnS	24.5

Iron in the PLS can be recovered via microbial iron oxidation and subsequent ferric iron mineral formation either prior to the metal sulfide formation or afterwards [74, 182].

Although the process operates under less acidic conditions than other common treatments, a reasonable amount of sulfuric acid (Eq. 8) is required, mainly for the acid dissolution of goethite. Therefore du Plessis et al. [46] suggested a circular process that allows the regeneration of sulfuric acid. Acid generation can be achieved by utilizing the ferric-rich liquor, produced in the iron oxidation step, in a packed-bed reactor containing sulfur and iron-reducing bacteria. The microorganisms reduce the ferric iron coupled to sulfur oxidation (Eq. 11) and produce sulfuric acid, which together with the colonized sulfur can be reintroduced into the reductive leaching process.

$$S^0 + 6\ Fe^{3+} + 4\ H_2O \rightarrow SO_4^{2-} + 6\ Fe^{2+} + 8\ H^+. \quad (11)$$

The amount of sulfuric acid produced by the regeneration is, however, restricted by the lower pH limit of the bacteria, in the case of *At. ferrooxidans* ~1.3. Additional acid required for the reductive leaching can either be produced from a sulfur-burning acid plant [89] or by schwertmannite/jarosite autoclave treatment which results in hematite and the release of acid [43].

Another factor that requires controlling the process is the production and removal of impurities, such as soluble manganese and magnesium sulfates produced during leaching of the laterite. Although the mild acidic conditions limit the dissolution of such compounds, their presence has a negative influence on the bacterial activity. du Plessis et al. [46] therefore integrated a bleed stream treatment (reviewed in Willis [182) which harnesses sulfate-reducing bacteria to remove sulfate and generate alkalinity, following separate recovery of $MnCO_3$ (a saleable product) and $MgCO_3$. The hydrogen sulfide produced during the process can again be used to precipitate metal sulfides from the pregnant leach solution.

Depending on the leaching kinetics, du Plessis et al. [46] suggest the process either be carried out in agitated tank reactors (rapid kinetics) or vat/submerged pond leaching set-ups (slow kinetics). The mild acidic conditions allow the use of a relatively low-cost building material and no strict anaerobic conditions are required because *At. ferrooxidans* is also able to oxidize sulfur and reduced iron under aerobic conditions and thereby burn up all the oxygen. The authors also

suggest recirculating unused sulfur which has the advantage of saving electron donor costs and reintroducing the biomass immobilized on the sulfur would increase the leaching rate and process efficiency.

The operation under mild acidic conditions and ambient temperature, as well as the subsequent selective precipitation of valuable metals is a major advantage of the Ferredox process over conventional processes. There are, however, also disadvantages in the Ferredox process that need to be investigated and improved such as the high interdependency of the large recirculation stream with the risk of dropping out one of the parts affecting the overall process. Biological processes compared with high-intensity processes are relatively slow and require longer residence times with the need for larger reactor sizes. Also more stringent process controls are required compared to abiotic processes, to ensure appropriate conditions for the bacteria.

Although the Ferredox process has mainly been described for limonitic ores, with goethite being one of the main host minerals for valuable metals, this process can also be advantageous for the reductive dissolution of other oxidized (ferric-iron based) ores. As outlined in Fig. 10, the modularization of the process allows the optional integration of variable treatment units depending on the processed ore until a complete core process is established. This modular set-up ensures controlling of costs depending on process certainty and metal production and also acts as a buffer against upsets in individual process units.

6.2 Selective Metal Recovery from Mine-Impacted Waters

Process waters, for example, resulting from mineral leaching, as well as waters draining abandoned metal mines are (extremely) acidic and rich in various transition metals, aluminum, and sulfate [113]. The acidic character of the waters allows the metals to stay in solution until further processing.

Conventional remediation of acidic mine waters by aeration (oxidation of, e.g., ferrous to ferric iron) and neutralization (addition of alkalizing chemical), results in a mixed metal sludge (metal hydroxides, carbonates, and co-precipitation of, e.g., arsenate), which requires special deposition in designated landfill sites [81]. Despite major drawbacks, including high costs for reagents and operation, conventional chemical approaches fail to recover valuable metals. Anaerobic biological approaches (compost bioreactors) where metals are immobilized also do not selectively recover metals but produce spent compost, which is a waste product [81] and poses long-term environmental consequences. Approaches using hydrogen sulfide, produced by neutrophilic, sulfate-reducing bacteria, for offline metal sulfide precipitation (in a separate vessel) have been described as an alternative method to hydroxide precipitation [164 and references therein]. Two commercial processes based on the offline precipitation (*ThioTeq*, operated by the Dutch company Paques BV, and *BioSulfide*, operated by the Canadian company BioteQ) have been developed on a large scale. Both systems consist of at least two

components, with separate biological (hydrogen sulfide formation) and chemical (metal sulfide precipitation) steps, which increase engineering and operation costs. Alternative active biological treatment systems, one using iron-oxidizing acidophilic bacteria for the selective recovery of iron [74, 79] and the other one using acidophilic sulfate-reducing bacteria, allowing selective recovery of metals as sulfides [108], have recently been described as laboratory-scale or pilot-scale systems. While the iron-oxidizing system operates under aerobic conditions and favors the precipitation of schwertmannite (a potentially valuable mineral), the sulfate-reducing system requires anaerobic conditions and produces metal sulfides. Both systems can be operated inline because the formation of clean products (free of other metals) has been shown for each system [74, 108]. The iron-oxidation system is only suitable for the recovery of iron, however, the sulfate-reducing system allows the selective recovery of various chalcophilic metals (e.g., zinc and copper) depending on the solubility products of their sulfite phases.

6.2.1 Selective Metal Recovery from Acidic Waters in a Sulfidogenic System

Conventionally biological sulfate-reducing systems utilize neutrophilic, sulfate-reducing bacteria, which are extremely sensitive to acidic solutions (such as the mine-affected waters). These systems only allow offline precipitation of metals from acidic mine waters in a separate tank, with the SRB-system functioning as a hydrogen-sulfide producing unit.

A novel acidophilic, sulfate-reducing system, using glycerol as the electron donor, has been developed and successfully tested on a laboratory scale for selective metal sulfide precipitation [108]. This system combines sulfate-reduction and metal sulfide precipitation in one vessel, which reduces the engineering complexity and operation costs. The bacteria are immobilized on porous glass beads in a 2.3-L (working volume), pH-controlled bioreactor which can be operated in a pH range of 2.2 and 5.0, whereby the quantitative composition of the bacterial community varies with pH. The sulfate-reducing community in the system includes "*Desulfosporosinus acidophilus"* M1, *"Desulfobacillus acidavidus"* strain CL4, and Firmicute CEB3, and is also often associated with the heterotrophic strain *Acidocella* PFBC and the iron/sulfur-oxidizer *At. ferrooxidans*. The sulfate-reducing bacteria produce hydrogen sulfide by reduction of sulfate or elemental sulfur coupled to an organic (e.g., glycerol) or inorganic (e.g., hydrogen) electron donor. The hydrogen sulfide produced occurs as S^{2-} in solution, the concentration of which is determined by its two dissociation constants (pK_a's: H_2S/HS^-, 7; HS^-/S^{2-}, ~12) and therefore regulated by the solution pH [160].

The system described by Ñancucheo and Johnson (2012) has been operated with various acidic mine waters containing elevated metal concentrations (e.g., 30 mM aluminum or 100 mM ferrous iron) and successfully applied for the selective recovery of zinc and copper. Flow rates of the system depend on the rate of sulfate reduction and therefore consumption of protons to activate the pH-controlled feed-liquor pump. The system can be configured depending on the type

of mine-affected water (e.g., type of metals and pH) by varying the internal pH or in combination with a pre-treatment for the removal of interfering metals (such as iron).

Processing of mine waters by acidophilic sulfate reduction not only has the advantage of being able to selectively recover valuable metals, but also facilitates the pH increase (proton-consuming reaction) and sulfate removal during the process. Another benefit of the acidophilic sulfidogenic system is the low building and operations costs, whereby the size of the reactor depends on the nature of the processed water (flow rate, metal concentration, and pH). The substrate (glycerol) used in the system, is with its current price of ~US$650/t a low-cost chemical, compared to metal prices of, for example, ~US$8,000/t for copper and $2,000/t for zinc, making the process even more attractive over conventional methods.

7 Bioleaching with Heterotrophic Microorganisms

Two large groups of bioleaching microorganisms are distinguished by their metabolism: autotrophic (here chemolithoautotrophic) and heterotrophic (chemoorganoheterotrophic) microorganisms. Bioleaching processes with autotrophic microorganisms were presented above; here leaching processes with heterotrophic bacteria and fungi are introduced. Some heterotrophic bacteria and fungi are known for their ability to leach metals especially from oxidic, siliceous, and carbonaceous materials. Unlike autotrophs, heterotrophic microorganisms utilize organic substances as a carbon and energy source. In laboratory research, model substrates (e.g., sugars combined with mineral solutions) are used for leaching experiments with heterotrophs. In practice, less expensive complex organic substrates would be applied, such as molasses or other food industry waste.

Heterotrophs can use manifold mechanisms to solubilize metals from ores and minerals. They excrete H_3O^+, complexing or chelating organic acids, for example, oxalate, citrate, gluconate, and lactate, but also amino acids, peptides, lipides, exopolysaccharides, enzyme complexes, or even cyanide ions, which solubilize the metals or other elements from solid materials [17, 183]. The concerted action of several of these substances can cause a synergistic effect on the solubilization of the metals. Another solubilization mechanism is the reduction or oxidation of metals and other element species by heterotrophic microorganisms. In the case of metal oxide ores, anaerobic microbial reduction and thereby solubilization of the metals is one of the most promising applications for industrial use (see Sect. 6).

The disadvantages of leaching with heterotrophs are the enhanced costs due to the need for organic substrates, possible contamination of the bioleaching process with undesired microorganisms, and enhanced safety measures due to the occurrence of potentially pathogenic organisms (e.g., fungal spores). These reasons might have prevented large industrial application of leaching processes until today.

In ancient times leaching processes with heterotrophic microorganisms were likely to have been applied for discoloring kaolin in China for the production of high-grade porcelain. Nevertheless, due to the variation of excreted leaching substances, the large number of potential bioleaching microorganisms as well as a broad available pH spectrum, bioleaching with heterotrophs has wide application potential for the recovery of valuable metals or the beneficiation of minerals.

7.1 Application Potential of Bioleaching with Heterotrophs to Ores, Minerals, and Waste

For several decades bioleaching processes with heterotrophs have been the subject of a large variety of research [18, 26, 77, 90, 183]. Even nonsulfidic and non-ferrous ores and minerals can be leached by heterotrophic microorganisms. Compared to bioleaching processes with acidophilic autotrophic microorganisms, bioleaching can even be performed with alkaline materials such as carbonate-rich copper ores, without the addition of acid. Furthermore, bioleaching processes with heterotrophs could also be used for the biobeneficiation of minerals, including silicates, kaolin, bauxites, or black shales. By using heterotrophic microorganisms, especially fungi, iron is removed from such materials and its quality is significantly enhanced [5, 27, 62, 162]. Examples of the application potential for bioleaching with heterotrophic microorganisms are:

- Copper oxides and carbonates
- Manganese oxides
- Refractory gold ores
- Refractory silver ores
- Oxidic nickel ores
- Cobalt ores
- Quartz sands and silicates
- Spodumene
- Zircon
- Bauxites, to remove undesirable minerals
- Zinc ores
- Silicate ores containing chromium, iron, and titanium.

Another application of bioleaching processes with heterotrophic microorganisms may be the recovery of metals from industrial and mining wastes [67, 109, Chap. 2]. A recovery of the metals might be feasible, which can help to close the presently incomplete cycles of anthropogenic metal usage. The microbial solubilized metals can be recovered by further concentration, cementation, extraction, precipitation, and electrolysis processes. In this way, the metal content of the waste materials could be reduced, and the bulk material could be disposed of with lower environmental risks, or be further used as a secondary raw material.

7.2 Bioremediation of Contaminated Sites

In addition to the application for raw materials supply, leaching processes with heterotrophs also have the potential for remediation of soils and sediments contaminated with heavy metals, including radionuclides. In some research studies it was shown that autotrophic as well as heterotrophic enrichment cultures can solubilize the contaminating metals to various extents, depending on several factors of the bioleaching process. Partially, the contaminants were removed to a large extent, so that the threshold values recommended for almost unrestricted use of the soil were achieved [14, 19, 185]. For example, the content of metal contamination in a fluvial tailings sediment could be reduced by leaching with heterotrophs in batch culture up to 18 % for Pb, 38 % for Cd, 100 % for Cr, 21 % Fe, 81 % Cu, 95 % Mn, and 54 % Zn. The investigation of such processes with the aim of decontamination of such sites and/or of their stabilization and immobilization may have high importance for future environmental measures when former mining sites are closed/decommissioned and remediation, recultivation, and renaturation need to be accomplished.

7.2.1 Bioremediation of Sites Contaminated with Radionuclides

Radionuclides can occur in soils, ores, and residues mainly as oxides, usually in crystalline form which are unsoluble and often co-precipitated with iron oxides. The oxides can be solubilized by enzymatic reduction processes and/or excretion of microbial metabolites such as organic acids and chelating agents [19, 63, 64, 86]. An in situ leaching process with application of indigenous heterotrophic soil organisms is feasible. The induced bioleaching process leads to an elution of the contaminants; hence an effective removal of the enriched seepage waters is very important to avoid further groundwater contamination. The leaching of the contaminated soil area helps to minimize and prevent long-term spontaneous formation of contaminated seepage waters. After the first bioremediation step, the contaminated area can be covered with a low permeable layer (e.g., loam or clay, marl); a topsoil layer can be added for humidity regulation (drought and fissure formation are prevented) and for mechanical stabilization a plant cover can be cultivated (prevention of erosion processes). The final topsoil layer can be meliorated and used for recultivation on the dump surface. Seepage water formation can be minimized with these measures, and a stabilization/immobilization of the contaminants is feasible [186, 187].

7.3 Environmental Impact of Weathering Processes with Heterotrophs

The microbial weathering of mineral surfaces, including anthropogenic disposed mineral materials, is a naturally occurring process. These microbial weathering processes occur on uncovered surfaces of mineral wastes and dumps, increased by enhanced mass transport processes [183]. Figure 11 shows the microbial weathering of a siliceous slag material by heterotrophs when organic matter is present. The siliceous material shows large fissures with distinct and visible erosion phenomena (see Fig. 11 right); a larger amount of secondary minerals such as calcite or organic salts (e.g., wedellite) was observed as fine-grained precipitates especially in the erosion flutes [183].

Microbial weathering of minerals also occurs in metal-contaminated soils and in sediments, where it can be amplified by mass transport processes, for example, stimulated by fluctuating water tables. The periodical change between saturated and unsaturated conditions in a metal-contaminated site seems to enhance microbial weathering cycles and the transport of solubilized metals to surface water and into groundwater [184].

Organic matter contained in these sites increases weathering processes with heterotrophs in a complex manner [185]. However, only little information is available about these processes, and especially about the manifold relations between autotrophic and heterotrophic communities at these sites [4, 82, 146, 188]. The cooperative interaction of these complex microbial communities seems to amplify the mobilization of toxic metals and metalloids [184, 188]. The observation of microbial weathering processes with heterotrophs is not limited to a neutral pH range; such processes can also occur in acidic (pH < 3) and alkaline (pH > 8) environments [149, 183–185].

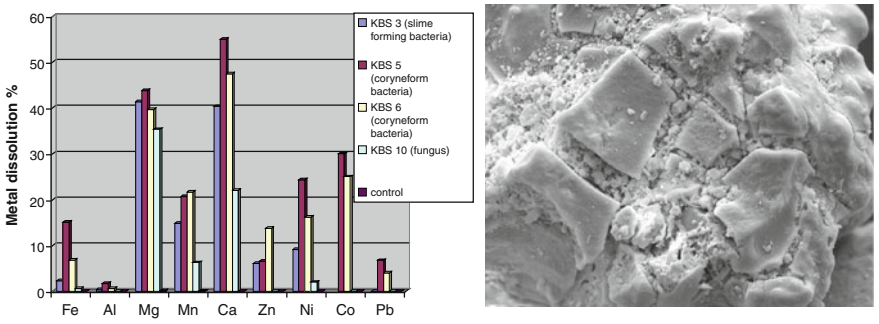

Fig. 11 *Left* Results of the microbial leaching of an alkaline slag material with several bacterial strains isolated from an alkaline slag dump. *Right* SEM image of the surface erosion of an alkaline slag particle after the microbial attack of one of the strains [183]

8 Conclusions

Biomining is increasingly being used in the mining industry for the production of copper and gold, but also for nickel, cobalt, zinc, and uranium. Dump/stockpile and heap bioleaching and stirred-tank bioleaching (or biooxidation) are the most important processes. Caving mining methods in combination with in situ bioleaching could play a major role in the future in order to increase the efficiency of copper mining, as well as the bioleaching of primary copper sulfides. With regard to the bioleaching of metals that are especially used in the electronic industry, as well as of mine and industrial waste (mine tailings, ash, etc.), promising laboratory methods and even pilot processes already exist. The newly developed Ferredox process (anaerobic bioleaching) enables the processing of silicate ores such as laterites and oxide ores such as manganese nodules, which is not possible on an industrial scale to date.

References

1. Acuña J, Rojas J, Amaro AM, Toledo H, Jerez CA (1992) Chemotaxis of *Leptospirillum ferrooxidans* and other acidophilic chemolithotrophs: comparison with the *Escherichia coli* chemosensory system. FEMS Microbiol Lett 96:37–42
2. Africa CJ, van Hille RP, Sand W Harrison STL (2013) Investigation and in situ visualization of interfacial interactions of thermophilic microorganisms with metal-sulphides in a simulated heap environment. Min Eng (online)
3. Ahmadi A, Schaffie M, Petersen J, Schippers A, Ranjbar M (2011) Conventional and electrochemical bioleaching of chalcopyrite concentrates by moderately thermophilic bacteria at high pulp density. Hydrometallurgy 106:84–92
4. Amaral Zettler LA, Gomez F, Zettler E, Keenan BG, Amils R, Sogin ML (2002) Eukaryotic diversity in Spain's river of fire. Nature 417:137
5. Ambikavedi VR, Lalithambika M (2000) Effect of organic acids on ferric iron removal from iron-stained kaolinite. Appl Clay Sci 16:133–145
6. Andrews GF (1988) The selective adsorption of thiobacilli to dislocation sites on pyrite surfaces. Biotechnol Bioeng 31:378–381
7. Bagdigian RM, Meyerson AS (1986) The adsorption of *Thiobacillus ferrooxidans* on coal surfaces. Biotechnol Bioeng 28:467–479
8. Balci N, Shanks WC III, Mayer B, Mandernack KW (2007) Oxygen and sulfur isotope systematics of sulfate produced during bacterial and abiotic oxidation of pyrite. Geochim Cosmochim Acta 71:3796–3811
9. Balci N, Mayer B, Shanks WC III, Mandernack KW (2012) Oxygen and sulfur isotope systematics of sulfate produced during abiotic and bacterial oxidation of sphalerite and elemental sulfur. Geochim Cosmochim Acta 77:335–351
10. Batty JD, Rorke GV (2006) Development and commercial demonstration of the BioCOP™ thermophile process. Hydrometallurgy 83:83–89
11. Bellenberg S, Vera M, Sand W (2011) Transcriptomic studies of capsular polysaccharide export systems involved in biofilm formation by *Acidithiobacillus ferrooxidans.* In: Guanzhou Q, Tao J, Wenqing Q, Xueduan L, Yu Y, Haidong W (eds) Biohydrometallurgy: biotech key to unlock mineral resources value, vol 1 (Proceedings of the 19th International Biohydrometallurgy Symposium, Changsha, China)

12. Bellenberg S, Leon-Morales CF, Sand W, Vera M (2012) Visualization of capsular polysaccharide induction of *Acidithiobacillus ferrooxidans*. Hydrometallurgy 129–130:82–89
13. Blake RC II, Shute EA, Howard GT (1994) Solubilization of minerals by bacteria: electrophoretic mobility of *Thiobacillus ferrooxidans* in the presence of iron, pyrite, and sulfur. Appl Environ Microbiol 60:3349–3357
14. Bock M, Bosecker K (1996) In: DECHEMA monographs, vol 133. VCH Verlagsgesell, Weinheim (in German)
15. Borg RJ, Dienes GJ (1992) The physical chemistry of solids. Academic press, Boston
16. Bosecker K (1986) Leaching of lateritic nickel ores with heterotrophic microorganisms. In: Salley J, McCready RGL, Wichlacz P (eds) Biohydrometallurgy International Symposium proceedings. CANMET, Ottawa
17. Bosecker K (1994) Mikrobielle Laugung (leaching). In: Praeve P, Faust U, Sittig W, Sukatsch DA (eds) Handbuch der Biotechnologie. R. Oldenbourg, Munich
18. Bosecker K (1997) Bioleaching: metal solubilization by microorganisms. FEMS Microbiol 20:591–604
19. Bosecker K (1999) Microbial leaching in environmental clean-up programmes In: Amils R, Ballester A (eds) Process Metallurgy 9B. Elsevier, Amsterdam
20. Brandl H (2001) Microbial leaching of metals. In: Rehm HJ, Reed, G (eds) Biotechnology, vol 10. Wiley, Weinheim
21. Brierley CL (2008) How will biomining be applied in future? Trans Nonferr Met Soc 18:1302–1310
22. Brierley JA (2008) A perspective on developments in biohydrometallurgy. Hydrometallurgy 94:2–7
23. Bridge TAM, Johnson DB (1998) Reduction of soluble iron and reductive dissolution of ferric iron minerals by moderately thermophilic iron-oxidising bacteria. Appl Environ Microbiol 64:2181–2186
24. Bridge TA, Johnson DB (2000) Reductive dissolution of ferric iron minerals by *Acidiphilium* SJH. Geomicrobiol J 17:193–206
25. Brock TD, Gustafson J (1976) Ferric iron reduction by sulphur- and iron-oxidizing bacteria. Appl Environ Microbiol 32:567–571
26. Brombacher C, Bachofen R, Brandl H (1997) Biohydrometallurgical processing of solids: a patent review. Appl Microbiol Biotechnol 18:577–587
27. Cameselle C, Ricert MT, Nunez MJ, Lema JM (2003) Iron removal from kaolin: comparison between "in situ" and "two-stage" processes. Hydrometallurgy 68:97–105
28. Clark ME, Batty JD, van Buuren CB, Dew DW, Eamon MA (2006) Biotechnology in minerals processing: technological breakthroughs creating value. Hydrometallurgy 83:3–9
29. Coto O, Galizia F, Hernandez I, Marrero J, Donati E (2008) Cobalt and nickel recoveries from lateritic tailings by organic and inorganic bio-acids. Hydrometallurgy 94:18–22
30. Coupland K, Johnson DB (2008) Evidence that the potential for dissimilatory ferric iron reduction is widespread among acidophilic heterotrophic bacteria. FEMS Microbiol Lett 279:30–35
31. Crundwell FK (1988) The influence of the electronic structure of solids on the anodic dissolution and leaching of semiconducting sulphide minerals. Hydrometallurgy 21:155–190
32. Das A, Mishra AK, Roy P (1992) Anaerobic growth on elemental sulfur using dissimilar iron reduction by autotrophic *Thiobacillus ferrooxidans*. FEMS Microbiol Lett 97:167–172
33. Deplanche K, Woods RD, Mikheenko IP, Sockett RE, Macaskie LE (2008) Manufacture of stable Pd and Au nanoparticles on native and genetically engineered flagella scaffolds. Biotechnol Bioeng 101:873–880
34. d'Hugues P, Foucher S, Galle'-Cavalloni P, Morin D (2002) Continuous bioleaching of chalcopyrite using a novel extremely thermophilic mixed culture. Int J Min Process 66:107–119
35. DiSpirito AA, Dugan PR, Tuovinen OH (1983) Sorption of *Thiobacillus ferrooxidans* to particulate material. Biotech Bioeng 25:1163–1168

36. Dold B (2008) Sustainability in metal mining: from exploration, over processing to mine waste management. Rev Environ Sci Biotechnol 7:275–285
37. Domic EM (2007) A review of the development and current status of copper bioleaching operations in Chile: 25 years of successful commercial implementation. In: Rawlings DE, Johnson DB (eds) Biomining. Springer, Berlin
38. Donati ER, Sand W (2007) Microbial processing of metal sulfides. Springer, Dordrecht
39. Donati E, Pogliani C, Boiardi JL (1997) Anaerobic leaching of covellite by *Thiobacillus ferrooxidans*. Appl Microbiol Biotechnol 47(6):636–639
40. Donati ER, Viera MR, Tavani EL, Giaveno MA, Lavalle TL, Chiacchiarini PA (2009) Biohydrometallurgy: a meeting point between microbial ecology, metal recovery processes and environmental remediation. In: Advanced Material Research, vol 71–73. Trans Tech Publications, Switzerland
41. Dopson M, Johnson DB (2012) Biodiversity, metabolism and applications of acidophilic sulfur-metabolizing microorganisms. Environ Microbiol 14:2620–2631
42. Druschel G, Borda M (2006) Comment on "Pyrite dissolution in acidic media" by Descostes M, Vitorge P, Beaucaire C. Geochim Cosmochim Acta 70:5246–5250
43. Dutrizac JE (1990) Converting jarosite residues into compact hematite products. J Met 42:36–39
44. Dutrizac JE, MacDonald RJC (1974) Ferric ion as a leaching medium. Min Sci Eng 6:59–100
45. du Plessis CA, Batty JD, Dew DW (2007) Commercial applications of thermophilic bioleaching. In: Rawlings DE, Johnson DB (eds) Biomining. Springer, Berlin
46. du Plessis CA, Slabbert W, Hallberg KB, Johnson DB (2011) Ferredox: a biohydrometallurgical processing concept for limonitic nickel laterites. Hydrometallurgy 109:221–229
47. Dziurla MA, Achouak W, Lam BT, Heulin T, Berthelin J (1998) Enzyme-linked immunofiltration assay to estimate attachment of *Thiobacilli* to pyrite. Appl Environ Microbiol 64:2937–2942
48. Edelstein DL (2011) 2009 Minerals yearbook: Copper, US geological survey. http://minerals.usgs.gov/minerals/pubs/commodity/copper/myb1-2009-coppe.pdf. Accessed July 2011
49. Edwards KJ, Rutenberg AD (2001) Microbial response to surface microtopography: the role of metabolism in localized mineral dissolution. Chem Geol 180:19–32
50. Edwards KJ, Schrenk MO, Hamers R, Banfield J (1998) Microbial oxidation of pyrite: experiments using microorganisms from an extreme acidic environment. Am Miner 83:1444–1453
51. Edwards KJ, Goebel BM, Rodgers TM, Schrenk MO, Gihring TM, Cardona MM, McGuire MM, Hamers RJ, Pace NR, Banfield JF (1999) Geomicrobiology of pyrite (FeS_2) dissolution: case study at Iron Mountain, California. Geomicrobiol J 16:155–179
52. Ehrlich HL (1999) Past, present and future of biohydrometallurgy. In: Amils R, Ballester A (eds) Process Metallurgy 9A. Elsevier, Amsterdam
53. Ehrlich HL (2000) Ocean manganese nodules: biogenesis and bioleaching possibilities. Miner Metall Process 17:121–128
54. Ehrlich HL (2002) Geomicrobiology. Marcel Dekker, New York
55. Evangelou VPB (1995) Pyrite oxidation and its control. CRC Press, Boca Raton
56. Florian B, Noel N, Thyssen C, Felschau I, Sand W (2011) Some quantitative data on bacterial attachment to pyrite. Miner Eng 24:1132–1138
57. Fuchs T, Huber H, Teiner K, Burggraf S, Stetter KO (1995) *Metallosphaera prunae*, sp. nov., a novel metal-mobilizing, thermoacidophilic archaeum, isolated from a uranium mine in Germany. Syst Appl Microbiol 18:560–566
58. Gehrke T, Telegdi J, Thierry D, Sand W (1998) Importance of extracellular polymeric substances from *Thiobacillus ferrooxidans* for bioleaching. Appl Environ Microbiol 64:2743–2747

59. Gehrke T, Hallmann R, Kinzler K, Sand W (2001) The EPS of *Acidithiobacillus ferrooxidans*: a model for structure-function relationships of attached bacteria and their physiology. Water Sci Technol 43:159–167
60. Gonzalez A, Bellenberg S, Mamani S, Ruiz L, Echeverria A, Soulere L, Doutheau A, Demergasso C. Sand W, Queneau Y, Vera M, Guiliani N (2012) AHL signaling molecules with a large acyl chain enhance biofilm formation on sulfur and metal sulfides by the bioleaching bacterium *Acidithiobacillus ferrooxidans*. Appl Microbiol Biotechnol. 97:3729–3737
61. Goodman AE, Babij T, Ritchie AIM (1983) Leaching of a sulphide ore by *Thiobacillus ferrooxidans* under anaerobic conditions. In: Rossi G, Torma AE (eds) Progress in biohydrometallurgy. Associazione Mineraria Sarda, Iglesia source, Italia
62. Groudev SN (1987) Use of heterotrophic microorganisms in mineral biotechnology. Acta Biotechnol 7: 299 – 306
63. Groudev SN, Georgiev PS, Spasova II, Komnitsas K (1999) In: Amils R, Ballester A (eds) Process metallurgy 9B. Elsevier, Amsterdam
64. Groudev S, Spasova I, Nicolova M, Georgiev P (2009) In situ bioremediation of contaminated soils in uranium deposits. Adv Mat Res 71–73:533–540
65. Grützner T (2001) Auswirkungen von *Acidithiobacillus ferrooxidans* auf die flotierbarkeit sulfidischer minerale. Diploma thesis, TU Clausthal, Clausthal-Zellerfeld, Germany
66. Hackl RP, Dreisinger DB, Peters E, King JA (1995) Passivation of chalcopyrite during oxidative leaching in sulfate media. Hydrometallurgy 39:25–48
67. Hahn M, Willscher S, Straube G (1993) Copper leaching from industrial wastes by heterotrophic microorganisms. In: Torma AE, Wey JE, Lakshmanan VI (eds) Proceedings of the international biohydrometallurgy symposium, Jackson Hole, Wyoming, USA
68. Hallberg KB, Johnson DB (2001) Biodiversity of acidophilic prokaryotes. Adv Appl Microbiol 49:37–84
69. Hallberg KB, González-Toril E, Johnson DB (2010) *Acidithiobacillus ferrivorans*, sp. nov.; facultatively anaerobic, psychrotolerant iron-, and sulfur-oxidizing acidophiles isolated from metal mine-impacted environments. Extremophiles 14:9–19
70. Hallberg KB, Hedrich S, Johnson DB (2011) *Acidiferrobacter thiooxydans*, gen. nov. sp. nov.; an acidophilic, thermo-tolerant, facultatively anaerobic iron- and sulfur-oxidizer of the family *Ectothiorhodospiraceae*. Extremophiles 15:271–279
71. Hallberg KB, Grail BM, du Plessis CA, Johnson DB (2011) Reductive dissolution of ferric iron minerals: a new approach for bio-processing nickel laterites. Miner Eng 24:620–624
72. Harneit K, Göksel A, Kock D, Klock JH, Gehrke T, Sand W (2006) Adhesion to metal sulfide surfaces by cells of *Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans*. Hydrometallurgy 83:245–254
73. Hawkes RB, Franzmann PD, O'hara G, Plumb JJ (2006) *Ferroplasma cupricumulans* sp. nov., a novel moderately thermophilic, acidophilic archaeon isolated from an industrial-scale chalcocite bioleach heap. Extremophiles 10:525–530
74. Hedrich S, Johnson DB (2012) A modular continuous flow reactor system for the selective bio-oxidation of iron and precipitation of schwertmannite from mine-impacted waters. Biores Technol 106:44–49
75. Hedrich S, Schlömann M, Johnson DB (2012) The iron-oxidizing proteobacteria. Microbiology 157:1551–1564
76. Hol A, van der Weijden RD, van Weert G, Kondos P, Buisman CJN (2012) Bio-reduction of elemental sulfur to increase the gold recovery from enargite. Hydrometallurgy 115–116:93–97
77. Hoque ME, Phili OJ (2011) Biotechnological recovery of heavy metals from secondary sources: an overview. Mat Sci Eng C 31:57–66
78. Hosseini MR, Schaffie M, Pazouki M, Schippers A, Ranjbar M (2013) A novel electrically enhanced biosynthesis of copper sulfide nanoparticles. Mat Sci Semicond Process 16:250–255

79. Janneck E, Arnold I, Koch T, Meyer J, Burghard D, Ehinger S (2010) Microbial synthesis of schwertmannite from lignite mine water and its utilization for removal of arsenic from mine waters and for production of iron pigments. In: Wolkersdorfer C, Freund (eds) Proceedings IMWA 2010 "Mine water and innovative thinking", Sydney, Canada
80. Johnson DB (2012) Reductive dissolution of minerals and selective recovery of metals using acidophilic iron- and sulfate-reducing acidophiles. Hydrometallurgy 127–128:172–177
81. Johnson D, Hallberg KB (2005) Acid mine drainage: remediation options. Sci Tot Environment 338:3–14
82. Johnson DB, Roberto FF (1997) In: Rawlings DE (ed) Biomining: theory, microbes and industrial processes. Springer, Berlin
83. Johnson DB, Joulian C, d'Hugues P, Hallberg KB (2008) *Sulfobacillus benefaciens* sp. nov., an acidophilic facultative anaerobic Firmicute isolated from mineral bioleaching operations. Extremophiles 12:789–798
84. Johnson DB, Bacelar-Nicolau P, Okibe N, Thomas A, Hallberg KB (2009) *Ferrimicrobium acidiphilum* gen. nov., sp. nov. and *Ferrithrix thermotolerans* gen. nov., sp. nov.: heterotrophic, iron-oxidizing, extremely acidophilic actinobacteria. Int J Syst Evol Microbiol 59:1082–1089
85. Julin DE, Tobie RL (1974) Block caving. In: Given IE (ed) SME mining engineering handbook. Society for Mining, Metallurgy, and Exploration, Englewood
86. Karavaiko GI, Groudev SN (1985) Biogeotechnology of metals. GKNT International Projects, Moscow
87. Kinzler K, Gehrke T, Telegdi J, Sand W (2003) Bioleaching: a result of interfacial processes caused by extracellular polymeric substances (EPS). Hydrometallurgy 71:83–88
88. Korehi H, Blöthe M, Sitnikova MA, Dold B, Schippers A (2013) Metal mobilization by iron- and sulfur-oxidizing bacteria in a multiple extreme mine tailings in the Atacama Desert, Chile. Environ Sci Technol 47:2189–2196
89. Kracik I (1996) Integrated acid plant design for pressure leaching plants for laterites. In: Proceedings of ALTA conference Ni/Co
90. Lee JC, Pandey BD (2012) Bio-processing of solid wastes and secondary resources for metal extraction: a review. Waste Manag (Oxford) 32:3–18
91. Lewis AE (2010) Review of metal sulphide precipitation. Hydrometallurgy 104:222–234
92. Little B, Ray B, Pope R, Franklin M, White DC (2000) *S*patial and temporal relationships between localised corrosion and bacterial activity on iron-containing substrata. In: Sequeira CAC (ed) Microbial corrosion. European Federation of Corrosion Publications no 29, Institute of Materials, London
93. Logan TC, Seal T, Brierley JA (2007) Whole-ore heap biooxidation of sulfidic gold-bearing ores. In: Rawlings DE, Johnson DB (eds) Biomining. Springer, Berlin
94. Lovley DR, Phillips EJP (1988) Novel mode of microbial energy metabolism: organic carbon oxidation coupled to dissimilatory reduction if iron or manganese. Appl Environ Microbiol 54:1472–1480
95. Lovley DR, Ueki T, Zhang T, Malvankar NS, Shrestha PM, Flanagan KA, Aklujkar M, Butler JE, Giloteaux L, Rotaru AE, Holmes DE, Franks AE, Orellana R, Risso C, Nevin KP (2011) *Geobacter*: the microbe electric′s physiology, ecology, and practical applications. Adv Microbiol Physiol 59:1–100
96. Lowson RT (1982) Aqueous oxidation of pyrite by molecular oxygen. Chem Rev 82:461–497
97. Luther GW III (1987) Pyrite oxidation and reduction: molecular orbital theory considerations. Geochim Cosmochim Acta 51:3193–3199
98. Marston C, Rodgers M (2009) Nickel recovery from lateritic ore in the presence of iron using bispicolylamine chelating resin. In: Budac JJ, Fraser R, Mihaylov I, Papangclakis VG, Robinson DJ (eds) Hydrometallurgy of Nickel and Cobalt (Proceedings of the 39th annual hydrometallurgy meeting, Sudbury, Ontario, Canada)

99. McCready RGI, Gould WD (1989) Bioleaching of uranium at Dennison mines. In: Biohydrometallurgy (Proceedings of the international symposium, Jackson Hole, Wyoming, USA)
100. McDonald RG, Whittington BI (2008) Atmospheric acid leaching of nickel laterites review (part I): sulphuric acid technologies. Hydrometallurgy 91:35–55
101. McGuire MM, Edwards KJ, Banfield JF, Hamers RJ (2001) Kinetics, surface chemistry, and structural evolution of microbially mediated sulfide mineral dissolution. Geochim Cosmochim Acta 65:1243–1258
102. Medvedev D, Stuchebrukhov AA (2001) DNA repair mechanism by photolyase: electron transfer path from the photolyase catalytic cofactor $FADH^-$ to DNA thymine dimer. J Theor Biol 210:237–248
103. Meyer G, Schneider-Merck T, Böhme S, Sand W (2002) A simple method for investigations on the chemotaxis of *A. ferrooxidans* and *D. vulgaris*. Acta Biotechnol 22:391–399
104. Monhemius AJ (1977) Precipitation diagrams for metal hydroxides, sulfates, arsenates and phosphates. Trans Inst Min Metall Sec C86:C202–C206
105. Morin DHR, d'Hugues P (2007) Bioleaching of cobalt-containing pyrite in stirred reactors: a case study from laboratory scale to industrial application. In: Rawlings DE, Johnson DB (eds) Biomining. Springer, Berlin
106. Moses CO, Nordstrom DK, Herman JS, Mills AL (1987) Aqueous pyrite oxidation by dissolved oxygen and by ferric iron. Geochim Cosmochim Acta 51:1561–1571
107. Narayanan KB, Sakthivel N (2010) Biological synthesis of metal nanoparticles by microbes. Adv Colloid Interface 156:1–13
108. Ñancucheo I, Johnson DB (2012) Selective removal of transition metals from acidic mine waters by novel consortia of acidophilic sulfidogenic bacteria. Microb Biotechnol 5:34–44
109. Nguyen HL, Willscher S, Fritsche W (1994) Mikrobielle Laugung von Schwermetallen aus Galvanikschlämmen. Chem Tech 46:201
110. NIST (2003) NIST critical selected stability constants of metal complexes database. In: NIST standard reference database 46, version 7.0. National Institute of Standards and Technology, Gaithersburg
111. Noël N, Florian B, Sand W (2010) AFM and EFM study on attachment of acidophilic leaching organisms. Hydrometallurgy 104:370–375
112. Nordstrom DK (1982) Aqueous pyrite oxidation and the consequent formation of secondary iron minerals In: Hossner LR, Kittrick JA, Fanning DF (eds) Acid sulfate weathering, pedogeochemistry and relationship to manipulation of soil minerals. Soil Science Society of America Press, Madison
113. Nordstrom DK (2000) Advances in the hydrogeochemistry and microbiology of acid mine waters. Int Geol Rev 42:499–515
114. Norris PR (2007) Acidophilic diversity in mineral sulfide oxidation. In: Rawlings DE, Johnson DB (eds) Biomining. Springer, Berlin
115. Norris PR, Davis-Belmar CS, Brown CF, Calvo-Bado LA (2011) Autotrophic, sulfur-oxidizing actinobacteria in acidic environments. Extremophiles 15:155–163
116. Ohmura N, Kitamura K, Saiki H (1993) Selective adhesion of *Thiobacillus ferrooxidans* to pyrite. Appl Environ Microbiol 59:4044–4050
117. Ohmura N, Sasaki K, Matsumoto N, Saiki H (2002) Anaerobic respiration using Fe^{3+}, S^0, and H_2 in the chemolithoautotrophic bacterium *Acidithiobacillus ferrooxidans*. J Bacteriol 184:2081–2087
118. Olson GJ, Brierley JA, Brierley CL (2003) Bioleaching review part B: progress in bioleaching: applications of microbial processes by the minerals industries. Appl Microb Biotechnol 63:249–257
119. Petersen J, Dixon DG (2007) Modeling and optimization of heap bioleach processes. In: Rawlings DE, Johnson DB (eds) Biomining. Springer, Berlin
120. Plumb JJ, Hawkes RB, Franzmann PD (2007) The microbiology of moderately thermophilic and transiently thermophilic ore heaps. In: Rawlings DE, Johnson DB (eds) Biomining. Springer, Berlin

121. Plumb JJ, Haddad CM, Gibson JAE, Franzmann PD (2007) *Acidianus sulfidivorans* sp. nov., an extremely acidophilic, thermophilic archaeon isolated from a solfatara on Lihir Island, Papua New Guinea, and emendation of the genus description. Int J Syst Evol Microbiol 57:1418–1423
122. Pronk JT, de Bruyn JC, Bos P, Kuenen JG (1992) Anaerobic growth of *Thiobacillus ferrooxidans*. Appl Environ Microbiol 58:2227–2230
123. Puhakka JA, Kaksonen AH, Riekkola-Vanhanen M (2007) Heap leaching of black schist. In: Rawlings DE, Johnson DB (eds) Biomining. Springer, Berlin
124. Rawlings DE (2002) Heavy metal mining using microbes. Annu Rev Microbiol 56:65–91
125. Rawlings DE, Johnson DB (2007) Biomining. Springer, Berlin
126. Rawlings DE, Johnson DB (2007) The microbiology of biomining: development and optimization of mineral-oxidizing microbial consortia. Microbiology 153:315–324
127. Rawlings DE, Dew D, du Plessis C (2003) Biomineralization of metal-containing ores and concentrates. Trends Biotechnol 21:38–44
128. Remonsellez F, Galleguillos F, Moreno-Paz M, Parro V, Acosta M, Demergasso C (2009) Dynamic of active microorganisms inhabiting a bioleaching industrial heap of low-grade copper sulfide ore monitored by real-time PCR and oligonucleotide prokaryotic acidophile microarray. Microbiol Biotechnol 2:613–624
129. Rimstidt JD, Vaughan DJ (2003) Pyrite oxidation: a state-of-the-art assessment of the reaction mechanism. Geochim Cosmochim Acta 67:873–880
130. Roden EE (2008) Microbiological controls on geochemical kinetics (2): case study on microbial oxidation of metal sulfide minerals and future prospects. In: Brantley SL, Kubicki J, White AF (eds) Kinetics of water–rock interactions, pp 417–467. Springer, New York
131. Rodriguez-Leiva M, Tributsch H (1988) Morphology of bacterial leaching patterns by *Thiobacillus ferrooxidans* on synthetic pyrite. Arch Microbiol 149:401–405
132. Rohwerder T, Gehrke T, Kinzler K, Sand W (2003) Bioleaching review part A: progress in bioleaching: fundamentals and mechanisms of bacterial metal sulfide oxidation. Appl Microbiol Biotechnol 63:239–248
133. Rossi G (1990) Biohydrometallurgy. McGraw-Hill, Hamburg
134. Rossi G (1993) Biodepyritization of coal: achievements and problems. Fuel 72:1581–1592
135. Saari P, Riekkola-Vanhanen M (2011) Talvivaara bio-heap leaching process. In: Proceedings of the conference on percolation leaching: the status globally and in southern Africa, Misty Hills Conference Centre, Cradle of Humankind, Gauteng, South Africa
136. Sampson MI, Phillips CV, Blake RC II (2000) Influence of the attachment of acidophilic bacteria during the oxidation of mineral sulfides. Min Eng 13:373–389
137. Sand W, Gehrke T (2006) Extracellular polymeric substances mediate bioleaching/biocorrosion via interfacial processes involving iron(III) ions and acidophilic bacteria. Res Microbiol 127:49–56
138. Sand W, Gehrke T, Hallmann R, Schippers A (1995) Sulfur chemistry, biofilm, and the (in)direct attack mechanism: a critical evaluation of bacterial leaching. Appl Microbiol Biotechnol 43:961–966
139. Sand W, Gehrke T, Hallmann R, Schippers A (1998) Towards a novel bioleaching mechanism. Min Pro Ext Met Rev 19:97–106
140. Sand W, Gehrke T, Jozsa P-G, Schippers A (2001) (Bio)chemistry of bacterial leaching: direct versus indirect bioleaching. Hydrometallurgy 59:159–175
141. Sanhueza A, Ferrer IJ, Vargas T, Amils R, Sánchez C (1999) Attachment of *Thiobacillus ferrooxidans* on synthetic pyrite of varying structural and electronic properties. Hydrometallurgy 51:115–129
142. Schippers A (2004) Biogeochemistry of metal sulfide oxidation in mining environments, sediments and soils. In: Amend JP, Edwards KJ, Lyons TW (eds) Sulfur biogeochemistry: past and present. Special paper 379. Geological Society of America, Boulder

143. Schippers A (2007) Microorganisms involved in bioleaching and nucleic acid-based molecular methods for their identification and quantification (Chap. 1). In: Donati ER, Sand W (eds) Microbial processing of metal sulfides. Springer, Dordrecht
144. Schippers A (2009) Biomining for metal extraction from ore and waste. Adv Min Solutions: AMS Online 2009(1):27–33
145. Schippers A, Sand W (1999) Bacterial leaching of metal sulfide proceeds by two indirect mechanisms via thiosulfate or via polysulfides and sulfur. Appl Environ Microbiol 65:319–321
146. Schippers A, Hallmann R, Wentzien S, Sand W (1995) Microbial diversity in uranium mine waste heaps. Appl Environ Microbiol 61:2930–2935
147. Schippers A, Jozsa P-G, Sand W (1996) Sulfur chemistry in bacterial leaching of pyrite. Appl Environ Microbiol 62:3424–3431
148. Schippers A, Rohwerder T, Sand W (1999) Intermediary sulfur compounds in pyrite oxidation: implications for bioleaching and biodepyritization of coal. Appl Microbiol Biotechnol 52:104–110
149. Schippers A, Bosecker K, Willscher S, Spröer C, Schumann P, Kroppenstedt RM (2002) *Nocardiopsis metallicus* sp. nov., a metal leaching actinomycete isolated from an alkaline slag dump. Int J Syst Evol Microbiol 52:2291–2295
150. Schippers A, Sand W, Glombitza F, Willscher S (2007) Biohydrometallurgy: from the single cell to the environment. In: Advanced Materials Research 20/21. Trans Tech Publications, Switzerland
151. Schippers A, Nagy AA, Kock D, Melcher F, Gock ED (2008) The use of FISH and *real-time* PCR to monitor the biooxidation and cyanidation for gold and silver recovery from a mine tailings concentrate (Ticapampa, Peru). Hydrometallurgy 94:77–81
152. Schippers A, Breuker A, Blazejak A, Bosecker K, Kock D, Wright DL (2010) The biogeochemistry and microbiology of sulfidic mine waste and bioleaching dumps and heaps, and novel Fe(II)-oxidizing bacteria. Hydrometallurgy 104:342–350
153. Shrihari RK, Modak JM, Kumar R, Gandhi KS (1995) Dissolution of particles of pyrite mineral by direct attachment of *Thiobacillus ferrooxidans*. Hydrometallurgy 38:175–187
154. Sillitoe RH, Perelló J (2005) Andean Copper Provinces: tectonomagamtic settings, deposit types, metallogeny, exploration and discovery. In: Hedenquist JW, Thompson JFH, Goldfarb R (eds) Economic geology one hundredth anniversary. Society of Economic Geologists
155. Simate GS, Ndlovu S (2008) Bacterial leaching of nickel laterites using chemoautotrophic microorganisms: identifying influential factors using statistical design of experiments. Int J Min Proc 88:31–36
156. Singer PC, Stumm W (1970) Acidic mine drainage: the rate-determining step. Science 167:1121–1123
157. Smart RSC, Jasieniak M, Prince KE, Skinner WM (2000) SIMS studies of oxidation mechanisms and polysulfide formation in reacted sulfide surfaces. Miner Eng 13:857–870
158. Sobral LGS, de Oliveira DM, de Souza CEG (2011) Biohydrometallurgical processes: a practical approach. Centre for Mineral Technology (CETEM), Ministry of Science, Technology and Innovation (MCTI), Rio de Janeiro, Brazil
159. Solari JA, Huerta G, Escobar B, Vargas T, Badilla-Ohlbaum R, Rubio J (1992) Interfacial phenomena affecting the adhesion of *Thiobacillus ferrooxidans* to sulphide mineral surfaces. Colloid Surf 69:159–166
160. Steudel R (2000) The chemical sulfur cycle. In: Lens P, Hulshoff Pol L (eds) Environmental technologies to treat sulfur pollution: principles and engineering. International Association on Water Quality, London
161. Stumm W, Morgan JJ (1996) Aquatic chemistry, chemical equilibria and rates in natural waters, 3rd edn. Willey, New York
162. Styriakova J, Styriak I, Kraus I, Hradil D, Grygar T, Bezdicka P (2003) Biodestruction and deferritizaion of quartz sands by *Bacillus* species. Miner Eng 16:709–713

163. Sugio T, Domatsu C, Munakata O, Tano T, Imai K (1985) Role of ferric ion-reducing system in sulphur oxidation of *Thiobacillus ferrooxidans*. Appl Environ Microbiol 49:1401–1406
164. Tabak HH, Govind R (2003) Advances in biotreatment of acid mine drainage and biorecovery of metals (2): membrane bioreactor system for sulfate reduction. Biodegradation 14:437–452
165. Third KA, Cord-Ruwisch R, Watling HR (2002) Control of the redox potential by oxygen limitation improves bacterial leaching of chalcopyrite. Biotech Bioeng 78:433–441
166. Thomas JE, Jones CF, Skinner WM, Smart RSC (1998) The role of surface sulfur species in the inhibition of pyrrhotite dissolution in acid conditions. Geochim Cosmochim Acta 62:1555–1565
167. Thomas JE, Skinner WM, Smart RSC (2001) A mechanism to explain sudden changes in rates and products for pyrrhotite dissolution in acid solution. Geochim Cosmochim Acta 65:1–12
168. Thurston RS, Mandernack KW, Shanks WC III (2010) Laboratory chalcopyrite oxidation by *Acidithiobacillus ferrooxidans*: oxygen and sulfur isotope fractionation. Chem Geol 269:252–261
169. Torma A (1978) Oxidation of gallium sulfides by *Thiobacillus ferrooxidans*. Can J Microbiol 24:888–891
170. Tributsch H (2001) Direct versus indirect bioleaching. Hydrometallurgy 59:177–185
171. Tributsch H, Bennett JC (1981) Semiconductor-electrochemical aspects of bacterial leaching (part 1): oxidation of metal sulphides with large energy gaps. J Chem Technol Biotechnol 31:565–577
172. Tributsch H, Bennett JC (1981) Semiconductor-electrochemical aspects of bacterial leaching (part 2): survey of rate-controlling sulphide properties. J Chem Technol Biotechnol 31:627–635
173. Vandevivere P, Kirchman DL (1993) Attachment stimulates exopolysaccharide synthesis by a bacterium. Appl Environ Microbiol 59:3280–3286
174. Vaughan DJ, Craig JR (1978) Mineral chemistry of metal sulphides. Cambridge University Press, Cambridge
175. Vera M, Krok B, Bellenberg S, Sand W, Poetsch A (2013) Shotgun proteomics study of early biofilm formation process of *Acidithiobacillus ferrooxidans* ATCC 23270 on pyrite. Proteomics 13:1133–1144
176. Vilinska AK, Rao KH (2009) Surface thermodynamics and extended DLVO theory of *Acidithiobacillus ferrooxidans* cells adhesion on pyrite and chalcopyrite. Open Colloid Sci J 2:1–14
177. von Aswegen PC, van Niekerk J, Oliveri W (2007) The BIOX^{TM} process for the treatment of refractory gold concentrates. In: Rawlings DE, Johnson DB (eds) Biomining. Springer, Berlin
178. von Wahl S (1990) Bergwirtschaft Band I. Verlag Glückauf, Essen
179. Watling HR (2006) The bioleaching of sulphide minerals with emphasis on copper sulphides: a review. Hydrometallurgy 84:81–108
180. Wellmer FW (2002) Leachable supergene base and precious metal deposits worldwide. Erzmetall 55:25–33
181. Williamson MA, Rimstidt JD (1994) The kinetics and electrochemical rate-determing step of aqueous pyrite oxidation. Geochim Cosmochim Acta 58:5443–5454
182. Willis B (2007) Downstream processing options for nickel laterite heap leach liquors. ALTA Conference Ni/Co, Proceedings
183. Willscher S, Bosecker K (2003) Studies on the leaching behaviour of heterotrophic microorganisms isolated from an alkaline slag dump. Hydrometallurgy 71:257–264
184. Willscher S, Clark TR, Cohen RH, Ranville JF, Smith KS, Walton-Day K (2003) Bioleach of a fluvial tailings deposit material indicates long term potential for pollution. In: Biohydrometallurgy: a sustainable technology in evolution (Proceedings of 15th International Biohydrometallurgical Symposium, 14–19 September, Athens, Greece

185. Willscher S, Pohle C, Sitte J, Werner P (2007) Solubilization of heavy metals from a fluvial AMD generating tailings sediment by heterotrophic microorganisms (part I): influence of pH and solid content. J Geochem Explor 92:177–185
186. Willscher S, Hertwig T, Frenzel M, Felix M, Starke S (2010) Results of remediation of hard coal overburden and tailing dumps after a few decades: Insights and conclusions. Hydrometallurgy 104:506–517
187. Willscher S, Mirgorodsky D, Jablonski L, Ollivier D, Merten D, Büchel G, Wittig J, Werner P (2013) Field scale phytoremediation experiments on a heavy metal and uranium contaminated site, and further utilization of the plant residues. Hydrometallurgy 131–132:46–53
188. Willscher S, Knippert D, Ihling H, Kuehn D, Starke S (2013) Underground degradation of lignite coal spoil material by a mixed microbial community under acid mine drainage conditions. In: Advanced Materials Research (submitted)
189. Xu Y, Schoonen MAA (2000) The absolute energy positions of conduction and valence bands of selected semiconductiong minerals. Am Miner 85:543–556
190. Yahya A, Hallberg KB, Johnson DB (2008) Iron and carbon metabolism by a mineral-oxidizing *Alicyclobacillus*-like bacterium. Arch Microbiol 189:305–312
191. Zhu J, Li Q, Jiao W, Jiang H, Sand W, Xia J, Liu X, Qin W, Qiu G, Hu Y, Chai L (2012) Adhesion forces between cells of *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans* or *Leptospirillum ferrooxidans* and chalcopyrite. Colloid Surfaces B Interface 94:95–100

Adv Biochem Eng Biotechnol (2014) 141: 49–107
DOI: 10.1007/10_2013_254

Published Online: 11 June 2014

Metal-Containing Residues from Industry and in the Environment: Geobiotechnological Urban Mining

Franz Glombitza and Susan Reichel

Abstract This chapter explains the manifold geobiotechnological possibilities to separate industrial valuable metals from various industrial residues and stored waste products of the past. In addition to an overview of the different microbially catalyzed chemical reactions applicable for a separation of metals and details of published studies, results of many individual investigations from various research projects are described. These concern the separation of rare earth elements from phosphorous production slags, the attempts of tin leaching from mining flotation residues, the separation of metals from spent catalysts, or the treatment of ashes as valuable metal-containing material. The residues of environmental technologies are integrated into this overview as well. The description of the different known microbial processes offers starting points for suitable and new technologies. In addition to the application of chemolithoautotrophic microorganisms the use of heterotrophic microorganisms is explained.

Keywords Ashes · Biohydrometallurgy · Bioleaching · Catalysts · Flotation residues · Industrial residues · Organic acids · Rare earth elements · Slags · Sludges · Tailings · Trace elements

Abbreviations

BDS	Bacterial Dried Substances
BOP	Basic Oxygen Process
B12	Cobalamin
CTR	Continuous Tank Reactor
DS	Dried Substances
DSZM	German Collection of Microorganisms and Cell Cultures
ESM	Electronic Scrap Material

F. Glombitza (✉) · S. Reichel
G.E.O.S. Ingenieurgesellschaft mbH, Schwarze Kiefern 2,
09633 Halsbrücke, Germany
e-mail: f.glombitza@geosfreiberg.de

S. Reichel
e-mail: s.reichel@geosfreiberg.de

EU	European Union
kt	Kilo tons (thousands of tons)
LD	Linz–Donawitz
LD Slag	Slag of the steel producing basic oxygen process (BOP) Linz–Donawitz process
MET	Methionine
MO	Microorganisms
n.d.	Not determined
PFS	Phosphorous Furnace Slag
pH	pH value (negative decade logarithm of the H^+ ion activity)
REE	Rare Earth Elements
REO	Rare Earth Oxides
TE	Trace Elements
THF	Tetrahydrofolic acid
TOC	Total Organic Carbon
UFZ	Helmholtz Centre for Environmental Research
w.s.	Water soluble

Contents

1 Introduction

Raw materials and energy are essential for economic existence. Even if no growth is expected and only maintenance of the economic force is aspired to, enormous amounts of basic materials and energy sources are required. The satisfaction of needs is based on the exploration and exploitation of new resources and energy deposits as well as on the attempt to recover raw materials via recycling processes. Recycling processes depend on the state of development of separation techniques which are still imperfect. Most exploitation technologies create secondary materials that still contain valuable substances as well as unusable residues. Older exploitation technologies, especially, produced unexploitable residues containing much higher concentrations of valuable substances in these residues than in some mineral ores. The increase of the efficiency of resource exploitation is recommended as a most important challenge by the OECD [1].

Such metal-containing residues were accumulated in huge amounts in the past. These are waste materials from the smelters, mainly slags, but on the other hand there are flotation residues from mineral ore processing deposited in tailings, the iron and aluminum containing red mud from aluminum production, slags and residues from phosphate and phosphorous production, sludges from electroplating plants, ashes from incineration processes of gas, oil, or lignite and coal power stations as well as of the municipal waste incineration plants, dusts from various dust removing and air filtration plants, and the manifold residues from wastewater treatment and biogas formation plants in addition to the sludges from rivers and harbors.

These residues are not only remnants of the past, but are still constantly obtained from various purification and incineration processes. They comprise, for example, the sludges from (drinking) water treatment plants as well as the ashes from various incineration processes and mainly slags from running smelters. In contrast, the number of recycled materials is, however, much greater than in the past and is continuously increasing. It includes electronic scraps, spent catalysts, composite materials, and the residues of young industries such as the photovoltaic

or the chip industry. The proportion of recycled goods and residues from other processes such as color television and electronic tube production decreases or is almost negligible in contrast to the increase of the former residues.

The concentration of valuable substances in all these residues can be increased by novel accumulation and separation processes. In addition to pyro- and hydrometallurgical extraction processes, there are many geomicrobiological processes applicable for the extraction of valuable substances by dissolving or transforming the inorganic matrix [2–5]. Most of the residues are oxides, hydroxides, phosphates, carbonates, or silicates that contain different important metals and trace elements, but cannot be used as an energy source for the microbial processes. The microbial decomposition is based on secondary processes such as the formation of acids or other water-soluble and/or volatile products related to different metabolic transformations. As there are numerous possible reactions based on various microbial processes, the challenge is to look for the most suitable solution to the problem [6–9].

Such reactions are the formation of inorganic acids, for example, sulphuric acid after the addition of sulphur or sulphides, but also the formation of nitric acid and carbonic acid which, in a second reaction, dissolve the inorganic materials, or the formation of organic acids that also dissolve the inorganic materials and act as chelating agents simultaneously. The dependence of industrial processes on natural biogeochemical cycles of matter is an important precondition for further sustainable development [1].

The acidolysis describes the destruction of the mineral matrix by inorganic acids inducing sulphuric acid, nitric acid, or carbonic acid, whereas organic acids simultaneously act as complexing agents. This process is designated as complexolysis. Additional extracellular substances such as siderophores also play a special role because they act as chelating substances with the ability to increase the solubility of metal oxides with small solubility products and enhance their bioavailability. Quite different mechanisms of microbial dissolution are based on the redoxolysis, the reduction and/or oxidation of cations in a mineral matrix, for example, Mn^{2+}, Mn^{4+}, Fe^{2+}, or Fe^{3+}. The microbial cyanide production induces their instability by the formation of water-soluble cyanide-containing complexes. In contrast to the acidic processes the latter process takes place under alkaline conditions. The transformation of metals into organometallic compounds via microbial processes such as methylation or ethylation and the formation of volatile metal organic compounds has scarcely been investigated or applied thus far. Microbial silicate solubilizing processes and the extraction of valuable materials from silicates should attain increased importance in the light of the increasing need of the global industrial society. They offer extensive possibilities for the extraction of rare elements.

The assessment of the European Union shows 14 elements and compounds that are expected probably to run short for European industries in the future. These elements and compounds are presented in Table 1.

Table 1 Elements and compounds with critical availability in the future

Antimony	Tungsten
Beryllium	Tantalum
Cobalt	Indium
Gallium	Platinum metals
Germanium	Rare earth elements
Magnesium	Fluorspar
Niobium	Graphite

Attempts to recycle materials and to recover valuable substances have been undertaken for many years, including the consideration of microbial processes [10–16]. A compilation of running or developed technologies for the separation of valuable substances from anthropogenic residues is demonstrated in Sects. 4–8.

The following examples give an idea of the huge possibilities concerning the exploitation of raw materials by means of biotechnological recycling technologies. This list is not exhaustive and complete due to the complexity of this issue. For example, it does not contain the various running metal recovery processes from mine wastes such as dumps and heaps [17–23].

General conditions that limit the application of a biotechnological process depend on the prevailing economic and technological situations and legislative regulations. Such preconditions are:

- Market price of the product and market demand as well as market development
- Product and capital costs
- Influence of political development and legal interpretation (politically driven development).

2 Overview of Appropriate Microbial Reaction Mechanisms

The extraction of valuable substances from the named residues typically requires the dissolution or partial destruction of oxides, hydroxides, phosphates, carbonates, or silicates that contain the wanted metals, valuable substances, and trace elements. Usually these residues do not contain energy-supplying agents for the microorganisms. Processes from natural cycles of matter have to be analyzed and applied causing destruction of materials thereby. This destruction is achieved by secondary processes such as the microbial formation of acids, or the formation of water-soluble and/or volatile products due to microbial transformation or complexing agents as well as by reducing processes that transfer cations in a lower oxidation state with better water solubility.

The following chapter provides a brief overview of the most important existing possibilities.

3 Acid Formation Processes (Acidolysis)

Microorganisms are able to produce inorganic and organic acids.

3.1 Microbial Processes Related to the Formation of Inorganic Acids

The most common process is the formation of sulphuric acid. Sulphur or sulphides from metal sulphides are oxidized to sulphuric acid by sulphur-oxidizing bacteria:

$$2\ S^{\circ} + 2\ H_2O + 3\ O_2 \longrightarrow 2\ H_2SO_4 \quad (1)$$

$$Me(Cu,\ Ni,\ Fe...)S_2 + 3.5\ O_2 + H_2O + 2\ H^+ \longrightarrow 2\ H_2SO_4 + Me^{2+}. \quad (2)$$

In addition to the formation of sulphuric acid, the formation of nitric acid has to be taken into account. Ammonia is oxidized via nitrite to nitrate by nitrifying archaea or bacteria such as *Nitrosomonas* and *Nitrobacter*:

$$2NH_3 + 3\ O_2 \longrightarrow 2\ NO_2^- + 2\ H_2O + 2\ H^+ \quad (3)$$

$$NO_2^- + 0.5\ O_2 \longrightarrow NO_3^-. \quad (4)$$

The formation of H_2CO_3 is based on the release of CO_2 during microbial growth:

$$C_{org} + \text{microorganisms} + N, P, O \longrightarrow \text{biomass} + CO_2 + H_2O + \text{Energy} \quad (5)$$

$$CO_2 + H_2O \longrightarrow H_2CO_3. \quad (6)$$

3.2 Microbial Processes Related to the Formation of Organic Acids

3.2.1 Fatty Acids

Fatty acids belong to the aliphatic monocarboxylic acids. Sources of such acids are organic compounds such as saturated aliphatic hydrocarbons, paraffins, or iso-paraffins. They are oxidized to the corresponding organic acids under aerobic conditions and lack of nitrogen. For example, yeasts or hydrocarbon-utilizing bacteria form hexadecanoic acid (palmitic acid) from hexadecane [24, 25]:

$$C_{16}H_{34} + 1.5\,O_2 \longrightarrow C_{15}H_{31}CO(OH) + H_2O. \quad (7)$$

3.2.2 Gluconic Acid

Glucose or glucose-containing substances are oxidized to gluconic acid by microorganisms under aerobic conditions and in a nitrogen-free medium:

$$C_6H_{12}O_6 + 0.5\ O_2 \longrightarrow C_5O_5H_{11}COOH. \tag{8}$$

3.2.3 Citric Acid

Citric acid is often produced by yeasts under stress and citric acid is released as a side product of the tricarboxylic acid cycle into the fermentation medium. Such stress factors can be the lack of nitrogen in the medium. The carbon-containing substrate cannot be transformed into biomass and is converted to CO_2 and citric acid:

$$C_{org} + \text{ microorganisms } + \text{ nutrients} \longrightarrow C_6H_8O_7 + CO_2. \tag{9}$$

The formation of citric acid by fungi is different from the processes in yeasts. Citric acid is often produced by *Aspergillus niger* under iron deficiency during cultivation.

3.2.4 Oxalic Acid

Oxalic acid is also an intermediate product or constituent of the tricarboxylic acid cycle. Another mechanism is responsible for the microbial formation of oxalic acid by fungi. Oxalic acid is excreted under alkaline conditions:

$$C_{org} + \text{ microorganisms } + \text{ nutrients } \xrightarrow{\textit{Alkaline Conditions}} \text{ biomass } + C_2H_2O_4 + CO_2. \tag{10}$$

The formation and expression of citric acid, isocitric acid, and oxalic acid as well as some other acids are well known for various fungi, bacteria, and yeasts.

4 Oxidizing and Reduction Processes (Redoxolysis)

Microbial growth is always linked to energy transfer, which includes electron delivering (donator) and electron receiving (acceptor) processes. Because different metal and nonmetal ions are involved in this electron transfer, different oxidizing and reducing processes exist, relevant for a leaching process.

4.1 Oxidation Processes

4.1.1 Oxidation of Ferrous Iron (Fe^{2+})

$$2\ Fe^{2+} + 0.5\ O_2 + 2\ H^+ \longrightarrow 2\ Fe^{3+} + H_2O. \quad (11)$$

The formed Fe^{3+} can be separated as iron hydroxyl sulphate in an acidic and sulphate-containing medium.

4.1.2 Oxidation of Manganese Ions (Mn^{2+})

$$2\ Mn^{2+} + O_2 + 2\ H_2O \longrightarrow 2\ MnO_2 + 4\ H^+. \quad (12)$$

The Mn^{4+} is mainly separated from solution as MnO_2 after the oxidation.

4.1.3 Oxidation of As^{3+} and Sb^{3+} (e.g., [26, 27])

$$As^{3+} + 0.5\ O_2 + 2\ H^+ \longrightarrow As^{5+} + H_2O. \quad (13)$$

As^{5+} forms an arsenate anion and can be separated as $[AsO_4]^{3-}$ by precipitation.

An analogous process takes place with the ions and compounds from trivalent antimony and the formation of $[SbO_4]^{3-}$ [28].

4.1.4 Oxidation of Uranium (U^{4+})

$$U^{4+} + O_2 + 2\ SO_4^{2-} \longrightarrow \left[UO_2(SO_4)_2\right]^{2-}. \quad (14)$$

Uranium(IV) is transferred to uranium(VI) directly by microorganisms or indirectly by an oxidizing agent and forms a uranylsulphate anion under acidic conditions with sulphuric anions [29].

4.2 Reduction Processes

Reduction is described as an electron receiving process. Many electron acceptors are known in combination with microbial actions, for example:

$$Cr^{6+} + 3\ e^- \longrightarrow Cr^{3+}. \quad (15)$$

Chromium(VI) ions are reduced and form highly soluble chromium(III) ions [30, 31].

The reduction of As^{5+} and Sb^{5+} is also known:

$$As^{5+} + 2\ e^- \longrightarrow As^{3+}. \quad (16)$$

Compared to As^{5+} compounds the compounds of As^{3+} often have a higher solubility. This was demonstrated, for example, under reducing conditions in wetlands where the As_2S_5 compounds are transformed into more soluble As_2S_3 compounds resulting in an increased concentration of arsenic in the treated water.

Ferric ions are enzymatically reduced by acidophilic or neutrophilic microorganisms under anaerobic conditions [32–35, Chap. 1]. Organic carbon, hydrogen, or sulfur compounds can act as the electron donor in this reaction:

$$Fe^{3+} + e^- \longrightarrow Fe^{2+}. \quad (17)$$

The reduction of Mn^{4+} to Mn^{2+} is similar to the reduction of ferric iron. It is enzymatically catalyzed, for example, by *Geobacter* or *Shewanella* species. It takes place under anaerobic conditions also in the presence of sulphate-reducing bacteria (SRB) via a chemical reduction with their metabolic product sulphide [36]:

$$Mn^{4+} + 2\ e^- \longrightarrow Mn^{2+}. \quad (18)$$

The reduction of uranium(VI) (anerobic conditions) is connected with the formation of uraninite, an insoluble uranium-containing mineral [37]:

$$U^{6+} + 2\ e^- \longrightarrow U^{4+}. \quad (19)$$

In addition to the direct enzymatic microbial reduction and oxidation of the cations an indirect reduction and oxidation occurs. This happens where the microbially formed products act as a reducing or oxidizing agent. The oxidizing action of Fe^{3+} on uranium(IV) to form uranium(VI) is such an example, and the formation of ferric iron again by a repeated microbial oxidation. Some other reactions focus on the relationship between Fe^{3+} and S^{2-}. Sulphide is oxidized to S° and Fe^{3+} is reduced to Fe^{2+}. Both substances are energy sources in a subsequent microbial process. A similar mechanism can be assumed between Fe^{3+} and As^{3+}.

The reduction of chromate is also important, because the toxicity of Cr^{3+} is much lower than that of Cr^{6+}. Such a reduction can be achieved by biologically produced hydrogen [38].

5 Alkalinity-Producing Reactions and Growth Under Alkaline Conditions (Alkalinolysis)

In addition to microbial processes under acidic conditions, there are processes under alkaline conditions [39, 40].

The alkaline conditions can be caused by chemical or microbial processes. Such a process is the carbonization of slags or other materials containing CaO reacting with CO_2 from the air and with water on the surface to $CaCO_3$ and $Ca(OH)_2$. This results in a high pH value (in part $\geq$10) and the hydrolysis of organic material into low-molecular–weight compounds serving as substrate for extreme alkaliphilic microorganisms. They form organic acids in subsequent metabolic processes as well as potential ligands that enhance the metal mobilization by complex formation [41]:

$$CaO + CO_2 \longrightarrow CaCO_3 \tag{20}$$

$$CaCO_3 + 2\,H_2O \longrightarrow Ca(OH)_2 + H_2CO_3 \longrightarrow Ca^{2+} + 2\,OH^- + H_2CO_3. \tag{21}$$

Another possibility exists if H^+ ions are removed and OH^- ions remain in the water. Such a basic reaction is the interaction of NH_3 with water or the dissociation of some carbonates. The microbial formation of NH_3 can be achieved by the transfer of organic nitrogen from amino acids [Eqs. (22) and (23)] or a hydrolysis of urea catalyzed by the enzyme urease and urea cleavage microorganisms (Eq. 24) as *Sarcina urea* [42, 43],

$$R - CHNH_2COOH + NAD^+ \longrightarrow R - CNH\,COOH + NADH_2 \tag{22}$$

$$R - CNHCOOH + H_2O \longrightarrow R - COCOOH + NH_3 \tag{23}$$

or:

$$NH_2CONH_2 + 2\,H_2O \longrightarrow 2\,NH_4^+ + CO_3^{2-} \tag{24}$$

A third possibility is the nitrate ammonification [44, 45],

$$NO_3^- + 8(H) + H^+ \longrightarrow NH_3 + 3H_2O \tag{25}$$

An example for the formation of carbonate is the transfer of orthoclase by CO_2,

$$2\,KAlSi_3O_8 + CO_2 + 2\,H_2O \longrightarrow H_4Al_2Si_2O_9 + K_2CO_3 + 4\,SiO_2 \tag{26}$$

and the delivery of OH^- in unbuffered systems

$$K_2CO_3 \longrightarrow 2\,K^+ + CO_3^{2-} \tag{27}$$

$$CO_3^{2-} + H_2O \longrightarrow HCO_3^- + OH^- \tag{28}$$

In addition, many metals form highly soluble complexes with $(OH)^-$ anions:

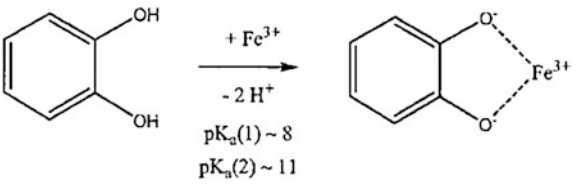

Fig. 1 Complex formation of iron by catechol

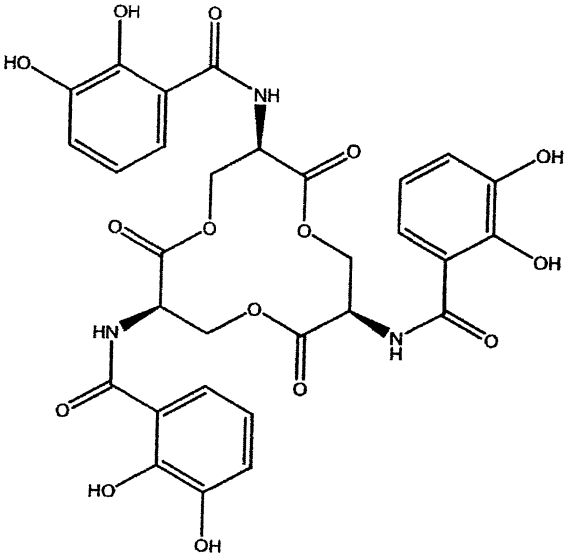

Fig. 2 Enterobactin

$$\mathrm{Me(OH)_n} + \mathrm{n(OH)^-} \longrightarrow \left[\mathrm{Me(OH)_{n+1}}\right]. \tag{29}$$

This can be observed for Al or Zn which form aluminates or zincates.

6 Complexolysis

6.1 Siderophores

Some microorganisms are able to produce organic compounds that form stable complexes with heavy metals. Well known are siderophores which form stable compounds with iron [46].

They are formed by bacteria and fungi, for example, by *Escherichia coli, Pseudomonas picketti (Ralstonia)*, or *Agrobacterium tumefaciens*. About 200 different natural siderophores are known. Because iron exists in insoluble compounds, the complexes often possess extremely high complex formation constants.

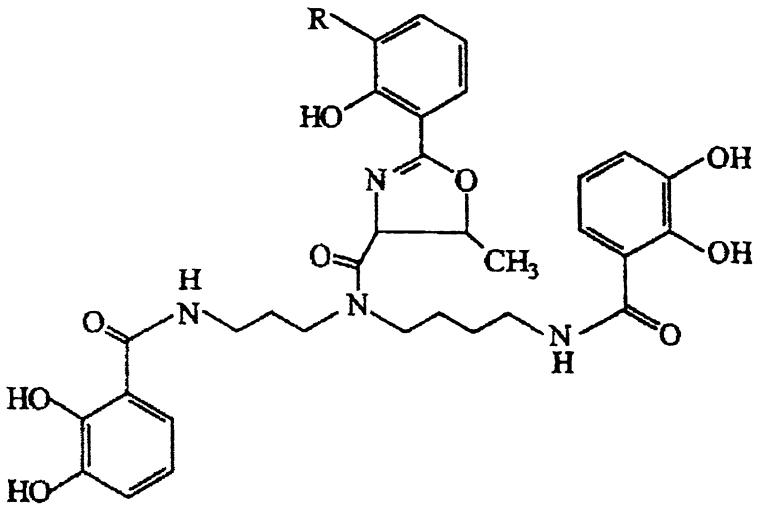

Fig. 3 Agrobactin

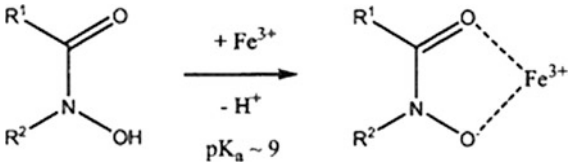

Fig. 4 Complex formation with hydroxamate

The stability of the complexes is very strong and in parallel is a precondition for the dissolution of iron oxides. This opens the possibility to leach iron-oxide–containing materials and to use them in microbial rust separation processes [47].

There are three different types of siderophores according to three main complexation mechanisms: catecholate, hydroxamate, and hydroxyl keto–carboxylate. The formation of a complex with catechol is demonstrated in Fig. 1.

Enterobactin and agrobactin are compounds related to catechol as demonstrated in Figs. 2 and 3. The formation of complexes with hydroxamate is shown in Fig. 4. Ferrichrome is one of the most known compounds of this type and is demonstrated in Fig. 5. The third kind of siderophore is based on the complex formation with alpha-keto-carboxylate (Fig. 6). This complex has a lower stability due to a lower pKa value.

Ferrichrome is the iron-containing compound as a representative of the hydroxamate compounds (Fig. 5).

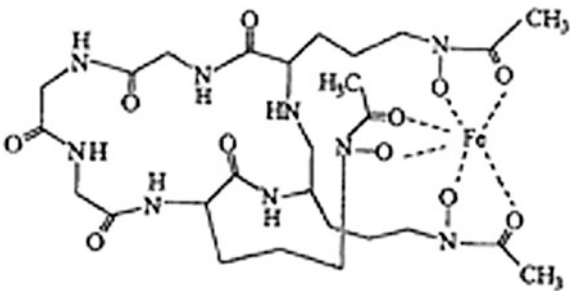

Fig. 5 Ferrichrome

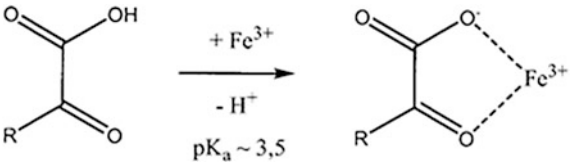

Fig. 6 Complex formation with alpha-keto-carboxylate

6.2 Cyanide Formation Processes

In addition to the iron complexation by siderophores, the formation of cyanide complexes is another quite different complex-forming process. Cyanide can be produced by microorganisms under alkaline conditions whereby glycine as a substrate and precursor is used [48–50]. This offers the possibility to form stable and water-soluble metal cyanide complexes. This is schematically demonstrated for the leaching of silver in the following equations.

$$C_{org.} + \text{microorganisms} + H_2N{-}CH_2\text{-}COOH \longrightarrow \text{biomass} + CO_2 + HCN \tag{30}$$

$$Me^{n+} + (n+1)CN^- \longrightarrow \left[Me^{n+}(CN)_{n+1}\right]^- \tag{31}$$

$$Ag^+ + 2\ CN^- \longrightarrow \left[Ag(CN)_2\right]^- \tag{32}$$

In addition to silver many other metals form stable cyanide complexes and thus can be leached.

7 Transformation and Formation of Metal Organic Compounds

7.1 Methylation/Ethylation

The formation of metal organic compounds by methylation and ethylation is a very interesting process [51]. An alkylation takes place directly by a microbial transfer of methyl or ethyl groups or indirectly by microbially formed metabolites followed

Table 2 Metal(loid) organic compounds from different sources [140]

Waste disposal	Sewage sludge	Low contaminated soil
$(CH_3)_2AsH$	$(CH_3)AsH_2$	$(CH_3)AsH_2$
$(CH_3)_3As$	$(CH_3)_2AsH$	$(CH_3)_2AsH$
$(CH_3)_2AsC_2H_5$	$(CH_3)_3As$	$(CH_3)_3As$
$(CH_3)_3Sb$	$(CH_3)_3Sb$	$(CH_3)_2BiH$
$(CH_3)_3Bi$	$(CH_3)_3Bi$	$(CH_3)_3Bi$
$(CH_3)_2Te$	$(CH_3)_2Te$	$(CH_3)_2Se$
$(CH_3)_2Hg$	$(CH_3)_4Sn$	$(CH_3)_2Se_2$
$(CH_3)_4Sn$		CH_3TeH
$(CH_3)_4Pb$		$(CH_3)_2Te$
		$(CH_3)_2Hg$
		$(CH_3)_4Sn$
		$(CH_3)_4Pb$

by cleavage under sunlight irradiation [52, 53]. The intracellular transfer of the methyl group is linked to the presence of methyl donors. Methyl donors are, for example, methyl-cobalamin (methyl B12), methionine (MET), and tetrahydrofolic acid (THF). This offers the possibility of separating heavy metals by microbially formed volatile compounds. Table 2 shows some microbially formed metal organic compounds in different habitats.

7.2 Mercury

A well-known mechanism is the transformation of Hg into methyl-, dimethyl-, or ethylmercury. In addition to the habitats listed in Table 2 it can be observed in sediments of harbors, rivers, or the sea:

$$\mathrm{Hg} + \mathrm{CH_3(Methyl{-}B12)} \longrightarrow \mathrm{(CH_3)\ Hg} \tag{33}$$

$$\mathrm{Hg} + 2\ \mathrm{CH_3(Methyl{-}B12)} \longrightarrow \mathrm{(CH_3)\ Hg\ (CH_3)} \tag{34}$$

$$\mathrm{Hg} + 2\ \mathrm{CH_3(Methyl{-}B12)} \longrightarrow \mathrm{(C_2H_5)\ Hg.} \tag{35}$$

The process occurs under anaerobic conditions and in the presence of sulphate-reducing bacteria.

The next equations demonstrate the formation of methyl-Hg with acetate as metabolite under sunlight irradiation (hν):

$$\mathrm{Hg^{2+} + CH_3COO^- + h\nu \longrightarrow CH_3Hg^+ + CO_2} \tag{36}$$

$$\mathrm{CH_3Hg^+ + CH_3COO^- + h\nu \longrightarrow (CH_3)_2Hg + CO_2.} \tag{37}$$

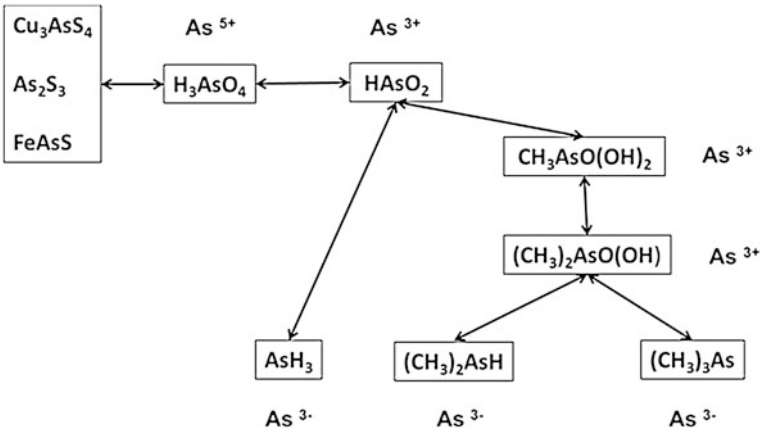

Fig. 7 Interactions of arsenic with microorganisms and change of the oxidation state [6]

7.3 Arsenic

Inorganic arsenic can be reduced by microorganisms and algae but also in vivo in mammals. The resulting formation of organic As compounds plays an important role in nature and the transformation can be used as a remediation process [54]. Similar processes are used for mercury-contaminated sites. Different microbially initiated processes are demonstrated in Fig. 7.

Another remarkable process is the alkylation of tin, because Sn^{4+} is very stable and forms oxides with a low water solubility. Hence, this is an important process, because it offers a new possibility of separating tin from ores and residues.

8 Preconditions for the Application of Microbial Leaching Processes

To select a suitable and effective leaching strategy, preconditions concerning the microbial growth conditions must be considered in addition to economic feasibility and legislative restriction. This means that leaching or dissolution under acidic conditions is possible, however,

– The Ca and Mg concentration has to be low in the raw material. In most cases Ca and Mg are bound as carbonate in minerals. This requires the destruction of the carbonate minerals as the first step. Ca reacts with sulphuric acid to form $CaSO_4$ in a second reaction and prevents a further leaching process by clogging the capillaries and consuming the sulphuric acid. The recommendation for the application of an acidic leaching process is that the total concentration of Ca and $MgCO_3$ be lower than 30 % [F. Glombitza, unpublished; 55].

The following is valid in general.

- The choice of a suitable pH value and the pH range determine the solubility of the dissolved ions. Therefore there are leaching processes under alkaline conditions but also under neutral, moderate acidophilic, and acidophilic conditions.
- Leaching is more effective, if many products of the leach solution can be exploited. A combination of the production of the leaching concentrate and the purification of the organic acid is much more effective in comparison with a pure acidic leaching process.
- Another strategy of leaching is the selection of some elements in a mineral matrix whose oxidation state can be changed by a microbial oxidizing or reducing process and cause the destabilization of the solid phase as a first step in the leaching process.

9 Residues from Mining and Metallurgical Industry

If we designate residues from the industry, we mostly subsume sludges, ashes, dusts, slags, and the residues from flotation processes, the so-called tailings. In the next chapter residues from the mining and metallurgical industry are described for the potential recovery of valuable substances. The chapter is divided in two sections. The first section concerns slags from running smelter processes. The second section refers to stored residues and slags from the past.

10 Residues from Smelters: Slags

10.1 Introduction

Slags are the residues of various pyrometallurgical processes and are obtained as a side product of zinc, lead, nickel, or copper production. Due to the decrease of mining activities and minimization of the number of smelter processes during the last decades disposal was covered with a water-impermeable top layer. Such sites are found in all old mining districts such as in Germany in the Harz Mountains, or in the Ore Mountains (Erzgebirge), for example, near Freiberg or in St. Egidien where a former nickel smelter operated in the past. The slags of these smelters are mainly silicates and oxides which always contain remarkable amounts of valuable substances. Other mining residues are products from the flotation tailings that are stored separately.

Table 3 gives examples of studies with residues from mining and metallurgical processes. It contains remarks concerning potential microbial leaching strategies of these materials and information about the main valuable substances. The

residues are the slags from different smelters and materials from flotation processes. The table lists the great variety of slags and residues. Some examples are from Germany, where different active smelters and many stored residues from the past exist. In addition, other authors are cited who have published investigations of and experiences from the leaching of such materials outside Germany.

10.2 Zinc Smelter Slags

The zinc recycling smelter in Freiberg, Germany, produces zinc from zinc-containing dusts of rotary kiln processes. In comparison to other plants the capacity is very high and amounts to an average of 50,000 t/a [56]. Previous analyses of the slags have demonstrated that neutrophilic and alkaliphilic microorganisms are able to extract metals including zinc, lead, and cadmium, whereas under acidic pH conditions zinc is extracted preferably.

Figure 8 demonstrates the leaching of zinc by *Acidithiobacillus* strains under acidic conditions and the attempts of a leaching of lead under alkaline conditions with an alkaliphilic mixed culture. A medium after Horikoshi was used for the cultivation of the alkaliphilic microorganisms [39]. The curves represent the average results for zinc and lead of different leaching experiments in shake-flasks. A notable concentration of zinc was measured. Only a few micrograms of cadmium were mobilized under alkaline conditions (not shown) but the concentration of lead ranged to some milligrams.

Another example for the treatment of slag resulting from a smelter process has been reported by Willscher et al. [57, Chap. 1]. They investigated the heterotrophic leaching of silicate-based and alkaline materials of slag from a smelter. About 38 % of Mn, 46 % of Mg, 68 % of Ca, 27 % of Zn, 15 % of Fe, 26 % of Ni, 40 % of Co, and 8 % of Pb could be dissolved. Other aspects include a strong alteration of the particle surfaces due to the microbial influence and the microbially mediated formation of secondary minerals such as calcite. The isolated microorganisms (e.g., the novel species *Nocardiopsis metallicus*; [58]), were alkali-tolerant strains with the ability to grow in the culture medium at a pH value of 11.

10.3 Lead Smelter: Slags

The treatment of slags from lead smelters is different from that from other base metal smelters. Some slags are deposited; other slags are used as the building material Berzelit® for the construction of disposals or roads [59]. Alternative investigations revealed that up to 70–80 % of the remaining metals in the slag could be leached with a mixed population of moderately thermophilic and acidophilic microbes [60, 61].

Table 3 Suitable microbial processes for leaching or destruction of slags and other mining residues

Residue/waste	Origin	Content/valuable substances	Treatment	References	Remarks
Slags	Lead/Zinc smelter	Al, Cu, Mn, Fe, Zn	Leaching with mixed culture isolate of moderate—thermophilic bacteria, *Bacillus* spp. *Sporosarcina* spp. *Pseudomonas* spp	[60, 61, 141]	Leaching efficiency of about 80 % in average, shaking flasks, pH 1.5, >60 °C electrochemical investigation of the metal solubilization
	Zinc smelter	Zn, Pb, Cd	Acidic and/or alkaline leaching	[142]	Solubilisation of Zn, Pb, and Cd under alkaline condition, Zn under acidic conditions
	Nickel smelter, nickel ore residues	Ni, Cr, Co, Fe	Acidic autotrophic or heterotrophic leaching	[71, 72, 74]	Residues from Ni—laterite, Ni extraction is influenced by the iron and solid concentration in the medium, 90 % at 2 % solids are possible
	Phosphor production	Ca, Fe, Al, Si, Mg, Mn, REE	Heterotrophic leaching of REE	[80; 81, 84]	Leaching of the slag was carried out with gluconic acid producing *A. methanolicus*
	Copper smelter slag	Cu, Ni, Co	Acidic leaching with *At. feroooxidans*, *At. thiooxidans*	[65, 66]	66 % Cu, 50 % Ni, 64 % Co are dissolved from particles of <75 Mm size slag
	Copper mining/ copper converter slag	Fe, Cu	Pulverized slag was leached with *At. ferrooxidans*	[69]	5 % pulp density, pH 1.9, 32 °C, Cu recovery is 77.5 %
	Steel producer	Fe, Ca, Si, V, TE	Alkaline heterotrophic leaching	[41, 67]	Heterotrophic leaching of LD slag by means of organic acids from wood,
	Waste incineration plant	Al, Cr, Cu, Ni, Pb, Zn	Leaching with acid producing autotrophic and heterotrophic bacteria and fungies	[143]	Extraction of Cu and Zn until 80–90 % depending on the process conditions

(continued)

Table 3 (continued)

Residue/waste	Origin	Content/valuable substances	Treatment	References	Remarks
Tailings/ flotation residues/ore residues	Tin mining	Sn	Leaching of sulphidic and oxidic tin minerals and flotation residues with *At. ferrooxidans*	[89–91]	Treatment of tin containing mine rals and residues with *At. ferrooxidans* and maximum concentration of tin from about 200 Mg/l
	Nickel mining	Ni, Cr, Co, Fe	Acidic leaching—autotrophic or heterotrophic	[21, 71, 72, 74]	Leaching of residues from Ni—laterite and lateritic ores, a delivery of Fe, Cr and Co takes place besides Ni

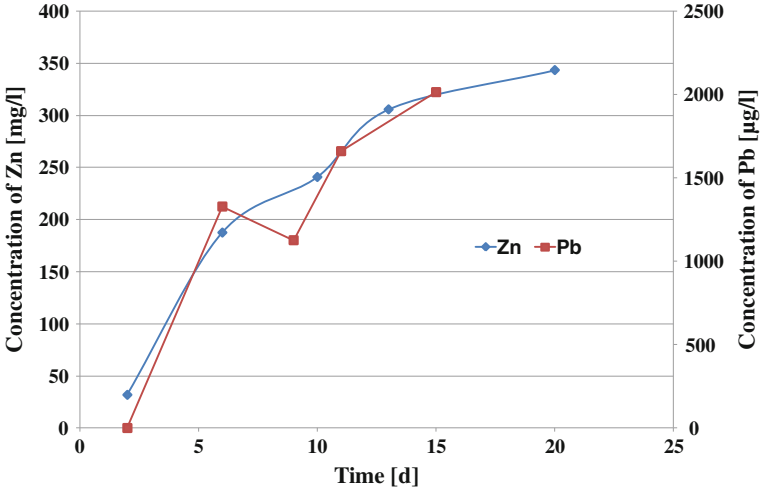

Fig. 8 Average values of the release of zinc from slags by an acidic leaching process with *Acidithiobacillus* and lead leached with alkaliphilic strains of microorganisms

10.4 Copper Smelter Slag

During smelting of copper concentrates, in addition to copper and precious metals, an iron silicate smelt is obtained in the Aurubis copper smelter, which is converted into iron silicate stones and a granulated material [62]. About 95 % of the iron silicate stones consist of olivin $(Mg,Fe)_2[SiO_4]$ which is used in water engineering [63, 64].

Inasmuch as the slags also contain other valuable elements, their recovery is conceivable according to the known and cited microbial dissolution investigations reported by Mehta et al. [65, 66], Karsson et al. [41], Willscher and Bosecker [67], Carranca et al. [68], or Baghery and Oliazadeh [69]. All these authors reported a successful leaching of similar material using various strains and conditions.

11 Slag Deposits: A New Possibility for Resources

11.1 Nickel-Containing Ores and Residues

An example for the treatment of smelter residues is the former nickel smelter in St. Egidien, Saxony, Germany. After closure of the mine in 1990, a so-called high-contaminated industrial area remained. This area contains about 2.3 million t of slags, tailings, and some other waste materials from industrial processes. The major contaminants are nickel, chromium, and cobalt compounds [70]. Leaching of this material seems to be possible using special leaching technologies.

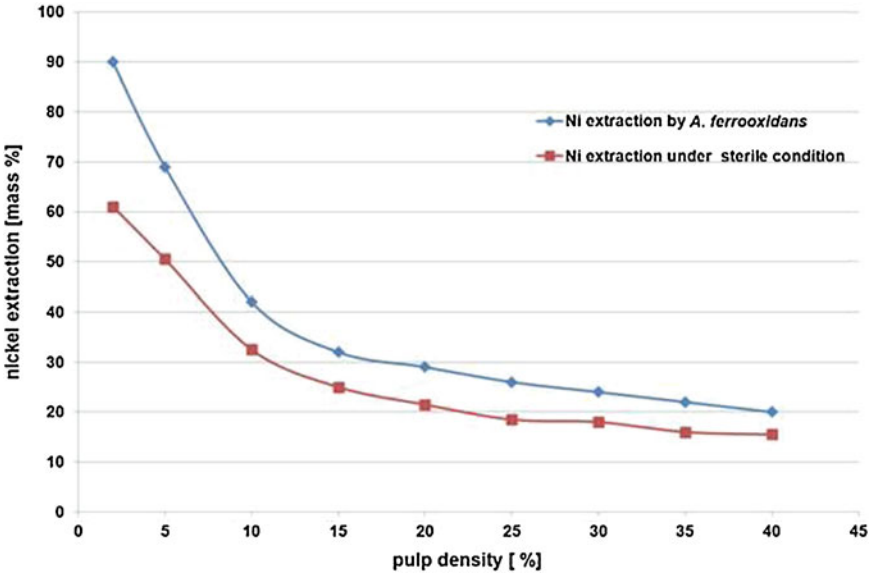

Fig. 9 Nickel extraction from hydrosilicate residues with and without microorganisms

Comprehensive investigations of nickel bioleaching from ores and residues were carried out [71, 72]. Because the nickel ore is a hydrosilicate with concentrations of 2,500–15,900 mg/kg Ni, 240–2,000 mg/kg Co, 2,400–7,000 mg/kg Cr and 1,900–9,000 mg/kg Mn and no energy-delivering substances for microbial leaching are available, a suitable leaching technology had to be developed. The leaching was carried out as submerged leaching [73] with different pulp densities with different strains of microorganisms. The influence of the ferrous iron concentration on the leaching process was investigated too. The results revealed a high extraction rate at a low solid concentration in the suspension and a decrease of the extraction efficiency with increasing solid concentration (Fig. 9). The figure displays the results of the leaching experiments with and without microorganisms and the investigations confirmed the positive influence of the bioleaching with *Acidithiobacillus ferrooxidans* on the nickel extraction. Figure 10 shows the attempt to increase the nickel extraction by increasing the concentration of ferrous iron. The results demonstrated a decrease of the nickel extraction due to an assumed precipitation of iron and a plugging of the porespace.

Table 4 shows the maximum concentrations and efficiency obtained with different microbial strains. The data result from separate investigations and are not related to each other. That means a concentration of 1,000 mg/l nickel in the leaching solution and a maximum average efficiency of 70 % were achieved [71].

In Fig. 11 the bioleaching of a nickel hydrosilicate with the extraction of iron, nickel, chromium, and cobalt is demonstrated. The process was driven by *Acidithiobacillus* strains with sulphur as the energy source and for sulphuric acid

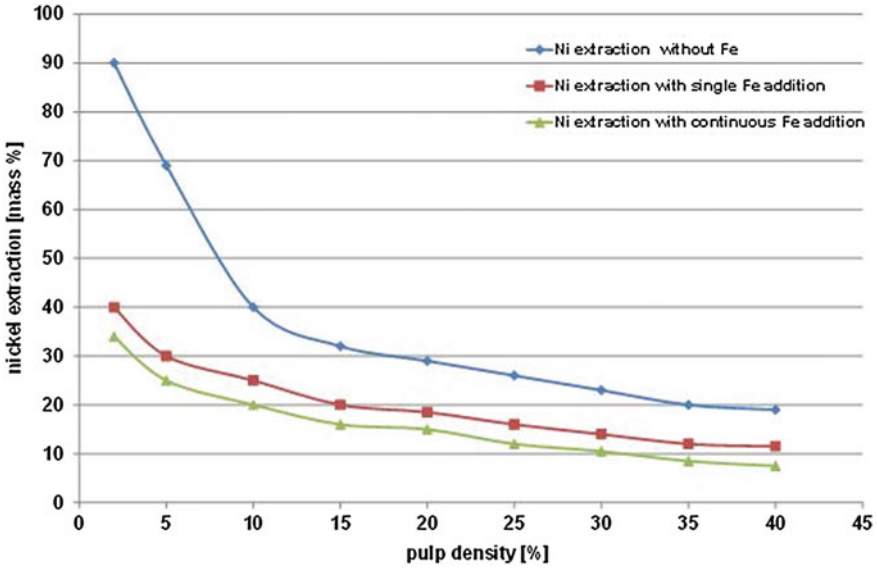

Fig. 10 Bioleaching of nickel from lateritic material with *A. ferrooxidans* and different Fe^{2+} supply

Table 4 Leaching effectivity and maximum metal concentrations in the leaching solution

Microorganisms/metals	*Acidithiobacillus ferrooxidans*	*Acetobacter methanolicus*	Yeast *Rhodotorula* sp.	Yeast *Candida*
Ni				
Concentration (mg/l)	1000	30	160	169
Max. effectivity (%)	70	30	19	20
Mn				
Concentration (mg/l)	112	40	480	460
Max. effectivity (%)	10	47	57	55
Cr				
Concentration (mg/l)	2.15	2.5	15	13.6
Max. effectivity (%)	5.5	6	4	3.4
Co				
Concentration (mg/l)	25	n.d.	3.8	77
Max. effectivity (%)	5.5	n.d.	19.4	38

formation simultaneously [74]. The maximum yielded nickel concentration was about 1 g/L. The iron concentration was much higher and averaged some grams per liter.

Similar investigations with microbially produced organic and inorganic acids were reported by Coto et al. [21]. Comprehensive investigations on the influencing factors proven by statistical methods were published by Simate and Ndlovu [75].

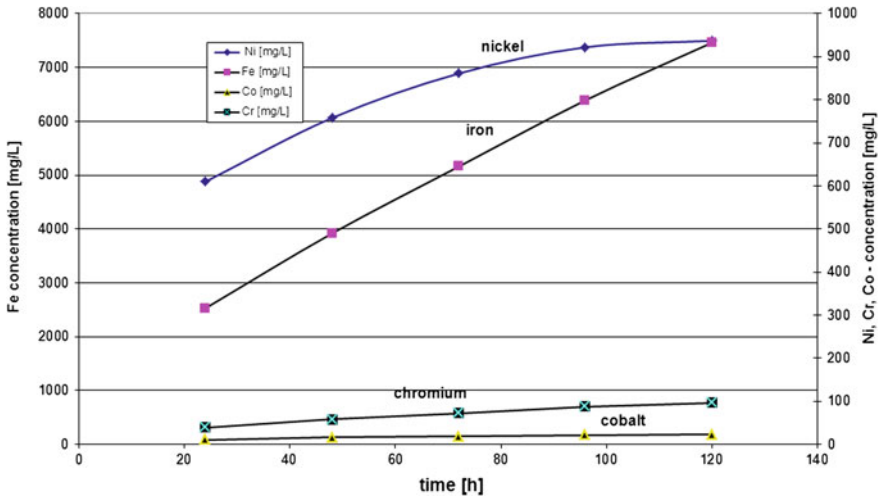

Fig. 11 Microbial acidic leaching of lateritic nickel ore [74]

A new technology for the leaching of nickel laterites involves the reduction of ferric iron ions under acidic anaerobic conditions with sulphur as electron donor. A cleavage of the mineral matrix caused by the reduction of ferric iron takes place and results in the dissolution of the metals [76, Chap. 1].

11.2 Rare Earth Elements Containing Residues

Slags usually consist of silicates and contain trace elements as well as rare earth elements. Such elements are very important for modern economics due to the needs of the electric and electronic industries. Covering the demand is really difficult because currently no economically recoverable primary mineral resources containing REE and trace elements exist. The importance of rare earth elements for a modern economy increases continuously. The EU demand for rare earth elements in 2008 was 23,013 t. About 90 % of the REE and REO are imported from China. Table 5 shows the amounts of Chinese production of rare earth oxides (REO) and of some single elements in 2006. Table 6 contains the global demand of REO in 2008 classified by different fields of application. It should be noted that China is not only a producer of REE but also a country with a big consumption [77].

Germany lacks high-grade REE deposits, therefore alternatives for the recovery of REE have been investigated in the past (and are still under investigation today).

One possibility is the separation of the REE from REE-containing residues. Such residues are various kinds of slags. This results in the analyses of slags and subsequent development of methods for the extraction of rare earth elements and trace elements from slags. An example is a slag obtained from the phosphorous production from apatite. Apatite contains between 0.4 and 1.2 % of REE and the

Table 5 Produced amounts of rare earth oxides and single metals in China in 2006 [77]

La_2O_3	CeO_2	Pr_6O_{11}	Nd_2O_3	Sm_2O_3	Eu_2O_3	Gd_2O_3	Tb_4O_7	Dy_2O_3	Er_2O_3	Y_2O_3	Sum
19730	22579	2297	11343	1586	368	4625	607	2311	954	9027	75427
La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Er	Y	
2034	–	–	7032	489	–	–	–	1280	–	–	10835

Data in t

Table 6 Overview of global application of REO from different industrial branches in 2008 related to the global production in % derived from [77]

Application	Amount (t)	%
Glass, polishing, ceramics	33,000–42,000	ca. 30
Magnets	21,000–27,000	ca. 20
Catalysts	20,000–25,000	ca. 20
Metal alloys, batteries	17,000–23,000	ca. 18
Phosphorous, luminescence	9,000	ca. 7
Others		ca. 5

phosphorous furnace slag (PFS), as a residue of this process, therefore contains these REE as well.

Table 7 demonstrates the composition of the slag and the distribution of different REE. The slag contains about 0.7 % REE consisting of more than 80 % light and about 12 % heavy REE as displayed in the righthand columns.

Hence, the separation of the REE from the slag was an important objective in the past. In addition to chemical processing, microbial processes were applied. The leaching of the slag was done chemically using waste hydrochloric acid or nitric acid, respectively. The first microbial investigations focused on the leaching of the slag using *Acidithiobacillus* strains to destroy the material by the formation of sulphuric acid. The formation of free sulphuric acid was, however, impossible due to the high concentration of calcium and the transfer of the sulphate ions into gypsum. A stable pH value in the acidic range could not be reached and the pH value was changed to alkaline conditions. Another attempt was the leaching with autotrophic microorganisms and Fe^{2+} as energy source for the growth of the microorganisms. Unfortunately, maintaining a stable acidic pH value was found to be difficult too.

The above-mentioned difficulties fostered research for alternative leaching techniques, for example, to connect the extraction of the rare earth and trace elements with the microbial formation of gluconic acid. The addition of an organic substrate was necessary because the slag doesn't contain any energy source for microorganisms [78–82, 83].

Gluconic acid is an important and most frequently used agent in the food industry and in healthcare. It can be separated by precipitation with some ions, for example, as Ca-gluconate from solution. This offers the chance to destroy the phosphorous furnace slag and to clean the organic acid.

Gluconic acid is microbially formed during the oxidation of glucose by different microorganisms in a nitrogen-free cultivation medium. The leaching process

Table 7 Composition of the phosphorous furnace slag (PFS) and the contained REE

Compound/ element	Concentration (%)	Composition REE (100 %)	Concentration (%)	Total (%)
REE	0.7	La_2O_3	22.3	
CaO	45.0	CeO_2	44.6	
SiO_2	41.0	Pr_6O_n	1.7	
F	2.9	Nd_2O_3	16.7	
Al_2O_3	3.5	Sm_2O_3	1.1	
Fe_2O_3	0.4	Eu_2O_3	1.7	88.1
P_2O_5	1.0	Gd_2O_3	5.6	
K_2O	1.0	Tb_4O_7	0.17	
Na_2O	0.4	Dy_2O_3	2.2	
MgO	0.5	Er_2O_3	1.7	
SO_3	0.5	Tm_2O_3	1.1	
MnO	0.2	Lu_2O_3	1.1	11.9

and the gluconic acid formation are carried out by various bacteria, for example, by *Acetobacter methanolicus* [84, 85]. Methanol is used as a suitable carbon source for the cultivation [82, 83]. The advantage is that the slag is destroyed by the formation of a water-insoluble calcium gluconate compound. The residue and the REE of the slag can be separated afterwards [86].

The process can be described by the following reaction equations [86].

$$1.0\ CaSiO_3 \times 1/9\ CaF_2(REE) + 2.19\ \text{gluconic acid} \longrightarrow 1.1\ Ca-\ \text{gluconate} + SiO_3^{2-} + REE + TE\ (\text{trace elements}) \quad (38)$$

or

$$1\ \text{tPFS} + 3.5\ \text{t gluconic acid} \longrightarrow 3.8\ \text{t gluconate} + 0.6\ \text{t}\ SiO_3^{2-} + 0.007\ \text{t}\ Se_2O_3 \quad (39)$$

It is generally believed that the leaching process is mainly influenced by the amount of formed gluconic acid and the amount of added slag. Because the calcium gluconate has a low solubility of approximately 3 g/l, the free gluconic acid in the leach solution decreases with a higher concentration of the slag. The formed amount of gluconic acid is important for the leaching process and depends on the precultivation conditions and the biomass concentration (Figs. 12 and 13). Precultivation conditions are the residence time of the cultivation process and the used carbon substrate or the biomass concentration [88].

Figure 12 shows that the highest concentration of gluconic acid is related to the highest amount of biomass and a short residence time during their cultivation. Figure 13 demonstrates the influence of different carbon sources on the concentration of formed gluconic acid. Methanol was the preferred substrate for a selective cultivation during the addition of slag to the medium, because providing glucose as a substrate at the technical scale that is used by a vast number of

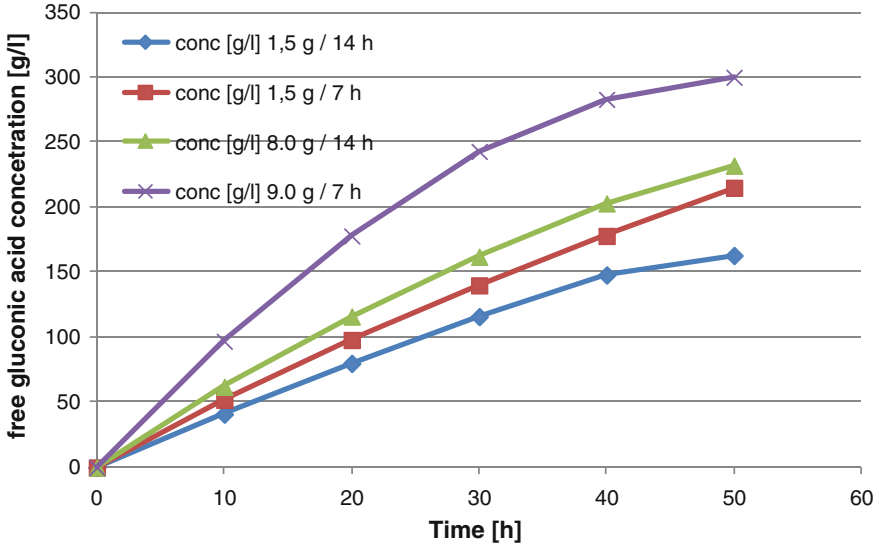

Fig. 12 Influence of the biomass concentration and residence time on the formed amount of gluconic acid with *A. methanolicus*

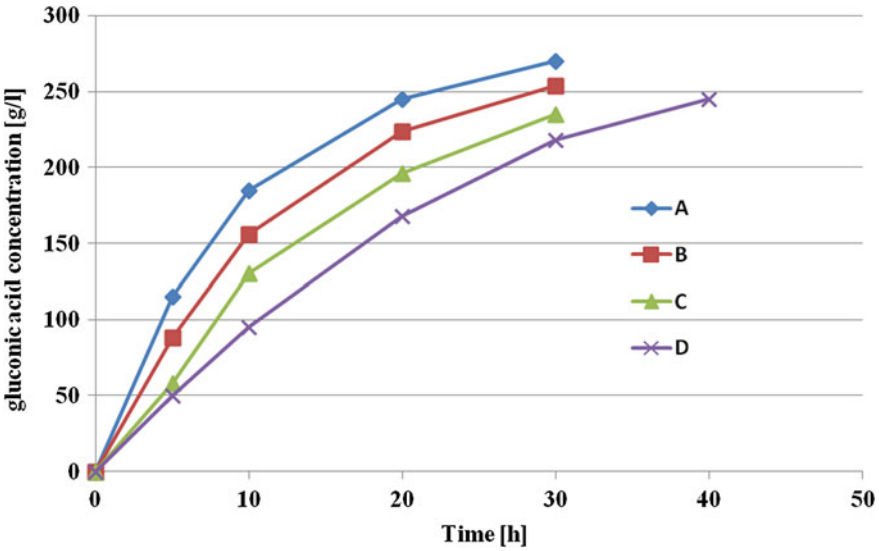

Fig. 13 Influence of the carbon source on the gluconic acid formation, *A* growth on glucose 5 g BDS/l; *B* growth on a mixture of methanol and glucose in a ratio 5:1; *C* growth on a mixture of methanol and gluconate in a ratio 5:1; *D* growth on methanol

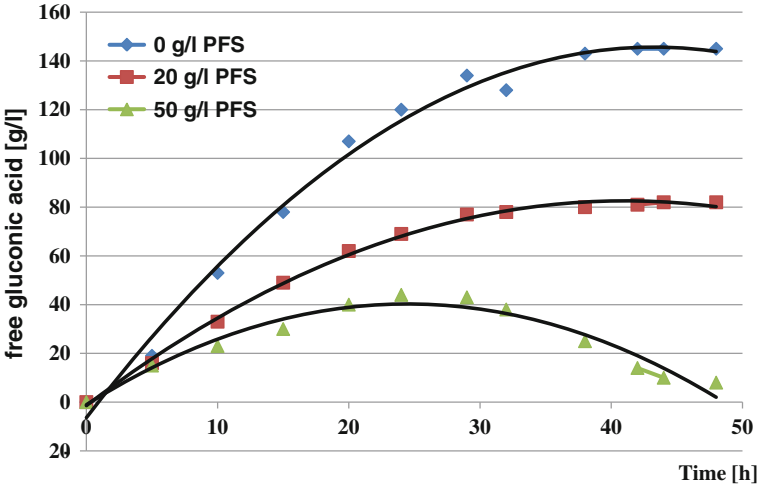

Fig. 14 Gluconic acid formation with different concentrations of PFS in the medium

microorganisms would have increased the risk of contamination by other microbial strains.

Figure 14 shows the decrease of free gluconic acid in the medium depending on the slag concentration. Inasmuch as the gluconic acid forms a stable compound with the Ca ions from the slag, a precipitation of calcium gluconate can be observed.

Figure 15 shows the amount of leached REE related to the total content of REE as a function of the formed concentration of gluconic acid. About 40 % of the REE were extracted from the slag at a gluconic acid concentration of 150 g/l.

Because the leaching system consisted of three different phases—two solid phases, namely the biomass and the slag and one liquid phase, the leaching solution—the distribution of the REE in these phases was investigated. Figure 16 demonstrates that more than 80 % of the leached REE were dissolved in the leaching solution and only 10–15 % of the REE were bound to the total biomass. The majority of REE were bound and stored in the biomass during the beginning of the leaching process. An increase of the REE concentration in the leaching solution can be observed after a saturation of the REE on the biomass. Comprehensive investigations of the sorption processes and the sorption capacity of the biomass confirmed this statement [78, 79]. Such a multiphase reaction system generates comprehensive difficulties due to the analytical determination and the establishment of a balance equation.

The results of such a discontinuous leaching process and the distribution of the REE in the leaching system as well as the dissolution of the slag as an example are shown in Table 8. Related to a volume of 1l the leaching system was characterized by 50 g slag with a concentration of 0.59 % REE according to a total amount of 0.259 g/l, an average biomass content of 5 g and 165 g glucose. The expected

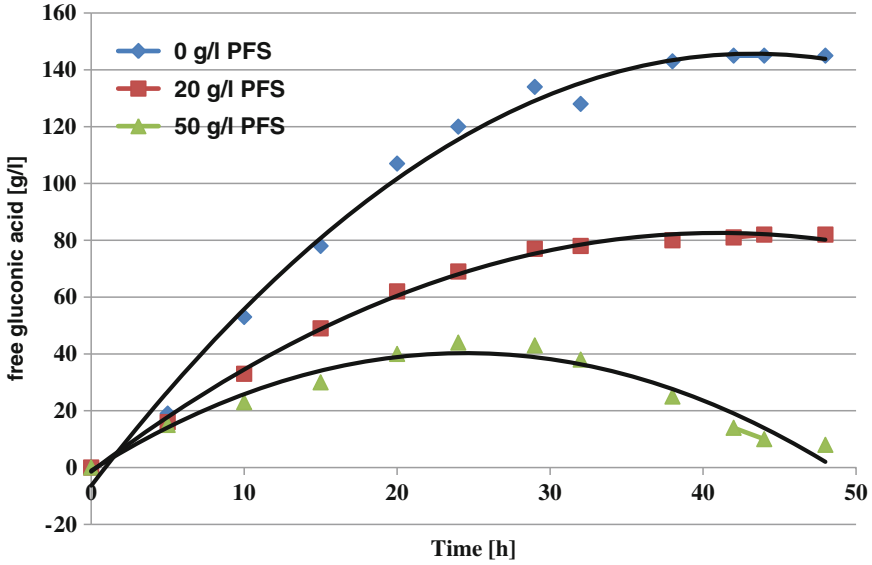

Fig. 15 Ratio of dissolved related to the total amount of rare earth elements depending on the produced amount of gluconic acid

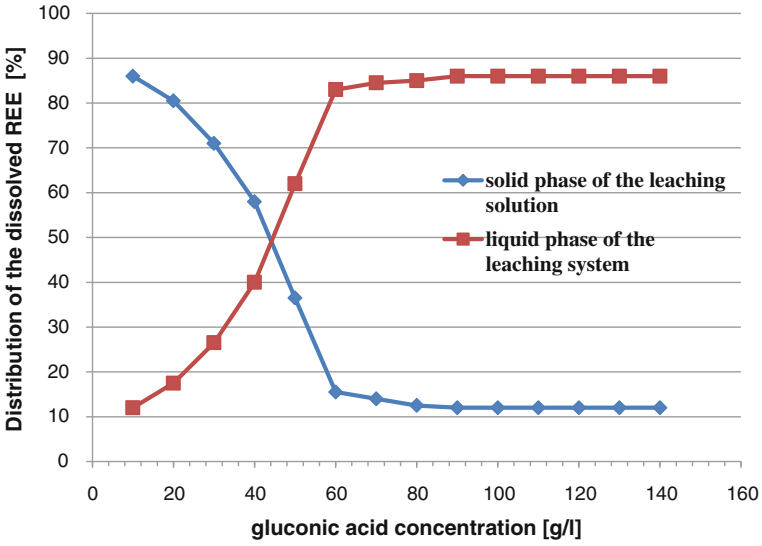

Fig. 16 Distribution of the leached REE in the leaching system

concentration of the formed gluconic acid was 140 g/l. The slag after the leaching was 19.1 g/l with a concentration of REE of 0.67 % (0.128 g/l). This means a leached amount of REE of about 56.6 % and a solubilized amount of slag of

Table 8 Results of the phosphorous furnace slag leaching process with *A. methanolicus* [80, 87]

	Dimension	Slag	After the leaching process		
			Slag	Biomass in the leaching solution	Dry residues of the leaching solution
Amount	g/l	50	19.1	19	119
REE	%	0.59	0.67	0.262	0.07
REE	g/l	0.295	0.128	0.05	0.083
Solublized slag:	%	61.8			
Leached SEE:	%	56.61			

61.8 %. The determination of the distribution of the leaching solution had given 19 g/l biomass containing solids with 0.262 % of REE or 0.05 g/l REE and 119 g/l: dry residues of the liquid phase with 0.07 % of REE or 0.083 g/l. Both phases contained 0.133 g/l REE according 79.6 % of the leached REE [87].

A huge amount of such slags is obtained from phosphorous production. About 120 kt/a were produced in Piesteritz over a long period and about 25 kt/a in Bitterfeld. The whole amount of slags produced in Germany can be assessed with 22.6 million t.

In addition to the REE-containing slags there are other residues from phosphoric acid production. In contrast to the slag this sludge contains gypsum because the production of phosphoric acid is achieved by the treatment of apatite with sulphuric acid. The calcium of the $Ca_3(PO_4)_2$ forms not very soluble gypsum with the sulphuric acid and the phosphoric acid is released. REE and trace elements are precipitated in the sludge. The produced and deposited amount of sludge is estimated to be 34 million t.

12 Flotation Residues: Tailings

Mining of ores is often linked to the generation of flotation residues which were stored in special tailing ponds or dams in the past. These residues mostly contain various valuable substances due to less-efficient separation techniques in the past. The ore processing mainly focused on the production of one product and by-product metals were not exploited during this time.

The long-lasting mining activities in the Ore Mountains ("Erzgebirge") created tailings with fine-grained residues of the tin flotation. The "Bielatal" tailing pond, Saxony, Germany, solely contains more than 10 million t of residues comprising 0.2 % of tin, 200 ppm of tungsten, 100 ppm of molybdenum, 200 ppm of bismuth, and additionally lithium, rubidium, manganese, titanium, cesium, and some trace elements [89]. This is a remarkable potential of resources. Table 9 displays the average concentrations of the target elements, Table 10 lists the concentration of the by-product elements in the flotation tailings, and Table 11 gives an overview of the structure and the composition of the material in the tailing pond.

Table 9 Main components of tin flotation tailings at "Bielatal," ore mountains (Erzgebirge), Saxony, Germany

	Concentration (mg/kg)		
Element	Average value	Min	Max
Sn	644	563	766
W	876	766	986
Bi	76	60	84
Mo	12.2	11.2	13.8

Table 10 ICP analyses of the concentration of some by-product elements of the flotation tailings

Element	Concentration (mg/kg)	Element	Concentration (mg/kg)
Ba	128	Ni	4
Be	12.5	Sc	7
Co	7	Sr	54
Cr	7	Ti	595
Cu	66	V	6
La	37	Y	47
Li	853	Zn	80
Mn	775	S total	<0.01 %

Table 11 Concentration of the main compounds of the flotation tailings

Compound	Percentage (%)	Compound	Percentage (%)
SiO_2	72.1	CaO	0.96
TiO_2	0.1	Na_2O	n.d.
Al_2O_3	14.5	K_2O	2.3
Fe_2O_3	4.36	P_2O_5	0.064
FeO	1.84	CO_2	0.33
MnO	0.1	H_2O	2.18
MgO	0.08		

The potential for microbial leaching of this material under acidic conditions was investigated with the aim of subsequent tin separation [89]. The leaching was carried out with a mixed culture of *Acidithiobacillus ferrooxidans* and Fe^{2+} as energy source. The treated amount of solid residues varied between 2 and 30 % in the leaching system. The pH value ranged from 2.4 to 1.7 and the initial Fe^{2+} concentration was between 10 and 12 g/l. Table 12 shows some results of the bioleaching processes. The first line in the table shows the content of solids in the medium; the second line displays the total amounts of tin in the solids related to the liquid phase. This is the potentially maximum leachable tin concentration. The next line includes information about the pH conditions and the added Fe^{2+} concentration at the beginning and the end of the leaching experiment. The last line shows the tin concentration in the leaching solution after the leaching process. Only a few micrograms of tin could be separated from the flotation residues.

The extraction of tin from different tin compounds was investigated earlier by Teh et al. [90, 91; see Fig. 17]. The authors investigated the microbial leaching of the quaternary synthetic sulphidic tin minerals stannite, kesterite, and stannoidite,

Table 12 Tin bioleaching of flotation residues from the Bielatal tailing pond

Pulp density (%)	2		5		20		30	
Maximum resulting tin concentration (mg/l)	40		100		400		600	
Process conditions	pH	Fe^{2+} (g/l)	pH	Fe^{2+} (g/l)	pH	Fe^{2+} (g/l)	pH	Fe^{2+} (g/l)
	2.4–1.7	10.3–0.2	2.4–1.7	10.98–0.23	2.4–1.8	11.2–9.0	2.4–1.7	12.35–7.98
Dissolved tin (µg/l)	90		120		130		215	

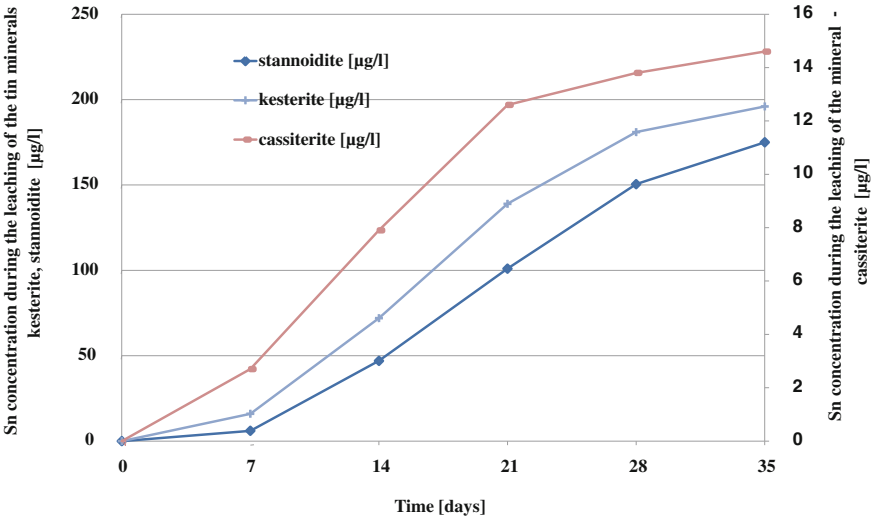

Fig. 17 Bioleaching results of several tin minerals after [90, 91]

and the oxidic minerals cassiterite and varlamoffite. The leaching was carried out with *At. ferrooxidans* at 32 °C, 0.5 % pulp density, pH 2.5, and a particle size smaller than 0.16 mm. Investigations under sterile conditions were carried out simultaneously.

An influence of *At. ferrooxidans* strains could be observed, but the resulting concentrations were in the same range as in the aforementioned investigations. The results from the leaching of the sulphidic tin minerals stannite, kesterite, and stanniodite show concentrations of dissolved tin sometimes higher than those of the leached cassiterite. But the highest observed concentration was about 200 µg/l. These results demonstrate that the concentration of dissolved tin was low and that bioleaching was without remarkable success.

Investigations concerning the behavior of tin revealed that Sn^{4+} is not stable in the leaching solution. Sn^{2+} acts as a reducing agent with a potential of +0.15 V and forms Sn^{4+}. This generates SnO_2 again which exhibits a very low solubility. Only 1.2×10^{-16} mass % are dissolved at 20 °C in water. But Sn^{4+} ions form stable chloride complexes $[SnCl_6]^{2-}$ under acidic conditions. This requires a chloride concentration between 1 and 3 mol/l to enhance the solubility of Sn in the leaching

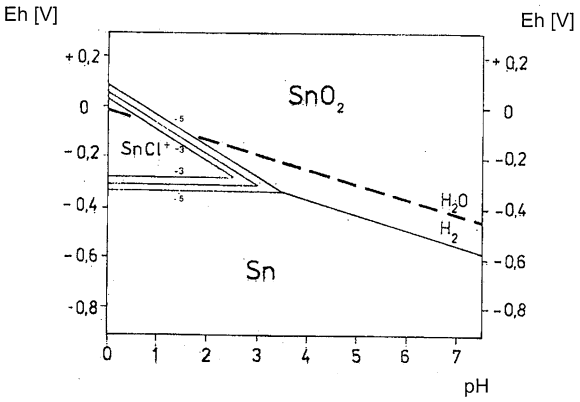

Fig. 18 pH Eh diagram of SnO_2 in the system Sn–Cl–H_2O (Sn concentration 10^{-3}, 10^{-4}, 10^{-5} mol/l, Cl concentration 10^{-1} mol/l), [89]

solution. The calculation of the stability of tin and chloride in an aqueous system at different pH and Eh values is displayed in Fig. 18 and revealed stable conditions at low pH values and a negative redox potential [89].

Therefore bioleaching with *At. ferrooxidans* strains was repeated after the adaption to higher concentrations of chloride under aerobic conditions. The highest chosen concentration of chloride anions was 5 g/l, whereas the highest concentration of tin in the leaching solution with a pulp density of 30 % was 6 mg/l, much higher than in the previous experiments.

Considering this relationship, a separation of tin seems to be possible by a microbial leaching process under anaerobic and acidic conditions with chloride anions in the solution or via biomethylation resulting in a tin organic compound such as dimethyl tin.

13 Sludges from Industry and Environment

13.1 Industrial Sludges

There are a huge number of different types and volumes of deposited metal-containing sludges. Table 13 summarizes some processes that demonstrate the treatment of metal-containing sludges. The sludges have different origins. They are residues from industry or from water treatment plants as well as sediments from rivers and harbors. A review concerning the separation of heavy metals from contaminated sludge for soil remediation is given by Babel and Dacera [92].

Today sludges from galvanic plants and pickling processes are reprocessed very often by chemical and physical techniques. For their treatment, microbial processes were developed but are currently not applied [93–95].

A waste product of high commercial interest is the iron- and titanium-containing red mud from aluminum production. These sludges are water insoluble. In addition to iron, aluminum, and titanium, they contain trace elements as well as

Table 13 Treatment of various metal-containing sludges

Residue/ Waste	Origin	Content/valuable substances	Treatment	References	Remarks
Sludges	Copper mining (Theisen sludge)	Zn, Pb, Sn, Cu	No successful treatment until now	[99]	Metals are bound in a sludge from the blast furnace gas washer containing bituminous carbon
	Electroplating plants	Cr, Cu, Ni, Zn	Leaching of the metal hydroxide	[144]	Leaching efficiency Zn 99 %, Ni 96 %, Cu 81 %, Cr 71 %
	Plating sludge	Zn, Cu, Ni, Pb, Cd, Cr	Leaching with *At. ferrooxidans*	[145]	97 % Zn, 96 % Cu, 93 % Ni, 84 % Pb, 67 % Cd, 34 % Cr are separated
	River/harbour sludges	Cd, Cu, Zn, Ni, Pb, Hg, As, Cr	Leaching of heavy metal polluted harbor sludge	[110]	Treatment consists of a two stage process, an anerobic sulphate reducing process followed by an autotrophic acidic leaching process with *Acidithiobacillus* and *Leptospirillum* strains
	River sludge	Zn, Mn, Cr, Cu, Pb, Ni, Cd	Chemical and microbiological leaching in solid bed and percolator system	[106, 107, 163]	Up to 70 % of the metals were solubilized depending on the leaching conditions (content of S°, influence of surfactants, irrigation rate and height of the solid bed)
	Canal sediments	Cu, Ni, Zn	Sediments of a canal in an industrial area were treated with biosurfactans	[146]	Different concentration of rhamnolipid were used for washing the sediments. 15 % of Cu and 5 % of Zn were solubilized
	Sand/Soil	Cr, Ni, Zn	Leaching of a heavy metal contaminated sand by a two stage process	[147]	Heavy metals were solubilized by *At. thiooxidans* and separateted from the leach liquor by sulphate reducing bacteria, 14.6 % Cr, 26.7 % Ni, 90.5 % Zn were solubilized, 2.2 % Cr, 54 % Ni, 28 % Zn were precipitated
		Cu, Zn, Cd	Alkaline heavy metal polluted soil was treated in situ by stimulating the activity of the indigenous soil microflora	[148]	The soil was treated with biodegradable organic materials and phosphate. Heavy metals were solubilized as complex with organic acids

Table 13 (continued)

Residue/ Waste	Origin	Content/valuable substances	Treatment	References	Remarks
	Sewage sludge	Cr, Cu, Ni, Pb, Zn	Metals stored in the sludge are leached with indigenous S- oxidizing *Acidithiobacilli* strains	[149]	50.6 % Cr, 87 % Cu, 94.4 % Ni, 41.2 % Pb, 99.5 % Zn were solubilized
		Cu, Al, Cr, Zn, Ni, Pb, Fe	Leaching of the sludge with *At. thiooxidans*	[162]	Leaching was carried out with high solid concentration (130 g/l), no remarkable influence of the solid concentration on the bioleaching, high dependance on pH condition, leaching efficiency 91 % Zn, 51 % Cu, 47 % Cr, 30 % Pb, 84 % Ni, 29 % Al
	Municipal sewage sludge	Cd, Cu	Separation of Cd and Cu with indigenous Fe and S oxidizing bacteria	[150]	Indigenous bacteria were selected and the sludge from a municipal treatment plant was leached, maximum leaching efficiency: 100 % Cd and 70 % Cu

silicic acid. An extraction of iron by microbial processes with a parallel dissolution of the silicate matrix also is possible. Other investigations include the microbial leaching of aluminum from red mud [96, 97]. A similar situation concerns the sludges of mines and drinking water treatment plants.

A special and interesting issue is the processing of residues from the former copper smelter in the Mansfeld region, Saxony-Anhalt, Germany. Copper production resulted in the generation of a special sludge, the so-called Theisen sludge. The Theisen sludge is a side product of the blast furnace flue dust washing plant, containing volatile organic bituminous substances as well as heavy metals. There was no technology for the treatment of the sludge after closing the plant in 1970, therefore the sludge was stored and the search for a suitable technology for the recovery failed until today. Between 200,000 and 450,000 t of sludge are stored in special dumps to avoid any risk to the environment [98–100]. The concentration of valuable substances is noteworthy. Theisen sludge contains about 18 % Zn, 14 % Pb, 1.2 % Cu, and 1.2 % Sn, in addition to trace and minor elements. Main components of the sludge are 17 % SiO_2, 16 % S, and 11 % C wherein the carbon originates from the bitumen. Recent investigations demonstrated the possibility of the extraction of metals from bituminous matter related to the production of copper from copper shale [101]. The organic matrix can be destroyed microbially, releasing and mobilizing metals and trace elements [102, 40, 103].

Sludges from wastewater treatment plants are rich in organic carbon and exhibit high concentrations of heavy metals due to the ability of the microorganisms to accumulate and store heavy metals by biosorption processes. High concentrations of zinc and copper were found up to several g/kg in the dried sewage sludge. The concentrations increase if the sewage sludge is used for biogas formation in digestion processes related to the utilization of organic carbon. The metals are precipitated as sulphides, carbonates, or hydroxides via reduction of sulphate to sulphide, under neutral conditions accompanied by the formation of CO_2 as a side product of the methanogens. The composition of the sludge determines the chosen microbial leaching process. As well as metals, sewage sludge is an interesting phosphate resource. According to the environmental database, the produced amount of sludge from biogas formation was about 17 million t in 1990. Of these 60 % were deposited and 10 % were introduced in the municipal waste incineration process [104].

13.2 River, Harbor, and Marine Sludges

Small suspended particles that bind metal cations due to their high sorption capacity are stored in rivers and storage reservoirs. They have to be removed periodically to clean the rivers and storage reservoirs. Storage of the suspended particles also takes place in rivers where a barrier exists and sediments are formed. These sediments are enriched with heavy metals and trace elements. Investigations of a 2.5-km segment of the river Elster near the town Leipzig, Germany, revealed

330,000 t of sediments with 1,300 t of zinc, 81 t of nickel, and 79 t of copper. A technique for recovering the metals from the sediments was successfully developed [105, 106]. Comprehensive investigations of the development of a treatment technology were conducted during the last 20 years in the environmental research center (UFZ). The bioleaching process was carried out as suspension and solid bed leaching after the treatment of the sludge. The treatment involved homogenization and analytical determination. The sludge was poor in inorganic carbon (below 0.05 %) and rich in organic matter (19 %) containing the following metals in mg/kg: 3,291 Zn, 954 Mn, 515 Cr, 322 Cu, 312 Pb, 286 Ni, and 36 Cd. Investigated parameters were, among others, the irrigation rate, the influence of sulphur addition, and the presence of surfactants [107]. Because freshly dredged sediment proved to be unsuitable for solid-bed leaching, due to its low permeability, an upstream process for sediment conditioning has been developed. This process involved the treatment of the sludge with helophytes converting it into a soil-like material [108]. After this pretreatment the bioleaching of the sludge was successful and 20,000 kg of the material were processed at large scale.

A special interaction exists between natural surface water and mining residues. Such fluvial materials (often tailings) are exposed to microbial oxidation processes and heavy or precious metals are mobilized. Willscher et al. [109, Chap. 1] investigated such processes and reported the bioleaching of fluvial residues of historical gold mining with autotrophic as well as heterotrophic microorganisms, detecting a quantitative separation of chromium and manganese during the heterotrophic process. The autotrophic leaching using an acidophilic mixed culture showed a quantitative separation of Cu, Al, Cr, and As.

The sludges in harbors bear similar characteristics. They are very often enriched in heavy metals and trace elements. A treatment of the sludges by leaching processes is also possible. Beolchini et al. [110] demonstrated a two-stage treatment. The processing involved anaerobic sulphate reduction followed by an acidic leaching process with the autotrophic bacteria *Acidithiobacillus* and *Leptospirillum* strains. The method is based on the transfer of heavy metals into sulphides under anaerobic conditions (Chap. 1) and a subsequent leaching of the sulphides under acidic conditions.

Marine sludges often exhibit high concentrations of heavy metals. The sludge is removed to ensure sufficient depth of shipping passages. Due to the anaerobic conditions the sulphate reduction is one of the driving processes and metals are precipitated as sulphides. On the other side huge amounts of carbonate exist as a result of the CO_2 formed. Therefore a successful bioleaching process depends on the ratio between sulphide and carbonate in the sludge.

The sludges of floodwaters are sometimes contaminated with heavy metals and stored as hazardous waste in disposals analogous to the river sludges. Thus a leaching process for the separation of these metals has to be considered.

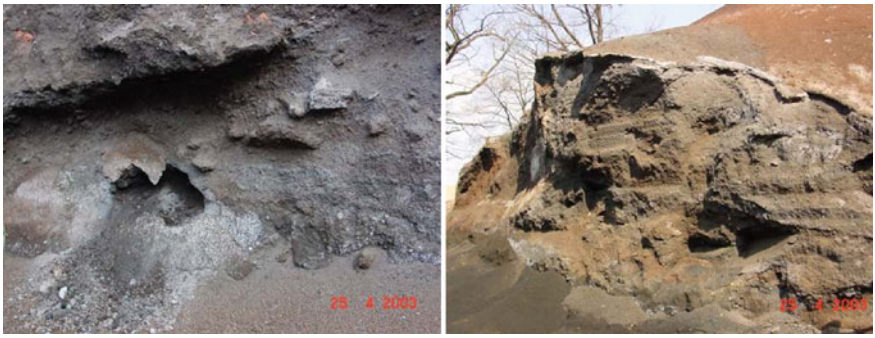

Fig. 19 Photos of a slag dump

14 Ashes and Dusts

14.1 Introduction

Many industrial processes are linked to the formation of dusts. They are separated from the air by special dust filter plants. Zinc-containing dusts are predominantly treated in smelters today and residues are stored in special disposals. The influence of different microbial strains on the release of some elected heavy metals was therefore investigated. Because of the neutral to alkaline pH conditions, in addition to acidophilic, also alkalophilic microorganisms were used for the bioleaching experiments, as well as humic acid and citric acid as organic leaching agents. Because the treated dusts are similar to slags, the results of some investigations are reported in Chap. 4.1.2

The pictures in Fig. 19 show a dump of stored residues from a zinc smelter and the influence of environmental and climate conditions on the deposited slag as visible by the changes in color.

Table 14 summarizes the characteristics of ashes and dusts from various incineration processes and from a copper-producing smelter in accordance with microbial leaching studies for the extraction of valuable substances.

14.2 Ashes from Lignite Electric Power Stations

Ashes are residues from incineration processes and contain various valuable elements. Table 15 shows a comparison of ashes from different lignite-fired power stations and power stations using other fuels. Unfortunately the data are incomplete, but they demonstrate the variation in the composition of the ashes and the huge number of valuable substances.

Table 14 Valuable substances of different ashes and kind of dusts

Residue/ Waste	Origin	Content/Valuable substances	Treatment	References	Remarks
Ashes	Lignite power station	Ca, Mg, Al, Si, Fe, Mn, Cu, Ni, Ga, Nb, Ge, Ti, REE	Heterotrophic leaching with acid producing microorganisms	[116]	Depending on the kind of lignite, solubilization depends on the amount of formed acid
	Fuel power station	V, Ni, Fe, S, Ca, Mg, Co, Ti, Al, Mo, Ba	No leaching tests are reported	[117]	Investigations were carried out for energy saving in the metallurgical industry
	Gas power station	V (2–10 %), Ni (1–3 %)	No leaching tests are reported	[117]	Ash is stored in disposals or used in smelter processes
	Waste incineration plant	Al, Cd, Cr, Cu, Ni, Pb, Sn, V, Zn	Leaching with *A. niger*	[151]	Depending on the kind of waste material, extraction of Cu, Pb 60–70 %, Al, Mn, Zn, 80–100 %
	Municipal solid waste incineration ash	Al, Ca, Cu, Fe, K, Mg, Mn, Pb, Sr, Zn	Fly ash was treated with *A. niger* at different pulp densities, spore and sucrose concentrations	[152]	Optimum conditions are: pulp density 2.7 %, sucrose 150 g/l (for gluconic acid formation)
Dusts	Copper containing dust	Cu, Fe as different sulphides and oxides (Cu conc. 23–36 %)	Bioleaching from copper flue dust with a mixed mesophilic culture of *At. ferrooxidans*, *At. thiooxidans* and *Leptospirillum*	[153]	Leaching carried out in CTR with a pulp density of 2–7 %, Cu extraction decreases from 92 to 87 %, with increasing density

Table 15 Comparison of elemental composition of ashes from different electric power stations

Element	Power station ash refinery residues (mg/kg)	Power station ash oil firing (mg/kg)	Power station ash lignite Leipzig (mg/kg)	Power station ash lignite Lusatia average values (mg/kg)
Vanadium	42,500	70,000	10	84.5
Iron	31,800	65,400	56,000	132,000
Nickel	21,600	32,400	16	37
Sulphur	46,900	199,800	11,900	17,200
Silicon	48,300	6,800	234,000	217,500
Calcium	11,500	5,100	164,000	113,600
Magnesium	2,000	1,100	18,000	26,100
Cobalt	400	300	4	10.50
Titanium	1,500	200		4,025
Phosphorus	2,000	400		590
Barium	0.15	1,300	3	535
Molybdenum	0.05	100		4.5
Aluminium	17,100	3,400	64,000	52,100
Chromium	200		27	95.5
Arsenic			3	19
Tungsten	200			<1
Copper			17	74
Manganese			1125	1,060
Strontium			1756	1,340
Zinc			29	70.5

Lignites are organic substances that often contain and accumulate metals. These metals remain in the ash after the incineration process and reach higher concentrations than in lignite due to the reduced volume. The annual production of lignite is about 300 million t in Germany. The amount of ash from lignite ranges from 10 to 20 % of lignite dry weight. This means the total amount of ash accounts for approximately 15 million t per year. In addition to the main element Si, the ashes consist of Ca, Mg, Al, and contain various trace elements and REE depending on the kind of lignite. Main components of lignite ashes [111] and the average typical composition of East German lignites from different regions are presented by Holzapfel [112]. Table 16 includes the concentrations of heavy metals and trace elements in different lignites and ashes.

Ashes are valuable resources. If the value of 1 t of ash according to the valid prices of the contained elements without the element Si is calculated, a price exceeding €500/ton ash results. A suitable mineral processing for the separation of the valuable substances needs to be explored. This can be done by the combination of physical chemical or biotechnological treatment techniques. During leaching experiments the solubilization of Ca, Mg, Fe, and Al was detected depending on the pH value and the kind of water used [113].

According to the focused elements the leaching can be carried out under neutral, acidic, or alkaline conditions. A separation of aluminum by microbially produced organic acids was investigated by Singer et al. [114] and Torma and

Table 16 Concentration of heavy metals and trace elements of different lignites and ashes in Germany (data in mg/kg [154]) With the kind approval of the publishing company

Origin	4th Lusatian coal stratum		2nd Lusatian coal stratum		Piskowitz	Puschwitz	Olbersdorf	Bornstedt/ Eisleben	Zipsendorf/ Zeitz	Etzdorf/ Eisleben	Holzwei*ß*ig/ Bitterfeld	Mittweida/ Chemnitz
Element	Ash	Lignite	Ash	Lignite	Ash	Lignite	Ash	Ash	Ash	Ash	Ash	Ash
Pb	4.8	1.3	1.4	0.1	13	<20	230	–	–	–	–	–
Ge	0.1	0.01	0.1	0.01	–	–	145	–	–	–	–	250
B	105	17	99	6	40	15	165	90	190	1,000	610	330
Cu	73	15	12	0.7	130	55	150	77	45	55	680	570
Ni	0.7	0.2	18	0.3	<4	<35	180	–	–	–	40	150
Mn	310	43	970	61	300	65	500	250	2,150	740	780	1,725
Cr	11	2.3	4.7	0.5	60	–	150	–	–	–	400	–
V	4.8	1.2	4.5	0.2	≤125	–	6,220	110	–	11	305	450
Ti	3,700	1,100	390	35	2,000	2,100	500	5,230	1,950	4,600	5,620	8,810
Sr	1,290	250	580	31	5	5	125	1,240	–	6,920	3,030	250
Ba	360	50	450	24	530	200	760	2,350	2,000	970	1,615	2,800

Table 17 Composition of a fly Ash from the lignite power station neurath [116] precleaned by Sieving [161]

Parameter/Element	Value
Dry residue (mass %)	99.8
TOC (mass %)	0.3
NH_4–N (mg/kg)	<0.5
SiO_2 (mg/kg)	265,000
Na_2O (mg/kg)	29,200
K_2O (mg/kg)	14,600
P total (mg/kg)	<100
SO_4^{2-} (mg/kg)	10,500
NO_3^- (mg/kg)	<50
Sulfide (mg/kg)	950
Ca (mg/kg)	152,000
Al (mg/kg)	85,400
Fe (mg/kg)	52,500
Mg (mg/kg)	34,700
Mn (mg/kg)	480
Va (mg/kg)	240
Ni (mg/kg)	110
Zn (mg/kg)	99
As (mg/kg)	36
Mo (mg/kg)	36
Co (mg/kg)	21
Wo (mg/kg)	12
Cd (mg/kg)	<2

Singh [115]. Krejcik [116] tried to solubilize all elements. Table 18 contains the composition of a fly ash from a lignite power station. Calcium, aluminum, iron, and magnesium are the main metal components in addition to silicon dioxide. For leaching of the fly ash different microbial strains can be used (Table 17).

Figure 20 shows the leaching of iron by the neutrophilic strain *Bacillus circulans* and the organic acid producing strain *Acetobacter methanolica* at different concentrations of ashes in the leaching solution [116].

The bioleaching efficiency decreases with an increasing amount of ash due to the constant amount of organic acid formed by *A. methanolica*. In the case of *B. circulans* the increased efficiency can be explained by the shift of the pH into the alkaline region and better growth conditions of the neutrophilic–alkaliphilic strain. The leaching results without microorganisms are denoted as control. Therefore a selection of the most suitable leaching conditions for each element is recommended.

14.3 Ash from Oil- and Gas-Fired Power Stations

Ashes from oil- and gas-fired power stations as well as the filter dusts of the crude oil processing industry contain mainly vanadium with concentrations of 2–10 % and nickel with a concentration of 1–3 %. They are mixed with sodium carbonate

Table 18 Valuable substances from water treatment plants

Origin	Content/valuable substances	Treatment	References	Remarks
Drinking water	Fe, Mn	Separation of Fe, Mn by microbial oxidation and precipitation	[131, 132]	Fe^{2+}, Mn^{2+} are microbially oxidized at neutral pH and separated as Fe^{3+} and Mn^{4+} hydroxide/oxide
Mine water	Separation of Mn^{2+} from contaminated water	In situ removal of Mn^{2+} from mine water but also drinking water and ground water with pH >4	[155]	A reactor using Mn^{4+} coated pebbles is used for the microbial Mn^{2+} oxidation and separation. Two fungi (*Pleosporales*) and one bacteria (*Bosea thiooxidans*) were isolated
	Iron	Continuous oxidation of Fe^{2+} with a moderate acidophilic mixed culture in a mine water treatment plant and precipitation as hydroxysulphate	[123, 124, 129,130]	The reaction takes place in a continuous flowed and aerated basin with carriers for the growth of the microorganisms
Surface treatment effluents	Separation of heavy metals from surface water	Treatment consists in an application of sulphate reducing bacteria to transform metals into sulphides	[156]	Microbial production of S^{2-} and precipitation of metals from surface water

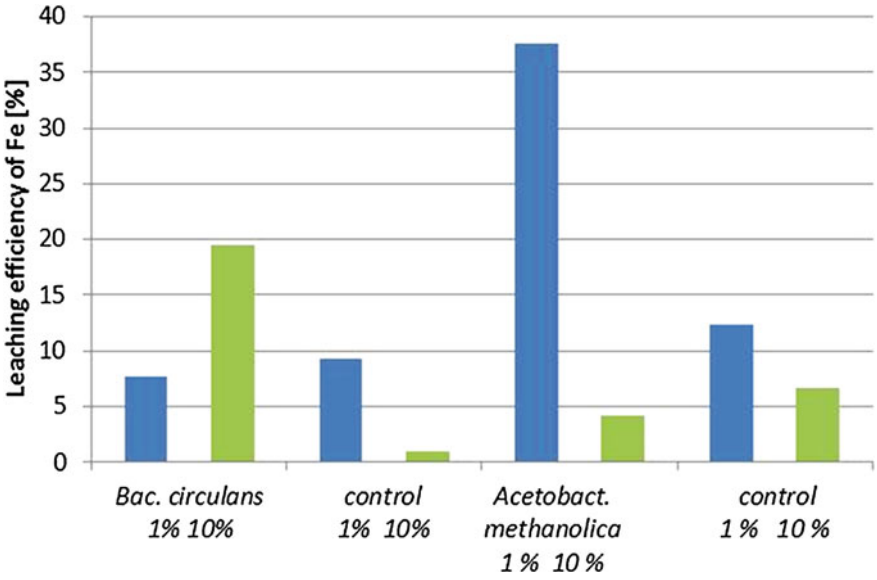

Fig. 20 Bioleaching of Fe from lignite combustion ash with *Acetobact. methanolica* and *Bac. circulans*, leaching conditions: *Acetobact. methanolica* strain DSZM: 5432, medium 569, *Bac. circulans* strain DSZM: 11, medium No 1, controls: ash plus sterilized nutrient solution, without microorganisms, pH adjusted with HCl

and melted in a drum furnace. As results, sodium vanadate ($Na_4V_2O_7$) and iron and nickel sulphide compounds (FeS, NiS) are produced. Vanadium concentrate with a high concentration of organic carbon is deposited. An annual available amount of 100,000 t has been calculated for Europe [117] and a microbial leaching to extract the metals might be reasonable.

14.4 Ashes from Waste Incineration Plants

The incineration of urban waste produces about 250 kg ash per ton of domestic waste. About 70 incineration plants for urban waste exist in Germany producing about 5 million t of ash from 20 million t of domestic waste per year with an increasing trend in the future [118, 119]. Only 8 % of the metals in the ash are recovered including only 1 % nonferrous metals. The major part of the ashes (75 %) is used in disposal and road construction. About 10 % are deposited [120]. Ashes from municipal waste incineration have a different composition depending on the kind of wastes. The concentration of nonferrous metals varies from 1,000–3,500 mg/kg for lead, 200–1,000 mg/kg for chromium, 1,000–10,000 mg/kg for copper, 100–500 mg/kg for nickel and 2,000–7,000 mg/kg for zinc [120, 121]. The copper concentration is in the range of the actually exploited copper ore bodies.

The improvement of the recovery of nonferrous metals can be achieved by an optimization of the downstream processing in combination with biotechnological techniques [122].

15 Residues from Water Treatment Plants

Because wastewater usually contains anions and cations and even heavy metals and some trace elements (TE), different technologies are used for separating these substances. Table 18 shows some examples for the separation of valuable substances from different water qualities.

A typical example for separating a metal in an industrial water treatment plant is the separation of iron from lignite mine water. The mine water is treated by aeration and the addition of lime milk to neutralize the water and oxidize the ferrous iron. The produced sludge contains trace elements as well as valuable substances. The sludge is stored in special deposits, transferred into flooded open cast mines, or transported into "dewatering fields," producing a dried iron-containing sludge.

A new technology separates the iron before lime addition by a microbial process [123–125]. The process is based on the microbial oxidation of ferrous iron to ferric iron at low pH and the formation of an iron hydroxy sulphate mineral, called schwertmannite [126, Chap. 2].

The formed iron compounds precipitate and can easily be separated due to the low solubility of schwertmannite in water. The mineral has got a higher purity in comparison to the lime containing iron hydroxide sludge [123, 124]. Figure 21 shows the formed schwertmannite crystals in the treated mine water.

According to the amount of dissolved iron in the mine water at this site, about 10,000 t of the mineral could be produced and utilized. Different research projects focused on the production of adsorbents or iron oxide pigments from schwertmannite [127]. A pilot technology has been applied for 10 years now and confirms the stability of the process [128]. Comprehensive investigations concerning the composition of the microbial population revealed a moderate acidophilic mixed population consisting of two groups of *Betaproteobacteria* accompanied by "*Ferrovum myxofaciens*" and strains related to *Gallionella ferruginea* as the main part [129, 130].

Another process concerns the separation of iron and manganese via neutrophilic iron and manganese oxidizing bacteria. These bacteria form a stable film at the surface of sand grains and oxidize the metal ions. In a second step a precipitation of ferric iron or Mn^{4+} takes place. This technology is applied in the treatment of drinking water or groundwater [131, 132].

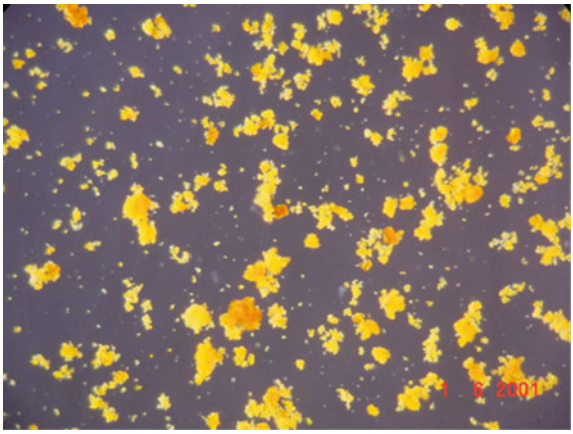

Fig. 21 Schwertmannite crystals in a treated mine water

16 Residues from the Electric and Electronic Industry and Spent Catalysts

Table 19 shows some microbial processes for the separation of heavy and precious metals from residues of the electric and electronic industry or from spent catalysts. A multitude of further recycable residues are composite materials such as laminated boards and laminated papers, as well as metal-containing plastics that can be separated by microbial processes. A current treatment technology for these materials includes the incineration and separation of the metals. An alternative process could be microbial treatment where the organic material is utilized or transformed into water-soluble compounds and the metals are released.

The significance of electronic scrap has increased. Its components can be dissolved and separated by different microbial acid-forming processes [16, 133]. Heterotrophic as well as autotrophic microorganisms can be used for these processes. For example about 90 % of copper, about 50 % of zinc and 17 % of manganese could be bioleached by an autotrophic mixed culture [134] (Table 19).

Some other metals, for examples, lead could be separated by precipitation. This category also includes car shredding residues.

The treatment of this material with cyanide-forming microorganisms offers a similar yet new possibility. This is valid in particular for the treatment of electronic scrap as well as for the treatment of precious-metal–containing catalysts [50, 135]. Analogue processes are possible with spent catalysts [16]. Hydrogenating catalysts are used in the chemical industry. The spent catalysts contain between 1.5 and 3.5 % Ni, 4–11 % Mo, 35–50 % Al, about 15 % of Fe, and 5–18 % Ca. But they contain between 3.3 and 6.6 % sulphur, mostly occurring as sulphide. In the past the recovery of nickel and molybdenum was investigated by Iske et al. [55]. Analogue investigations using strains of *Aspergillus* demonstrated yields of 78 % of nickel, 82 % molybdenum, and 85 % aluminum [136].

Table 19 Composition of valuable substances in catalysts and residues of the electronic industry

Residue/waste	Origin	Content/valuable substances	Treatment	References	Remarks
Catalysts	Motor car	0.1 % Pt, Cu	Pt leaching by CN^-—forming microorganisms	[135]	*C. violaceum* are able to solubilize Cu and Pt
	Hydrogenating catalysts	Al, Ni, Mo, Fe, Ca	Leaching of Ni, Mo by moderately acidophilic autotrophic microorganisms	[55]	Separation of Ni and Mo from Ca, Fe, Al
	Spent refinary catalysts	Ni, V, Mo	Bioleaching of pretreated refinery catalysts—a mix of iron and sulphur oxidizing bacteria was used	[157]	The recovery by the Fe oxidizing bacteria was 90, 80, 54 % for Ni, V, Mo respectively, recovery using the S oxidizing bacteria was 88, 94, 46 % (Ni, V, Mo)
Residues/scrap from the electric/ electronic industries	Electronic scrap (ESM)	35 % Sn, 65 %Pb, Zn, Al, Mn, >70 % Fe, Ni, Cu were mobilized	Leaching with *Aspergillus niger*, pulp density of 0.1–2.0 %	[133]	Two step leaching process due to the inibitory effects of toxic metals
	Shreddered electronic scrap	Ag, Au, Pt	Solubilization of the precious metals by different $(CN)^-$—forming microorganisms from electronic scrap	[50]	Au was mobilized as dicyanoaurate $[Au(CN2)]^-$, Ag as $[Ag(CN2)]^-$ and Pt as $[Pt(CN)]^-$
	Electrical appliance	Al, Cd, Co, Cr, Cu, Ni, Pb, Sn, W, Zn	Separation of nickel by bacteria and fungi	[158]	*Thiobacillus sp.* and fungies were grown in presence of computer scrap, scrap concentration >10 g/l inhibited the growth, an adaptation up to 100 g/l was possible, leaching efficiency of Cu and Sn were 65 %, Al, Pb, Ni, Zn >95 % at scrap concentration 5–10 g/l

Table 19 (continued)

Residue/ waste	Origin	Content/valuable substances	Treatment	References	Remarks
	Printed circuit board	Cu Zn, Cr, Ni, Pb, Mn	Leaching with a mixed culture of acidophilic Fe and S oxidizing and acidophilic heterotrophic bacteria	[134]	4 % of crushed circuit boards are treated in shake flasks with the mixed culture, all metals were removed from the boards but sometimes precipitated in the liquor
		Cu	Recovery of copper from printed circuit boards by leaching with *At. ferrooxidans*	[159]	The leached amount of Cu increased with the addition of Fe^{2+}, max. Fe^{2+} was 5.2 g/l. The recovery of Cu was 37 % without citric acid and >80 % in the presence of citric acid
	Secondary sources	Pt group elements	Separation of PGM from leaching liquor by bioreduction with pretreated *E. coli* cells	[160]	Pd(II), Pt(II), Pt(IV), Rh(II) were enzymatically reduced and precipitated as metals under acidic conditions by a hydrogenase from *E. coli*
	paint pigments of colour TV	Ca, Zn, Al, Y, Eu, Gd	Leaching with Fe and S oxidzing acidophilic microorganisms	[139]	Investigations of different composed colour films and pure fluorescent substance Y_2O_2S
	Printed circuit boards	Cu, Pb, Sn, Zn, Cr, Ni, Mn	Leaching of crushed circuit boards with acidophilic iron and sulphur oxidizing and acidophilic heterotrophic strains	[134]	Average leaching efficiencies: 81–99 % Cu, 40–48 % Zn, 5–11 % Ni, 0.15 % Pb, 6–18 % Mn

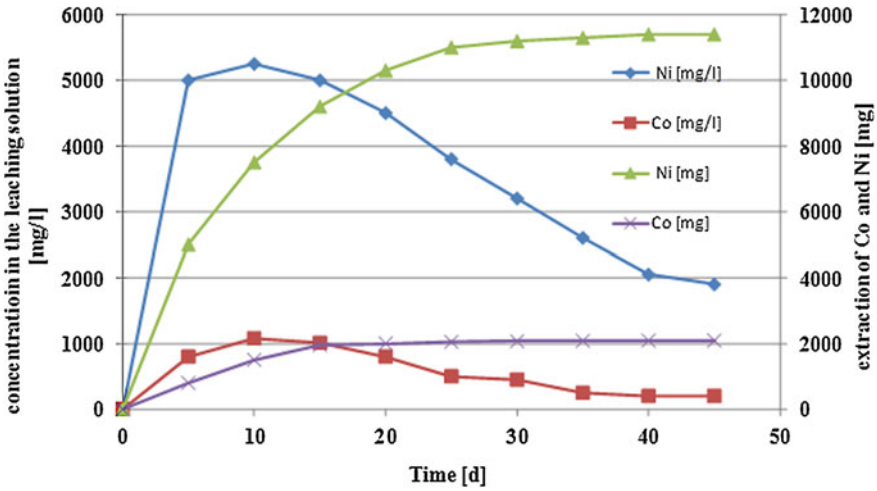

Fig. 22 Ni and Mo bioleaching from a hydrogenating catalyst [55]

The aforementioned bioleaching of Ni and Mo from spent catalysts was carried out by moderate acidophilic autotrophic microorganisms. Various mixed cultures isolated from the storage place of the catalysts as well as a moderate acidophilic *Acidithiobacillus* population isolated from copper shale leaching processes were used. The pH optimum of these strains was in the range of 4–5. This range was chosen, because the solubilization of iron, calcium, and aluminum is prevented at moderate acidic conditions.

The leaching was carried out in shake flasks, in columns (each filled with 1 kg material) and in a stirred tank reactor [55, 137]. The investigated material consisted of dust from the catalysts and the catalysts pill. Figure 22 displays the change of the nickel and molybdenum concentration in the leaching solution and the total amount of metals separated from the catalyst in the column leaching experiment. The highest concentration of nickel accounted for more than 5 g/l and the concentration of molybdenum for 1.2 g/l. The leaching efficiency was 50–60 % for nickel and 3.3–12.5 % for molybdenum. The leaching was carried out under saturated and unsaturated conditions in the column. A batch system represented the unsaturated conditions where 50 % of the leaching solution was exchanged by a fresh solution every 6, 12, 19, 30, and 47 h, to prevent an inhibition of the microbial process caused by the high nickel concentration. However, separate investigations of the influence of nickel on the microbial growth revealed much higher concentrations of up to 16 g/l without an inhibiting effect on the adapted microorganisms [138].

Other residues and dusts are the REE and trace elements containing dusts from colour TVs or fluorescent tubes with a concentration of 8–15 % of yttrium, 1–2 % of europium and some gadolinium, furthermore zinc, aluminum, cadmium, and iron. The leaching was investigated with oxidizing and acidic acid generating

microbial processes. The aim was to destroy the material and separate Eu, Gd, and Y. The results demonstrated the possibility to leach the sulphide-containing material and to concentrate yttrium in the pregnant leaching solution with up to 500 mg/l [139].

New industrial residues are wastes and nonconforming products of the photovoltaic industry with trace elements, for which suitable recycling technologies have not yet been investigated properly.

Further sources for recoverable valuable substances are old municipal waste disposals. The content of the valuable substances of ash disposals is adequate to the composition of the fuel but with higher concentrations. If the waste material contains a high fraction of organic compounds, the concentration of metals increases during the transformation of carbon into methane. A separation of the metals by different leaching processes is possible in both cases.

Examples for the treatment of other different catalysts and residues from the electric and electronic industry are summarized in Table 19. Because these materials are not sulphides or do not contain Fe^{2+}, a leaching mechanism based on the formation of inorganic and organic acids or complexing agents has to be developed. Nevertheless, all these tables reveal the huge diversification of valuable substances containing residues and the large number of treatment processes.

References

1. OECD (2001) The application of biotechnology to industrial sustainability. OECD Publication Service Paris, 16 Nov 2001
2. Ehrlich HL, Newmann DK (2009) Geomicrobiology. CRC—Press Taylor & Francis Group LLC. ISBN 13-978-0-8493-7906-2
3. Brandl H (2001) Microbial leaching of metals. In: Rehm HJ (ed) Biotechnology, vol 10. Wiley- VCH, Weinheim, pp 191–224
4. Donati ER, Sand W (2007) Microbial processing of metal sulfides. Springer, Berlin. ISBN 978-1-4020-5588-1 (HB)
5. Rawling DE, Johnson DB (2007) Biomining. Springer, Heidelberg. ISBN 978-3-642-07115-7
6. Ehrlich HL (1996) Geomicrobiology 3rd rev, edn. Marcel Decker Inc., New York. ISBN 0-82457-9541-5
7. Rossi G (1990) Biohydrometallurgy. McGraw-Hill, Hamburg, New York. ISBN 3-89028-781-6
8. Torma AE (1988) Leaching of metals. In: Rehm HJ, Reed G (eds) Biotechnology, vol 6B. VCH Verlagsgesellschaft, Weinheim, pp 367–399
9. Köhler M, Völsgen F (1998) Geomikrobiologie—Grundlagen und Anwendungen. Wiley—VCH, Weinheim, New York. ISBN 3-527-30083-X
10. Ebner HG (1978) Metal recovery and environmental protection by bacterial leaching of inorganic waste materials. In: Murr LE, Torma AE, Brierley JA (eds) Metallurgical applications of bacterial leaching and related microbiological phenomena. Proceedings of the international symposium at the New Mexico institute of mining and technology, Socorro N.M. Academic Press, New York, NY, pp 195–206
11. Bosecker K (1987) Microbial recycling of mineral waste products. Acta Biotechnol 7:487–497

12. Bosecker K (1997) Bioleaching: metal solubilization by microorganisms. FEMS Microbiol Rev 20:591–604
13. Brombacher C, Bachofen R, Brandl H (1997) Biohydrometallurgical processing of solids: a patent review. Appl Microbiol Biotechnol 18:577–587
14. Jain N, Sharma DK (2004) Biohydrometallurgy for nonsulfidic minerals—a review. Geomicrobiol J 21:135–144
15. Hoque ME, Phili OJ (2011) Biotechnological recovery of heavy metals from secondary sources—an overview. Mat Sci Eng C 31:57–66
16. Lee J-C, Pandey BD (2012) Bio-processing of solid wastes and secondary resources for metal extraction—a review. Waste Manag 32:3–18
17. Hsu CH, Harrison RG (1995) Bacterial leaching of zinc and copper from mining wastes. Hydrometallurgy 37:169–179
18. Yaraghi A, Khodadai A, Shojasadati SA (2005) Recovery of zinc from low grade ores by *Asperigillus niger*. In: Proceedings of the 20th world mining congress 7–11 Nov 2005, Tehran/Iran. "Mining and Sustainable Development"
19. Zandevakili S, Ranjbar M, Manafi Z (2005) Column bioleaching of copper flotation tailings from Sarcheshmeh. In: Proceedings of the 20th world mining congress 7–11 Nov 2005, Tehran/Iran. "Mining and Sustainable Development"
20. Morin DHR, d'Hugues P (2007) Bioleaching of cobalt-containing pyrite in stirred reactors: a case study from laboratory scale to industrial application. In: Rawlings DE, Johnson DB (eds) Biomining. Springer, Berlin
21. Coto O, Galizia F, Hernandez I, Marrero J, Donati E (2008) Cobalt and nickel recoveries from lateritic tailings by organic and inorganic bio-acids. Hydrometallurgy 94:18–22
22. Schippers A, Nagy AA, Kock D, Melcher F, Gock ED (2008) The use of FISH and real-time PCR to monitor the biooxidation and cyanidation for gold and silver recovery from a mine tailings concentrate (Ticapampa, Peru). Hydrometallurgy 94:77–81
23. Kamradt A, Borg G, Schaefer J, Kruse S, Fiedler M, Romm P, Schippers A, Gorny R, du Bois M, Bieligk C, Liebetrau N, Nell S, Friedrich B, Morgenroth H, Wotruba H, Merkel C (2012) An integrated process for innovative extraction of metals from Kupferschiefer mine dumps, Germany. CIT Chemie Ingenieur Technik 84:1694–1703
24. Stichel E (1978) Untersuchungen zum Einfluss individueller n–Alkane auf den Prozess der Erdölkonvertierung durch Hefen. Dissertation – Akademie der Wissenschaften, Institut für technische Chemie Leipzig, Germany
25. Fritsche W (1990) Abbau aliphatischer Kohlenwasserstoffe. In: Mikrobiologie – Uni—Taschenbuch UTB 1561, Gustav Fischer Verlag Jena, pp 228–229. ISBN 3-334-002 36-5
26. Battaglia-Brunet F, Dictor MC, Garrido F, Michel C, Joulian C, Bourgeois F, Baranger P, Itard Y, Morin D (2005) Remediation processes using biological as (III) oxidation. In: Harrison STL, Rawlings DE, Petersen J (eds) Proceedings of the 16th international biohydrometallurgy symposium, IBS 2005, Cape Town, pp 343–350, September 2005. ISBN 1-920051-17-1
27. Lyalikova NN (1972) Oxidation of trivalent antimony to higher oxides as an energy source for the development of a new autotrophic organism Stibiobacter gen. Dokl Akad Nauk SSSR Ser Biol 205:1228–1229
28. Lyalikova NN (1974) *Stibiobacter senarmontii*: a new antimony—oxidizing microorganism. Microbiologiya 43:941–948
29. Tuovinen OH (1985) Some characteristics of iron- oxidizing thiobacilli isolated from uranium mine leach liquors. In: Karavaiko GI, Groudev SN (eds) Biogeotechnology of metals. Proceedings of the international seminar and international training course, United Nations environment programme Moscow (1985), Centre of internat, Projekts GKNT Moscow, pp 56–63
30. Matsumoto N, Hirano S, Ohmura N (2007) Enrichment of chromium reducing bacteria using potential controlled electrochemical cultivation. Adv Mater Res 20–21:505–508. In: Schippers A, Sand W, Glombitza F, Willscher S (eds) Biohydrometallurgy: from the single cell to the environment. Proceedings of the 17th international biohydrometallurgy

symposium, Frankfurt/Main Germany. Trans Tech Publications LTD., Switzerland, UK, USA, 2–5 Sept 2007. ISSN 1022-6680
31. Goulhen F, Gloter A, Guyot F, Bruschi M (2005) Bioremediation of polluted soils and effluents using sulfate reducing bacteria: molecular mechanisms of the metal resistance. In: Harrison STL, Rawlings DE, Petersen J (eds) Proceedings of the 16th international biohydrometallurgy symposium, IBS 2005 Cape Town, pp 391–399, September 2005. ISBN 1-920051-17-1
32. Johnson DB, Hallberg KB (2008) Carbon, iron and sulfur metabolism in acidophilic microorganisms. Adv Microbiol Physiol 54:202–256
33. Coupland K, Johnson DB (2008) Evidence that the potential for dissimiatory ferric iron reduction is widespread among acidophilic heterotrophic bacteria. FEMS Microbiol Lett 279:30–35
34. Schippers A, Breuker A, Blazejak A, Bosecker K, Kock D, Wright DL (2010) The biogeochemistry and microbiology of sulfidic mine waste and bioleaching dumps and heaps, and novel Fe(II)-oxidizing bacteria. Hydrometallurgy 104:342–350
35. Hedrich S, Schlömann M, Johnson DB (2012) The iron-oxidizing proteobacteria. Microbiology 157:1551–1564
36. Lovley DR, Chapelle FH (1995) Deep surface microbial processes. Rev Geophys 33:365–381
37. Lovley DR, Phillips E, Gorby YA, Landa ER (1991) Microbial reduction of uranium. Nature 350:413–416
38. Humphries AC, Penfolder DW, Forster CF, Macaskie LE (2003) Chromate reduction by immobilized cells of *Desulfovibrio vulgaris* using biologically produced hydrogen. In: Tsezos M, Hatzikioseyian A, Remoundaki E (eds) Biohydrometallurgy: a sustainable technology in evolution (First edition 2004). Proceedings of the 15th international biohydrometallurgy symposium (IBS), National technical University of Athens, Athens, Greece, pp 525–531, 14–19 Sept 2003. ISBN 960-88415-1-8
39. Horikoshi K (1991) Microorganisms in Alkaline Environment, Kodansha, Tokyo. VCH Weinheim, New York. ISBN 3-572-28200-9
40. Sklodowska A, Matlakowska R (2007) Bioleaching of metals in neutral and slightly alkaline environment. In: Donati ER, Sand W (eds) Metal processing of metal sulfides. Springer, New York, pp 121–129. ISBN 978-1-4020-5588-1
41. Karsson S, Sjöberg V, Grandin A (2011) Heterotrophic leaching of LD slag—formation of organic ligands. In: Rüde TR, Freund A, Wolkersdorfer C (eds) Conference proceedings of 11th international mine water association congress: mine water—managing the challenges, IMWA 2011, Aachen, Germany, pp 371–374. ISBN 978-1-897009-47-5
42. Avakyan ZA, (1985) Microflora of rock and its role in the leaching of silicate minerals. In: Karavaiko GI, Groudev SN (eds) Biogeotechnology of metals. Centre of international Projects GKNT, Moscow, pp 175–194
43. Alexander M (1977) Introduction to soil microbiology, 2nd edn. Wiley, New York
44. Jørgensen BB, Sörensen J (1985) Seasonal cycles of O_2, NO_3- and SO_4^{2-} reduction in estuarine sediments: the significance of an NO_3- reduction maximum in the spring. Mar Ecol Prog Ser 24:65–74
45. Jørgensen BB, Sørensen J (1988) Two annual maxima of nitrate reduction and denitrification in estuarine sediment (Norsmine Fjord Denmark). Mar Ecol Prog Ser 48:147–154
46. Neilands JB (1995) Siderophores: structure and function of microbial iron transport compounds. J Biol Chem 270:26723–26726
47. Dausmann S, Kunz PM, Paul M, Willrett M, Zaehner H (1996) WO patent/1996/009423, B1 30. 05. 1996, Organically—based agent for removing rust from surfaces, production and use of said agent
48. Brandl H, Stagars M, Faramarzi MA (2003) A novel type of microbial metal mobilization: cyanogenic bacteria and fungi solubilize metals as cyanide complexes. In: Tsezos M, Hatzikioseyian A, Remoundaki E (eds) Biohydrometallurgy: a sustainable technology in

evolution (First edition 2004). Proceedings of the 15th international biohydrometallurgy symposium (IBS), National technical University of Athens, Athens, Greece, pp 457–463, 14–19 Sept 2003. ISBN 960-88415-1-8
49. Faramarzi MA, Stagars M, Pensini E, Krebs W, Brandl H (2004) Metal solubilization from metal containing solid materials by cyanogenic Chromobacterium violaceum. J Biotechnol 113:321–326
50. Brandl H, Lehman St, Faramarzi MA (2007) Mobilization of silver, gold and platinum from solid materials by HCN—forming microorganisms. Adv Mater Res 20–21:50–53. In: Schippers A, Sand W, Glombitza F, Willscher S (eds) Biohydrometallurgy: from the single cell to the environment, proceedings of the 17th international biohydrometallurgy symposium, Frankfurt/Main Germany. Trans Tech Publications LTD., Switzerland, UK, USA, 2–5 Sept 2007. ISSN 1022-6680
51. Feldmann J, Hirner AV (1995) Occurence of volatile metal and metalloid species in landfill and sewage gases. Int J Environ Anal Chem 60:339–359
52. Brinkmann FE, Olson GJ (1987) Global biomethylation of the elements: its role in the biosphere translated to new organometallic chemistry and biotechnology. In: Craig PJ, Glockling F (eds) The biological alkylation of heavy elements, Royal Society of Chemistry, special publication no. 66. Proceedings of a conference, London, pp 168–196, 17–18 Sept 1987. ISBN 0–85186–716–2
53. Craig P J, Glockling F (1987) The biological alkylation of heavy elements, Royal Society of Chemistry, Special Publication No. 66, Proceedings of a Conference, London, 17–18 Sept 1987. ISBN 0-85186-716-2
54. Feller KA (1993) Biologische Arseneliminierung aus kontaminiertem Erdreich, Vortrag auf dem ISSC- Seminar Nr. 93/0351 „ Biotechnologische Verfahren zur Abwasser und Bodenreinigung unter besonderer Berücksichtigung Bundeswehr – spezifischer Probleme, 15–16 Feb 1993, Günnewig Bristol Hotel Bonn – personal communication
55. Iske U, Glombitza F, Huber H, Stetter K-O (1991) Leaching of carbonate and/or alkali containing ore materials by chemolithoautotrophic moderate acidophilic bacteria, environmental biotechnology. In: Verachtert H, Verstraete VZEW W (eds) Proceedings of the international symposium, Ostende Belgium, Part II. Koninklijke Vlaamse Ingenieursvereniging, pp 501–504
56. BUS (2012) http://www.B.U.S.com. Zinkrecycling Freiberg. Accessed Nov 2012
57. Willscher S, Clark TR, Cohen RH, Ranville JF, Smith KS, Walton-Day K (2003) Bioleach of a fluvial tailings deposit material indicates long term potential for pollution. In: Tsezos M, Hatzikioseyian A, Remoundaki E (eds) Biohydrometallurgy: a sustainable technology in evolution (First edition 2004). Proceedings of the 15th international biohydrometallurgy symposium (IBS), National technical University of Athens, Athens, Greece, pp 497–505, 14–19 Sept 2003. ISBN 960-88415-1-8
58. Schippers A, Bosecker K, Willscher S, Spröer C, Schumann P, Kroppenstedt RM (2002) Nocardiopsis metallicus sp. nov., a metal leaching actinomycete isolated from an alkaline slag dump. Int J Syst Evol Microbiol 52:2291–2295
59. Berzelius (2012) Metall GmbH. http://www.berzelius.de
60. Cheng Y, Guo Z, Liu X, Yin H, Qiu G, Pan F, Liu H (2009) The bioleaching feasibility of Pb/Zn smelting slag and community characteristics of indigenous moderate—thermophilic bacteria. Bioresour Technol 100:2737–2740
61. Guo Z, Zhang L, Cheng Y, Xiao X, Pan F, Jiang K (2010) Effects of pH, pulp density and particle size on solubilization of metals from a Pb/Zn smelting slag using indigenous moderate thermonphilic bacteria. Hydrometallurgy 104:25– 31
62. Aurubis Umweltreport (2011) Gemeinsam schützen, gemeinsam verbessern. In: Aurubis AG (ed) Hamburg (www.aurubis.com). Gutenberg beuys feindruckerei, Hannover, Druck
63. Aurubis (2012) http://de.wikipedia.org. Accessed Nov 2012
64. Schmukat A, Duester L, Ecker D, Schmid H, Heil C, Heininger P, Ternes TA (2012) Leaching of metal(loid)s from a construction material: influence of the particle size, specific surface area and ionic strength. J Hazard Mater 227–228:257–264

65. Mehta KD, Pandey BD, Premchand (1997) Bioleaching of copper, nickel, cobalt from a copper converter slag by *Thiobacillus ferrooxidans*. NMI Tech J 39:59–70
66. Mehta KD, Pandey BD, Premchand (1999) Bio—assisted leaching of copper, nickel and cobalt from copper converter slag. Mater Trans JIM (Japan Institute of Metals) 40:214–221
67. Willscher S, Bosecker K (2003) Studies on the behavior of heterotrophic microorganisms isolated from alkaline slag dump. Hydrometallurgy 71:257–264
68. Carranca F, Romero R, Mazuelos A, Iglesias N, Forcat O (2009) Biorecovery of copper from converter slag: slags characterization and exploratory ferric leaching tests. Hydrometallurgy 97:39–45
69. Baghery SA, Oliazadeh M (2003) Bioleaching of copper converter slag using *A. ferrooxidans*. In: Tsezos M, Hatzikioseyian A, Remoundaki E (eds) Biohydrometallurgy: a sustainable technology in evolution (First edition 2004). Proceedings of the 15th international biohydrometallurgy symposium (IBS), National technical University of Athens, Athens, Greece, pp 507–511, 14–19 Sept 2003. ISBN 960-88415-1-8
70. Altlasten Aktuell (2002) No 9, Informationen zur Altlastenbehandlung. In: Sachsen (ed) Freistaat Sachsen, Landesamt für Umwelt und Geologie. Sächsisches Druck und Verlagshaus AG Dresden, Druck
71. Volland B, Pollmer K, Binde G (1988) Versuche zur mikrobiologischen Laugung von Nickel aus Nickelhydrosilikaten 25.01.1988 Forschungsbericht der Bergakademie Freiberg, Sektion Geowissenschaften zum Rahmenthema "Grundlagen der Nutzung biotechnologischer Verfahren zur Gewinnung und Veredlung mineralischer Rohstoffe"
72. Voland B, Pollmer K, Goldbach E, Matthes H (1989) Untersuchungen zur mikrobiologischen Laugung von Nickel aus Nickelhydrosilikaten, Forschungsbericht der Bergakademie Freiberg, Sektion Chemie, WB anorganische Chemie, Sektion Geowissenschaften WB Geochemie/Mineralogie, September 1989
73. Schippers A, Bosecker K (2005) Bioleaching: analysis of microbial communities dissolving metal sulfides. In: Barredo J-L (ed) Methods in Biotechnology, vol 18: microbial processes and products. Humana Press Inc., Totowa, pp 405–412. ISBN 1-58829-548-6
74. Pinka J (2007) Entwicklung und Optimierung eines neuen Verfahrens zur Gewinnung von Nickel und Kobalt aus Nickelhydrosilikaten (Development and optmization of a new process for the production of Ni and Co from Ni hydroslikates) Projektentwurf 0852 V 7353 A PtJ G.E.O.S.(unpublished material)
75. Simate GS, Ndlovu S (2008) Bacterial leaching of nickel laterites using chemoautotrophic microorganisms. Identifying influential factors using statistical design of experiments. Int J Miner Process 88:31–36
76. du Plessis CA, Slabbert W, Hallberg KB, Johnson DB (2011) Ferredox: a biohydrometallurgical processing concept for limonitic nickel laterites. Hydrometallurgy 109:221–229
77. Schüler D, Buchert M, Liu R, Dittrich S, Merz C (2011) Study on rare earths and their recycling, Final report for the Greens/EFA group in the European Parliament, Öko-Institut e.V., Darmstadt, January 2011
78. Bullmann M, Iske U, Glombitza F, Dietrich B (1987) Wechselwirkungen zwischen seltenen Erdenmetallen und Mikroorganismen. Acta Biotechnol 7:409–412
79. Glombitza F, Iske U, Bullmann M, Dietrich B (1987) Bacterial leaching of zircon mineral for obtaining trace and rare earth elements, Biohydrometallurgy, pp 407–20. In: Norris PR, Kelly DP (eds) Proceedings of the international symposium, Warwick, Kew Surry, UK
80. Glombitza F, Iske U, Bullmann M (1988) Mikrobielle Laugung von Seltenen Erdmetallen und Spurenelementen. Bio-Engineering/Forschung und Praxis, München 4:37–43
81. Iske U, Bullmann M, Glombitza F, Babel W, Miethe D, Dietze H-J, Becker S (1985) Verfahren zur mikrobiellen Laugung von Seltene Erdmetalle enthaltenden Phosphatmineralien und Abprodukten, Patentschrift: DD 249 156 A3, 04. 04. 1985, 02. 09. 1987
82. Iske U, Bullmann M, Glombitza F (1987) Organoheterotrophe Laugung resistenter Materialien. Acta Biotechnol 7:401–407

83. Iske U, Richter K, Wünsche L, Karbaum K, Glombitza F, Klappach G, Weichert D, Meyer J (1989) Verfahren zur Kultivierung von Mikroorganismen, Patentschrift DD 263 662 A3 29. 05. 1973; 11. 01. 1989
84. Iske U, Bullmann M, Dietze H-J, Becker S (1985) Verfahren zum mikrobiellen Aufschluss metallhaltiger silikatischer und/oder oxidischer Rohstoffe hoher chemischer Resistenz, Patentschrift: DD 250 235 A3, 04. 04. 1985, 08. 10. 1987
85. Uhlig H, Karbaum K, Steudel A (1986) *Acetobacter methanolicus* sp. nov., an acidophilic facultatively methylotrophic bacterium. Int J Syst Bacteriol 36:317–322
86. Dittrich B, Bullmann M, Iske U, Glombitza F (1988) Verfahren zur Abtrennung von Seltenen Erdelementen aus calciumhaltigen Fermentationslösungen, Patentschrift DD 259 212 A1, 30. 03.1987; 17. 08. 1988
87. Bullmann M, Iske U, Becker S, Dietze H-J, Dietrich B, Glombitza F, (1986) Grundlagenuntersuchungen zur Technologie der Gewinnung von Seltenen Erden durch mikrobielle Laugung - Report, Akademie der Wissenschaften, Institut für Biotechnologie Leipzig 05.09.1986
88. Miethe D, Iske U (1985) Versuche zur Erhöhung der Glucoseoxidation bei methylotrophen Mikroorganimen. Akademie der Wissenschaften, Institut für Biotechnologie Leipzig, Arbeitsbericht Nr 51/85 vom 20. 11. 1985
89. Stichel E, Ondruschka J, Glombitza F, Tischendorf G (1987) Microbial leaching of residues of the tin mining processes, report: institute of biotechnology and central institute of physics of the earth. Academy of Sciences of the GDR (03.02.1987)
90. Teh GH, Schwartz W, Amstutz CC, (1981) Geomicrobiological leaching of tin minerals by *Thiobacillus ferrooxidans* and organic agents, Zeitschr. Allgem. Mikrobiol. 21:157–167
91. Teh GH, Schwartz W, Amstutz GC, (1982) Geomicrobiological leaching of tin minerals by *Thiobacillus ferrooxidans* and organic agents. In: Amstutz GC (ed) Ore Genesis—the state of the art. Springer, Berlin, Heidelberg, pp 701–725
92. Babel S, Dacera DDM (2006) Heavy metal removal from contaminated sludge for land application: a review. Waste Manag 26:988–1004
93. Karwowska E (2010) Copper and zinc bioleaching from galvanic sludge in mixed microbial cultures. In: Dudzinska P (ed) Environmental engineering III. Taylor & Francis Group, London, p 291. ISBN 978-0-415-54882-3
94. Blase F, Meyer A, Nguyen HL, Willscher S, Baumann KH, Fritsche W, (1996) Untersuchungen zur heterotrophen Laugung eines ausgewählten Galvanikschlammes. In: Proceedings of DECHEMA- Jahrestagungen 1996, Biotechnologie, DECHEMA e. V. Frankfurt/Main, pp 541–542
95. Nguyen HL, Willscher S, Fritsche W (1994) Mikrobielle Laugung von Schwermetallen aus Galvanikschlämmen. Chem Tech 46:201
96. Vanchon P, Tyagi RD, Auclair J-C, Wilkinson J (1994) Chemical and biological leaching of aluminium from red mud. Environ Sci Technol 28:26–30
97. Ghorbani Y, Oliazadeh M, Shahverdi, AR (2009) Microbiological leaching of Al from the waste of Bayer process by some selective fungi. Iran J Chem Chem Eng 28:109–115
98. Sanierungsverbund e.V. Mansfeld (1993) Theisenschlamm, Manuskriptsammlung zum Fachkolloquium Theisenschlamm am 07. 12. 1993. In: Sanierungsverbund e.V. Mansfeld (ed) im Auftrag des Ministeriums für Umwelt und Naturschutz des Landes Sachsen-Anhalt März 1994
99. Lorenz R (1993) Erfahrungen, Versuche und Konzepte zur Verwertung von Theisenschlamm. In: Theisenschlamm, Manuskriptsammlung zum Fachkolloquium Theisenschlamm. Sanierungsverbund e.V. Mansfeld, Saxony-Anhalt, Germany, pp 1–16
100. Landesanstalt für Altlastenfreistellung des Landes Sachsen-Anhalt (2002) Altlastensanierung in Sachsen-Anhalt. Gehrig Verlagsgesellschaft mbH, Merseburg, Germany
101. Bioshale (2007) Final Report of the EU Project "Search for a sustainable way of exploiting black shale ores using biotechnologies—Bioshale" (2007) Ref. Number: STRP 50 57 10–1 Bioshale Contract number NMP2–CT–2004-505710 Time: 2004–2007, Project Co-ordinator–BRGM

102. Sklodowska A, Matlakowska R (2007) Biodegradation of organic matter and release of heavy metals from the copper bearing black shale of Fore Sudetic Monocline (Poland). Adv Mater Res 20–21:238–239. In: Schippers A, Sand W, Glombitza F, Willscher S (eds) Biohydrometallurgy: from the single cell to the environment. Proceedings of the 17th international biohydrometallurgy symposium, Frankfurt/Main Germany. Trans Tech Publications LTD., Switzerland, UK, USA, 2–5 Sept 2007. ISSN 1022-6680
103. Sadowski Z, Szubert A, Maliszewska I, Jazdzyk E (2007) A view on the organic matter and metalloporphyrins biodegradation as characteristic components of black shale ores. Adv Mater Res 20–21:95–99. In: Schippers A, Sand W, Glombitza F, Willscher S (eds) Biohydrometallurgy: from the single cell to the environment. Proceedings of the 17th international biohydrometallurgy symposium, Frankfurt/Main Germany. Trans Tech Publications LTD., Switzerland, UK, USA, 2–5 Sept 2007. ISSN 1022-6680
104. Umweltdatenbank (2012) www.Umweltdatenbank.de/lexikon/Klaerschlamm.htm
105. Peretzki K (2012) Flusssedimente als Rohstoffquelle. In: Transferbrief Leipzig 1/2012—Wirtschaft und Wissenschaft. AGIL GmbH Leipzig (ed) Germany
106. Zehnsdorf A, Hoffmann P, Seidel H, Schlenker U, Müller R (2011) Reinigung schwermetallhaltiger Sedimente mithilfe von Pflanzen und Mikroorganismen, Überführung des Konditionierungsverfahrens in die Praxis. KW Korresponenz Wasserwirtschaft 4:558–563
107. Seidel H, Ondruschka J, Morgernstern P, Wennrich R, Hoffmann P (2000) Bioleaching of heavy metal—contaminated sediments by indigenous *Thiobacillus* spp: metal solubilization and sulfur oxidation in the presence of surfactants. Appl Microbiol Biotechnol 54:854–857
108. Löser C, Zehnsdorf A, Hoffmann P, Seidel H (1999) Conditioning of heavy-metal—polluted river sediments by helophytes. Int J Phytorem 1:339–359
109. Willscher S, Pohle C, Sitte J, Werner P (2007) Solubilisation of heavy metals from a fluvial AMD generating tailings sediment by heterotrophic microorganisms. Part I: Influence of pH and solid content. J Geochem Exploration 92:177–185
110. Beolchini F, Ubaldini S, Passariello B, Gül N, Türe D, Veglio F, Danovaro R, Dell'Anno A (2007) Bioremediation of dredged sediments polluted by heavy metals. Adv Mater Res 20–21. In: Schippers A, Sand W, Glombitza F, Willscher S (eds) Biohydrometallurgy: from the single cell to the environment. Proceedings of the 17th international biohydrometallurgy symposium, Frankfurt/Main Germany. Trans Tech Publications LTD., Switzerland, UK, USA, pp 307–310, 2–5 Sept 2007. ISSN 1022-6680
111. Effenberger H (2000) Dampferzeugung. VDI Springer, Berlin, Heidelberg. ISBN 3-540-64175-0
112. Holzapfel T (1993) Stand der Industrie-Verbundforschungsvorhaben zur Verwertung von Aschen aus Braunkohlen-Kraftwerken in der BRD und Möglichkeiten der Verwertung dieser Kraftwerksrückstände. In: "Abfallwirtschaft und Altlastensanierung" Projektträgerschaft BMFT im Umweltbundesamt (ed): Verwertung von Braunkohlenaschen in den neuen Bundesländen. Vorträge und Diskussionsbeiträge aus dem Arbeitsgespräch am 18. 03. 1993 im Umweltbundesamt [Berlin]: Umweltbundesamt, Projektträgerschaft des BMFT Abfallwirtschaft und Altlastensanierung
113. Pinka J (1994) chemische und biochemische Prozesse bei der Wechselwirkung von Wässern mit abgelagerten Braunkohlefilteraschen in Bergbaufolgelandschaften. Dissertation TU Bergakademie Freiberg, Fakultät für Mathematik und Naturwissenschaften 09.06. 1994
114. Singer A, Navrot J, Shapira R (1982) Extraction of aluminium from fly-ash by commercial and microbiologically-produced citric acid. Eur J Appl Microbiol Biotechnol 16:228–230
115. Torma AE, Singh AK (1993) Acidolysis of coal fly ash by Aspergillus niger. Fuel 12:1625–1630
116. Krejcik S (2012) Cultivation of microorganisms for heterotrophic leaching of lignite filter ash. Thesis Bachelor of Science, TU Bergakademie Freiberg, Freiberg, Germany
117. Hopf N, Bimüller A, Poehlmann E, Sattelberger S (2006) Energieeinsparung in der Metallurgie am Beispiel der energieeffizienten Aufschmelzung von Vanadiumkonzentraten, Abschlussbericht EULV15 zum Kooperationsvorhaben des Forschungs- und Entwicklungs-

zentrums für Sondertechnologien (FES) mit der GfE Metalle und Materialien GmbH, Nürnberg, Forschungs- und Entwicklungszentrum für Sondertechnologien, Siemensstraße 3 – 5, 91126 Rednitzhembach
118. Greinert J (2012) Vermarktung von MVA-Schlacken—Erfahrungen aus Hamburg. In: Thomé-Kozmiensky KJ und Goldmann D (eds) Recycling und Rohstoffe—Bd. 5. TK Verlag, Neuruppin, pp 819–836
119. Briese D, Duill B, Westholm H (2012) Der Markt für MVA-Schlacken. In: Thomé-Kozmiensky KJ und Goldmann D (eds) Recycling und Rohstoffe—Bd. 5. TK Verlag, Neuruppin, S. 811–817
120. Alwast H, Riemann A (2010) Verbesserungen der umweltrelevanten Qualitäten von Schlacken aus Abfallverbrennungsanlagen, UBA-Texte, 50/2010. Umweltbundesamt, Dessau-Roßlau, p 87
121. ITAD (2009) Interessengemeinschaft der Thermischen Abfallbehandlungsanlagen Deutschland e.V.: Zusammengestellte Daten aus Mitgliederbefragungen 2006 und 2008
122. Ishigaki T (2011) Bioleaching of metals from solid waste incineration ash. In: Soda S, Yamashita M, Ike M (eds) Handbook of metal biotechnology, applications for environmental conservation and sustainability—Chap. 9. Pan Stanford Publishing Ltd., Singapore 2012, pp 101–110. ISBN 978-981-4267-98-4
123. Glombitza F, Janneck E, Arnold I, Rolland W, Uhlmann W (2007) Eisenhydroxysulfate aus der Bergbauwasserbehandlung als Rohstoff, Sonderheft FA Rohstoffwirtschaft. In: Consulting—Erfahrungen und Kontakte für Neuanfänge, Untertägiger Bergbau auf Industrieminerale in Deutschland, Heft 110 der Schriftenreihe der GDMB Gesellschaft für Bergbau, Metallurgie, Rohstoff- und Umwelttechnik, GDMB e.V. Clausthal Zellerfeld, 2007, pp 31–40. ISSN 0720-1877, ISBN 3-935797-35-4
124. Janneck E, Arnold I, Koch Th, Meyer J Burghardt D, Ehinger S (2010) Microbial synthesis of schwertmannite from lignite mine water and its utilization for removal of arsenic from mine waters and for production of iron pigments. In: Wolkersdorfer Ch, Freund A (eds) Mine water & innovative thinking. Canada (CBU Press), Sydney, Nova Scotia, pp 131–135
125. Burgos WD, Borch Th, Troyer LD, Luan F, Larson LN, Brown JF, Lambson J, Shimizu M (2012) Schwertmannite and Fe oxides formed by biological low–pH Fe(II) oxidation versus abiotic neutralization: impact on trace metal sequestration. Geochimica et Cosmochimica Acta 76:29–44
126. Bigham J M, Carlson LL, Murad L, (1994) Schwertmannite, a new iron oxyhydroxysulphate from Pyhasalmi, Finland, and other localities. Mineral Mag 58:641–648
127. Gaal GE, Kaja J (2009) Nanoparticle products from new mineral resources in Europe, Pro Mine Project, Coordinator: GTK Geological survey of Finland, Referenz number: FP7–NMP-2008-Large 2 Grant agreement number: 228559, Starting date 01 May 2009. http://promine.gtk.fi
128. Janneck E, Burghardt D, Martin M, Damian C, Schöne G, Meyer J, Peiffer S (2011) From waste to valuable substance: utilization of schwertmannite and lignite filter ash for removal of arsenic and uranium from mine drainage. In: Rüde TR, Freund A, Wolkersdorfer C (eds) 11th international mine water association congress: mine water—managing the challenges. IMWA (2011) Conference proceedings. Aachen, Germany, pp 359–364. ISBN 978-1-897009-47-5
129. Heinzel E, Hedrich S, Janneck E, Glombitza F, Seifert J, Schlömann M (2009) Bacterial diversity in a mine water treatment plant. Appl Env Microbiol 75:3858–3861
130. Heinzel E, Janneck E, Glombitza F, Schlömann M, Seifert J (2009) Population dynamics of iron-oxidizing communities in pilot plants for the treatment of acid mine waters. Environ Sci Technol 43:6138–6144
131. Czekalla C, Mevius W, Hanert H (1995) Quantitative removal of iron and manganese by microorganisms in rapid sand filters (in situ investigations), Water Supply vol 3, Berlin "B" pp 111–123, Pergamon Press Ltd., Great Britain
132. Mouchet P (1992) From conventional to biological removal of iron and manganese in France. J AWWA April:158–167

133. Ten WK, Ting YP (2003) Bioleaching of electronic scrap material by *Aspergillus niger*. In: Tsezos M, Hatzikioseyian A, Remoundaki E (eds) Biohydrometallurgy: a sustainable technology in evolution (First edition 2004). Proceedings of the 15th international biohydrometallurgy symposium (IBS), National technical University of Athens, Athens, Greece, pp 137–146, 14–19 Sept 2003. ISBN 960-88415-1-8
134. Willscher S, Katzschner M, Jentzsch K, Matys S, Pöllmann H (2007) Microbial leaching of metals from printed circuit boards. Adv Mater Res 20–21:99–102. In: Schippers A, Sand W, Glombitza F, Willscher S (eds) Biohydrometallurgy: from the single cell to the environment. Proceedings of the 17th international biohydrometallurgy symposium, Frankfurt/Main Germany. Trans Tech Publications LTD., Switzerland, UK, USA, 2–5 Sept 2007. ISSN 1022-6680
135. Brandl H, Lehmann S, Faramarzi MA, Martinelli D (2008) Biomobilization of silver, gold and platinum from solid materials by HCN-forming microorganisms. Hydrometallurgy 94:14–17
136. Santhiya D, Ting Y-P (2006) Use of adapted Aspergillus niger in bioleaching of spent refinery processing catalyst. J Biotechnol 121:62–74
137. BMFT (1991) Projektträger FZ Jülich GmbH BEO, Bericht zum BMFT Projekt "Mikrobielle und mikrobiell gestützt chemische Laugungsprozesse für Erze und Abprodukte mit hohem Karbonat und Calziumgehalt", Dezember 1991, FKZ 0319742 A
138. Glombitza F (1991) Mikrobielle und mikrobiell gestützte chemische Laugungsprozesse für Erze und Abprodukte mit hohem Karbonat- und Kalziumgehalt, Bericht zum BMFT Projekt FKZ 0 319 742 A, Institut für Biotechnologie Leipzig, Universität Regensburg Lehrstuhl für Mikrobiologie, 30 Sept 1991
139. Innovent e.V. Jena (1995) Umwelt- und gesundheitsgerechtes Verfahren zur Rückgewinnung von Wertstoffen aus Bildröhrenbeschichtungen DBU Projekt FKZ 03809, 18. 09. 1995–22. 12. 1999 Bericht: Laugung von thermisch abgetrennten Bildschirmbeschichtungen zur Rückgewinnung Seltener Erden und Flotationsergebnisse, Löffler R, Glombitza F, Irmscher A, Karnatz F (1997) Projektkennblatt der Deutschen Bundesstiftung Umwelt vom 11. 08. 2009
140. Thomas F, Diaz-Bone R A, Meyer A, Michalke K, Hensel R (2012) Methylierte Metall(oid)e. Biospektrum 18:487–489
141. Jiang K, Guo Z, Xiao X, Wei X (2012) Effect of moderately thermophilic bacteria on metal extraction and electrochemical characteristics for zinc smelting slag in bioleaching system. Trans Nonferrous Met Soc China 22:3120–3125
142. Fischer H, Glombitza F, Patzig A (2003) Einschätzung der mikrobiellen Solubilisierung von Schlacke (estimation of the microbial leaching of slag), Report G.E.O.S., 20 June 2003
143. Krebs W, Brombacher C, Bosshard PP, Bachofen R, Brandl H (1997) Microbial recovery of metals from solids. FEMS Microbiol Rev 20:605–617
144. Brandl H (2002) Metal: microbe—interactions and their biotechnological applications for mineral waste treatment. Recent Res Develop Microbiol 6:571–5894
145. Bayat B, Sari B (2010) Comparative evaluation of microbial and chemical leaching processes for heavy metal removal from dewatered metal plating sludge. J Hazard Mater 174:736–769
146. Mulligan CN, Dahrazma B (2003) Extraction of copper from mining residues and sediments by addition of rhamnolipids. In: Tsezos M, Hatzikioseyian A, Remoundaki E (eds) Biohydrometallurgy: a sustainable technology in evolution (First edition 2004). Proceedings of the 15th international biohydrometallurgy symposium (IBS), National technical University of Athens, Athens, Greece, pp 253–260, 14–19 Sept 2003. ISBN 960-88415-1-8
147. Cabrera G, Gomez JM, Cantero D (2007) Integrated system to biological solubilization and precipitation of heavy metals. Adv Mater Res20–21: 279–282. In: Schippers A, Sand W, Glombitza F, Willscher S (eds) Biohydrometallurgy: from the single cell to the environment. Proceedings of the 17 international biohydrometallurgy symposium,

Frankfurt/Main Germany. Trans Tech Publications LTD., Switzerland, UK, USA, 2–5 Sept 2007. ISSN 1022-6680
148. Groudeva V, Doychea A, Krumova K, Groudev S (2007) Bioremediation in situ of an alkaline soil polluted with heavy metals. Adv Mater Res20–21:287–290. In: Schippers A, Sand W, Glombitza F, Willscher S (eds) Biohydrometallurgy: from the single cell to the environment. Proceedings of the 17th international biohydrometallurgy symposium, Frankfurt/Main Germany. Trans Tech Publications LTD., Switzerland, UK, USA, 2–5 Sept 2007. ISSN 1022-6680
149. Villar LD, Garcia O Jr (2003) Effects of total-solids concentration on metal bioleaching from sewage sludge. In: Tsezos M, Hatzikioseyian A, Remoundaki E (eds) Biohydrometallurgy: a sustainable technology in evolution (First edition 2004). Proceedings of the 15th international biohydrometallurgy symposium (IBS), National technical University of Athens, Athens, Greece, pp 559–565, 14–19 Sept 2003. ISBN 960-88415-1-8
150. Matlakowska R, Sklodowska A (2003) Isolation and evaluation of indigenous iron- and sulphur- oxidizing bactera for heavy metal removal from sewage sludge. In: Tsezos M, Hatzikioseyian A, Remoundaki E (eds) Biohydrometallurgy: a sustainable technology in evolution (First edition 2004). Proceedings of the 15th international biohydrometallurgy symposium (IBS), National technical University of Athens, Athens, Greece, pp 265–276, 14–19 Sept 2003. ISBN 960-88415-1-8
151. Bosshard PB, Bachofen R, Brandl H (1996) Metal leaching of fly ash from municipal waste incineration by A*spergillus niger*. Environ Sci Technol 30:3066–3070
152. Xu TJ, Ting YP (2003) Optimization study on bioleaching of municipal solid waste incineration fly ash by *Aspergillus niger*. In: Tsezos M, Hatzikioseyian A, Remoundaki E (eds) Biohydrometallurgy: a sustainable technology in evolution (First edition 2004). Proceedings of the 15th international biohydrometallurgy symposium (IBS), National technical University of Athens, Athens, Greece, pp 329–336, 14–19 Sept 2003. ISBN 960-88415-1-8
153. Bakhtiary F, Atashi H, Zivdar M, Seied Baghery SA (2007) Bioleaching of a mixed copper dust emanating from copper smelter. Adv Mater Res 20–21:143–148. In: Schippers A, Sand W, Glombitza F, Willscher S (eds) Biohydrometallurgy: from the single cell to the environment. Proceedings of the 17th international biohydrometallurgy symposium, Frankfurt/Main, Germany. Trans Tech Publications LTD., Switzerland, UK, USA, 2–5 Sept 2007. ISSN 1022-6680
154. Rösler HJ, Lange H (1972) Geochemical tables. Leipzig, Germany. ISBN 0444408940
155. Mariner R, Johnson DB, Hallberg KB (2007) Development of a novel biological system for removing manganese from contaminated water. Adv Mater Res 20–21: 267–270. In: Schippers A, Sand W, Glombitza F, Willscher S (eds) Biohydrometallurgy: from the single cell to the environment. Proceedings of the 17th international biohydrometallurgy symposium, Frankfurt/Main, Germany. Trans Tech Publications LTD., Switzerland, UK, USA, 2–5 Sept 2007. ISSN 1022-6680
156. Battaglia-Brunet F, Foucher S, Denamur A, Chevard S, Morin D, Ingnatiadis I (2003) Development of a bio-process using sulphate-reducing bacteria to remove metals from surface treatment effluents. In: Tsezos M, Hatzikioseyian A, Remoundaki E (eds) Biohydrometallurgy: a sustainable technology in evolution (First edition 2004). Proceedings of the 15th international biohydrometallurgy symposium (IBS), National technical University of Athens, Athens, Greece, pp 549–557, 14–19 Sept 2003. ISBN 960-88415-1-8
157. Kim DJ, Mishra D, Ralph DE, Ahn JG, Rhee YH (2007) Application of mesophilic mixed microorganisms for recovery of valuable metals from spent refinery catalysts. Adv Mater Res 20–21:119–125. In: Schippers A, Sand W, Glombitza F, Willscher S (eds) Biohydrometallurgy: from the single cell to the environment. Proceedings of the 17th international biohydrometallurgy symposium, Frankfurt/Main Germany. Trans Tech Publications LTD., Switzerland, UK, USA, 2–5 Sept 2007. ISSN 1022-6680

158. Brandl H, Bosshard R, Wegmann M (2001) Computer—munching microbes; metal leaching from electronic scrap by bacteria and fungi. Hydrometallurgy 59:319–326
159. Cho KS, Choi MS, Hong JH, Kim DS, Ryu HW, Kim DJ, Sohn JS, Park KH (2003) Microbial recovery of copper from printed circuit boards of waste computer by *Acidithiobacillus ferrooxidans*. In: Tsezos M, Hatzikioseyian A, Remoundaki E (eds) Biohydrometallurgy: a sustainable technology in evolution (First edition 2004). Proceedings of the 15th international biohydrometallurgy symposium (IBS), National technical University of Athens, Athens, Greece, pp 631–637, 14–19 Sept 2003. ISBN 960-88415-1-8
160. Murray AJ, Mikheenko IP, Goralska E, Rowson NA, Macaskie LE (2007) Biorecovery of platinum group metals from secondary sources. Adv Mater Res 20–21:651–654. In: Schippers A, Sand W, Glombitza F, Willscher S (eds) Biohydrometallurgy: from the single cell to the environment. Proceedings of the 17th international biohydrometallurgy symposium, Frankfurt/Main Germany. Trans Tech Publications LTD., Switzerland, UK, USA, 2–5 Sept 2007. ISSN 1022-6680
161. Bauer M, Gassen N, Stanjek H, Peiffer S (2011) Carbonation of lignite fly ash at ambient T and P in a semi-dry reaction system for CO_2 sequestration. Appl Geochem 26:1502–1512
162. Cho KS, Moon HS, Yoo NY, Ryu HW (2003) Leaching characteristics of heavy metals from sewage sludge by *Acidithiobacillus thiooxidans* MET. In: Tsezos M, Hatzikioseyian A, Remoundaki E (eds) Biohydrometallurgy: a sustainable technology in evolution (First edition 2004). Proceedings of the 15th international biohydrometallurgy symposium (IBS), National technical University of Athens, Athens, Greece, pp 613–620, 14–19 Sept 2003. ISBN 960-88415-1-8
163. Löser C, Zehnsdorf A, Hoffmann P, Seidel H (2007) Chemosphere 66: 1699–1705

Adv Biochem Eng Biotechnol (2014) 141: 109–172
DOI: 10.1007/10_2013_265

Published Online: 20 December 2013

Bioremediation of Mine Water

Robert Klein, Judith S. Tischler, Martin Mühling and Michael Schlömann

Abstract Caused by the oxidative dissolution of sulfide minerals, mine waters are often acidic and contaminated with high concentrations of sulfates, metals, and metalloids. Because the so-called acid mine drainage (AMD) affects the environment or poses severe problems for later use, treatment of these waters is required. Therefore, various remediation strategies have been developed to remove soluble metals and sulfates through immobilization using physical, chemical, and biological approaches. Conventionally, iron and sulfate—the main pollutants in mine waters—are removed by addition of neutralization reagents and subsequent chemical iron oxidation and sulfate mineral precipitation. Biological treatment strategies take advantage of the ability of microorganisms that occur in mine waters to metabolize iron and sulfate. As a rule, these can be grouped into oxidative and reductive processes, reflecting the redox state of mobilized iron (reduced form) and sulfur (oxidized form) in AMD. Changing the redox states of iron and sulfur results in iron and sulfur compounds with low solubility, thus leading to their precipitation and removal. Various techniques have been developed to enhance the efficacy of these microbial processes, as outlined in this review.

Keywords Acid mine waters · Active treatment system · Bioreactor design · Microbial iron oxidation · Microbial sulfate reduction · Passive treatment system · Remediation

Abbreviations

ALD	Anoxic limestone drain
AMD	Acid mine drainage
ARD	Acid rock drainage

R. Klein · J. S. Tischler · M. Mühling (✉) · M. Schlömann (✉)
Institute of Biosciences, TU Bergakademie Freiberg, Leipziger Str. 29, 09599 Freiberg Germany
e-mail: Martin.Muehling@ioez.tu-freiberg.de

M. Schlömann
e-mail: Michael.Schloemann@ioez.tu-freiberg.de

ASPAM	Algal sulfate reducing ponding process for the treatment of acidic and metal wastewaters
COD	Chemical oxidation demand
CSTR	Continuously stirred tank reactor
DAS	Dispersed alkaline substrate
E°	Standard electrode potential
Eh	Redox potential
EGSB	Expanded granular sludge blanket (reactor)
HRAP	High-rate algal ponds
HRT	Hydraulic retention time
IASRS	In-adit-sulfate-reducing system
INAP	International network for acid prevention
NF	Nanofiltration
OLC	Open limestone channel
PAA	Polyacrylic acids
PLB	Pulsed limestone bed
PVA	Polyvinyl alcohol boric acid
RAPS	Reducing and alkalinity producing systems
RO	Reverse osmosis
SAPS	Successive alkalinity producing system
SHMP	Sodium hexametaphosphate
SRP	Sulfate-reducing prokaryotes
SRT	Sludge (biomass) retention time
TDS	Total dissolved solids
UASB	Upflow anaerobic sludge blanket (reactor)

Contents

1 Characteristics of Mine Waters

Coal and ore minerals have been mined by mankind for thousands of years. Due to these anthropogenic mining activities, sulfide minerals are exposed to oxygen and water, resulting in the oxidative dissolution of these minerals [13]. One ubiquitous sulfide mineral is pyrite (FeS_2), which is oxidized in a complex series of reactions. The net process of pyrite oxidation (1) results in the formation of ferrous iron, sulfate, and acid:

$$2FeS_2 + 2H_2O + 7O_2 \rightarrow 2Fe^{2+} + 4SO_4^{2-} + 4H^+ \quad (1)$$

Pyrite oxidation is further promoted by ferric iron, which serves as an oxidant (2) [13, 213]. Ferric iron, in turn, is generated by iron oxidation catalyzed by acidophilic microorganisms occurring in the mine water, because ferrous iron is chemically stable under acidic conditions [138, 221].

$$FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+ \quad (2)$$

Apart from iron, sulfate, and protons, other elements such as arsenic, cadmium, cobalt, copper, lead, and zinc are also released during pyrite oxidation because these elements often occur in association with pyrite [13]. Moreover, the acidic pH also means that heavy metals and metalloids are particularly well dissolved (Table 1). These waters are known as acid mine drainage (AMD) or acid rock drainage (ARD), which represent a severe environmental problem caused by worldwide mining activities.

Upon oxidative dissolution of metal-bearing minerals, the fate of the metals and counter ions is further influenced by their redox state. For instance, iron is mainly present in its reduced form (ferrous iron) due to its redox potential ($E^0(Fe^{3+}/Fe^{2+}) = +0.77$ V) [231]. Sulfur, in contrast, occurs in its oxidized form (sulfate) as a consequence of its low redox potential ($E^0({SO_4}^{2-}/H_2S) = -0.22$ V) [231]. Thus, both elements tent to occur in AMD in their most mobile forms.

Besides acting as oxidant for pyrite oxidation, ferric iron may also precipitate. Thus, acidic mine waters are often characterized by red-brownish iron-bearing minerals like schwertmannite (idealized formula: $Fe_8O_8(OH)_6(SO_4)$), jarosite ($MFe_3(SO_4)_2(OH)_6$, $M = Na^+$, K^+, ${NH_4}^+$, ${H_3O}^+$), ferrihydrite ($5Fe_2O_3 \times 9H_2O$), lepidocrocite (γ-FeOOH), and goethite (α-FeOOH) [30, 135, 168, 169, 248]. The minerals occurring in mine water depend mainly on the pH. Ferrihydrite and

Table 1 Physicochemical parameters of various acid mine drainage sites

Mine	Type of mine	pH	T (°C)	E_h (mV)	Concentration (mg/l)									
					Fe^{2+}	SO_4^{2-}	Mn	Cu	Zn	Al	Cd	Pb	Co	Ni
Open pit Nochten (Saxony, D)[a]	Lignite	4.9	17	270	630	2,700	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Open pit Garzweiler (North Rhine-Westphalia, D)[b]	Lignite	2.2	n.d.	n.d.	5,600[r]	24,780	120	n.d.	81	656	0.29	<0.2	11.7	42.2
Dump Berrenrath (North Rhine-Westphalia, D)[b]	Lignite	4.4	n.d.	n.d.	3,210[r]	6,003	15	n.d.	4.41	70	n.d.	n.d.	9.6	2.75
King's Mine (N)[c]	Copper	3.71	n.d.	n.d.	6.7[r]	139	0.25	3.76	11	4.25	0.02	<0.05	0.02	0.03
Río Tinto (ES)[d, s]	Metals, sulfide ore	2.0	n.d.	665	2,369	n.d.	49.8	20.4	20.4	n.d.	n.d.	n.d.	n.d.	11.3
Mynydd Parys (Wales, UK)[e]	Copper	2.7	8.8	420	473	1,552	5	35	50	n.d.	n.d.	n.d.	n.d.	n.d.
Wheal Jane (Wales, UK)[f]	Copper	3.6	n.d	n.d.	130	350	20	2	130	50	n.d.	n.d.	n.d.	n.d.
Ynysarwed (Wales, UK)[f]	Coal	6.2	n.d.	n.d.	140	460	n.d.	n.d.	n.d.	20	n.d.	n.d.	n.d.	n.d.
Cae Coch (Wales, UK)[g]	Pyrite	2.5	n.d.	n.d.	1,460[r]	5.110	3.05	0.16	0.94	84.21	n.d.	n.d.	n.d.	n.d.
Iron Mountain (California, USA)[h]	Polymetallic	~2.5	42	n.d.	34,500	760,000	23	4,160	23,500	1,420	211	11.9	5.3	3.7
Leviathan Mine (California, USA)[h]	Sulfur	1.85	14	n.d.	2,150	11,200	9.32	9.64	2.62	623	0.338	0.037	5.07	13
Cameron Mine (Pennsylvania, USA)[h]	Coal	4	13.6	n.d.	8.2	510	6.1	0.0014	0.36	5.4	0.0017	0.0016	0.18	0.24
Gum Boot (Pennsylvania, USA)[i]	Coal	4.1	12	n.d.	48.5	95	2.8	n.d.	n.d.	1.4	n.d.	n.d.	n.d.	n.d.
Dalseong Mine (Sangwon-ri, KP)[j]	Tungsten	5.4	14.2	137	195.57[r]	1,996	63.54	8.98	16.24	13.67	n.d.	n.d.	n.d.	n.d.
Xiang Mountain (Anhui Province, CN)[k]	Pyrite	2.9	20.2	583	0.4	1.09	0.24	48.0	n.d.	1,020	n.d.	n.d.	n.d.	n.d.
Yun-Fu Mine (Guangdong, CN)[l]	Pyrite	3.0	28.1	n.d.	670[r]	2,881	19	0.006	7.2	n.d.	0.01	0.04	n.d.	0.3
Dabaoshan Mine (Guangdong, CN)[m]	Limonite, pyrite	2.3	20	n.d.	6	n.d.	50.55	13.9	55.86	429.1	0.59	3.13	0.65	0.85

(continued)

Table 1 (continued)

Mine	Type of mine	pH	T (°C)	E_h (mV)	Concentration (mg/l) Fe^{2+}	SO_4^{2-}	Mn	Cu	Zn	Al	Cd	Pb	Co	Ni
Witbank Colliery (Mpumalanga, SA)[n]	Coal	2.7	n.d.	n.d.	122[r]	910	5.9	n.d.	n.d.	81	n.d.	n.d.	n.d.	n.d.
Ojancos flotation plant (Copiapó, RCH)[o]	Pyrite, chalcopyrite	7.55	9.2	n.d.	n.d.	2,836	0.36	0.12	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Rio Malo (El Indio Mine, RCH)[p]	Gold	4.8	n.d.	n.d.	7.9[r]	8,270	4.2	5.7	1.8	n.d.	n.d.	n.d.	n.d.	n.d.
El Teniente Mine (RCH)[q]	Copper	9.6	n.d.	256	2.4[r]	1,739	0.003	0.028	0.013	0.69	n.d.	n.d.	n.d.	0.021

[a] [101], [b] [274], [c] [122], [d] [72], [e] [92], [f] [121], [g] [13], [h] [178], [i] [220], [j] [48], [k] [95], [l] [236], [m] [280], [n] [25], [o] [60], [p] [59], [q] [229]

[r] No differentiation between ferrous and ferric iron was made

[s] Data presented originate from the origin 1 of Rio Tinto (station RT1; for details, see [72])

n.d. not determined

goethite are formed at pH > 6.5, whereas the iron oxyhydroxysulfate schwertmannite dominates the mineral phase in waters with pH 2.5–4.5 [28, 30]. Thus, schwertmannite is typically found in AMD [29, 98, 125, 135, 214]. However, it is not a pure mineral phase: Schwertmannite is a metastable secondary mineral, and changes in the water chemistry (e.g. increase or decrease of pH, changes in the concentration of monovalent cations) lead to a transformation into jarosite or goethite [2, 30, 177, 258].

Due to their chemistry, acid mine waters are optimal niches for some microorganisms. These microorganisms occurring in AMD were reviewed in detail by Baker and Banfield [12], Johnson and Hallberg [123], and Hallberg [90]. Briefly, microorganisms detected could be assigned to eight bacterial and two archaeal divisions [12]. Bacteria typically found are *Acidithiobacillus* spp., *Leptospirillum* spp., "*Ferrovum*" spp., *Ferrimicrobium acidiphilum*, *Alicyclobacillus* spp., *Sulfobacillus* spp., *Desulfotomaculum* spp., *Desulfobacter* spp., *Acidiphilium* spp., *Acidocella* spp., and *Acidobacterium capsulatum*. *Ferroplasma* spp. and *Sulfolobus* spp. are typical archaea occurring in AMD.

A variety of inorganic electron donors and electron acceptors are present in AMD for microbial energy generation, although ferrous iron (electron donor) and ferric iron and sulfate (electron acceptors) are the dominating species. Therefore, it is not surprising that most of the microorganisms are able to oxidize iron or to reduce iron and sulfate. Heterotrophic acidophiles have been detected in minor percentages [123].

Caused by low pH and high loads of contaminants, AMD is a worldwide environmental problem. An example of the relevance of pollution by mine waters is provided by the mining area of Lusatia (Germany), where it is estimated that by 2015 the discharged sulfate load into River Spree will rise up to 130,000 tons per year [229]. The river itself is an important source of drinking water for a number of cities, including Berlin, where up to 80 % of the drinking water is produced from surface water [229]. Although the adverse effects of high sulfate concentrations on freshwater biodiversity have so far not been reported [229], the need for treatment of the water bodies and the corrosive effects on concrete buildings and other structures in or near high sulfate burdened water bodies still cause additional costs to individuals and the public as a whole. In addition, ferric iron hydroxide precipitates now reach River Spree and tributaries near Berlin. These precipitates result from the input of ferrous iron into freshwater bodies which, upon dilution with natural river water, are abiotically oxidized to ferric iron due to pH increases. This problem has now come to the attention of the public, which is putting pressure on responsible government departments because the pollution will likely lead to major consequences for aquatic life.

National and international laws require the treatment of AMD. Apart from conventional chemical treatment methods, biotechnological approaches that take advantage of the ability of some microorganisms to metabolize iron or sulfate have also been in the focus of research [124, 129, 133, 225]. Although this article reviews biotechnological approaches for the remediation of AMD, it is different from previous publications in two ways. Firstly, AMD treatment using microbial

iron oxidation catalyzed by acidophilic iron-oxidizing microorganisms has not been discussed in previous reviews. Secondly, apart from some more recent research not summarized before, this review also provides an extensive literature analysis on microbial sulfate reduction in an attempt to reveal process parameters that influence the performance of this biotechnological application.

2 Overview of Remediation Strategies

The ideal remediation strategy is to prevent the formation of AMD. Prevention is conventionally achieved by compaction, coverage, or underwater deposition [106, 124, 145, 178, 212]. A further strategy to prevent AMD is the so-called coating technology [68, 124]. Phosphate is added as apatite, which leads to the immobilization of iron and lead as ferric phosphate and pyromorphite, respectively [52, 277]. Due to the precipitation of ferric phosphate, the oxidant of the sulfide mineral dissolution, ferric iron, is removed and the sulfide mineral is coated simultaneously. Thus, the sulfide mineral oxidation is slowed down [124].

Prevention of AMD, however, may not always be feasible, thus requiring discharge of mine water to be prevented and the water to be treated. Remediation techniques for AMD are designed to reduce its volume; change the pH; lower the levels of dissolved metals and sulfate; oxidize or reduce the contaminants; or collect, dispose, or isolate the mine water or any metal-rich sludge generated [156]. The strategy used for mine water treatment is site-specific and depends on the water quantity and quality, the occurring pollutants, and the space available [90, 156].

Although organic pollutants in bioremediation strategies are usually degraded to carbon dioxide, inorganic contaminants are in general removed from the aqueous phase by sorption or by formation of insoluble minerals due to the transformation of their redox state [47, 90, 152, 188, 191, 199]. Because AMD is mainly contaminated with metals and sulfate, various techniques for the removal of these contaminants have been developed in recent years. These technologies can be divided into *abiotic* and *biotic* remediation strategies, depending on whether biological processes, which are generally performed by microorganisms, play a role or not (Fig. 1). For both approaches, active and passive systems have been developed for the treatment of AMD [124, 225]. In addition, (bio)sorption provides another means of treating AMD, which is distinct from the former approaches because it does not alter the redox state of metals and sulfate via oxidative and reductive processes, respectively (Fig. 1).

Conventional active treatment technologies use chemical treatment to increase the pH of AMD, leading to the formation of insoluble metal complexes and their subsequent precipitation [225]. Additionally, physical processes such as nanofiltration, reverse osmosis, and ionic exchange can also be used for the removal of metals and sulfate. In essence, these active abiotic treatment technologies are highly efficient, but only at high costs due to the high energy demand and large amounts of chemicals and expensive devices required for the process [124, 156, 226]. Over the

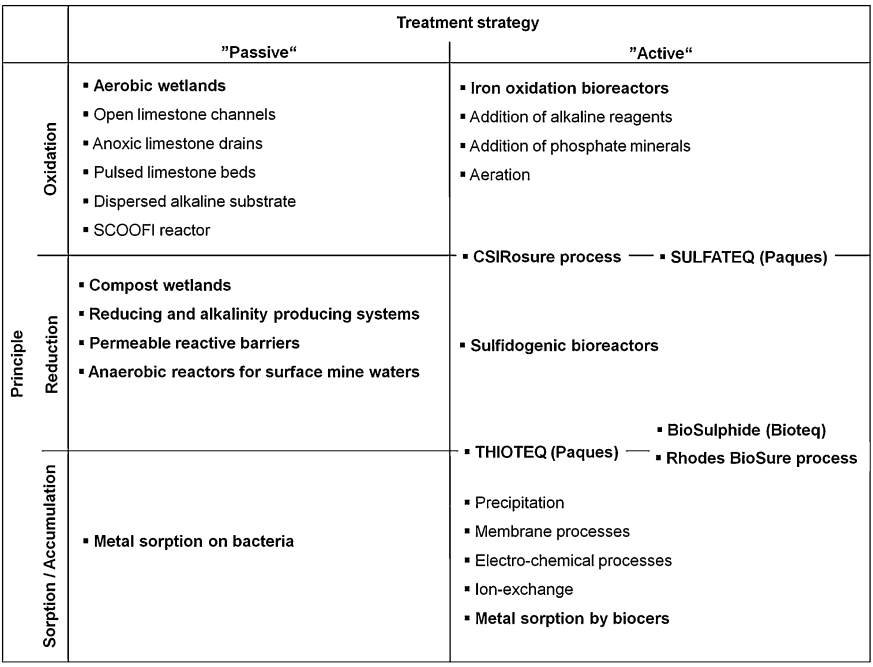

Fig. 1 Strategies to treat mine waters using oxidative, reductive, and accumulation processes. Biotic remediation techniques are marked in *bold*

last three decades, a number of bioreactor designs have been developed, which aim at higher efficiency and better rates achieved by natural microbial sulfate reduction; therefore, this represents an inexpensive approach. These bioreactor-based approaches are defined here as active biotic treatment systems because they also offer control of the process but, again, only in exchange of higher capital and operational costs. In contrast, passive treatments utilize natural water flow, chemical and biological processes to reduce dissolved metal concentrations and to neutralize acid [113, 124, 156]. Consequently, the running costs for such approaches are below those associated with active treatment technologies [124].

3 Abiotic Remediation

The common active abiotic strategy to remove metals from mine waters is neutralization by addition of neutralizing chemicals or dissolution of limestone [225]. Since the beginning of the twentieth century, AMD has been treated by addition of alkaline reagents (e.g. lime, calcium carbonate, sodium carbonate, sodium hydroxide) [9, 56, 226]. Consequently, the pH increases and the chemical oxidation of ferrous iron is accelerated. Ferric iron then precipitates as iron oxides and

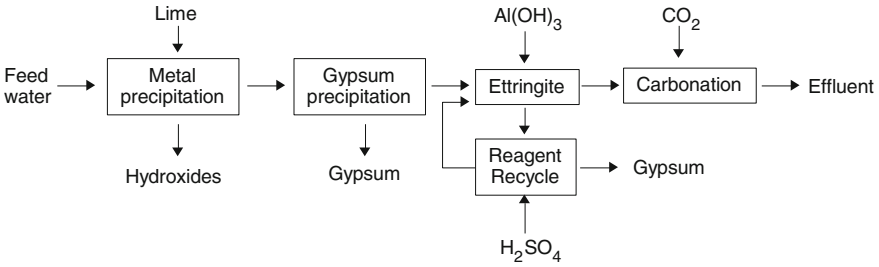

Fig. 2 Basic scheme of the SAVMIN process (based on [246])

iron hydroxides, which are contaminated with metals from the solution resulting from co-precipitation as carbonates and hydroxides or adsorption to the iron precipitate [7, 8]. The oxidation rate, especially for the removal of iron, can be improved by aeration using venture, cascade, or trickle filter; by mechanical aeration; or by addition of oxidants (e.g. calcium hypochlorite, hydrogen peroxide, potassium permanganate) [225, 281]. Due to its high water content and low density, the iron sludge is voluminous [42, 163] and chemically unstable, resulting in the release of adsorbed metals [8, 42, 163]. Therefore, the sludge has to be disposed or recycled so that possibly released heavy metals do not affect the environment. The disposal results in an increase of the operational cost of the mine water treatment [8, 42].

The application of lime to raise the pH of the AMD water not only leads to the precipitation of metals but also of sulfate (gypsum precipitation):

$$Na_2SO_4 + Ca(OH)_2 \rightarrow CaSO_4 + 2NaOH \quad (3)$$

Despite the relatively low costs of lime, this broad range application, however, only achieves the saturation state of gypsum in the treated water (depending on the ionic strength of the solution between 1.6 and 1.8 g/l; [35]). The problem hereby is that the solubility of gypsum is several times the concentration permitted by national (e.g. Germany, Austria, USA) and European law (250 mg/l) for freshwater bodies. Water with such sulfate concentrations is also unsuitable for reuse as process water or in cooling systems due to the corrosive effects of the residual sulfate.

To overcome the problem of high residual gypsum concentration after precipitation, different alternative processes, such as the cost-effective sulfate removal process or SAVMIN (Fig. 2) process, were developed. Both treatment technologies are based on ettringite ($Ca_6Al_2(SO_4)_3(OH)_{12}{\cdot}26H_2O$) precipitation at alkaline pH (11, 12), which is achieved through the addition of inorganic aluminum compounds and lime to AMD water. Because ettringite has a low solubility in aqueous solutions, both processes lead to sulfate concentrations of <200 mg/l [110, 246].

Another approach, which exploits the precipitation of poorly soluble sulfate compounds and also leads to the remaining sulfate concentration (0.24 mg/l) being within the legal limits, uses soluble barium salts (barium chloride, barium carbonate, barium sulfide) to form barite ($BaSO_4$) [103, 161]. However, barium sulfate

precipitation results in additional costs due to the toxicity of barium salts and the associated need for appropriate disposal or thermal regeneration and recovery of the barium from the waste products [110, 246].

In an alternative to limestone dissolution, metals are removed actively by addition of other chemical reagents. Fly ash has been successfully tested in preliminary studies to remove lead, copper, zinc, cadmium, nickel, cobalt, molybdenum, and chromium [11]. In laboratory studies, the use of fly ash in combination with carbon dioxide has been shown as promising treatment strategy, because this lead to an increase in pH and a decrease in the concentrations of various metals occurring in AMD [211]. Simultaneously, this approach may also be used for carbon dioxide sequestration [211].

Other active treatment technologies are based on pressure-driven membrane processes which, in combination with appropriate membranes, can be used for the separation of particles or ions of various sizes: *reverse osmosis* (in combination with membranes separating particles or ions <0.001 μm), *nanofiltration* (0.001–0.01 μm), *ultrafiltration* (0.01–0.1 μm), and *microfiltration* (0.1–0.45 μm). Furthermore, because these membranes can be selective for both cations and anions, these techniques are applicable to both metal and sulfate removal from AMD [70, 195, 225, 255]. However, a major challenge associated with the use of membrane processes for the treatment of highly polluted waters is the potential for fouling and scaling on the surface of the membranes. Therefore, membrane techniques in general require pretreatment of the mine water (i.e. filtration) to remove particulate matter and adjustment of the pH [41, 176, 181]. The continuous application of pressure as the driving force is the main contributor to the relatively high operating costs for membrane-based processes. Additionally, the resulting concentrated solutions still require further treatment [166]. To overcome the limitations of conventional membrane-based techniques, modifications have been made to the original process leading, for example, to the development of the seeded reverse osmosis process and the slurry precipitation and recycle reverse osmosis process; apart from their lower energy consumption, these approaches also require little to no pretreatment of the water (Fig. 3). To specifically counteract scaling in electrodialysis, the process was modified. The major modification was the regular change of the polarity of the electrodes in the system, therefore changing the direction of ion flow, which, in turn, releases ions that accumulate on the membrane surface (electrodialysis reversal).

Modern approaches for ion exchange processes use anionic, cationic, and chelating ion exchange resins (organic polymers) or inorganic resins (zeolites) with various active groups for the exchange of ions. The problems caused by scaling at high concentrations of Ca^{2+} and SO_4^{2-} have partially been overcome through the development of the GYP-CIX process, which removes Ca^{2+} and other cations by binding to a selective resin in a first step, followed by treatment of the remaining solution with a resin for the binding of anions, thus also removing SO_4^{2-}. This approach therefore avoids precipitation and accumulation of gypsum on the resins. Regeneration of the resins for cations (mainly calcium) and anions (mainly sulfate) results in precipitation of gypsum due to the use of sulfuric acid

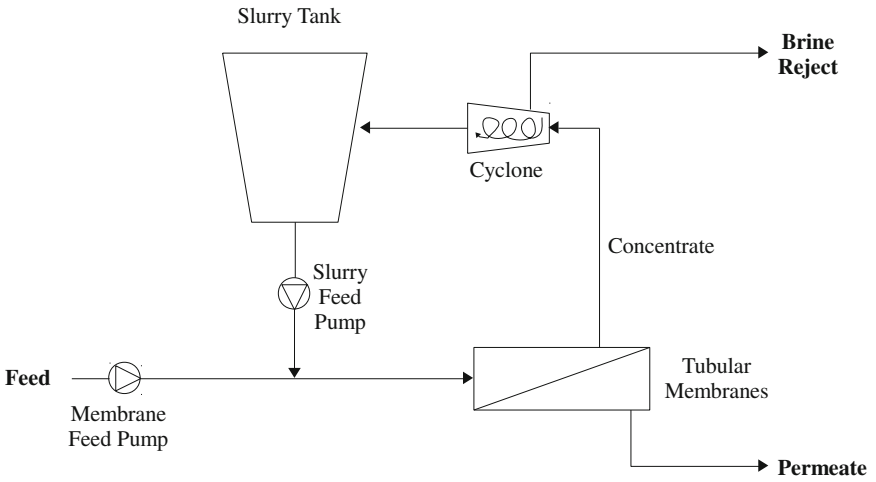

Fig. 3 Basic Scheme of the slurry precipitation and recycle reverse osmosis process (based on [126])

(resin for cation exchange) and calcium hydroxide solution (resin for anion exchange), respectively [149]. This approach has already been demonstrated to be successful at pilot plant scale with the sulfate load of the effluent of the Berkeley Pit (Butte, Montana, USA) being reduced from 8,000 to 200 mg/l [203]. Further modifications of the GYP-CIX process aimed at better removal of dissolved metals and precipitated minerals during pretreatment of the water, which, as a consequence, increases the loading capacity of the ion exchange resin and, again, also protects it against scaling (e.g. [70, 155]).

Although membrane and ion-exchange processes produce water of high purity, both approaches also lead to large volumes of concentrated mine water, which again results in additional costs for further treatment or disposal. This, in turn, limits their application in mining due to the combination of high operational costs and large volumes of acidic drainage water.

A variety of other methods, which originally were developed for the treatment of industrial waste waters, may also prove applicable to counter high sulfate concentrations of AMD waters or as an additional step to some of the above outlined approaches (e.g. precipitative softening reverse osmosis, enhanced membrane systems, two-pass nanofiltration, forward osmosis, capacitive deionization). However, experimental tests for such an application have not yet been performed [27, 35, 245].

Generation of alkalinity by dissolution of limestone has been used in passive oxidative remediation strategies since the 1970s [186, 187]. The basic principle of this process is the channeling of mine waters through a bed of crushed limestone, resulting in an increase of the pH and in the acceleration of the chemical iron oxidation [286]. In open limestone channels (OLC), ferric iron formed precipitates on the limestone, which may reduce the efficiency up to 42 % and thus may

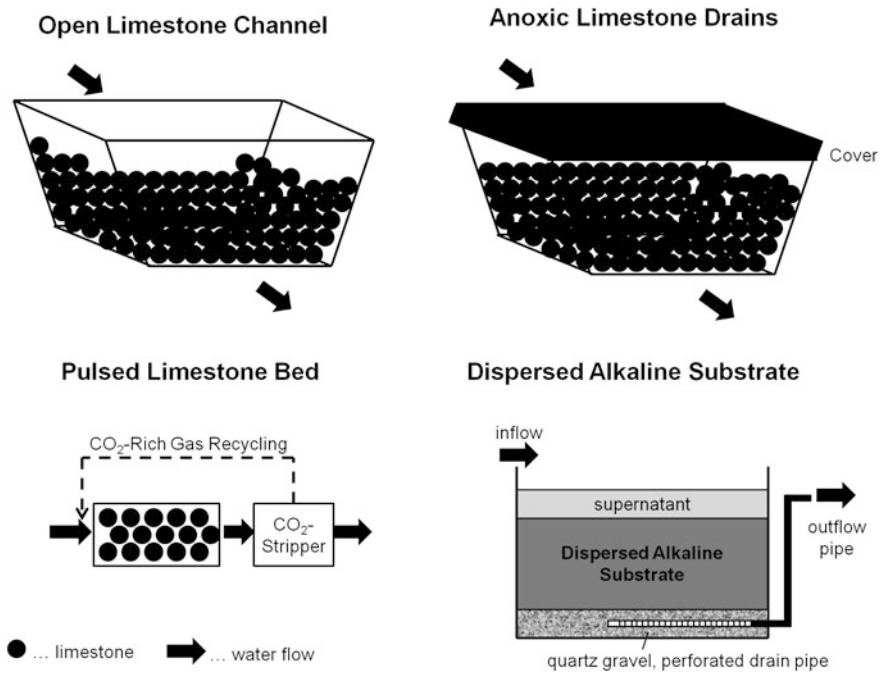

Fig. 4 Schematic diagrams of passive treatment systems for abiotic remediation of acid mine drainage (modified from [205, 225, 259])

prevent further reactions and dissolution of the limestone [93, 285, 286]. To overcome the coating of limestone with ferric precipitates in OLCs, anoxic limestone drains (ALD), pulsed limestone bed (PLB), and dispersed alkaline substrate (DAS) strategies have been developed (Fig. 4) [93, 206, 224, 259]. Due to the coverage of the limestone channel with plastic and impermeable soil or sediment, in ALD anoxic conditions are achieved; thus ferrous iron remains in solution and encrustation of limestone with ferric iron phases is avoided [224]. Consequently, ALDs are not suitable for the treatment of AMD waters with elevated concentrations of ferric iron, aluminum, and oxygen [202, 222, 260]. In the PLB process, mine water is aerated with carbon dioxide. Consequently, the acidity of the water is increased and the limestone dissolution is accelerated, which minimizes the coating of the limestone [93, 259]. The DAS is a fine-grained reagent (e.g. calcite) mixed with a coarse inert matrix (e.g. wood chips). The small grain size prevents a passivation, because the grains are dissolved before a thick layer of precipitate is formed [205, 206].

Another abiotic passive oxidative approach to reduce the ferrous iron concentration is the surface-catalyzed oxidation of ferrous iron described by Younger [279]. Ferrous iron is adsorbed on ferric hydroxides occurring in mine waters and is oxidized in situ due to dissolved oxygen [279].

In contrast to abiotic remediation strategies, bioremediation has only more recently been the subject of research and development. The selection of biotic processes for the remediation of the high concentrations of metals and sulfate in AMD is dictated by the specific chemistry of the pollutants. As outlined previously, the main pollutants, iron and sulfur, tend to occur in their most mobile forms—reduced (ferrous iron) and oxidized (sulfate) forms, respectively [231]. Transferring them into their oxidized (ferric iron) and reduced (sulfide) states, respectively, will decrease their solubility in AMD which, in turn, leads to precipitation and removal of iron containing minerals and metal sulfides. From this follows that bioremediation using oxidative and reductive metabolic activities of microorganisms utilizing iron and sulfate to gain energy, respectively, represents a sensible strategy. The application of the process for bioremediation will be discussed in the following sections.

4 Biotic Remediation Using Oxidative Processes

Ferrous iron oxidation by acidophilic iron-oxidizing bacteria is the major oxidative process in AMD waters [80, 90, 124]. Secondly, arsenic oxidation is obviously mediated microbially as well, because *Thiomonas* spp., which are able to oxidize arsenite and reduced sulfur compounds, were isolated from mine waters [21, 22, 53, 124]. Subsequently, the formed arsenate may be adsorbed on ferric minerals present in AMD [6, 44].

4.1 Microbial Iron Oxidation

The oxidation of ferrous iron to ferric iron is dependent on the concentrations of protons (pH) and dissolved oxygen (4). Although ferrous iron is chemically stable in strongly acidic, oxygen-saturated waters, abiotic iron oxidation is fast in oxygen-saturated waters with circumneutral pH [221]. The oxidation rate is first-order with respect to ferrous iron and oxygen concentration and second-order with respect to the concentration of protons [231]. Additionally, the oxidation rate ($k = 3 \times 10^{12}$ mol/l/min at 20 °C) depends on the temperature (5) [231].

$$4Fe^{2+} + O_2 + 4H^+ \rightarrow 4Fe^{3+} + 2H_2O \tag{4}$$

$$\frac{-d\left[Fe^{2+}\right]}{dt} = \frac{k * \left[O^2\right] * \left[Fe^{2+}\right]}{\left[H^+\right]^2} \tag{5}$$

At pH $\leq$ 3.5 and at interfaces of aerobic and anoxic zones with neutral pH, ferrous iron is oxidized by acidophilic iron-oxidizing microorganisms (e.g. *Acidithiobacillus ferrooxidans*) or by microaerophilic neutrophilic iron-oxidizing bacteria such as *Gallionella ferruginea* and *Leptothrix* spp., respectively [99, 138].

Recently, iron oxidation mechanisms of iron-oxidizing microorganisms were reviewed by Bonnefoy and Holmes [39] and Ilbert and Bonnefoy [109]. The pathway of microbial iron oxidation has not yet been identified for all iron oxidizers, but it seems to differ between neutrophilic and acidophilic iron-oxidizing microorganisms as well as within both groups. The model of iron oxidation of the well-known acidophilic iron oxidizer *At. ferrooxidans* suggests an electron transfer from ferrous iron via cytochrome c and the copper-bearing protein rusticyanin A through the periplasm to the cytochrome aa_3-oxidase in the inner membrane. There, electrons are used for the reduction of oxygen to water [111, 174, 197]. The energy yield from iron oxidation is low [111, 174]. Kelly [136] determined that *At. ferrooxidans* has to oxidize 71 mol of ferrous iron to fix 1 mol of carbon dioxide.

Furthermore, the growth of acidophilic iron-oxidizing bacteria may be limited by the availability of essential nutrients in mine-impacted waters. Phosphate occurs only in traces in acidic mine waters [13, 257], because phosphate precipitates as iron phosphates or is adsorbed to ferric minerals present [10, 231]. The resulting low bioavailability of phosphate manifests itself as a reduction of the growth rate, of the iron oxidation rate, and of the CO_2 fixation rate as described for *At. ferrooxidans* [215, 201].

Most of the microorganisms living in AMD are autotrophs; thus, CO_2 is an essential nutrient. Due to the poor solubility of CO_2 under acidic conditions, acidophilic iron-oxidizing bacteria may be limited by CO_2 under atmospheric conditions [231]. The growth rate of *At. ferrooxidans* and thus the oxidation rate have been shown to be enhanced by aeration with CO_2-enriched air [17, 89].

A third important nutrient is nitrogen. Mine waters are often nitrate-rich as a consequence of the use of N-based explosives in former mining activities [13]. The growth of iron-oxidizing bacteria has been reported to be inhibited by elevated nitrate concentrations [37, 242]. The more important nitrogen source is ammonium. A lack of ammonium can result in a reduction of bacterial activity [243]. Due to the absorption of ammonia from the atmosphere by acidic media, traces of ammonia will often be present in acid mine waters [242] and may be sufficient for iron oxidation [243].

Limiting effects of nutrients, such as the ones outlined here, should be considered with respect to the performance of biotic treatment techniques. In some cases, a greater availability of the nutrients may increase the efficiency of the bioreactors (described in Sect. 4.3).

4.2 Aerobic Wetlands

Net alkaline mine waters can be treated passively in aerobic wetlands, which consist of a shallow trench planted with macrophytes (Fig. 5) [97, 279]. Although abiotic iron oxidation is accelerated at near-neutral pH [221], iron oxidation may be increased by the presence of biological components by a factor of 10 [262]. The neutrophilic iron-oxidizing bacteria oxidize iron at interfaces of aerobic and

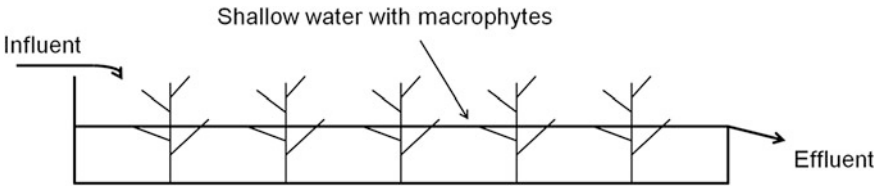

Fig. 5 Schematic diagram of aerobic wetland (from [279])

anaerobic zones (e.g. *G. ferruginea*) or use organically complexed iron (*Leptothrix* spp.) [124]. As a result of the plantation of macrophytes (e.g. *Phragmites australis*, *Typha latifolia*, *Juncus effusus*), the operation of the aerobic wetland is improved. In addition to achieving a regulation of the water flow, the iron oxidation is enhanced by the oxygen flow to the roots and by iron uptake of the macrophytes [23].

In addition, iron arsenic is removed in aerobic wetlands as arsenate by adsorption to ferric minerals formed [6, 44, 91] or by the formation of scorodite ($FeAsO_4$) [124].

4.3 Iron Oxidation Bioreactors

Ferrous iron is chemically stable at pH $\leq$ 3.5; thus, iron occurring in acidic mine waters can be removed biotically [221]. Therefore, iron oxidation bioreactors were developed as an active treatment approach. Due to the low energy yield by ferrous iron oxidation and the associated low cell yield, immobilization of bacterial cells on a solid matrix has been considered. In the last decade, various materials have been tested concerning their suitability as a support matrix. Most of the studies cultivated *At. ferrooxidans* in packed-bed bioreactors (Fig. 6). Using various materials as the matrix, bacterial cells were immobilized successfully and iron was oxidized with removal efficiencies of up to 99 % (Table 2).

However, the various studies are hardly comparable, because different process parameters such as medium, iron concentration of the medium, and retention time were used. The studies revealed that the oxidation rate and the removal efficiency are dependent on the material of the matrix, the iron concentration, and the microorganisms used. Via cultivation of *At. ferrooxidans* in packed-bed bioreactors filled with activated carbon, 95 % of ferrous iron was removed with an oxidation rate of 52 g/l/h, which is the maximum oxidation rate reported among the papers cited in Table 2 [86]. Nemati and Webb [173] and Kahrisi et al. [128] have reported an inhibitory effect of elevated iron concentrations on the oxidation rate and the removal efficiency. Furthermore, microorganisms have different oxidation capacities under given process conditions [207]. The isolate particular bacterial strain of Ferrovum, for example (which is related to the new, currently not validly described species "*Ferrovum myxofaciens*"), had higher oxidation rates in packed-bed bioreactors than *At. ferrivorans* NO37 (formerly assigned to *At. ferrooxidans*) under

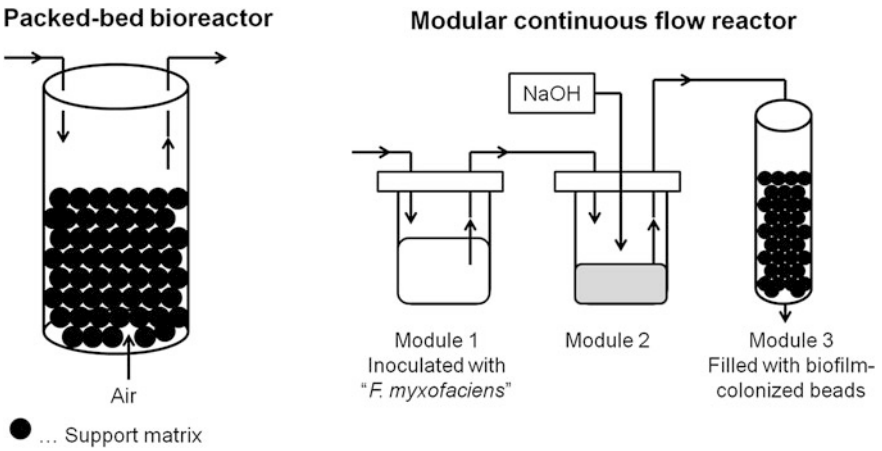

Fig. 6 Schematic layout of packed-bed bioreactors (adapted from [207]) and schematic construction of the modular continuous flow reactor suggested by Hedrich and Johnson [100]

comparable conditions [207]. Thus, new iron-oxidizing bacteria might have a greater potential for remediation of AMD than the well-known *At. ferrooxidans*. Mixed cultures containing various iron-oxidizing microorganisms seem to be more efficient for mine water treatment than pure cultures [207].

The approaches outlined previously have been tested in laboratory scale. At the opencast pit Nochten (Lusatia, Germany), a plant to treat mine waters via microbial iron oxidation with subsequent ferric iron precipitation has been realized on a pilot scale (Fig. 7) [80, 101]. The plant was constructed to pretreat the acidic mine water and thus to lower the amount of lime and costs for conventional treatment [101]. The biological iron oxidation is mediated by a microbial community from mine water dominated by "*Ferrovum*" spp. and *Gallionella* relatives [101, 102]. In this pilot plant, ferric iron precipitates mainly as the iron-oxyhydroxsulfate schwertmannite [98]. Because this mineral contains sulfur and (Leerzeichen fehlt) iron, the concentration of sulfate in the water is lowered to some extent. Depending on retention time and sulfate concentration in the inflow, 10–90 % of iron and up to 10 % sulfate are removed in the process [102, 114]. To improve the efficiency, schwertmannite formed in the plant could be recirculated to increase the oxidation capacity of the pilot plant, because viable cells have been detected in schwertmannite precipitated on carrier material occurring in the plant and in stored schwertmannite [115, 236]. A further strategy to increase the oxidation capacity is to overcome the low bioavailability of phosphate by phosphate addition [237].

As an alternative to packed-bed bioreactors, Hedrich and Johnson [100] suggested a modular flow system for the remediation of AMD (Fig. 6). The first module of the system is a continuous bioreactor inoculated with a "*Fv. myxofaciens*" culture from mine water of an abandoned copper mine in northwest Wales. Approximately 80 % of the ferrous iron was oxidized after the first step. In the second module, the pH of the mine water was raised from 2.3 to 3.5 by the addition of sodium hydroxide

Table 2 Comparison of process parameters of several iron oxidation bioreactor systems described in the literature

Bioreactor type	Support matrix	Microorganism used	Medium	pH	$[Fe^{2+}]_{in}$ (g/l)	Reactor volume (l)	Retention time (h)	Oxidation rate (g/l/h)	Removal efficiency (%)	Reference
Packed-bed	Calcium alginate	*At. ferrooxidans*	Synthetic medium	1.5	6.7	0.04	5.5	0.6	90	[145]
Packed-bed	Glass beads (710–1,180 μm)	*At. ferrooxidans*	Synthetic medium	1.3	6.7	0.05	3.3	3.5	~60	[86]
	Glass beads (425–600 μm)	*At. ferrooxidans*	Synthetic medium	1.3	6.7	0.05	10	8.1	~90	
	Ion-exchange resin	*At. ferrooxidans*	Synthetic medium	1.3	6.7	0.05	0.5	29.3	~70	
	Activated carbon	*At. ferrooxidans*	Synthetic medium	1.3	6.7	0.05	0.7	52	~95	
Circulating packed-bed	Polyurethane foam	*At. ferrooxidans*	Synthetic medium	2.3	8.8	0.4	3.2	1.6	60	[5]
Trickle packed-bed	Polyurethane foam	*At. ferrooxidans*	Synthetic medium	2.3	8.8	0.25	1.1	4.4	~55	[117]
Packed-bed	Polyurethane foam	*At. ferrooxidans*	Synthetic medium	1.7	5	0.45	0.2	34.3	~40	[174]
	Polyurethane foam	*At. ferrooxidans*	Synthetic medium	1.7	10	0.45	0.2	32	~20	
	Polyurethane foam	*At. ferrooxidans*	Synthetic medium	1.7	20	0.45	0.4	10	~10	
Packed-bed	Nickel alloy fiber	*At. ferrooxidans*	Synthetic medium	1.4–2.0	8.8	1.35	4	0.5	93	[81]
Packed-bed	Sand	*At. ferrooxidans*	Synthetic medium	2	8.8	0.188	1.5	0.3	95–99	[278]
Packed-bed	PVA	*At. ferrooxidans*	Synthetic medium	1.7	8.8	0.365	2.5	3.1	95	[154]

(continued)

Table 2 (continued)

Bioreactor type	Support matrix	Microorganism used	Medium	pH	$[Fe^{2+}]_{in}$ (g/l)	Reactor volume (l)	Retention time (h)	Oxidation rate (g/l/h)	Removal efficiency (%)	Reference
Packed-bed	PVA-boric acid	*At. ferrooxidans*	Synthetic medium	1.7	8.8	0.365	3.6	1.9	97	[155]
Packed-bed	Polyethylene	*At. ferrooxidans*	Synthetic medium	2–2.2	33.4	2	2.5	2.9	>95	[168]
Packed-bed	Ceramic beads	*At. ferrooxidans*	Synthetic medium	1.6	13.9	0.22	1.3	4.2	70	[127]
Packed-bed	Chitosan	*At. ferrooxidans*	Synthetic medium	1.8	8.8	0.1	6.7	1.1	~85	[76]
	Chitosan cross-linked	*At. ferrooxidans*	Synthetic medium	1.8	8.8	0.1	5	1.5	~85	
Packed-bed	Porous glass beads	*At. ferrivorans*	Synthetic AMD water	2.0	8.8	0.5	3.7	1.4	98–99	[209]
	Porous glass beads	*L. ferrooxidans*	Synthetic AMD water	2.0	8.8	0.5	3.7	1.5	98–99	
	Porous glass beads	*Ferrimicrobium* sp.	Synthetic AMD water	2.0	0.3	0.5	3.7	1.6	98–99	
	Porous glass beads	*"Fv. myxofaciens"*	Synthetic AMD water	2.2	0.3	0.5	3.7	1.6	98–99	
	Porous glass beads	*At. ferrivorans*	Synthetic AMD water	2.0	0.3	0.5	0.8	1.0	98–99	
	Porous glass beads	*L. ferrooxidans*	Synthetic AMD water	2.0	0.3	0.5	0.8	1.0	98–99	
	Porous glass beads	*Ferrimicrobium* sp.	Synthetic AMD water	2.0	0.3	0.5	0.8	1.0	98–99	
	Porous glass beads	*"Fv. myxofaciens"*	Synthetic AMD water	2.2	0.3	0.5	0.8	1.7	98–99	

(continued)

Table 2 (continued)

Bioreactor type	Support matrix	Microorganism used	Medium	pH	$[Fe^{2+}]_{in}$ (g/l)	Reactor volume (l)	Retention time (h)	Oxidation rate (g/l/h)	Removal efficiency (%)	Reference
Packed-bed	Monolith	*At. ferrooxidans*	Synthetic medium	1.6	3.5	0.5	0.5	6.7	~95	[128]
	Monolith	*At. ferrooxidans*	Synthetic medium	1.6	6	0.5	0.5	7	~60	
	Monolith	*At. ferrooxidans*	Synthetic medium	1.6	16	0.5	12.5	0.9	~70	
	Monolith	*At. ferrooxidans*	Synthetic medium	1.6	21.3	0.5	14	0.6	~40	
Pilot-plant Nochten	Plastic straps	Iron-oxidizing community	Mine water	3.0[a]	0.3–0.4	10,000	7–8	0.01–0.03	10–90	[102]
Continuous stirred-tank of a modular system (module 1)	–	*"Fv. myxofaciens"*	Synthetic AMD water	2.3	0.3	1.5	2	0.1	~80	[100]

[a] Operating pH of the system

Synthetic medium contained mineral salts and iron

AMD acid mine drainage, *PVA* polyvinyl alcohol boric acid

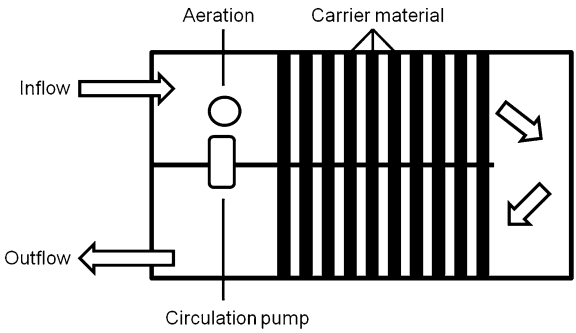

Fig. 7 Schematic diagram of the mine-water treatment pilot plant located at the open-cast pit Nochten (Germany) (adapted from [101])

and of a flocculating agent, resulting in the precipitation of ferric iron formed in module 1. The residual ferrous iron was eliminated by in situ oxidation mediated by "*Fv. myxofaciens*" and subsequent ferric iron precipitation in a trickle packed-bed bioreactor as module 3 [100].

One major problem of iron oxidation bioreactors is the blockage of the system as a consequence of the formation of ferric minerals. One possibility to antagonize the blockage is that packed-bed bioreactors are performed at a pH value <1.5 to minimize the amount of the ferric mineral formed (e.g. [86]). A further strategy has been realized in the treatment plant located at the openpit Nochten. A chain cleaner has been installed to remove the schwertmannite from the bottom of the plant [115].

In contrast to iron sludge formed during the abiotic treatment, schwertmannite can be dewatered relatively easily and used for industrial applications as pigment for dyes or ceramics [14]. Due to its potential to adsorb metals, metalloids, phosphate, and fluoride, schwertmannite can be applied in mine water treatment as adsorbent as well [65, 66, 116, 200, 263]. It has been shown to eliminate arsenate, cadmium, chromate, copper, lead, and zinc from mine water by co-precipitation or adsorption [44, 200, 263].

5 Biotic Remediation Using Reductive Processes

Apart from precipitation and physical removal, only reductive processes are available to eliminate sulfate from mine water due to the fact that sulfate is the most oxidized form of sulfur. The biological sulfate reduction by prokaryotic microorganisms provides an approach that can be operated at relatively low costs [124]. In essence, the process uses microorganisms that cover their energy demand from redox reactions whereby sulfate serves as the electron acceptor, thus being eliminated from the medium or mine water (6). Moreover, this process also consumes protons while producing alkalinity and hydrogen sulfide (H_2S or HS^-), which leads to neutralization of the acidic pH and the precipitation of heavy metals (e.g. iron, copper, zinc, cadmium, lead, mercury, nickel, molybdenum) and

metalloids (arsenic, antimony) from the mine water in form of metal sulfides (MS; Eq. 7).

$$SO_4^{2-} + 8e^- + 8H^+ \rightarrow H_2S + 2H_2O + 2OH^- \quad (6)$$

However, it should be noted that the precipitation of metal sulfides may again lead to the release of protons.

$$M^{2+} + HS^- \leftrightarrow MS(s) + H^+ \quad (7)$$

Nevertheless, in practice the net change in acidity is directed towards higher pH and increasing alkalinity which, in turn, leads to additional precipitation of metals through the formation of metal hydroxides (e.g. in the case of aluminum and iron) or as carbonates (e.g. in the case of zinc, manganese, and iron). However, hydroxide and carbonate precipitation of metals represents only a minor fraction as compared to precipitation as metal sulfides; therefore, it has not yet been evaluated as an alternative for the targeted removal of metals by reductive processes.

For the selection of an appropriate treatment option, the efficiency of the process should be evaluated on the basis of a number of criteria, in particular the chemistry of the influent (the mine water) and effluent water and the prioritized aim of the treatment [54, 124]. Other factors that should be considered when choosing a treatment option are: (i) the characteristics of solid byproducts (sludge), such as their volume, toxic potential, long-term stability, and disposal cost; (ii) the availability of necessary operational resources (e.g. chemicals, energy); (iii) the possible recovery of valuable products (e.g. metals); (iv) capital and operation costs; (v) necessary land surface area for the treatment plant (especially in the case of passive treatment systems, such as constructed wetlands); (vi) performance criteria of the process (e.g. the volumes of water that can be treated by the technology); (vii) reliability, long-term stability, and expandability of the process; (viii) personnel requirements; and (ix) legal regulations [54, 124].

If the prevailing conditions are suitable for the application of biological sulfate-reducing processes, the decision between active and passive treatment options still remains. Although active methods are mostly implemented using bioreactors, passive methods use naturally occurring processes in so-called constructed wetlands.

5.1 Basic Aspects of Microbial Sulfate Reduction

Detailed reviews on the basics of biological sulfate reduction and its application in treatment processes have been published previously (e.g. [18, 94, 124, 141, 147, 171, 219]). Therefore, only a brief summary of the most important aspects are provided in the following.

Prokaryotic microorganisms capable of reducing sulfate have been discovered among both *Bacteria* and *Archaea,* whereby bacteria represent all but one of the 40 genera of known sulfate-reducing prokaryotes (SRPs). Overall, the currently

known SRPs belong to four bacterial and two archaeal phyla [171]: (i) the gram-negative, mesophilic *Proteobacteria,* whereby all of the known SRP are part of the *Deltaproteobacteria* (e.g. *Desulfobacter postgatei*, *Desulfovibrio desulfuricans*), (ii) the gram-positive endospore-forming *Firmicutes* (e.g. *Desulfotomaculum acetoxidans*, *Desulfosporosinus orientis*), (iii) the thermophilic *Thermodesulfobacteria* (e.g. *Thermodesulfobacterium commune*), (iv) the *Nitrospirae* (the thermophilic species of the genus *Thermodesulfovibrio*), (v) the thermophilic and hyperthermophilic *Crenarchaeota* (e.g. *Thermocladium modestius* and *Caldivirga maquilingensis*, respectively),and (vi) the hyperthermophilic *Euryarchaeota* (e.g. *Archaeoglobus fulgidus*, *A. veneficus*).

Sulfate-reducing microorganisms of these phyla also differ in terms of their capacity to oxidize organic substances either completely to CO_2 (e.g. *Desulfobacter, Desulfonema, Desulfobacterium*) or incompletely mainly to acetate (e.g. *Desulfomicrobium* and the majority of the genera *Desulfovibrio* and *Desulfotomaculum*: [78, 171]. The end product of the dissimilatory reduction of sulfate is, however, in all cases the fully reduced form of sulfur, hydrogen sulfide.

The dissimilatory reduction of sulfate is a multistep biochemical process. Following the active uptake of sulfate into the cell by primary and secondary transport systems, sulfate is activated in an adenosine triphosphate (ATP)-consuming step by the ATP sulfurylase, whereby the intermediate adenosine-5-phosphosulfate (APS) is formed:

$$SO_4^{2-} + ATP \rightarrow APS + PP_i \quad (8)$$

This energetically costly step is necessary because sulfate is a relatively inert molecule with an $E^{\circ\prime}$ of −516 mV for the redox couple sulphate–sulphite; thus, it is too negative for the intermediary electron providers NADH ($E^{\circ\prime} = -314$ mV) or ferredoxin ($E^{\circ\prime} = -398$ mV) that seem to be widely distributed among sulfate-reducing bacteria [171]. APS is then reduced to sulfite by the APS reductase, which then is reduced by a dissimilatory sulfite reductase to sulfide, though details of this process are still to be resolved [171]. The energy yielded by this process (sulfate reduction with lactate as electron donor: $\Delta G_o' = -80$ kJ/mol) is small compared to aerobic respiration (e.g. aerobic oxidation of glucose: $\Delta G_o' = -1{,}140$ kJ/mol), but typically (depending on the substrate and the active species) higher than that obtained from methanogenesis (e.g. for acetoclastic methanogenesis: $\Delta G_o' = -31$ kJ/mol methane, for the reduction of carbon (dioxide) with hydrogen as electron donor: $\Delta G_o' = -135$ kJ/mol methane). The latter is important for the application of SRP in bioremediation because it provides SRP with the metabolic potential to outcompete methanogenic archaea, which in some cases are able to use the same substrate [171].

In the absence of sulfate, SRP may also use other compounds as electron acceptors, such as elemental sulfur, thiosulfate, sulfite, polythionates, polysulfides, fumarate, nitrate, dimethylsulphoxide, or metal ions such as Mn(IV), Fe(III), UO^{2+} or As(V) [147, 157, 158, 179], whereby organic molecules are oxidized (e.g. pyruvate is transformed to acetate, CO_2 and H_2) [78].

Both autotrophic and heterotrophic lifestyles are known among SRPs with CO_2 (autotrophic) or a range of organic compounds (heterotrophic) as the source of carbon (for review, see [150]. Examples of electron donors and carbon sources that have been analyzed in terms of their suitability for the application of microbial sulfate reduction for bioremediation are lactate, ethanol, acetate, glucose, corn steep [228, 269], ethanol, glycerol [140], methanol [79], complex organic substrates such as animal manure, mushroom compost, and saw dust [283], synthesis gas [239, 250], and activated sludge [43]. Industrial waste products such as tannery effluent [40], glycerol-methanol waste from biodiesel production [284], and dairy waste water [273] were also tested as alternative electron donors and carbon sources. For the purpose of comprehensiveness, it should be mentioned in this context that autotrophic growth by hydrogen-utilizing species solely with CO_2 as the source of carbon is not always possible because some also require an additional organic carbon source for growth [33, 270]. For example, Widdel [270] showed in experiments with *Desulfovibrio* species that only one-third of the cell mass originated from CO_2, whereas two thirds were derived from the additionally added acetate.

The natural environment of SRPs is typically that near the transition zone between anaerobic and aerobic conditions or anaerobic environments, such as those provided by marine and fresh water sediments, moorlands, anaerobic sewage sludge, thermal springs, and oil reservoirs [147, 179]. There, SRPs are usually associated with microorganisms possessing a fermentative metabolism, which provide appropriate electron donors for the SRPs (e.g. degraded organic compounds such as organic acids, ethanol, alkanes, aromatic compounds, or hydrogen). In the case of oxygen intrusion into the system, the microbial community that also harbors fermentative bacteria is then able to reduce the dissolved oxygen concentration due to the degradation of organic substrates, thus restoring anoxic conditions [150]. Moreover, because a number of SRPs are able to use hydrogen as an electron donor, they effectively reduce its concentration which, in turn helps the fermenting microorganisms to continue with their metabolic activity [147]. Living under anoxic conditions also avoids competition with faster-growing aerobic microorganisms for essential nutrients [58, 143].

Dissimilatory sulfate reduction of clonal cultures of SRPs is only known under anaerobic reducing conditions with a redox potential of approximately −100 to −200 mV [105, 118], although some species may tolerate oxygen for a short time period or are even able to live under microaerophilic conditions. There, oxygen or nitrate can serve as electron acceptors for members of the genus *Desulfovibrio*, but sustainable growth or sulfate reduction in the presence of dissolved oxygen has only been reported for mixed cultures with aerobically living microorganisms [210, 179].

One problem that impedes the application of sulfate-reducing microorganisms in the treatment of AMD waters is their sensitivity towards low pH [120, 140]. This can be caused by the influence of the pH on the inhibition potential of organic acids and the distribution of two sulfur species, the nondissociated $H_2S_{(aq)}$ and the dissociated HS^-or S^{2-} [105, 108, 141]: the lower the pH, the higher is the proportion

of the nondissociated form of hydrogen sulfide. The uncharged $H_2S_{(aq)}$ is able to penetrate the cell membrane, where it releases a proton due to the more neutral intracellular environment of the acidophilic SRP, thus leading to a damage to or even breakdown of the proton gradient [108]. The exact concentration of $H_2S_{(aq)}$ that can be tolerated by SRP is still unclear; various studies report a range from 40–80 mg H_2S/l to even 1,000 mg/l [247]. However, it seems that SRPs are less affected by high $H_2S_{(aq)}$ concentrations than methanogenic archaea [184], thus providing an advantage for SRPs over methanogens.

A possible reason for the reports on the observed wide range of tolerance limits towards $H_2S_{(aq)}$ may be provided by the fact that $H_2S_{(aq)}$ cannot be regarded as an isolated parameter since it also affects other constituents of AMD waters. For example, the presence of high concentrations of sulfide ions leads to the removal of dissolved metal ions due to the precipitation as metal sulfides. This may prove to either be beneficial or detrimental for microbial growth, depending on whether metal concentrations are toxic to SRPs (or, in the case of essential trace elements, limiting for SRPs). Furthermore, other factors, such as the type of sludge (containing SRPs) present in bioreactors, also seem to influence the tolerance of SRP towards $H_2S_{(aq)}$. For example, granular sludge seems to be less affected by $H_2S_{(aq)}$ than suspended sludge [108], which is likely to be a consequence of the sludge functioning as protective barrier between the SRP and the $H_2S_{(aq)}$.

Despite the fact that SRPs were the first anaerobic microorganisms (*Desulfovibrio desulfuricans*, formerly *Spirillum desulfuricans*) to be isolated [24], it was only as recent as the 1990s that microbial sulfate reduction was described in acidic environments (e.g. [38, 88, 217, 244]). Further reports on the isolation of acidophilic SRPs soon followed (e.g. [1, 3, 50, 137, 140, 142, 164]. However, successfully isolated pure strains from AMD waters are still rare or not validated and so far are limited to the bacteria [61].

Reasons for the rare detection of SRPs in AMD waters are thought to be associated with the lack of organic substrate and the competition with iron-reducing bacteria for electron donors [142].

5.2 Passive Treatment Systems

Alternative low-cost approaches to the abiotic systems mentioned previously may be provided by promoting the naturally occurring biological processes that lead to a removal of dissolved metals, sulfate, and acidity from the drainage water (Table 3). The treatment of AMD in biological passive systems is mainly based on processes that lead to an increase in the alkalinity and pH of the solution and to the removal of metals and/or sulfate.

Some of these passive treatment systems are intended to simulate natural wetlands and their natural potential for the remediation of polluted waters. The first examples of such constructed wetlands were reported in the early 1980s [238, 274]. Prerequisites for the successful performance of constructed wetlands

Table 3 Properties, applications, and main reactions of various treatment methods for metal- and/or sulfate-bearing waters

Passive system		Preferred water	Reactions
Anoxic limestone drain	Abiotic	Acidic water with high loads of ferrous iron	Increase of alkalinity and pH, precipitation and settling of metals in subsequentaerobic settling pond
Anaerobic wetland	Abiotic + biotic	Acidic water and/or high sulfate concentrations	Removal of oxygen, sulfate reduction, alkalinity addition
Reducing and alkalinity producing system	Abiotic + biotic	Acidic water	Removal of oxygen, increase of alkalinity and pH, precipitation and settling of metals in subsequent settling pond
Sulfate-reducing pond	Abiotic + biotic	Acidic and neutral water	Sulfate reduction, increase of alkalinity and pH, metal precipitation
Diversion wells	Abiotic	Acidic water with low dissolved metal content	Increase of alkalinity
Limestone channel	Abiotic	Acidic water	Increase of alkalinity and pH
Limestone sand	Abiotic	Acidic water, low dissolved metal content, small streams	Increase of alkalinity and pH
Settling pond	Mainly abiotic	Acidic[a] and alkaline water	Aeration, precipitation of metals, settling of suspended solids
Aerobic wetland	Abiotic + biotic	Neutral and alkaline water, not for high dissolved iron concentrations[b]	Aeration, precipitation of metals, settling/filtration of suspended solids, biosorption

Western PA Coalition for Abandoned Mine Reclamation, 2008; Wolkersdorfer and Bowell 274]

[a] To remove ferrous iron from acidic water, a pretreatment is necessary to increase pH of the water

[b] Precipitation of high amounts of iron concentration leads to a rapid filling of the pond

are, apart from the presence of SRPs, sufficient organic material, which forms the carbon source for respiring aerobic bacteria to consume dissolved oxygen. Additionally, organic material also provides substrate for fermenting microorganisms, whose metabolites can be used by the sulfate-reducing organisms as electron donors for the reduction of sulfate. A readily soluble or easily degradable organic substrate will be consumed fast, thus not ensuring a long-term supply. This, however, is easily overcome through the use of a mixture of quickly and slowly degradable organic substances.

Sufficient iron (or other metal ions) will be necessary for the sequestration of the resulting sulfide, although sulfides can also be retained as organic sulfide species (e.g. aryl sulfides, thiols, thiophenes) as is the case in low-iron systems, such as bogs or peat lands [181]. Thiols, for example, are formed by nucleophilic substitution of alkyl halides, alkyl sulfonates, or sulfates with hydrogen sulfides. The precipitation of dissolved metals (with a few exceptions, such as Al^{3+}) results in the liberation of protons (see Eq. 7) [280]. Because the reduction of sulfate produces more alkalinity (bicarbonate) than acidity, the generated protons are compensated for, thus maintaining net alkaline conditions [156]. Finally, metals are also removed by sorption and/or uptake by the vegetation.

As for the abiotic systems, the selection of a passive treatment approach is also dependent on the specifics of the mine water and of the site. However, apart from hydrochemical features, flow rate and local topography also need to be considered [96, 156, 190]. Extensive engineering guidelines for the construction of passive remediation systems have been compiled by the PIRAMID Consortium [190].

Passive systems using microbial sulfate reduction for the treatment of AMD waters also include the injection of appropriate substrates into the groundwater layer, permeable reactive barriers, infiltration beds, anoxic ponds, anaerobic wetlands, and anaerobic reducing and alkalinity producing systems (RAPS) or successive alkalinity producing systems (SAPS) [124, 275].

5.2.1 Substrate Injection, Reactive Barriers, and Funnel-and-Gate Systems

One approach to promote the growth and activity of sulfate-reducing bacteria in situ involves injection of organic substrates into the groundwater that is to be treated. It remains open for discussion as to whether such an approach should be assigned to the repertoire of passive treatment systems. However, it clearly involves an active introduction of chemicals into the underground, although no further control or input of energy is intended.

A pilot-scale application of the injection of organic substances was performed at the Skado dam in the lignite mining region of Lusatia (Germany). The sulfate- and iron-containing groundwater was channeled through a barrier, pumped up, mixed with glycerol, and reinjected into the underground to promote the growth and activity of SRPs for the removal of sulfate, the neutralization of the acidity, and the immobilization of dissolved iron as sulfide [27]. This application resulted in a complete neutralization of the groundwater, and sulfate removal rates of

30–60 % were achieved [27]. Other substrates for SRPs used for similar in situ applications were soybean oil and sodium lactate [189] and returned milk from a dairy farm [119]. The use of these substrates also leads to an increase of the pH and the reduction of sulfate and dissolved metals concentrations.

Reactive permeable barriers are underground installations set in the flow path of contaminated groundwater filled with reactive material appropriate for the specific application. Apart from the addition of limestone to increase the pH of the groundwater and to support the precipitation of dissolved metals [124], the reactive barrier may also be packed with organic material as a carbon source and electron donor for SRPs to gain energy and to produce sulfide for metal precipitation. Furthermore, dissolved oxygen can be minimized by the corrosive effect on zero-valent iron within the reactive barrier; this may also be relevant in cases of low-metal-containing water because the concentration of dissolved iron is increased, thus leading to an improved precipitation of biologically produced H_2S [62, 77, 235]. Further improvements to the remediation performance can be achieved through implementation of additional reactive barriers, as has been done for the remediation of AMD water from the uranium deposit in Curilo, Western Bulgaria [87].

An example of such a successful application is provided by the construction of a full-scale permeable reactive barrier to treat an acidic metal (200–1,000 mg/l Fe, max. 30 mg/l Ni) and sulfate (2,000–5,000 mg/l SO_4) bearing groundwater [26]. The nickel contamination originated from the Nickel Rim Mine Site (Sudbury, Ontario). Analyses of SRP in the reactive barrier, which consisted of municipal compost (20 vol%), leaf mulch (20 vol%), woodchips (9 vol%), gravel (50 vol%), and limestone (1 vol%), showed a 10,000-fold higher biomass and a 10-fold greater enzymatic activity compared to the upstream aquifer. Two years after the start of the treatment, 80 % of the iron content was removed, alkalinity increased from <50 to >500 mg/l ($CaCO_3$), and the concentration of nickel decreased to <0.05 mg/l.

However, it must be stressed in the context of in situ bioremediation approaches that they can only be judged as being successful if long-term stability of the precipitated metal sulfides is achieved. This effectively requires permanent anaerobic conditions, because contact with oxygen leads to the minerals being oxidized and the metals liberated again into solution [62].

5.2.2 Constructed Passive Bioreactors with Organic Support Matrix

Passive bioreactors mainly use packings of complex organic material as support matrix for the growth of microorganisms and as subtrate for reductive microbial processes. Examples for this treatment system are the so-called anaerobic wetlands or combined compost/limestone system, such as RAPS, SAPS, and the vertical flow reactor. The latter are constructed with additional alkaline material (e.g. limestone) to utilize the advantages of an anaerobic wetland combined with the provision of

additional alkalinity for the system (as is the case with anoxic limestone drains, described previously) for the treatment of net-acidic mine water [280].

The main differences between aerobic (Fig. 5) and anaerobic wetlands are connected to the subsurface flow of the mine water in anaerobic wetlands and the increased height of the organic layer. Anaerobic wetlands are preferred for the treatment of net-acidic, sulfate-rich, and metal-rich waters [124, 156], whereby the main biological reactions are sulfate reduction, methanogenesis, and ammonification (nitrate ammonification, ammonification during mineralization of organic material). The removal of metals represents the most effective application of anaerobic wetlands.

The construction of the ponds for combined compost/limestone systems usually comprises a layer of limestone at the bottom (0.5–1.0 m deep), an overlay of organic material (0.15–0.60 m deep) and drainage pipes for the effluent solution. The influent first percolates through the organic substrate, in which the dissolved oxygen is consumed and ferric iron is precipitated. This prevents the precipitation of dissolved iron (which would otherwise cover the underlying limestone) and also leads to favorable conditions for SRPs which, in turn, thrive in the lower part of the organic layer where they reduce dissolved sulfate. The resulting sulfides again react with dissolved metals to metal sulfides. As a result of the biological activity and the dissolution of the limestone, the alkalinity and the pH of the water are increasing [124, 275, Western PA Coalition for Abandoned Mine Reclamation website).

Overall, combined systems seem to have a higher alkalinity production, resulting in higher treatment efficiency, and also a more stable performance than anaerobic wetlands on their own [280].

An example of a pilot plant-scale RAPS was installed to treat iron-rich mine water from an abandoned fluorspar mine near Gernrode in the Harz Mountains of Germany [96]. The pilot plant consisted of a settling pond (aeration and settling of precipitated iron hydroxides), a RAPS pond (lime stone, horse manure as organic substrate), and an aerobic wetland pond for the oxidation, precipitation, and retention of metals. The concentration of dissolved Fe_{total} was reduced from 20 to 5 mg/l, the pH increased from 5.5 to 7.0, and the acid neutralizing capacity rose from 0.2 to 0.7 mmol/l.

Another example of a multistage process for the biological treatment of mining waters was designed by Deusner [57]. In a first stage, the necessary organic substrates (electron donor) were produced by microbial fermentation of silage. The effluent rich in organics was, following an adjustment of the pH, transferred into a fixed bed reactor (an active biological system, as described later) for microbial sulfate reduction of the mine drainage water and concomitant precipitation of dissolved metals as sulfides. In a final treatment step, the effluent was further treated to remove remaining hydrogen sulfide and organic compounds prior to its discharge into the natural waterways. This last step involved, for example, oxidation of sulfide through addition of MnO_2 or Fe_2O_3 and the aerobic degradation of residual organic compounds.

Passive systems filled with organic material may, however, be affected by decreasing treatment efficiency over time. A potential explanation for this decreasing efficiency may be offered by blockage of the pores within the substrate layer through biofilm growth and precipitation of metal sulfides which, in turn, results in lower hydraulic conductivity of the wetland. A solution to the problem is provided by upflow ponds, in which the influent enters the pond via a distribution pipe at the bottom (continuous outflow is achieved via perforated pipes in the upper part; [51]).

The algal sulfate-reducing ponding process for the treatment of acidic and metal wastewaters (ASPAM) directly utilizes algal biomass harvested from an aerobic high-rate algal pond (HRAP) as organic substrate for subsequent biological sulfate reduction in a facultative pond reactor (i.e. comprising aerobic and anaerobic zones) [204]. The sulfide produced there then leads to the precipitation of the metal ions. HRAPs are also considered for applications as a final polishing step of effluent from the facultative pond reactor due to biosorption of residual dissolved metals to algal biomass and for oxidative sulfide removal. An example of a treatment plant based on the ASPAM system was built in Wellington (South Africa) for the processing of tannery waste water [204].

5.2.3 Advantages and Disadvantages of Biological Passive Treatment Systems

Passive treatment systems, such as aerobic and anaerobic wetlands, use natural chemical and biological processes for the removal of dissolved heavy metals, sulfates, and acidity from contaminated waters. In theory, the biomass is generated exclusively through biological processes, with sunlight providing the required energy. These systems are self-regulating and should therefore be stable in the long-term [147], although their full-scale application with real industrial or mine water regularly demonstrates the need for maintenance. Apart from the large footprint required for passive systems, in comparison to abiotic and active biological systems, they also suffer from low sulfate reduction rates and, in particular, lack of control. Additionally, systems such as constructed wetlands can only be used for the treatment of AMD in areas where a constant water supply can be assured. The drying out of a wetland can result in the degradation of the organic substrate, the oxidation of immobilized sulfides, and the release of metals followed by the formation of sulfuric acid during the next rain fall [156].

However, because passive systems are overall low-cost alternatives to the more expensive active systems, they deserve further considerations and investment into future development, whereby a combination with other approaches may represent a worthwhile strategy to be explored. Other means of improving the performance and reliability of passive systems may focus on designs that result in the ponds neither freezing up during the cold seasons nor building up sludge (e.g. metal sulfides), as well as the use of appropriate vegetation [62]. For example, the negative influence of varying and very low temperatures on sulfate reduction may be overcome by

locating SAPS units directly within mine shafts (an in-adit sulfate-reducing system), thus ensuring nearly constant environmental temperatures [118].

However, although passive systems appear to be simple in their design, such modifications may affect the complex network of chemical and biological interactions and of processes that are currently not fully understood. For example, changes to internal hydraulic conditions or the organic carbon cycle in anaerobic wetlands have likely an impact on microbial diversity and abundance [275].

5.3 Active Treatment Systems Using Biological Sulfate Reduction

Although the aim of this review is to provide a comprehensive overview of strategies proposed for the remediation of high–sulfate-containing mine waters, it has a specific focus on the performance of active biological treatment options using a diverse set of reactor designs. These are therefore discussed in more detail.

5.3.1 Reactor Design

A vast variety of reactor designs have been reported for the application of microbial sulfate reduction [120]. Several of these will be discussed in more detail. A common feature to almost all of these reactor systems is the immobilization or retention of the SRP within the bioreactor. SRPs are generally rather slow-growing microorganisms that, if washed out by the stream of mine water passing through the system, would render the process inefficient.

The formation of a biofilm appears to be the most efficient means of decreasing the discharge of biomass out of and enhancing the biomass concentration within the bioreactor [175]. This is of particular importance in the case of high flow rates or high sulfate loads [172, 230]. Retaining biomass via the recycling of discharged biomass back into the reactor provides an additional means of maintaining high biomass concentrations [252].

The performance and sulfate reduction rates of continuous flow reactor systems are influenced by a number of parameters, of which particular attention should be paid to two process parameters: the sludge retention time (SRT: biomass retention in the reactor) and the hydraulic retention time (HRT: average time for water within the reactor). Long SRTs generally lead to stable processes and low sludge production in the bioreactor; they can be achieved by biofilm formation of the microbial biomass on appropriate carrier material within the reactor system. Although short HRTs effectively mean that only small reactor volumes are required, thus reducing investment costs, care must be taken when selecting the optimum HRT. If the residence time of the mine water is too short, it may lead to potential overload of the reactor system [79, 85]. Optimal SRTs and HRTs may be achieved by firstly using long HRTs to support the growth of sufficient biomass

within the reactor system and organisms within the biofilm to adapt to the environmental conditions. Following this initial period, the HRT leading to the optimum sulfate reduction rates is then experimentally explored [79, 85].

The basic design of *continuously stirred tank reactors* (CSTRs) is used in many applications, although this design suffers from high washout of biomass, which will negatively affect the efficiency of the treatment process. If long HRTs or small loading rates are not an option to prevent wash out of biomass, then the separation and recycling of the biomass from the effluent (e.g. by the use of anaerobic contact processes with biomass recycling, the use of centrifugation, or the use of flocculants) will be required to maintain high biomass content within the reactor system. Examples of applications of CSTRs are laboratory-scale experiments for the precipitation of zinc with biologically produced sulfide [67] or the development of a high-rate sulfate-reduction process using a "self-regulating bioreactor concept" for improved process performance [185].

Gas-sparged reactor systems were among the very first designs used for microbial sulfate reduction experiments with hydrogen and carbon dioxide as the electron donor and source of carbon, respectively [232]. This reactor system does not use immobilization of the biomass; therefore, it requires large reactor volumes for sustaining a reasonable biomass for the process to be effective [232]. A further characteristic of the gas-sparged reactor system lies in the collection of residual gas at the top of the reactor, which then is cleaned and repressurized for subsequent recycling back into the reactor. The recycling is necessary due to the large volumes of gas required and the fact that hydrogen has a low solubility in water. This, in turn, means that the system suffers from high energy costs for repressuring of the gas and low mass transfer of the substrate to the bacterial cells. A kind of gas-sparged bioreactor for the in situ sulfate reduction was established by Bilek and Wagner [36]. Hydrogen was used as electron donor for autotrophic sulfate reduction in a groundwater aquifer. The sulfide was used for the upstream precipitation of iron ions and excess sulfide was subsequently stripped with CO_2 and oxidized.

To overcome this disadvantage, *permeable membrane systems* have been developed, which permit the supply of hydrogen and carbon dioxide via membranes placed within the reactor system. This design leads to SRPs forming a biofilm at the outer surface of these membranes, at the sites of highest concentrations of electron donors and carbon source. In contrast to gas-sparged reactor systems, the hydrogen sulfide does not mix with the pressurized substrate gas (i.e. hydrogen sulfide cannot pass the membrane), thus avoiding the need for energy-consuming gas separation and recompression [232]. This reactor system has already been tested with real AMD water at laboratory and pilotscales using various membrane materials, process temperatures, and sulfate loading rates [69, 232]. Another modification of the permeable membrane system uses a silicone-based membrane to separate the SRP from the metal-bearing solution within the bioreactor [49].

Airlift- or gas-lift reactors are particularly useful in applications where strong shearforces negatively affect the reaction process because their specific design

provides good mixing of the solution and, hence, good mass transfer. However, because the biomass is maintained in a suspended state, only low biomass concentrations as compared to fixed-biomass reactors are achieved. Furthermore, as for the gas-sparged reactor systems, the gas-lift reactors also suffer from low solubility of the hydrogen, which limits mass transfer of the electron donor [253, 254].

The *upflow anaerobic sludge blanket* (UASB) reactor represents one of the most used fluidized-bed reactor designs. Specific operating conditions—in particular, a constant upflow of the mine water inside the reactor and high concentrations of organic substrate—lead to the microorganisms aggregating in solid particles with a diameter of 1–5 mm. Due to their settling properties, these sludge granules are retained within the reactor, where they form a moving sludge bed near the bottom of the reactor, which, in turn, permits high flow and loading rates while achieving good sulfate reduction rates [182].

UASB reactors are also used as part of a two-stage process for the biological production of sulfide to precipitate lead [104], for example, or for the treatment of drainage water of a municipal waste incineration bottom ash landfilling [223]. Further examples of the application of UASB reactors have been provided by Lenz et al. [148], who compared the performance of UASB reactor under methanogenic and sulfate-reducing conditions to remove selenium oxyanions from synthetic water. Weijma et al. [266] used expanded granular sludge bed reactors (see next paragraph) for the optimization of microbial sulfate reduction performance with thermophilic microorganisms at 65 °C, thus favoring sulfate reduction over methanogenesis, in particular when the chemical oxidation demand COD/SO_4^{2-} ratio was lowered from values >1.0 to 0.34 g/g.

A further development based on the UASB design is the *expanded granular sludge blanket* (EGSB) process. This process is based on the same principles as the UASB process, but differs in terms of design geometry, process parameters, and construction materials. These modifications result in a partial expansion of the sludge bed due to higher liquid and gas velocities, thus ensuring better contact between the water and the sludge [75]. De Smul et al. [55] examined the effects that varying ratios of COD/SO_4^{-} and temperature might have on the sulfate reduction performance of an EGSB reactor. To increase the amount of sulfate-reducing sludge and the rate of sulfide formation in an UASB reactor, Gonçalves et al. [82] developed a process ("bioactivation") in which lactate was used as the initial carbon source; this was subsequently gradually replaced by molasses, leading to a 100-fold increase of biomass.

A means to overcome the problems typically associated to packed-bed reactors (channeling, gas trapping, and precipitate covering the biofilm) is realized in *fluidized-bed reactors,* in which the carrier material is maintained in suspension, thus providing good mass transfer for the substrate and large surface area for biofilm formation [172]. The high biomass content of the reactor systems means that high sulfate loading rates can be applied while maintaining high sulfate removal rates from the water. However, continuous energy input to keep the carrier material

fluidized is only achieved at higher operating costs compared to other reactor designs [182].

A less energy-demanding design to overcome preferred flow paths, gas trapping, and precipitate covering the biofilm is provided by *moving-bed sand filters*. This design is, in essence, based on the continuous or semi-continuous cycling of the carrier material (sand) for the biofilm within the reactor. To limit the thickness of the biofilm and to remove precipitated metal compounds (e.g. metal sulfides) from the biofilm, the sand is moved upward in an inner tube, with an internal air or liquid lift as the driving force whereby part of the biofilm and the precipitated metal compounds are removed and separated [196].

Anaerobic hybrid reactors use aspects of both UASB and packed-bed reactor designs (e.g. cross-flow plastic media as biomass carrier) with a layer of granular sludge in the lower part of the reactor and solid material in the upper part, thus improving the filtration of the upstreaming waste water [83, 208].

The *anaerobic baffled reactor* is another modification of the UASB technology in which the reactor interior is divided by baffles into several compartments. This design is intended to increase the SRT within the reactor through the use of baffles set in the flow path of the water. Although anaerobic baffled reactors are mainly used for the treatment of organic-rich waste waters, they also appear suitable for the treatment of sulfate-rich waters with SRP as the inoculum and under appropriate conditions (for a review, see [15]). In this context, studies investigating the influence of the ratio of COD to sulfate within anaerobic baffled reactors have already been reported [256].

The main characteristic and advantage of *packed-bed reactors* is connected to the retention of the biomass in the reactor and a higher biomass concentration compared to suspended systems which, as mentioned above, is of particular importance in the case of slow-growing microorganisms like SRPs [172]. Immobilization of the microorganisms is achieved using a variety of solid carrier materials (e.g. glass particles, sand, ground rocks, plastic, complex organic materials). Packed-bed reactors can be operated at various flow modes (e.g. horizontal, upflow, or downflow mode). Possible problems specific to packed-bed reactors are the formation of preferred flow paths through the carrier material, biologically produced gas (hydrogen sulfide, carbon dioxide, methane) being trapped within the porous space between the carrier material, and the fact that precipitated material (e.g. metal sulfides) may block the flow paths inside the reactor or cover the biofilm, which then leads to a reduction of mass transfer of electron donor molecules [140].

Despite this, packed-bed bioreactors appear to represent the more successful designs among the bioreactor systems used for microbial sulfate reduction. For example, a pilot-scale anaerobic packed-bed reactor was used by Silva et al. [220] for the treatment of mine water with very high sulfate concentrations (up to 35 g SO_4^{2-}/l). The highest sulfate removal reached in the discontinuous and semi-continuous experiments was 97 %. Moreover, the highest sulfate reduction rate (65 g/l per day) reported so far (Table 4) has also been achieved using a packed-bed biofilm column [230], although no details have been provided on the HRT at

Table 4 Comparison of published data from laboratory and pilot-scale experiments for biological sulfate reduction

Reactor design	Temperature (°C)	Substrate	Hydraulic retention time (h)	pH	Sulfate reduction rate (g/l per day)	Reference
Upflow packed-bed reactor—coarse sand	25	Lactate	16.2	3.5	0.0003	[120]
High-rate anaerobic reactors with carrier material	?	Acetate	240	?	0.035	[112]
Upflow packed-bed reactor (lab scale)	Room temperature	(leaf mulch, saw dust and other)	?	5.5–6.5	0.074	[261]
High-rate anaerobic reactors with carrier material	?	Ethanol, acetate	240	?	0.098	[112]
Upflow packed-bed reactor—coarse sand	25	Lactate	16.2	6.0	0.1	[120]
Upflow packed-bed reactor (lab scale)	Room temperature	Leaf mulch and saw dust	?	5.5–6.5	0.12	[261]
Upflow packed-bed reactor-Lab scale (waste material)	25	Organic waste	480	6.8	0.12	[46]
CSTR (lab scale)	35	Acetate, peptone	90	8.0	0.17	[165]
Packed-bed reactor-Pilot-scale (plastic ballast rings)	35	Acetate	60	7.0	0.31	[151]
Upflow packed-bed reactor—coarse sand	25	Lactate	16.2	4.5	0.48	[120]
Packed-bed reactor	31	Molasses	20	?	0.5	[159]
Upflow packed-bed reactor (porous glass beads)	?	Ethanol, lactate, glycerol	49.3	4.0	0.5	[140]
High-rate anaerobic reactors with carrier material	?	Acetate	12	?	0.54	[112]
Fluidized-bed reactor with clarifier	?	Sucrose	51	4.0–6.0	0.6	[84]
Downflow packed-bed reactor	Room temperature	Lactate	6.6	4.2	0.76	[240]

(continued)

Table 4 (continued)

Reactor design	Temperature (°C)	Substrate	Hydraulic retention time (h)	pH	Sulfate reduction rate (g/l per day)	Reference
CSTR (lab scale)	35	Acetate, peptone	60	8.0	0.77	[165]
Packed-bed reactor—sand	33	Methanol	20	2.7	0.84	Klein et al. (unpubl.)
Packed-bed reactor—glass beads	22	Lactate	28.6	7.0	0.96	[20]
Downflow packed-bed reactor	Room temperature	Methanol	6.6	4.2	0.96	[240]
Packed-bed (pelletized ash)—pilot-scale	35	H_2/CO	?	?	1.2	[64]
Gas-Lift bioreactor (lab scale)	30	H_2/CO_2	24	5	1.25	[32]
High-rate anaerobic reactors with carrier material	?	Ethanol, acetate	12	?	1,3	[112]
Fluidized-bed reactor with clarifier	?	Sucrose	15.8	4.0–6.0	1.5	[84]
Expanded-granular-sludge-blanket (lab scale)	55	Ethanol	3.5–4.0	7.75–8.35	1.5	[55]
Gas-lift reactor (lab scale)	54–52	CO/H_2	3	6.9	1.6	[222]
Downflow packed-bed reactor	Room temperature	Methanol	6.6	4.2	1.61	[240]
UASB (lab scale)	20	Primary sewage sludge	20.5	5.9	1.7	[193]
CSTR (lab scale)	35	Acetate, peptone	36	8.0	1.8	[165]
Packed-bed reactor—sand	33	Methanol	12	4.5	1.8	Klein et al. (unpubl.)
Porous ceramic carriers, fixed bed (lab scale)	Room temperature	Methanol	20	?	2.1	[79]
Fluidized-bed reactor (lab scale)	35	(lactate) ethanol	16	5.0–>2.5	2.3	[130]

(continued)

Table 4 (continued)

Reactor design	Temperature (°C)	Substrate	Hydraulic retention time (h)	pH	Sulfate reduction rate (g/l per day)	Reference
UASB (lab scale)	35	Primary sewage sludge	18	6.0	2.4	[193]
Packed-bed (Raschig rings)—(lab scale)	35	CO	?	?	2.4	[64]
Completely mixed reactor with clarifier	?	Ethanol	12	?	2.5	[84]
Packed-bed reactor ("special packing")	30	H_2/CO_2	?	6.0	2.6	[71]
Packed-bed reactor ("special packing")	30	H_2/CO_2	–	6.0	2.7	[71]
Sludge-bed reactor	31	Molasses	15	?	2.7	[159]
UASB (lab scale)	35	Primary sewage sludge	13.5	7.2	2.8	[192]
Packed-bed reactor ("special packing")	30	H_2/CO_2	–	6.0	2.88	[71]
Packed-bed reactor ("special packing")	30	H_2/CO_2	50	6.0	3.1	[71]
Porous ceramic carriers, Packed-bed (lab scale)	Room temperature	Methanol	12	?	3.1	[79]
Packed-bed (on site, pilot scale)	?	Methanol	4.2	2.9	3.2	[79]
Fluidized-bed reactor	?	Ethanol	6.1	?	3.3	[84]
Packed-bed (k-carrageenan gel matrix)	30	Sewage sludge	6.8	6.85	3.9	[216]
Bioreactor (pilot scale)	?	Acetate (start), molasses	12	?	4.0	[160]
Expanded-granular-sludge-blanket (lab scale)	33	Ethanol	3.5–4.0	7.75–8.35	4	[55]
Fluidized-bed reactor (lab scale)	33	Ethanol	6.5	3.1	4.3	[131]
Completely mixed reactor with clarifier	?	Ethanol	7.2	?	4.5	[84]
Completely mixed reactor	?	Ethanol	7.2	?	4.8	[84]
Packed-bed reactor ("special packing")	30	H_2/CO_2	21.6	6.0	4.8	[71]
Packed-bed reactor—biomass support particles	22	Lactate	5.3	7.0	4.8	[20]
Stirred reactor with SRP flocs	30	Sewage sludge	66.6	7.0	4.8	[216]
Packed-bed reactor (lab scale)	?	Ethanol	5.6	?	4.9	[84]

(continued)

Table 4 (continued)

Reactor design	Temperature (°C)	Substrate	Hydraulic retention time (h)	pH	Sulfate reduction rate (g/l per day)	Reference
Gas-Lift bioreactor (lab scale)	30	H_2/CO_2	24	5	4.9	[32]
UASB reactor (lab scale)	35	Ethanol	120–20.4	7–10	6.0	[134]
Two-stage liquid–solid fluidized-bed reactor (lab scale)	30	Ethanol	Down to 5.1	6.3	6.3	[172]
Completely-mixed reactor with clarifier	?	Ethanol	4.8	?	6.6	[84]
Submerged anaerobic membrane reactor	33	Acetate, ethanol	9	7.2	6.6	[249]
Expanded-granular-sludge-blanket (lab scale)	33	Ethanol	3.5–4.0	7.7–8.3	7	[55]
UASB (pilot scale)	30	Ethanol (nutrients, flocculants)	4	3.2	7	[16]
Granular sludge bed reactor (lab scale)	30	Acetate, protepionate, butyrate	?	7.0 and 8.0	9.1	[179]
Expanded-granular-sludge-blanket	33	Acetate	2.5–1.9	7.9	10	[63]
Complete-mixed reactor	Room temperature	Sucrose, ethanol	48–8	4.3	12.4	[162]
UASB (lab scale)	32	Acetate	2.5	8.3	14	[170]
Packed-bed reactor—sand	22	Lactate	2.7	7.0	16.3	[20]
Membrane bioreactor (lab scale)	30	Formate/hydrogen	30 d	5	18	[34]
Packed-bed reactor—sand	22	Lactate	2.7	7.0	19.2	[20]
Gas lift reactor (lab scale)	30	H_2/CO_2	2.25	7.0	30	[251]
Packed-bed reactor—sand	22	Lactate	0.5	7.0	41	[20]
Packed bed (BioSep as carrier material)	30	Sewage sludge	3.4	6.85	46	[216]
Packed bed (polyurethane foam)—lab scale	35	Acetate	?	7.5–8.5	55.3	[230]
Packed bed (sintered glass beads)—lab scale	35	Acetate	?	7.5–8.5	65	[230]

CSTR continuously stirred tank reactors, *SRP* sulfate-reducing prokaryotes, *UASB* upflow anaerobic sludge blanket reactor

which the reactor was operated. However, relevant process parameters of this study might have been the seeding of the bioreactor system with two strains of SRPs (*Desulfotomaculum acetoxidans* strain DSM 771, *Desulfobacter postgatei* strain DSM 2034), the use of acetic acid as substrate, and the removal of H_2S via a gas-stripping column [230].

5.3.2 Modifications to the General Design of a Process Using Microbial Sulfate Reduction

Although the various reactor types appear to result in varying performance of the sulfate reduction process (see below), sulfate reduction rates are also greatly influenced by the individual approach taken for the treatment of the mine water. The criteria on which specific treatment strategies are chosen depend on the characteristics of the treatment site, the mine or industrial waste water, and the particular aim of the process (e.g. sulfate removal, metal precipitation).

The one-stage process is the simplest strategy, which aims for sulfate reduction and metal precipitation within the same reactor. The necessary electron donor for the reduction of the sulfate is mixed into the inflowing mine water stream. This design represents the most cost-effective solution among the active biological systems. Particular problems associated with this approach may occur if acidic mine or industrial waters containing a high metal load are treated because these factors are known to inhibit or to be toxic to microorganisms, including SRPs. Additionally, the formation of large amounts of metal sludge covering the biofilm may also result in reduced activity due to limited diffusion of nutrients and substrate molecules to the SRPs [233]. However, the removal of metal sludge also results in loss of biomass [232].

To improve the efficiency of sulfate reduction and metal precipitation, several reactors can be placed in series or part of the effluent can be used to dilute the inflowing mine water, thus increasing the pH and reducing dissolved metal concentrations. However, such modifications also mean higher investment and operation costs. Due to the simple design and the direct contact of the microorganisms with the mine water, it is necessary for the sulfide concentration within the reactor to be sufficiently high to counteract the negative effects of sudden increases in dissolved metals in the mine water. However, elevated sulfide concentrations have also been shown to negatively affect the performance of microbial sulfate reduction processes (see below).

Two-stage processes have been developed in response to the limitations specific to the one-stage approaches. The characteristic of the two-stage approach lies in the metal precipitation step using recycled sulfide-rich effluent or gas from the spatially separated sulfate-reducing reactor [233]. The sulfate-rich solution containing low concentrations of metals is then treated for the sulfate load and the production of sulfide. If only the H_2S-bearing gas from the sulfate reduction stage is recycled for the precipitation of the metals, no additional alkalinity is introduced, which allows selective recovery of the metals. For example, copper already

precipitates as CuS at very low pH values (<pH 1) where other metal sulfides are still dissolved (e.g. [100, 107]). Two-stage processes have been implemented in industrial applications to produce hydrogen sulfide from water containing high sulfate and metal concentrations (e.g. SULFATEQ; Paques, Balk, The Netherlands; [182]).

Although a two-stage process requires higher investment and operation costs than are necessary for a one-stage process, the separation of sulfate reduction and the precipitation provides important advantages over the latter. Apart from providing a means to avoid sulfide inhibition of the SRPs, it also allows selective precipitation of metals and individual control and improvement of the conditions for sulfate and metal removal.

6 Evaluation of Process Parameters that Influence the Biological Sulfate Reduction Rate in Bioreactors

The extent of the scientific literature addressing the issue of microbial sulfate reduction and its application in bioremediation and in industrial processes for the treatment of polluted mine water might present an opportunity to extract relevant correlations between process parameters and process performance. Therefore, a set of 75 experiments (Table 4) has been analyzed, with the aim of detecting parameters that might be the key to high sulfate reduction rates and hence technical performance of the treatment system.

6.1 Influence of the Hydraulic Retention Time

In most (56 out of 75) of the experiments screened for this analysis, carrier material (sand, glass beads, plastic materials, etc.) had been added to the reactor system to achieve immobilization of biomass, such as in the form of biofilm on the carrier material. Another approach uses specific conditions within the reactor to achieve formation of aggregates consisting of biomass and organic compound (e.g. granular sludge in UASB reactors). Apart from a reduction of the costs due to smaller reactor volumes being required, the immobilization of the biomass also permits the decoupling of HRT and cell retention time, because it drastically reduces the risk of biomass washout, particularly at shorter HRTs.

The comparison of the HRTs used in the various experiments to the resulting sulfate reduction rates reveals that shorter HRTs generally lead to higher sulfate reduction rates (Fig. 8). In particular, HRTs shorter than 4 h result in an increase of the reduction rates (e.g. [20, 63, 170, 222, 251]). A number of factors may contribute directly or indirectly to this observation and help to explain the correlation between decreasing HRT and increasing sulfate reduction rates. This section discusses some of these factors in more detail.

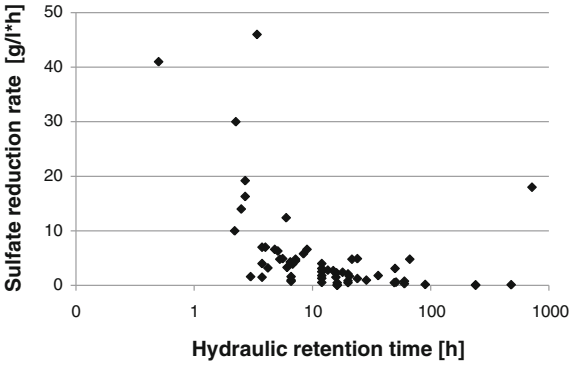

Fig. 8 Relationship between volumetric sulfate reduction rates and hydraulic retention time used in bench- and pilot-scale experiments (data of a total of 61 experiments provided in Table 4)

Assuming a constant volume of the bioreactor, the HRT is reduced by increasing the volumetric inflow rate into the reactor. Given a constant sulfate and electron donor concentration (unless the electron donor is dosed separately) in the inflowing water, the loading rates for sulfate and for the electron donor and carbon source are increased, which, in turn, means that the sulfate and substrate flow into the bioreactor per unit of time increase. This is likely to lead to an improved supply of nutrients, energy, and carbon source to the microorganisms, which then results in higher sulfate reduction rates [20]. However, it should be noted in this context that at least one study reported that long HRTs (e.g. 40 h) resulted in a better substrate (acetate) use by SRP relative to methanogens [4].

Higher flow velocity caused by increased volume inflow rates of water into the bioreactor can also lead to an improved mass transfer between the liquid phase and the biofilm due to greater turbulence and a reduction in the thickness of the laminar zone directly above the biofilm. A higher flow velocity may further assist in inhibiting competing microorganisms (e.g. methanogens), which are known to require longer HRTs (e.g. HRTs in the range of 24 h) (e.g. [220, 282]).

However, higher inflow rates and shorter HRTs also mean that the time available to the microorganisms to reduce sulfate to sulfide is shorter. If the increase in the loading rate (sulfate, electron donor) is higher than the increase of the (microbial) reaction rates, then this usually leads to an increase in sulfate concentration and residual organic content (the electron donor/organic substrate) in the outflow of the bioreactor.

Reviewing the literature also revealed a high level of variation in the rates of sulfate reduction reported for identical HRTs. For example, for an HRT between 2.2 and 2.7 h, sulfate reduction rates ranging from 10 to 30 g SO_4^{2-}/l per day have been reported (Table 4). This indicates that in addition to the key parameter of HRT, other factors also have a high impact on the performance of sulfidogenic bioreactors.

Finally, the highest sulfate reduction rate (up to 65 g SO_4^{2-}/l per day) within the dataset has been reported by Stucki et al. [230]. Unfortunately, this report does not provide any details on the HRT used in the experiment.

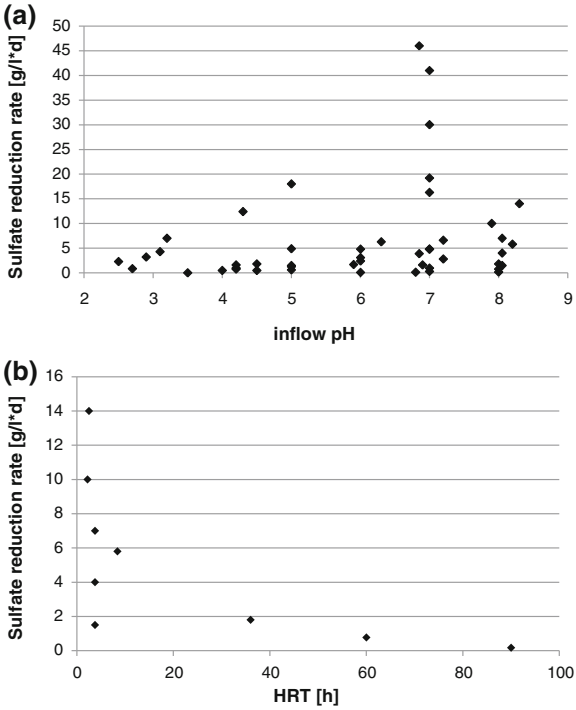

Fig. 9 Maximum sulfate reduction rates in relation to the pH of the feed stream (**a**) or as a function of the used hydraulic retention time (HRT) (**b**). The analysis of **a** is based on 47 experiments from Table 4 for which the relevant information on the pH range was available. **b** shows in detail those experiments within the narrow pH range of 7.9–8.3 for potential reasons causing the big differences in the sulfate reduction rate revealed in **a**

6.2 Influence of the pH

The majority of the experiments (28 of 47) with information concerning the pH of the treated mine or industrial water was undertaken using water of pH 6 or higher, whereas fewer investigations (19 out of 47) were carried out with acidic waters (pH < 6). This may reflect the fact that only recently the potential of acidophilic sulfate reducers has been recognized (e.g. [50, 140, 164, 194, 241]) and that the optimal pH of most known sulfate-reducing microorganisms are within the neutral pH range.

The overall indication from the literature is that with increasing pH (in the range from 3.5 to 8.5; see Table 4) of the inflowing mine water, higher sulfate reduction rates are being achieved (Fig. 9a). However, the comparison of the various experiments also revealed that this general trend is accompanied by a large number of exceptions (Fig. 9a). In particular, at a pH of approximately 7, the sulfate reduction rates range from 0.12 to 46.0 g SO_4^{2-}/l per day, whereas at lower or higher pH the fluctuation between individual experiments is less extensive. This, in turn, raises the question as to the factors responsible for these variations in sulfate reduction rates at specific pH.

Further analyses were therefore undertaken to reveal potential correlations of the sulfate reduction rate to the HRT for two sets of data, both defined by a narrow

pH range (6.8–7.2 and 7.9–8.3); these were chosen because the largest set of experiments are within these pH ranges (13 and 9, respectively). The results of this comparison again expose the relevance of the HRT on the reactor performance because the general trend indicates that shorter HRTs result in higher sulfate reduction rates (Fig. 9b).

Unfortunately, the available datasets for experiments on the treatment of acidic waters are small. However, based on the experimental data available, it can be deduced that a decrease of the pH of the mine water generally leads to lower sulfate reduction rates (Fig. 9a), presumably due to the increasing inhibition of SRPs (e.g. [31, 120, 271]) (as already discussed in Sect. 5.1). However, several publications revealed that the application of the microbial sulfate reduction to acidic mine waters is possible [34, 50, 73, 120, 140, 241] due to the presence of acidophilic sulfate reducing microorganisms.

6.3 Influence of Temperature

The majority of the experiments (65 of 75) were carried out under mesophilic conditions (20–35 °C), whereas only a minority (7) of the experiments tested thermophilic temperatures (50–65 °C). This may reflect the fact that the majority of the known sulfate-reducing microorganisms have a temperature optimum in the mesophilic range between 20 and 40 °C [19, 45], with only a few reports (e.g. [139, 198, 209]) on psychrophilic (<15 °C) and some others (e.g. [55, 222, 253, 265]) on thermophilic (54–94 °C) SRPs. It might be expected that rates of biochemical reactions increase with increasing temperature within the tolerance limits of the corresponding enzymes and organisms. However, the experiments using thermophilic process conditions (52–65 °C; e.g. [55, 222]) indicate that higher process temperatures, independent of the HRT applied, do not always lead to increased sulfate reduction rates (Table 4; Fig. 10). In addition, the application of thermophilic SRPs is likely to cause additional costs because such a process requires preheating of the mine water stream in order to ensure the high activity of the thermophilic sulfidogenic microorganisms.

Due to the limited number of experiments for the more extreme temperature ranges (i.e. 50–65 °C; Fig. 10), we will consider only the experiments that were carried out within the mesophilic temperature range (i.e. 22–35 °C). Within this range of temperature, the performance of the sulfidogenic process does not seem to be affected obviously (Fig. 10), and other factors (e.g. pH, HRT) appear to have a greater impact on the sulfate reduction rate. However, care must be taken in interpreting these observations due to the small number of experiments available for the specific temperatures tested (e.g. only two experiments for 31 °C and one for 32 °C). These experiments are unlikely to represent the total range of variation in sulfate reduction in the temperature range suitable for mesophilic microorganisms. Additionally, there is a high fluctuation in the sulfate reduction rates at a given temperature (Fig. 10). All of the reduction rates at a process temperature of

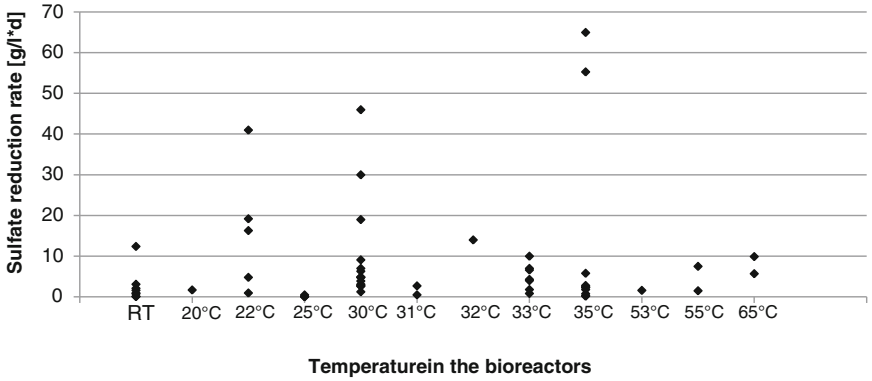

Fig. 10 Maximum sulfate reduction rates in relation to the temperature in the sulfidogenic bioreactors. The analysis is based on data of a total of 56 experiments provided in Table 4 (*RT* room temperature)

33 and 35 °C are as low as those achieved at 22 or 30 °C. Due to the higher operating costs while using higher bioreactor temperatures, it is doubtful that higher process temperatures sufficiently improve sulfate reduction rates.

The extent to which parameters other than the HRT or the sulfate feed concentrations and volumetric loading rates influence the sulfate reduction rate at a given temperature (Table 4) is even less understood. For example, the variation in sulfate reduction rates observed in experiments that were carried out at 30 °C does not correlate to reactor design (experiments at 30 °C were taken for this comparison due to the relatively high number of reports available). The three highest sulfate reduction rates at 30° C were achieved using a packed-bed, a gas-lift and a membrane bioreactor. Similarly, the substrates used for these experiments also varied (H_2/CO_2, sewage sludge, ethanol). The two highest sulfate reduction rates (30 and 46 g/l per day) at a temperature of 30 °C were achieved at short HRTs (2.25 and 3.4 h, respectively), again supporting the notion that short HRT ($\leq$4 h) represents the most obvious parameter that positively correlates with sulfate reduction rate. However, using a membrane bioreactor run at 30 °C Bijmans et al. [34], reported a sulfate reduction rate of 18 g/l per day with an HRT of 30 days. This is in stark contrast to the previous findings that long HRTs tend to lead to a lowering of the sulfate reduction rate. However, it should be noted in this context that, based on the available information, such an approach would only be applicable to mine water with very high sulfate loads.

6.4 Influence of the Bioreactor Design

Improvements to the performance and efficiency of the active microbial sulfate reduction process at the laboratory and pilot-plant scale has mainly focused on modifications to bioreactor designs. This is reflected in the variety of reactor types

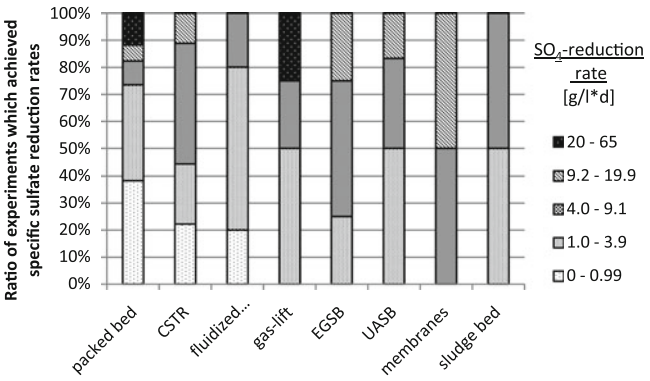

Fig. 11 Ratio of experiments using various bioreactor designs. Data are retrieved from Table 4

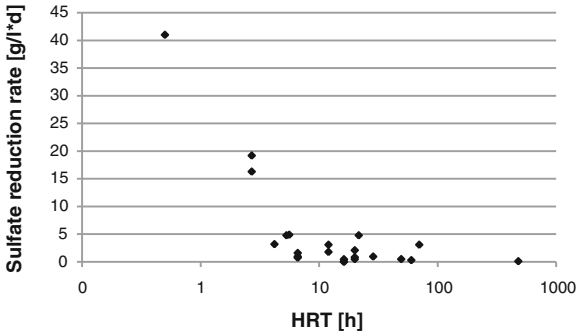

Fig. 12 Relationship between the hydraulic retention time (HRT) and the sulfate reduction rates reported for packed bed reactors. Data are from Table 4

found among the experiments screened in this literature analysis (6 of the 75 reports analyzed did not provide detailed information on the reactor design): upflow or downflow packed-bed reactor (34 reports), CSTR (9), fluidized-bed reactor (5), gas-lift reactor (4), EGSB (4), ASB (6), membrane bioreactor (2) and sludge-bed reactor (2).

The comparison between reactor design and reactor performance reveals that bioreactors with immobilized biomass, such as the packed-bed reactor, generally lead to higher reduction rates (between 20 and 65 g SO_4^{2-}/l per day) than what is achieved using other reactor types (Fig. 11). The better performance is likely to be connected to the higher biomass content due to its immobilization and the decoupling of HRT and cell retention time. Indeed, plotting the HRT applied in the 23 experiments using packed-bed reactor designs against the sulfate reduction rate revealed the general trend that shorter HRTs lead to higher sulfate reduction rates (Fig. 12), thus reflecting the overall tendency within the dataset (see above).

Gas-lift reactors also performed well (Fig. 11), probably due to the good mass transfer and mixing ability of the reactor interior, which is characteristic for the reactor type and known to improve the efficiency. Some reactor designs (e.g. gas-lift

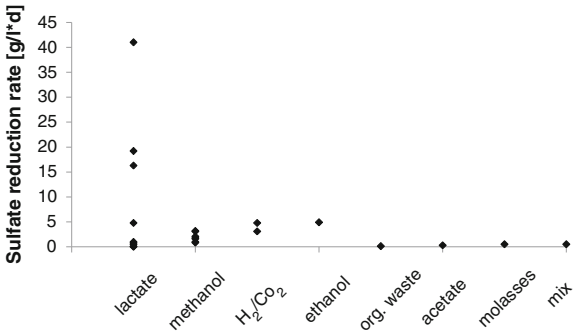

Fig. 13 Relationship between the use of substrates and sulfate reduction rates in experiments using packed bed bioreactors. Data are from Table 4 (*mix* several substrates as electron donor used in the experiment)

reactor, membrane, or sludge-bed reactor) were used only in few experiments. Care should be taken when drawing general conclusions from this because the observation may not be representative for the reactor performance in general.

6.5 Influence of the Substrate

Sulfate-reducing microorganisms are able to use a variety of organic electron donors and substrates, which have a major influence on the sulfate-reducing activity for two main reasons. Firstly, the various molecules serving as electron donor for the sulfate reduction provide varying amounts of electrons per molecule that is oxidized. This, in turn, determines the amount of electron donor molecules that are required for the reduction of sulfate. Additionally, the electron donor may be oxidized completely or incompletely by the sulfate-reducing microorganism (see above).

Based on the information provided by the 75 experiments screened in this review, it is again difficult to draw general conclusions on the influence of the various electron donors typically used in sulfidogenic bioreactors on the sulfate reduction process due to the relatively low number of experiments. Nevertheless, these experiments still indicate that the electron donor also plays an important role in the sulfate reduction process (Fig. 13). However, the use of a particular electron donor does not always lead to particularly high sulfate reduction rates, thus underlining the complexity of the system. For example, while experiments using lactate as the electron donor resulted in the highest sulfate reduction rates, there were also reports of particularly low rates (Table 6, Fig. 13). Moreover, as mentioned previously, lactate proved to be not suitable as an electron donor when acidic mine water were to be treated due to its function as organic acid under those conditions (pH range of 3.5–6.0: Table 6). The highest sulfate reduction reported among the 75 experiments analyzed in this review were, however, generally achieved using organic acids as electron donors (Table 5) because the mine waters treated in those experiments were circumneutral. Other parameters that seem to have a negative impact on the performance of the sulfidogenic bioreactors with

Table 5 Summary of the highest reported sulfate reduction rates (>10 g/l per day) and the substrates used in those bioreactor experiments

Substrate	Sulfate reduction rate (g/l*d)	Hydraulic retention time (h)	pH	Reference
Acetate	10	2.5–1.9	7.9	[63]
Sucrose, ethanol	12.4	6.0	4.3	[162]
Acetate	14	2.5	8.3	[169]
Lactate	16.3	2.7	7.0	[20]
Formate/hydrogen	18	720	5.0	[34]
Lactate	19.2	2.7	7.0	[20]
H_2/CO_2	30	2.25	7.0	[251]
Lactate	41	0.5	7.0	[20]
Sewage sludge	46	3.4	6.85	[216]
Acetate	55.3	?	7.5–8.5	[230]
Acetate	65	?	7.5–8.5	[230]

Table 6 Summary of the sulfate reduction rates, the hydraulic retention times, the carrier material, and the pH of the inflow in experiments using lactate as electron donor

Reference	Reactor design	Temperature (°C)	e-Donor	Hydraulic retention time (h)	pH	Sulfate-reduction rate (g/l*d)
[20]	Packed bed reactor—sand	22	Lactate	0.5	7.0	41
[20]	Packed bed reactor—sand	22	Lactate	2.7	7.0	19.2
[20]	Packed bed reactor—sand	22	Lactate	2.7	7.0	16.3
[20]	Packed bed reactor—biomass support particles	22	Lactate	5.3	7.0	4.8
[20]	Packed bed reactor—glass beads	22	Lactate	28.6	7.0	0.96
[240]	Downflow packed bed reactor—depleted org. substrate	Room temperature	Lactate	6.6	4.2	0.76
[120]	Upflow packed bed reactor—coarse sand	25	Lactate	16.2	4.5	0.48
[120]	Upflow packed bed reactor—coarse sand	25	Lactate	16.2	6.0	0.1
[120]	Upflow packed bed reactor—coarse sand	25	Lactate	16.2	3.5	0.0003

lactate as the electron donor were carrier materials other than sand (glass beads, "biomass support particles," depleted organic substrate: [20, 240]) and long HRTs (Table 6).

However, lactate does not represent a likely substrate in an industrial process due to costs associated with it and, as mentioned, would not be applicable to acidic mine drainage waters. In contrast to this, sewage sludge may represent the most cost-effective electron donor for the treatment of neutral pH waters, and a mixture of sucrose and ethanol was identified as the best performing electron donor for acidic mine waters with a pH > 4.5 [162].

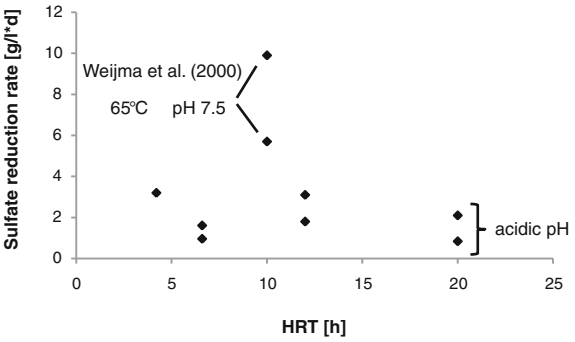

Fig. 14 Sulfate reduction rates in relation to the hydraulic retention time (HRT) used in bioreactor experiments with methanol as electron donor. Data are from Table 4

Methanol was not among the best performing electron donors, with reduction rates below 10 g/l per day (Fig. 14). This may, however, be explained by the fact that the majority of those experiments were undertaken with acidic mine waters since methanol often serves as an alternative to organic acids at acidic pH (see above). In these cases the reduction rates were below 4 g/l*d (Fig. 14). Other reasons for the generally low sulfate reduction rates at mesophilic temperatures if methanol is used as substrate are likely linked to the fact that methanol appears to only support low growth rates and low sulfate reduction rates [150]. Additionally, there seems to be a strong competition between sulfate reducers, methanogens, and homoacetogens for methanol at mesophilic temperatures [267]. This limitation can be overcome when thermophilic sulfate-reducing microorganisms are being used (Fig. 14; [249, 265]). Higher temperatures (50–65 °C) during the biological sulfate reduction with methanol as an electron donor seem to promote higher metabolic activity and faster growth rates [264]. High reaction temperatures have the additional advantage that methanogens, which also compete for methanol as an electron donor, are outcompeted by sulfate-reducing microorganisms [249].

One explanation for the high fluctuation of sulfate reduction rates found among the 75 experiments may be provided by the findings of a comparison of the sulfate reduction rates in dependence of various sulfate feed concentrations and volumetric loading rates [20]. These results indicate that higher feed concentrations of sulfate resulted in lower sulfate reduction rates at the same sulfate volumetric loading rates. Furthermore, increasing loading rates had a positive effect on the sulfate reduction rate, up to a threshold value above which the sulfate loading rate leads to decreasing sulfate reduction rates. Similar results were reported by Genschow et al. [74].

6.6 Other Factors that May Affect the Sulfate Reduction Rate

As mentioned above, hydrogen sulfide is the end product of microbial sulfate reduction and can have an inhibiting effect on the activity of the SRP. Depending on the microbial community (e.g. pure or mixed culture, origin of the inoculum)

and chemical conditions (in particular the pH, which governs the ratio of the various sulfide species), several inhibitory concentrations were reported in the literature, ranging from 40 mg/l [230] to values as high as 1,000 mg/l [247].

To ensure that hydrogen sulfide does not negatively impact the sulfate reduction process, biologically produced hydrogen sulfide was removed from the bioreactor in some of the experimental reports analyzed here. This stripping of hydrogen sulfide, which was mostly achieved by applying nitrogen gas to the reactor, always resulted in an increase of the sulfate reduction rate. For example, the partial removal of hydrogen sulfide from the bioreactor by Bijmans [31] led to an increase in the sulfate reduction rate from 1.25 g/l per day to 4.9 g/l per day. The highest sulfate reduction rate of 65 g/l per day reported within the dataset of 75 experiments [230] was again only achieved when the concentration of free H_2S was limited to 40–50 mg/l, whereas [251] observed the highest reduction rate (30 g/l per day) by limiting the free H_2S concentration to values below 450 mg/l.

In conclusion, the analysis of 75 experiments investigating the impact of various factors on the sulfate reduction indicates an important role of several process parameters, but did not reveal an individual parameter that, when changed, results in a drastic improvement in the performance of the process. In-depth multivariate statistical analyses to further disentangle the complex association between the various process parameters and the achieved performance of the process may also prove to be impracticable due to the limited information provided for many of the experiments. Moreover, direct comparisons between the experiments carried out by the various research groups are potentially misleading because probably each of those experiments used a unique microbial assemblage within the bioreactor. The differences in the composition of those complex microbial communities also underlines the difficulty in predicting the outcome caused by changes to this intricate process. Nevertheless, it has become clear that circumneutral pH and short HRTs with the biologically formed H_2S being removed from the bioreactor are particularly beneficial to the performance of the bioreactor-based microbial sulfate reduction process. Additionally, bioreactors designed to retain the microbial biomass in form of an immobilized biofilm on carrier material also appear to positively influence the performance of the process, particularly in combination with short HRTs. A similar recommendation cannot be deduced concerning the substrate, although lactate appears to be the best performing. However, in practice the price of lactate may drastically increase the operational costs of the process and, hence, be a hindrance to its use.

7 Perspectives

Biotechnological treatment strategies developed in recent years are environmentally sustainable but often not economically feasible. Thus, further developments are necessary in the future to make biotic mine water remediation economically more attractive.

A main reason for the lack of economic feasibility is associated with the complexity of biotic remediation systems in comparison with abiotic treatment methods. Chemical, biological, and technical aspects have to be considered. Varying water chemistry and process parameters, such as retention time, influence microbial growth. Against prior expectations, *At. ferrooxidans* plays a minor role in microbial communities of mine waters (e.g. [102]). Microorganisms dominating the microbial communities of acid mine waters (e.g. "*Ferrovum*" sp.) often have higher oxidation rates than the well-known *At. ferrooxidans* and, thus, they may be interesting for biotic remediation [207]. These bacteria should therefore be isolated and characterized to better understand their physiological requirements and optimal growth conditions. This, in turn, will reveal and quantify their potential for applications in mine water treatment. Apart from the use of new iron-oxidizing bacteria, the treatment efficiency may be further increased through the combination of iron oxidation bioreactors with abiotic remediation strategies. Such an approach consisting of an aerobic wetland, a compost reactor, and a rock filter has been used at a pilot plant to remediate mine water discharged from the Wheal Jane mine in Cornwall [91]. The economy of iron oxidation bioreactors may additionally be improved by the development of ferric mineral formed during microbial iron oxidation for commercial use. For example, preliminary studies have already demonstrated possible applications of schwertmannite, which is typically formed in AMD [14, 65].

As for the oxidative approach, the major challenge for the widespread and routine application of microbial sulfate reduction for the elimination of sulfate from AMD also appears to be associated with the complexity of the process. For example, the process is sensitive to changes in the water chemistry, with sudden inputs of high levels of metals toxic to microorganisms potentially causing a breakdown. On the other hand, it is yet unclear as to whether high sulfide production causes a limitation of trace elements required for cellular functioning. Preliminary experiments in our laboratory have been carried out to test this hypothesis using copper, as copper sulfide has a particularly low solubility even at acidic pH. The results revealed that the addition of low levels (up to 2 mg/l) of copper (added as $CuCl_2$) to a microbial consortium of SRP resulted in higher sulfate reduction rates (unpublished results), thus indicating that copper may indeed be limiting. Continuous removal of hydrogen sulfide formed by the microbial activity therefore appears to be of particular relevance since, apart from its direct toxicity to the microbial cell (see above), it also indirectly exerts growth limitation on the microbial consortium within the bioreactor and hence on the performance of the process. Further examples for the complexity associated with the application of the microbial sulfate reduction process for bioremediation of AMD is the competition between SRP and other microorganisms that use the same substrate(s) or microbially produced breakdown products thereof.

Acidity and high temperatures may be the key to support SRP over methanogenesis, which also requires anoxic process conditions. Acidophilic [1, 3, 50, 137, 140, 142, 165] and thermophilic (e.g. [132]) SRPs have been isolated and their application been demonstrated for the sulfidogenic process. Using acidophilic

SRPs additionally permits to specifically recover some of the metals due to pH-dependent differences in the solubility of the various metal sulfides [100] or, to a lesser extent, metal hydroxides. As prices for some of these metals rise, this approach may provide a realistic option to improve the economic viability of microbial bioremediation strategies, similar to the use of schwertmannite resulting from microbial iron oxidation. In this context, it is worthwhile to discuss once more the potential benefits of combining abiotic and biotic strategies for the remediation of AMD. Recovery of metals through metal sulfide precipitation in bioreactors should, for example, be more relevant if the metal content is sufficiently high, which could be achieved via membrane-based filtration techniques (e.g. nanofiltration).

Apart from reliable performance, the main requirement of the process still remains to be the sulfate reduction rate, which must manifest itself in a difference between the sulfate concentration of inflowing AMD and treated water leaving the bioreactor that is sufficient for subsequent discharge into natural waterways. Achieving such an elimination of sulfate within a manageable retention time of the mine water appears to be only feasible with active treatment systems. However, the huge volume of AMD within pit lakes in former or active mining areas means that bioreactor-based designs are unlikely to provide a solution to the problem. Here, improvement of in situ strategies using microbial sulfate reduction seems to be the only available option for bioremediation to play a major role.

References

1. Abicht HK, Mancini S, Karnachuk OV, Solioz M (2011) Genome Sequence of *Desulfosporosinus sp.* OT, an acidophilic aulfate-reducing bacterium from copper mining waste in Norilsk, Northern Siberia. J Bacteriol 193:6104–6105
2. Acero P, Ayora C, Torrentó C, Nieto J (2006) The behavior of trace elements during schwertmannite precipitation and subsequent transformation into goethite and jarosite. Geochim Cosmochim Acta 70:4130–4139
3. Alazard D, Joseph M, Battaglia-Brunet F, Cayol J-L, Ollivier B (2010) *Desulfosporosinus acidiphilus sp. nov.*: a moderately acidophilic sulfate-reducing bacterium isolated from acid mining drainage sediments. Extremophiles 14:305–312
4. Alphenaar PA, Visser A, Lettinga G (1993) The effect of liquid upward velocity and hydraulic retention time on granulation in UASB reactors treating wastewater with a high sulfate content. Bioresour Technol 43:249–258
5. Armentia H, Webb C (1992) Ferrous sulphate oxidation using *Thiobacillus ferrooxidans* cells immobilised in polyurethane foam support particles. Appl Microbiol Biotechnol 36:697–700
6. Asta MP, Cama J, Martínez M, Giménez J (2009) Arsenic removal by goethite and jarosite in acidic conditions and its environmental implications. J Hazard Mater 171:965–972
7. Aubé B, Zinck J (1999) Comparison of AMD treatment processes and their impact on sludge characteristics. In: Proceedings for Sudbury ‘99, Mining and the Environment II, pp 261–270
8. Aubé B, Zinck J (2003) Lime treatment of acid mine drainage in Canada. Brazil-Canda seminar on mine rehabilitation, Florianópolis, Brazil, 1–3 Dec 2003

9. Auld E, Campbell J (1919) Method of treating mine-water (US Patent 1,310,382)
10. Bache B (1964) Aluminium and iron phosphate studies relating to soils: II. Reactions between phosphate and hydrous oxides. J Soil Sci 15:110–116
11. Bäckström M, Sartz L (2011) Mixing of acid rock drainage with alkaline ash leachates—fate and immobilisation of trace elements. Water Air Soil Poll 222:377–389
12. Baker BJ, Banfield JF (2003) Microbial communities in acid mine drainage. FEMS Microbiol Ecol 44:139–152
13. Banks D, Younger P, Amesen R, Iversen E, Banks SB (1997) Mine-water chemistry: the good, the bad and the ugly. Environ Geol 32:157–174
14. Barham R (1997) Schwertmannite: a unique mineral, contains a replaceable ligand, transforms to jarosites, hematites, and/or basi iron sulfate. J Mater Res 12:2751–2758
15. Barber WP, Stuckey DC (1999) The use of the anaerobic baffled reactor (ABR) for wastewater treatment: a review. Water Res 33:1559–1578
16. Barnes LJ, Janssen FJ, Sherren J, Versteegh JH, Koch RO, Scheeren PJH (1991) A new process for the microbial removal of sulfate and heavy metals from contaminated waters extracted by a geohydrological control system. Chem Eng Res Des 69:184–186
17. Barron JL, Lueking DR (1990) Growth and maintenance of *Thiobacillus ferrooxidans* cells. Appl Environ Microbiol 56:2801–2806
18. Barton LL, Fauque GD (2009) Biochemistry, physiology and biotechnology of sulfate-reducing bacteria. Adv Appl Microbiol 68:41–98
19. Barton LL, Hamilton WA (2007) Sulphate-reducing bacteria: environmental and engineered systems. Cambridge University Press, Cambridge
20. Baskaran V, Nemati M (2006) Anaerobic reduction of sulfate in immobilised cell bioreactors, using a microbial culture originated from an oil reservoir. Biochem Eng J31:148–159
21. Battaglia-Brunet F, Dictor MC, Garrido F, Crouzet C, Morin D, Dekeyser K, Clarens M, Baranger P (2002) An arsenic(III)-oxidizing bacterial population: selection, characterisation and performance in reactors. J Appl Microbiol 93:656–667
22. Battaglia-Brunet F, Joulian C, Garrido F, Dictor MC, Morin D, Coupland K, Johnson DB, Hallberg KB, Baranger P (2006) Oxidation of arsenite by *Thiomonas* strains and characterisation of *Thiomonas arsenivorans* sp. nov. Anton Leeuw 89:99–108
23. Batty LC, Younger PL (2002) Critical role of macrophytes in achieving low iron concentrations in mine water treatment wetlands. Environ Sci Technol 36:3997–4002
24. Beijerinck WM (1895) Über *Spirillum desulphuricans*als Ursache von Sulphatreduktion. Zentralbl Bakteriol Parasitk Infekt AbtII 1:49–59
25. Bell F, Bullock S, Hälbich T, Lindsay P (2001) Environmental impacts associated with an abandoned mine in the Witbank Coalfield, South Africa. Int J Coal Geol 45:195–216
26. Benner SG, Blowes DW, Gould WD, Herbert RBJR, Ptacek CJ (1999) Geochemistry of a permeable reactive barrier for metals and acid mine drainage. Environ Sci Technol 33:2793–2799
27. Benthaus and Totsche (2011) Innovative Wasserbehandlung - Pilot- und Demo Vorhaben. Presentation held at 5. Fachkonferenz "Wasserwirtschaftliche Maßnahmen in der Bergbaufolgelandschaft" (01.03.2011), Leipzig/Germany
28. Bigham JM, Schwertmann U, Murad E, Carlson L (1990) A poorly crystallized oxyhydroxysulfate of iron formed by bacterial oxidation of Fe(II) in acid mine waters. Geochim Cosmochim Ac 54:2743–2758
29. Bigham JM, Carlson L, Murad E (1994) Schwertmannite, a new iron oxyhydroxysulphate from Pyhäsalmi, Finland, and other localities. Mineral Mag 58:641–648
30. Bigham JM, Schwertmann U, Traina SJ, Winland RL, Wolf M (1996) Schwertmannite and the chemical modeling of iron in acid sulfate waters. Geochim Cosmochim Acta 60:2111–2121
31. Bijmans MFM (2008) Sulfate reduction under acidic conditions for selective metal recovery. Ph.D. Thesis, Wageningen University, Wageningen, The Netherlands

32. Bijmans MFM, Dopson M, Ennin F, Lens PNL, Buisman CJN (2008) Effect of sulfide removal on sulfate reduction at pH 5 in a hydrogen fed gas-lift bioreactor. J Microbiol Biotechn 18:1809–1818
33. Bijmans MFM, Peeters TWT, Lens PNL, Buisman CJN (2008) High rate sulfate reduction at pH 6 in a pH-auxostat submerged membrane bioreactor fed with formate. Water Res 42:2439–2448
34. Bijmans MFM, Dopson M, Peeters TWT, Lens PNL, Buisman CJN (2009) Sulfate reduction at pH 5 in a high-rate membrane bioreactor: reactor performance and microbial community analyses. J Microbiol Biotechn 19:698–708
35. Bilek F (2012) Reinigungsverfahren von Grundwasser und Oberflächengewässern – Endbericht Februar 2012. Published by Sächsisches Landesamt für Umwelt Landwirtschaft und Geologie
36. Bilek F, Wagner S (2010) Testing autotrophic sulfate reduction to treat sulfate rich groundwaters highly contaminated by mining activities. In: Schirmer M, Hoehn E, Vogt T (eds) GQ10: Groundwater quality management in a rapidly changing world. IAHS Publ. 342 (2011) ISBN 978-1-907161-16-2, pp 512 + xvi. Proceedings of the 7th international groundwater quality conference held in Zurich, Switzerland, 13–18 June 2010
37. Blight KR, Ralph DE (2008) Aluminium sulphate and potassium nitrate effects on batch culture of iron oxidising bacteria. Hydrometallurgy 92:130–134
38. Blodau C, Hoffmann S, Peine A, Peifer S (1998) Iron and sulfate reduction in the sediments of acidic mine lake 116 (Brandenburg, Germany): rates and geochemical evaluation. Water Air Soil Poll 108:249–270
39. Bonnefoy V, Holmes DS (2012) Genomic insights into microbial iron oxidation and iron uptake strategies in extremely acidic environments. Environ Microbiol 14:1597–1611
40. Boshoff G, Duncan J, Rose PD (2004) Tannery effluent as a carbon source for biological sulphate reduction. Water Res 38:2651–2658
41. Bowell RJ (2004) A review of sulfate removal options for mine waters. In: Mine water 2004—Proceedings of the International Mine Water Association symposium vol 2, pp 75–91
42. Brown M, Barley B, Wood H (2002) Minewater treatment. Technology, application and policy. IWA Publishing, London
43. Burgess JE, Stuetz RM (2002) Activated sludge for the treatment of sulphur-rich wastewaters. Miner Eng 15:839–846
44. Carlson L, Bigham JM, Schwertmann U, Kyek A, Wagner F (2002) Scavenging of As from acid mine drainage by schwertmannite and ferrihydrite: a comparison with synthetic analogues. Environ Sci Technol 36:1715–1719
45. Castro HF, Williams NH, Ogram A (1999) Phylogeny of sulfate-reducing bacteria. FEMS Microbiol Ecol 31:1–9
46. Chang IS, Shin PK, Kim BH (2000) Biological treatment of acid mine drainage under sulphate-reducing conditions with solid waste materials as substrate. Water Res 34:1269–1277
47. Chen B, Utgikar VP, Harmon SM, Tabak HH, Bishop DF, Govind R (2000) Studies on biosorption of zinc(II) and copper(II) on *Desulfovibrio desulfuricans*. Int Biodeterior Biodegradation 46:11–18
48. Choo CO, Lee JK (2002) Mineralogical and geochemical controls on the formation of schwertmannite and goethite in the wetland at Dalseong tungsten mine, Korea. Geosci J 6:281–287
49. Chuichulcherm S, Nagpal S, Peeva L, Livingston A (2001) Treatment of metal-containing wastewaters with a novel extractive membrane reactor using sulfate-reducing bacteria. J Chem Technol Biot 76:61–68
50. Church CD, Wilkin RT, Alpers CN, Rye RO, McCleskey RB (2007) Microbial sulfate reduction and metal attenuation in pH 4 acid mine water. Geochem Trans 8:1–10
51. Cohen RRH (2006) Use of microbes for cost reduction of metal removal from metals and mining industry waste streams. J Clean Prod 14:1146–1157

52. Conca JL, Wright J (2006) An Apatite II permeable reactive barrier to remediate groundwater containing Zn, Pb and Cd. Appl Geochem 21:1288–1300
53. Coupland K, Battaglia-Brunet F, Hallberg KB, Dictor MC, Garrido F, Johnson DB (2003) Oxidation of iron, sulfur and arsenic in mine waters and mine wastes: an important role for novel *Thiomonas* spp. In: Tsezos M, Hatzikioseyian A, Remoudaki E (eds) Biohydrometallurgy: a sustainable technology in evolution. National Technical University of Athens, Zografou, pp 639–646
54. Department of Water Affairs and Forestry (2007) Best practice guideline H4: Water Treatment
55. de Smul A, Goethals L, Verstraete W (1999) Effect of COD to sulfate ratio and temperature in expanded-granular-sludge-blanket reactors for sulfate reduction. Process Biochem 34:407–416
56. Deul M, Mihok EA (1971) Limestone neutralization of dilute acid waste waters (US Patent 3,617,560)
57. Deusner C (2003) Entwicklung einer Technologie zur langzeitstabilen biologischen Reinigung schwermetallbelasteter Bergbauwässer. Phd. Thesis, Fakultät Maschinenwesen, Technischen Universität Dresden
58. Dilling W, Cypionka H (1990) Aerobic respiration in sulfate-reducing bacteria. FEMS Microbiol Lett 71:123–127
59. Dittmar T (2004) Hydrochemical processes controlling arsenic and heavy metal contamination in the Elqui river system (Chile). Sci Total Environ 325:193–207
60. Dold B, Fontboté L (2002) A mineralogical and geochemical study of element mobility in sulfide mine tailings of Fe oxide Cu–Au deposits from the Punta del Cobre belt, northern Chile. Chem Geol 189:135–163
61. Dopson M, Johnson DB (2012) Biodiversity, metabolism and applications of acidophilic sulfur-metabolizing microorganisms. Environ Microbiol 14:2620–2631
62. Doshi SM (2006) Bioremediation of acid mine drainage using sulfate-reducing bacteria. Study prepared for U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response, Office of Superfund Remediation and Technology Innovation, Washington, DC
63. Dries J, de Smul A, Goethals L, Grootaerd H, Verstraete W (1998) High rate biological treatment of sulfate-rich wastewater in an acetate-fed EGSB reactor. Biodegradation 9:103–111
64. du Preez LA, Maree JP (1994) Pilot-scale biological sulphate and nitrate removal utilising producer gas as energy source. Water Sci Technol 30:275–285
65. Eskandarpour A, Sassa K, Bando Y, Okido M, Asai S (2006) Magnetic removal of phosphate from wastewater using schwertmannite. Mater Trans 47:1832–1837
66. Eskandarpour A, Onyango M, Ochieng A, Asai S (2008) Removal of fluoride ions from aqueous solution at low pH using schwertmannite. J Hazard Mater 152:571–579
67. Esposito G, Veeken A, Weijma J, Lens PNL (2006) Use of biogenic sulfide for ZnS precipitation. Sep Purif Technol 51:31–39
68. Evangelou V (1998) Pyrite chemistry: the key for abatement of acid mine drainage. In: Geller A, Klapper H, Salomons W (eds) Acidic mining lakes: acid mine drainage, limology and reclamation. Springer, Berlin, pp 197–222
69. Fedorovich V, Greben M, Kalyuzhnyi S, Lens PNL, Hulshoff-Pol L (2001) Use of hydrophobic membranes to supply hydrogen to sulphate reducing bioreactors. Biodegradation 11:295–303
70. Feng D, Aldrich C, Tan H (2000) Treatment of acid mine water by use of heavy metal precipitation and ion exchange. Miner Eng 13:623–642
71. Foucher S, Battaglia-Brunet F, Ignatiadis I, Morin D (2001) Treatment by sulfate-reducing bacteria of chessy acid-mine drainage and metals recovery. Chem Eng Sci 56:1639–1645
72. García-Moyano A, González-Toril E, Aguilera Á, Amils R (2012) Comparative microbial ecology study of the sediments and the water column of the Río Tinto, an extreme acidic environment. FEMS Microbiol Ecol 81:303–314

73. Garcíaa C, Moreno DA, Ballester A, Blázquez ML, González F (2001) Bioremediation of an industrial acid mine water by metal-tolerant sulphate-reducing bacteria. Miner Eng 14:997–1008
74. Genschow E, Hegemann W, Maschke C (1996) Biological sulfate removal from tannery wastewater in a two-stage anaerobic treatment. Water Res 30:2072–2078
75. George R, Zoutberg GR, de Been P (1997) The Biobed® EGSB (Expanded Granular Sludge Bed) system covers shortcomings of the upflow anaerobic sludge blanket reactor in the chemical industry. Water Sci Technol 35:183–187
76. Giaveno A, Lavalle L, Guibal E, Donati E (2008) Biological ferrous sulfate oxidation by *A. ferrooxidans* immobilised on chitosan beads. J Microbiol Methods 72:227–234
77. Gibert O, de Pablo J, Cortina JL, Ayora C (2002) Treatment of acid mine drainage by sulphate-reducing bacteria using permeable reactive barriers: a review from laboratory to full-scale experiments. Rev Environ Sci Biotechnol 1:327–333
78. Gibson GR (1990) Physiology and ecology of the sulphate-reducing bacteria—a review. J Appl Bacteriol 69:769–797
79. Glombitza F (2001) Treatment of acid lignite mine flooding water by means of microbial sulfate reduction. Waste Manag 21:197–203
80. Glombitza F, Janneck E, Arnold I, Rolland W, Uhlmann W (2007) Eisenhydroxysulfate aus der Bergbauwasserbehandlung als Rohstoff. In: Wellmer F, Schilka W (eds) Consulting-Erfahrungen und Kontakte für Neuanfänge; Untertägiger Bergbau auf Industrieminerale in Deutschland; Vorträge der beiden Tagungen des Fachausschusses Rohstoffwirtschaft der GDMC Gesellschaft für Bergbau, Metallurgie, Rohstoff- und Umwelttechnik e.V. GDMB-Medienverlag, Clausthal-Zellerfeld, pp 31–40
81. Gómez JM, Cantero D, Webb C (2000) Immobilisation of *Thiobacillus ferrooxidans* cells on nickel alloy fibre for ferrous sulfate oxidation. Appl Microbiol Biotechnol 54:335–340
82. Goncalves MMM, Leite SGF, Santanna JR GL (2005) The bioactivation procedure for increasing the sulphate-reducing bacteria in a UASB reactor. Braz J Chem Eng (online) 22:565–571
83. Greben H (2007) Focus on CSIR research in pollution waste: cellulose degradation, volatile fatty acid formation and biological sulphate removal operating and anaerobic hybrid reactor. 2007 Stockholm World Water Week, 13–17 Aug 2007, p 2
84. Greben HA, Maree JP (2000) The effect of reactor type and hydraulic residence time on biological sulphate and sulphide removal rates. Presented at the WISA 2000 Biennial conference, Sun City, South Africa, 28 May–1 June 2000
85. Greben HA, Matshusa MP, Maree JP (2005) The biological sulphate removal technology. In: Proceedings of the 9th international mine water congress, Oviedo, Spain, 5–7 Sept 2005
86. Grishin S, Tuovinen OH (1988) Fast kinetics of Fe^{2+} oxidation in packed-bed reactors. Appl Environ Microbiol 54:3092–3100
87. Groudev S, Geprgiev P, Spasova I, Nicolova M, Angelov A, Diels L (2005) Cleanup of acid mine drainage by means of a pilot-scale passive system. In: Annual of the University of Mining and Geology "St. Ivan Rilski" 48 (Part I—Geology and Geophysics)
88. Gyure RA, Konopka A, Brooks A, Doemel W (1990) Microbial sulfate reduction in acidic (pH 3) strip-mine lakes. FEMS Microbiol Lett 73:193–201
89. Haddadin J, Morin D, Ollivier P, Fick M (1993) Effect of different carbon dioxide concentrations on ferrous iron and pyrite oxidation by a mixed culture of iron and/or sulfur-oxidizing bacteria. Enzyme Microb Technol 15:832–841
90. Hallberg KB (2010) New perspectives in acid mine drainage microbiology. Hydrometallurgy 104:448–453
91. Hallberg KB, Johnson DB (2003) Passive mine water treatment at the former Wheal Jane tin mine, Cornwall: important biogeochemical and microbiological lessons. Land Contam Reclam 11:213–220
92. Hallberg KB, Coupland K, Kimura S, Johnson DB (2006) Macroscopic streamer growths in acidic, metal-rich mine waters in North Wales consist of novel and remarkably simple bacterial communities. Appl Environ Microbiol 72:2022–2030

93. Hammarstrom JM, Sibrell PL, Belkin HE (2003) Characterisation of limestone reacted with acid-mine drainage in a pulsed limestone bed treatment system at the Friendship Hill National Historical Site, Pennsylvania, USA. Appl Geochem 18:1705–1721
94. Hansen TA (1994) Metabolism of sulfate-reducing prokaryotes. Anton Leeuw 66:165–185
95. Hao C, Zhang L, Wang L, Li S, Dong H (2012) Microbial community composition in acid mine drainage lake of Xiang Mountain sulfide mine in Anhui Province, China. Geomicrobiol J 29:886–895
96. Hasche-Berger A, Wolkersdorfer C (2005) Pilot scale RAPS-system in Gernrode/Harz Mountains. In: Merkel BJ, Hasche-Berger A (eds) Uranium in the environment. Springer, Heidelberg, pp 317–328
97. Hedin RS, Nairn R, Kleinmann R (1994) Passive treatment of coal mine drainage. U.S. Bureau of Mines I.C. 9389, 35 pp
98. Hedrich S, Lünsdorf H, Kleeberg R, Heide G, Seifert J, Schlömann M (2011) Schwertmannite formation adjacent to bacterial cells in a mine water treatment plant and in pure cultures of *Ferrovum myxofaciens*. Environ Sci Technol 45:7685–7692
99. Hedrich S, Schlömann M, Johnson DB (2011b) The iron-oxidizing proteobacteria. Microbiology 157:1551–1584
100. Hedrich S, Johnson DB (2012) A modular continuous flow reactor system for the selective bio-oxidation of iron and precipitation of schwertmannite from mine-impacted waters. Bioresour Technol 106:44–49
101. Heinzel E, Hedrich S, Janneck E, Glombitza F, Seifert J, Schlömann M (2009) Bacterial diversity in a mine water treatment plant. Appl Environ Microbiol 75:858–861
102. Heinzel E, Janneck E, Glombitza F, Schlömann M, Seifert J (2009) Population dynamics of iron-oxidizing communities in pilot plants for the treatment of acid mine waters. Environ Sci Technol 43:6138–6144
103. Hlabela P, Maree J, Bruinsma D (2007) Barium carbonate process for sulphate and metal removal from mine water. Mine Water Environ 26:14–22
104. Hoa TTH, Liamleam W, Annachhatre AP (2007) Lead removal through biological sulfate reduction process. Bioresour Technol 98:2538–2548
105. Hockin SL, Gadd GM (2007) Bioremedation of metals and metalloids by precipitation and cellular binding. In: Barton LL, Hamilton WA (eds) Sulphate-reducing bacteria—environmental and engineered systems. Cambridge University Press, Cambridge, pp 405–434
106. Höglund LO, Herbert R Jr (eds) (2004) MiMi-performance assessment main report.MiMi 2003:3. The MISTRA-programme MiMi, Mitigation of the environmental impact from mining waste, MiMi Print, Luleå, Sweden, 2004. http://www.mistra-research.se/program/mimi/guide/home/documentation/mimiperformanceassessmentmainreport.4.c791f4103209a06ec80008117.html
107. Huisman JL, Schouten G, Schultz C (2006) Biologically produced sulphide for purification of process streams, effluent treatment and recovery of metals in the metal and mining industry. Hydrometallurgy 83:106–113
108. Hulshoff-Pol LW, Lens PNL, Stams AJM, Lettinga G (1998) Anaerobic treatment of sulphate-rich wastewaters. Biodegradation 9:213–224
109. Ilbert M, Bonnefoy V (2013) Insight into the evolution of the iron oxidation pathways. Biochim Biophys Acta Bioenerg 1827:161–175
110. INAP—International Network for Acid Prevention (2009) Global Acid Rock Drainage Guide (GARD Guide). http://www.gardguide.com/. Accessed 16 June 2013
111. Ingledew WJ (1982) *Thiobacillus ferrooxidans*. The bioenergetics of an acidophilic chemolithotroph. Biochim Biophys Acta, Rev Bioenerg 683:89–117
112. Isa Z, Grusenmeyer S, Verstraete W (1986) Sulfate reduction relative to methane production in high-rate anaerobic digestion: microbiological aspects. Appl Environ Microb 51:580–587
113. Jage C, Zipper C, Noble R (2001) Factors affecting alkalinity generation by successive alkalinity-producing systems. J Environ Qual 30:1015–1022

114. Janneck E, Glombitza F, Terno D, Wolf M, Patzig A, Fischer H, Rätzel G, Herbach K (2008) Umweltfreundliche biotechnologische Gewinnung von Eisenhydroxysulfaten aus der Bergbauwasserbehandlung und deren Verwertung als Roh- und Grundstoff in der keramischen, Baustoffe produzierenden sowie Farben und Pigmente herstellenden Industrie zur Kosten-, Rohstoff- und Ressourceneinsparung, Teilprojekt 1: Koordination sowie Anlagenbetrieb und Produktherstellung. BMBF-Research report, FKZ: 01RI5013
115. Janneck E, Arnold I, Koch, T, Meyer J, Burghardt D, Ehinger S (2010) Microbial synthesis of schwertmannite from lignite mine water and its utilisation for removal of arsenic from mine waters and for production of iron pigments. In: Wolkersdorfer C, Freund A (eds) Mine water & innovative thinking. Proceedings of the International Mine Water Association symposium 2010. Cape Breton University Press, Sydney
116. Janneck E, Burghardt D, Martin M, Damian C, Schöne G, Meyer J, Peiffer S (2011) From waste to valuable substance: utilisation of schwertmannite and lignite filter ash for removal of arsenic and uranium from mine drainage. In: Rüde T, Freund A, Wolkersdorfer C (eds) 11th International Mine Water Association congress—mine water—managing the challenges, pp 359–364
117. Jensen AB, Webb C (1994) A trickle bed reactor for ferrous sulphate oxidation using *Thiobacillus ferrooxidans*. Biotechnol Tech 8:87–92
118. Ji SW, Kim SJ (2008) Lab-scale study on the application of in-adit-sulfate-reducing system for AMD control. J Hazard Mater 160:441–447
119. Jin S, Fallgren PH, Morris JM, Cooper JS (2008) Source treatment of acid mine drainage at a backfilled coal mine using remote sensing and biogeochemistry. Water Air Soil Poll 188:205–212
120. Jong T, Parry DL (2006) Microbial sulfate reduction under sequentially acidic conditions in an upflow anaerobic packed bed bioreactor. Water Res 40:2561–2571
121. Johnson DB (2003) Chemical and microbiological characteristics of mineral spoils and drainage waters at abandoned coal and metal mines. Water Air Soil Poll 3:47–66
122. Johnson DB, Rolfe S, Hallberg KB, Iversen E (2001) Isolation and phylogenetic characterisation of acidophilic microorganisms indigenous to acidic drainage waters at an abandoned Norwegian copper mine. Environ Microbiol 3:630–637
123. Johnson DB, Hallberg KB (2003) The microbiology of acidic mine waters. Res Microbiol 154:466–473
124. Johnson DB, Hallberg KB (2005) Acid mine drainage remediation options: a review. Sci Total Environ 338:3–14
125. Jönsson J, Persson P, Sjöberg S, Lövgren L (2005) Schwertmannite precipitated from acid mine drainage: phase transformation, sulphate release and surface properties. Appl Geochem 20:179–191
126. Juby GJG, Shutte CF (2000) Membrane life in a seeded-slurry reverse osmosis system. Water SA26:239–248
127. Junfeng Y, Guoliang L, Wei C (2007) Ferrous sulphate oxidation using *Acidithiobacillus ferrooxidans* cells immobilised in ceramic beads. Chem Biochem Eng Q 21:175–179
128. Kahrizi E, Alemzadeh I, Vossoughi M (2009) Bio-oxidation of ferrous iron by *Acidithiobacillus ferrooxidans* in a monolithic bioreactor. J Chem Technol Biotechnol 84:504–510
129. Kaksonen AH, Puhakka JA (2007) Sulfate reduction based bioprocesses for the treatment of acid mine drainage and the recovery of metals. Eng Life Sci 7:541–564
130. Kaksonen AH, Riekkola-Vanhanenc M-L, Puhakka JA (2003) Optimization of metal sulphide precipitation in fluidized-bed treatment of acidic wastewater. Water Res 37:255–266
131. Kaksonen AH, Franzmann PD, Puhakka JA (2004) Effects of hydraulic retention time and sulfide toxicity on ethanol and acetate oxidation in sulfate-reducing metal-precipitating fluidized-bed reactor. Biotechnol Bioeng 86:332–343

132. Kaksonen AH, Spring S, Schumann P, Kroppenstedt RM, Puhakka JA (2006) *Desulfotomaculum thermosubterraneum* sp. nov., a thermophilic sulfate-reducer isolated from an underground mine located in a geothermally active area. Int J Syst Evol Microbiol 56:2603–2608
133. Kalin M, Fyson A, Wheeler WN (2006) The chemistry of conventional and alternative treatment systems for the neutralization of acid mine drainage. Sci Total Environ 366:395–408
134. Kalyuzhnyi SV, de Leon FC, Rodriguez MJ (1997) Biological sulfate reduction using ethanol as electron donor. Microbiologiya 66:687–693
135. Kawano M, Tomita K (2001) Geochemical modeling of bacterially induced mineralization of schwertmannite and jarosite in sulfuric acid spring water. Am Mineral 86:1156–1165
136. Kelly DP (1978) Bioenergetics of chemolithotrophic bacteria. In: Bull A, Meadow P (eds) Companion to microbiology: selected topic for further study. Longman, London
137. Kimura S, Hallberg KB, Johnson DB (2006) Sulfidogenesis in low pH (3.8–4.2) media by a mixed population of acidophilic bacteria. Biodegradation 17:57–65
138. Kirby CS, Thomas H, Southam G, Donald R (1999) Relative contributions of abiotic and biological factors in Fe(II) oxidation in mine drainage. Appl Geochem 14:511–530
139. Knoblauch C, Jørgensen BB (1999) Effect of temperature on sulphate reduction, growth rate and growth yield in five psychrophilic sulphate reducing bacteria from Arctic sediments. Environ Microbiol 65:4230–4233
140. Kolmert A, Johnson DB (2001) Remediation of acidic waste waters using immobilised, acidophilic sulfate-reducing bacteria. J Chem Technol Biot 76:836–843
141. Koschorreck M (2008) Microbial sulphate reduction at a low pH. FEMS Microbiol Ecol 64:329–342
142. Koschorreck M, Wendt-Potthoff K, Geller W (2003) Microbial sulfate reduction at low pH in sediments of an acidic lake in Argentina. Environ Sci Technol 37:1159–1162
143. Krekeler D, Teske A, Cypionka H (1998) Strategies of sulfate-reducing bacteria to escape oxygen stress in a cyanobacterial mat. FEMS Microbiol Ecol 25:89–96
144. Lancy ED, Tuovinen OH (1984) Ferrous ion oxidation by *Thiobacillus ferrooxidans* immobilised in calcium alginate. Appl Microbiol Biotechnol 20:94–99
145. Ledin M, Pedersen K (1996) The environmental impact of mine wastes—roles of microorganisms and their significance in treatment of mine wastes. Earth Sci Rev 41:67–108
146. Lens PNL, Vallero M, Esposito G, Zandvoort M (2002) Perspectives of sulfate reducing bioreactors in environmental biotechnology. Rev Environ Sci Biotechnol 1:311–325
147. Lens PNL, Vallero M, Esposito G (2007) Bioprocess engineering of sulphate reduction for environmental technology. In: Barton LL, Hamilton WA (eds) Sulphate-reducing bacteria—environmental and engineered systems. Cambridge University Press, Cambridge, pp 383–404
148. Lenz M, van Hullebusch ED, Hommes G, Corvini PFX, Lens PNL (2008) Selenate removal in methanogenic and sulfate-reducing upflow anaerobic sludge bed reactors. Water Res 42:2184–2194
149. le Robinson RE, Barnard R, Riche FJ (1998) The treatment of acid effluent from the Grootvlei Mine using novel IX techniques. J S Afr I Min Metall 98:343–352
150. Liamleam W, Annachhatre AP (2007) Electron donors for biological sulfate reduction. Biotechnol Adv 25:452–463
151. Lin Y, Lee K (2001) Verification of anaerobic biofilm model for phenol degradation with sulfate reduction. J Environ Eng 127:119–125
152. Liu H, Chen B, Lan Y, Cheng Y (2004) Biosorption of Zn(II) and Cu(II) by the indigenous *Thiobacillus thiooxidans*. Chem Eng J 97:195–201
153. Long Z, Huang Y, Cai Z, Cong W, Ouyang F (2003) Biooxidation of ferrous iron by immobilised *Acidithiobacillus ferrooxidans* in poly(vinyl alcohol) cryogel carriers. Biotechnol Lett 25:245–249
154. Long Z, Huang Y, Cai Z, Cong W, Ouyang F (2004) Immobilisation of *Acidithiobacillus ferrooxidans* by a PVA–boric acid method for ferrous sulphate oxidation. Process Biochem 39:2129–2133

155. Lopez O (2009) Green technologies for sulphate and metal removal in mining and metallurgical effluents. In: Wiertz J, Moran C (eds) ENVIROMINE 2009—Proceedings of the I. International seminar on environmental issues in the mining industry, 30 Sept–2 Oct 2009, Santiago, Chile
156. Lottermoser B (2010) Mine wastes—characterisation, treatment and environmental impact. Springer, Berlin, p 400
157. Lovley DR, Roden EE, Phillips EJP, Woodward JC (1993) Enzymatic iron and uranium reduction by sulfate-reducing bacteria. Mar Geol 113:41–53
158. Macy JM, Santini JM, Pauling BV, O'Neill AH, Sly LI (2000) Two new arsenate/sulfate-reducing bacteria: mechanisms of arsenate reduction. Arch Microbiol 173:49–57
159. Maree JP, Strydom WF (1987) Biological sulphate removal from industrial effluent in an upflow packed bed reactor. Water Res 21:141–146
160. Maree JP, Hulse G, Dods D, Schutte CE (1991) Pilot plant studies on biological sulphate removal from industrial effluent. Water Sci Technol 23:1293–1300
161. Maree JP, Hlabela P, Nengovhela R, Geldenhuys AJ, Mbhele N, Nevhulaudzi T, Waanders FB (2004) Treatment of mine water for sulphate and metal removal using barium sulphide. Mine Water Environ 23:195–203
162. Maree JP, Netshidaulu I, Strobos G, Nengovhela R, Geldenhuys AJ (2004b) Integrated process for biological sulfate removal and sulphur recovery. In: Proceedings of the 2004 Water Institute of Southern Africa (WISA) biennial conference, pp 929–940. http://www.ewisa.co.za/literature/files/163.pdf. 15 June 2013
163. McDonald DM, Webb J, Musgrave R (2006) The effect of neutralisation method and reagent on the rate of Cu and Zn release from Acid Rock Drainage treatment sludges. In: Barnhisel RI (ed) 7th International Conference on Acid Rock Drainage (ICARD), 26–30 March 2006, St. Louis, MO. American Society of Mining and Reclamation, Lexington, pp 1198–1219
164. Meier J, Piva A, Fortin D (2012) Enrichment of sulfate-reducing bacteria and resulting mineral formation in media mimicking pore water metal ion concentrations and pH conditions of acidic pit lakes. FEMS Microbiol Ecol 79:69–84
165. Moosa S, Nemati M, Harrison STL (2002) A kinetic study on anaerobic reduction of sulphate, part I: effect of sulphate concentration. Chem Eng Sci 57:2773–2780
166. Mortazavi S (2008) Application of membrane separation technology to mitigation of mine effluent and acid drainage—MEND Report 3.15.1. http://www.mend-nedem.org/reports/files/3.15.1.pdf. Accessed 15 June 2013
167. Mousavi SM, Yaghmaei S, Jafari A (2007) Influence of process variables on biooxidation of ferrous sulfate by an indigenous *Acidithiobacillus ferrooxidans.* Part II: Bioreactor experiments. Fuel 86:993–999
168. Murad E, Rojik P (2003) Iron-rich precipitates in a mine drainage environment: influence of pH on mineralogy. Am Mineral 88:1915–1918
169. Murad E, Rojik P (2004) Jarosite, schwertmannite, goethite, ferrihydrite and lepidocrocite: the legacy of coal and sulfide ore mining. 3rd Australian New Zealand Soils Conference, Sydney
170. Muthumbi W, Boon N, Boterdaele R, de Vreese I, Top EM, Verstraete W (2001) Microbial sulfate reduction with acetate: process performance and composition of the bacterial communities in the reactor at different salinity levels. Appl Microbiol Biot 55:787–793
171. Muyzer G, Stams AJM (2008) The ecology and biotechnology of sulphate-reducing bacteria. Nature Rev Microbiol 6:441–454
172. Nagpal S, Chuichulcherm S, Peeva L, Livingston A (2000) Microbial sulfate reduction in a liquid–solid fluidized bed reactor. Biotechnol Bioeng 70:370–380
173. Nemati M, Webb C (1996) Effect of ferrous iron concentration on the catalytic activity of immobilised cells of *Thiobacillus ferrooxidans*. Appl Microbiol Biotechnol 46:250–255
174. Nemati M, Harrison S, Hansford GS, Webb C (1998) Biological oxidation of ferrous sulphate by *Thiobacillus ferrooxidans*: a review on the kinetic aspects. Biochem Eng J 1:171–190

175. Nicolella C, van Loosdrecht MCM, Heijnen JJ (2000) Wastewater treatment with particulate biofilm reactors. J Biotechnol 80:1–33
176. Nitsche C (2001) Tagebauseen: Wasserbeschaffenheit und wassergütewirtschaftliche Sanierung – Konzeptionelle Vorstellungen und erste Erfahrungen. Hg. v. Landesumweltamt Brandenburg (LUA), Potsdam (Studien und Tagungsberichte, Band 35)
177. Nordstrom DK (2011) Mine waters: acidic to circumneutral. Elements 7:393–398
178. Nordstrom DK, Alpers CN (1999) Geochemistry of acid mine waters. In: Plumlee GS, Logsdon MJ (eds) The environmental geochemistry of mineral deposits: reviews in economic geology, vol 6A. Society of Economic Geologists, Inc, Littleton, pp 133–160
179. Ollivier B, Cayol J-L, Fauque G (2007) Sulphate-reducing bacteria from oil field environment and deep-sea hydrothermal vents. In: Barton LL, Hamilton WA (eds) Sulphate-reducing bacteria—environmental and engineered systems. Cambridge University Press, Cambridge, pp 305–328
180. Omil F, Lens P, Hulshoff-Pol LW, Lettinga G (1997) Characterization of biomass from a sulfidogenic, volatile fatty acid-degrading granular sludge reactor. Enzyme Microb Tech 20:229–236
181. Orem W (2007) Sulfur contamination in the Florida Everglades: initial examination of mitigation strategies. U.S. Geological Survey Open-File Report 2007-1374
182. Ottow JCG, Bidlingmaier W (1997) Umweltbiotechnologie. Gustav Fischer Verlag, Stuttgart
183. PAQUES (2013) http://en.paques.nl/pageid=64/SULFATEQ%E2%84%A2.html. Accessed 15 June 2013
184. Parravicini V, Svardal K, Kroiss H (2006) Anaerobe biologische Sulfatentfernung aus Industrieabwässern am Beispiel einer Viskosefabrik. Oesterr Wasser Abfallwirtsch 58:19–25
185. Paulo PL, Kleerebezem R, Lettinga G, Lens PNL (2005) Cultivation of high-rate sulfate reducing next term sludge by pH-based electron donor dosage. J Biotechnol 118:107–116
186. Pearson F, McDonnell A (1975) Limestone barriers to neutralize acidic streams. J Env Eng Div 101:425–440
187. Pearson F, McDonnell A (1975) Use of crushed limestone to neutralize acid wastes. J Env Eng Div 101:139–158
188. Pérez Silva RM, Ábalos Rodríguez A, De Oca Gómez Montes JM, Cantero Moreno D (2009) Biosorption of chromium, copper, manganese and zinc by *Pseudomonas aeruginosa* AT18 isolated from a site contaminated with petroleum. Bioresour Technology 100: 1533–1538
189. Phifer MA, Sappington FC, Millings MR, Turick CE, McKinsey PC (2003) D-Area sulfate reduction, DIW-1 organic application field study (U), Prepared for the U.S. Department of Energy
190. PIRAMID Consortium (2003) Engineering guidelines for the passive remediation of acidic and/or metalliferous mine drainage and similar wastewaters. University of Newcastle Upon Tyne, Newcastle Upon Tyne UK, European Commission 5th Framework RTD Project no. EVK1-CT-1999-000021: Passive in situ remediation of acidic mine/industrial drainage (PIRAMID)
191. Pollmann K, Raff J, Merroun M, Fahmy K, Selenska-Pobell S (2006) Metal binding by bacteria from uranium mining waste piles and its technological applications. Biotechnol Adv 24:58–68
192. Poinapen J, Wentzel MC, Ekama GA (2009) Biological sulphate reduction with primary sewage sludge in an upflow anaerobic sludge bed (UASB) reactor—Part 1: feasibility study. Water SA (Online) 35:525–534
193. Poinapen J, Ekama GA, Wentzel MC (2009) Biological sulphate reduction with primary sewage sludge in an upflow anaerobic sludge bed (UASB) reactor—Part 3: performance at 20°C and 35°C. Water SA 35:543–552
194. Praharaj T, Fortin D (2004) Indicators of microbial sulfate reduction in acidic sulfide-rich mine tailings. Geomicrobiol J 21:457–467

195. Preuß V, Koch T, Schöpke R, Koch R, Rolland W (2007) Weitergehende Gruben wasserreinigung - Sulfatentfernung mittels Nanofiltration. In: Merkel BJ, Schaeben H, Wolkersdorfer C, Hasche A (eds) Behandlungstechnologien für bergbaubeeinflusste Wässer + GIS-Geowissenschaftliche Anwendungen und Entwicklungen. Wiss. Mittl. Inst. f. Geologie, Nr. 35/2007, pp 19–25
196. Pümpel T, Macaskie LE, Finlay JA, Diels L, Tsezos M (2003) Nickel removal from nickel plating waste water using a biologically active moving-bed sand filter. Biometals 16:567–581
197. Quatrini R, Appia-Ayme C, Denis Y, Jedlicki E, Holmes DS, Bonnefoy V (2009) Extending the models for iron and sulfur oxidation in the extreme acidophile *Acidithiobacillus ferrooxidans*. BMC Genomics 10:394–413
198. Rabus R, Brüchert V, Amann J, Könneke M (2002) Physiological response to temperature changes of the marine, sulfate-reducing bacterium *Desulfobacterium autotrophicum*. FEMS Microbiol Ecol 42:409–417
199. Raff J, Soltmann U, Matys S, Selenska-Pobell S, Böttcher H, Pompe W (2003) Biosorption of uranium and copper by biocers. Chem Mater 15:240–244
200. Regenspurg S, Peiffer S (2005) Arsenate and chromate incorporation in schwertmannite. Appl Geochem 20:1226–1239
201. Ribeiro D, Maretto D, Noguiera F, Silva M, Campos F, Domont G, Poppi R, Ottoboni L (2011) Heat and phosphate starvation effects on the proteome, morphology and chemical composition of the biomining *Acidithiobacillus ferrooxidans*. World J Microbiol Biotechnol 27:1469–1479
202. Robbins EI, Cravotta CA III, Savela CE, Nord GL Jr (1999) Hydrobiogeochemical interactions in 'anoxic' limestone drains for neutralization of acidic mine drainage. Fuel 78:259–270
203. Robertson AM, Everett D, du Plessis P (1993) Sulfate removal by GYP-CIX process following lime treatment. SUPERFUND XIV conference, 30/11-2/12/93, Washington, USA
204. Rose PD, Boshoff GA, van Hille RP, Wallace LCM, Dunn KM, Duncan JR (1998) An integrated algal sulphate reducing high rate ponding process for the treatment of acid mine drainage wastewaters. Biodegradation 9:247–257
205. Rötting TS, Caraballo MA, Serrano JA, Ayora C, Carrera J (2008) Field application of calcite Dispersed Alkaline Substrate (calcite-DAS) for passive treatment of acid mine drainage with high Al and metal concentrations. Appl Geochem 23:1660–1674
206. Rötting TS, Thomas RC, Ayora C, Carrera J (2008) Passive treatment of acid mine drainage with high metal concentrations using dispersed alkaline substrate. J Environ Qual 37:1741–1751
207. Rowe OF, Johnson DB (2008) Comparison of ferric iron generation by different species of acidophilic bacteria immobilised in packed-bed reactors. Syst Appl Microbiol 31:68–77
208. Sabumon PC (2008) Development of enhanced sulphidogenesis process for the treatment of wastewater having low COD/SO_4^{2-} ratio. J Hazard Mater 159:616–625
209. Sagemann J, Jørgensen BB, Greef O (1998) Temperature dependence and rates of sulfate reduction in cold sediments of Svalbard, Arctic Ocean. Geomicrobiol J 15:85–100
210. Sass H, Cypionka H (2007) Response of sulphate-reducing bacteria to oxygen. In: Barton, LL, Hamilton WA (eds) Sulphate reducing bacteria—environmental and engineered systems. Cambridge University Press, Cambridge, pp 167–183
211. Schipek M, Merkel BJ (2009) Mine water treatment—results using CO_2 and dumped fly ash in AMD affected lakes. Freiberg Online Geology 24:38–42
212. Schippers A, Breuker A, Blazejak A, Bosecker K, Kock D, Wright TL (2010) The biogeochemistry and microbiology of sulfidic mine waste and bioleaching dumps and heaps, and novel Fe(II)-oxidizing bacteria. Hydrometallurgy 104:342–350
213. Schippers A, Hedrich S, Vasters J, Drobe M, Sand W, Willscher S (2013) Biomining: metal revovery from ores with microorganisms. In: Schippers A, Glombitza F, Sand W (eds) Advances in biochemical engineering/biotechnology, Geobiotechnology. Springer, Berlin

214. Schwertmann U, Bigham JM, Murad E (1995) The first occurrence of schwertmannite in a natural stream environment. Eur J Mineral 7:547–552
215. Seeger M, Jerez CA (1993) Response of *Thiobacillus ferrooxidans* to phosphate limitation. FEMS Microbiol Rev 11:37–42
216. Selvaraj PT, Little MH, Kaufman EN (1997) Biodesulfurization of flue gases and other sulfate/sulfite waste streams using immobilised mixed sulfate-reducing bacteria. Biotechnol Progr 13:583–589
217. Sen AM, Johnson DB (1999) Acidophilic sulphate-reducing bacteria: candidates for bioremediation of acid mine drainage. In: Amils R, Ballester A (eds) Biohydrometallurgy and the environment toward the mining of the 21st century. process metallurgy 9A. Elsevier, Amsterdam, pp 709–718
218. Senko JM, Wanjugi P, Lucas M, Bruns MA, Burgos WD (2008) Characterisation of Fe(II) oxidizing bacterial activities and communities at two acidic Appalachian coalmine drainage-impacted sites. ISME J 2:1134–1145
219. Sheoran AS, Sheoran V, Choudhary RP (2010) Bioremediation of acid-rock drainage by sulphate-reducing prokaryotes: a review. Miner Eng 23:1073–1100
220. Silva AJ, Varesche MB, Foresti E, Zaiat M (2002) Sulphate removal from industrial wastewater using a packed-bed anaerobic reactor. Process Biochem 37:927–935
221. Singer P, Stumm W (1970) Acidic mine drainage: the rate-determining step. Science 167:1121–1123
222. Sipma J, Osuna MB, Lettinga G, Stams AJM, Lens PNL (2007) Effect of hydraulic retention time on sulfate reduction in a carbon monoxide fed thermophilic gas lift reactor. Water Res 41:1995–2003
223. Sivula LJ, Väisänen AO, Rintala JA (2007) Treatment of leachate from MSWI bottom ash landfilling with anaerobic sulphate-reducing process. Water Res 41:835–841
224. Skousen J (1991) Anoxic limestone drains for acid mine drainage treatment. Green Lands 21:30–35
225. Skousen J, Rose A, Geidel G, Foreman J, Evans R, Hellier W (1998) A handbook of technologies for avoidance and remediation of acid mine drainage. The National Mine Land Reclamation Center, Morgantown, West Virginia
226. Skousen J, Sexstone A, Ziemkiewicz P (2000) Acid mine drainage control and treatment. In: Barnhisel RI, Darmody R, Daniels W (eds) Reclamation of drastically disturbed lands. American Society of Agronomy; Crop Science Society of America; Soil Science Society of America, Madison, Wisconsin, pp 131–168
227. Smuda J, Dold B, Spangenberg JE, Pfeifer H (2008) Geochemistry and stable isotope composition of fresh alkaline porphyry copper tailings: implications on sources and mobility of elements during transport and early stages of deposition. Chem Geol 256:62–76
228. Song Y-C, Piak B-C, Shin H-S, La S-J (1998) Influence of electron donor and toxic materials on the activity of sulfate reducing bacteria for the treatment of electroplating wastewater. Water Sci Technol 38:187–194
229. Sonntag H (2007) Problem Sulfat in der Spree - Stand der Diskussion und aktuelle Trends. In: Proceedings oft he 58. Berg- und Hüttenmännischer Tag - Behandlungstechnologien für bergbaubeeinflusste Wässer/GIS- Geowissenschaftliche Anwendungen und Entwicklungen, pp 151–156
230. Stucki G, Hanselmann KW, Hürzeler RA (1993) Biological sulfuric acid transformation: reactor design and process optimization. Biotechnol Bioeng 41:303–315
231. Stumm W, Morgan J (1981) Aquatic chemistry: an introduction emphasizing chemical equilibria in natural waters, 2nd edn. Wiley-Interscience, New York
232. Tabak HH, Govind R (2003) Advances in biotreatment of acid mine drainage and biorecovery of metals: 2. Membrane bioreactor system for sulfate reduction. Biodegradation 14:437–452
233. Tabak HH, Scharp R, Burckle J, Kawahara FK, Govind R (2003) Advances in biotreatment of acid mine drainage and biorecovery of metals: 1. Metal precipitation for recovery and recycle. Biodegradation 14:423–436

234. Tan GL, Shu WS, Zhou WH, Li XL, Lan CY, Huang LN (2009) Seasonal and spatial variations in microbial community structure and diversity in the acid stream draining across an ongoing surface mining site. FEMS Microbiol Ecol 70:121–129
235. Thiruvenkatachari R, Vigneswaran S, Naidu R (2008) Permeable reactive barrier for groundwater remediation. J Ind Eng Chem 14:145–156
236. Tischler JS, Wiacek C, Janneck E, Schlömann M (2013) Microbial abundance in the schwertmannite formed in a mine water treatment plant. Mine Water Environm. doi: 10.1007/s10230-013-0250-8
237. Tischler JS, Wiacek C, Janneck E, Schlömann M (2013) Bench-scale study of the effect of phosphate on an aerobic iron oxidation plant for mine water treatment, Water Res. doi: 10.1016/j.watres.2013.09.049
238. Todd J, Reddick K (1997) Acid mine drainage. Groundwater Pollution Primer, Civil Engineering Department, Virginia Tech
239. Touze S, Battaglia-Brunet F, Ignatiadis I (2008) Technical and economical assessment and extrapolation of a 200-dm^3 pilot bioreactor for reduction of sulphate and metals in acid mine waters. Water Air Soil Poll 187:15–29
240. Tsukamoto TK, Miller GC (1999) Methanol as a carbon source for microbiological treatment of acid mine drainage. Water Res 33:1365–1370
241. Tsukamoto TK, Killion HA, Miller GC (2004) Column experiments for microbiological treatment of acid mine drainage: low-temperature, low-pH and matrix investigations. Water Res 38:1405–1418
242. Tuovinen OH, Niemelä S, Gyllenberg H (1971) Effect of mineral nutrients and organic substances on the development of *Thiobacillus ferrooxidans*. Biotechnol Bioeng 13:517–527
243. Tuovinen OH, Panda F, Tsuchiya H (1979) Nitrogen requirement of iron-oxidizing Thiobacilli for acidic ferric sulfate regeneration. Appl Environ Microbiol 37:954–958
244. Ulrich GA, Martino D, Burger K, Routh J, Grossman EL, Ammerman JW, Suflita JM (1998) Sulfur cycling in the terrestrial subsurface: commensal interactions, spatial scales, and microbial heterogeneity. Microbial Ecol 36:141–151
245. U.S. Department of the Interior, Bureau of Reclamation (2009) Reclamation-managing water in the west: Southern California Regional Brine-Concentrate Management, Study—Phase I, Lower Colorado Region. http://www.usbr.gov/lc/socal/planning.html. Accessed 16 June 2013
246. Usinowicz PJ, Monzyk BF, Carlton L (2006) Technical and economic evaluation and selection of sulfate ion removal technologies for recovery of water from mineral concentrate transport slurry. In: Proceedings of the Water Environment Federation, WEFTEC 2006, pp 139–153
247. Utgikar VP, Harmon SM, Chaudhary N, Tabak HH, Govind R, Haines JR (2002) Inhibition of sulfate-reducing bacteria by metal sulfide formation in bioremediation of acid mine drainage. Environ Toxicol 17:40–48
248. Valente T, Gomes C (2009) Occurrence, properties and pollution potential of environmental minerals in acid mine drainage. Sci Total Environ 407:1135–1152
249. Vallero MVG, Camarero E, Lettinga G, Lens PNL (2004) Thermophilic (55–65°C) and extreme thermophilic (70–80°C) sulfate reduction in methanol and formate-fed UASB reactors. Biotechnol Progr 20:1382–1392
250. van Houten RT, Lettinga G (1996) Biological sulphate reduction with synthesis gas: microbiology and technology. In: Wijffels RH, Buitelaar RM, Bucke C, Tramper J (eds) Immobilised cells: basics and applications. Elsevier Science B.V., Amsterdam
251. van Houten RT, Hulshoff-Pol LW, Lettinga G (1994) Biological sulfate reduction using gas-lift reactors fed with hydrogen and carbon dioxide as energy and carbon source. Biotechnol Bioeng 44:586–594
252. van Houten RT, van der Spoel H, van Aelst AC, Hulshoff-Pol LW, Lettinga G (1996) Biological sulfate reduction using synthesis gas as energy and carbon source. Biotechnol Bioeng 50:136–144

253. van Houten RT, Yun SY, Lettinga G (1997) Thermophilic sulfate and sulphite reduction in lab-scale gas-lift reactors using H_2 and CO_2 as energy and carbon source. Biotechnol Bioeng 55:807–814
254. van Houten BH, Roest K, Tzeneva VA, Dijkman H, Smidt H, Stams AJM (2006) Occurrence of methanogenesis during start-up of a full-scale synthesis gas-fed reactor treating sulfate and metal-rich wastewater. Water Res 40:553–560
255. Visser TJK, Modise SJ, Krieg HM, Keizer K (2001) The removal of acid sulphate pollution by nanofiltration. Desalination 140:79–86
256. Vossoughi M, Shakeri M, Alemzadeh I (2003) Performance of anaerobic baffled reactor treating synthetic wastewater influenced by decreasing COD/SO_4 ratios. Chem Eng Process 42:811–816
257. Walton C, Johnson DB (1992) Microbiological and chemical characteristics of an acidic stream draining a disused copper mine. Environ Pollut 76:169–175
258. Wang H, Bigham JM, Tuovinen OH (2006) Formation of schwertmannite and its transformation to jarosite in the presence of acidophilic iron-oxidizing microorganisms. Mater Sci Eng C 26:588–592
259. Watten BJ (1999) Process and apparatus for carbon dioxide pretreatment and accelerated limestone dissolution for treatment of acidified water (U.S. Patent No. 5,914,046)
260. Watzlaf G, Schroeder K, Kairies C (2000) Long-term performance of anoxic limestone drains. Mine Water Environ 19:98–110
261. Waybrant KR, Ptacek CJ, Blowes DW (2002) Treatment of mine drainage using permeable reactive barriers: column experiments. Environ Sci Technol 36:1349–1356
262. Weber KP, Werker A, Gehder M, Senger T, Legge RL (2010) Influence of the microbial community in the treatment of acidic iron-rich water in aerobic wetland mesocosms. Bioremediat J14:28–37
263. Webster J, Swedlund P, Webster K (1998) Trace metal adsorption onto an acid mine drainage iron(III) oxyhydroxysulfate. Environ Sci Technol 32:1361–1368
264. Weijma J, Stams AJ (2001) Methanol conversion in high-rate anaerobic reactors. Water Sci Technol 44:7–14
265. Weijma J, Hulshoff-Pol LW, Stams AJM, Lettinga G (2000) Performance of a thermophilic sulfate and sulfite reducing high rate anaerobic reactor fed with methanol. Biodegradation 11:429–439
266. Weijma J, Bots EAA, Tandlinger G, Stams AJM, Pol LWH, Lettinga G (2002) Optimisation of sulphate reduction in a methanol-fed thermophilic bioreactor. Water Res 36:1825–1833
267. Weijma J, Chi TM, Hulshoff-Pol LW, Stams AJM, Lettinga G (2003) The effect of sulfate on methanol conversion in mesophilic upflow anaerobic sludge bed reactors. Process Biochem 38:1259–1266
268. Western PA Coalition for Abandoned Mine Reclamation website: http://www.wpcamr.org/. 27 Sept 2013
269. White C, Gadd GM (1996) A comparison of carbon/energy and complex nitrogen sources for bacterial sulphate-reduction: potential applications to bioprecipitation of toxic metals as sulphides. J Ind Microbiol 17:116–123
270. Widdel F (1988) Microbiology and ecology of sulfate-and sulfur-reducing bacteria. In: Zehnder A (ed) Biology of anaerobic microorganisms. Wiley, New York
271. Willow MA, Cohen RR (2003) pH, dissolved oxygen, and adsorption effects on metal removal in anaerobic bioreactors. J Environ Qual 32:1212–1221
272. Wisotzky F (1994) Untersuchungen zur Pyritoxidation in Sedimenten des Rheinischen Braunkohlereviers und deren Auswirkungen auf die Chemie des Grundwassers. Besondere Mitteilungen zum Deutschen Gewässerkundlichen Jahrbuch 58
273. Wolicka D (2008) Biotransformation of phosphogypsum in wastewaters from the dairy industry. Bioresour Technol 99:5666–5672
274. Wolkersdorfer C, Bowell R (2005) Contemporary reviews of mine water studies in europe. Mine Water Environ Suppl Mater 24:1–76

275. Wolkersdorfer C, Younger PL (2002) Passive Grubenwasserreinigung als Alternative zu aktiven Systemen. Grundwasser – Zeitschrift der Fachsektion. Hydrogeologie 7:67–77
276. Wood TA, Murray KR, Burgess JG (2001) Ferrous sulphate oxidation using *Thiobacillus ferrooxidans* cells immobilised on sand for the purpose of treating acid mine-drainage. Appl Microbiol Biotechnol 56:560–566
277. Xu Y, Schwartz FW (1994) Lead immobilisation by hydroxyapatite in aqueous solutions. J Contam Hydrol 15:187–206
278. Yang Y, Wan M, Shi W, Peng H, Qiu G, Zhou J, Liu X (2007) Bacterial diversity and community structure in acid mine drainage from Dabaoshan Mine, China. Aquat Microb Ecol 41:141–151
279. Younger P (2000) The adoption and adaptation of passive treatment technologies for mine waters in the United Kingdom. Mine Water Environ 19:84–97
280. Younger PL (2004) Wetland treatment of mine waters. In: Prokop G, Younger PL, Roehl KE (eds) Groundwater management in mining areas—Proceedings of the 2nd IMAGE-TRAIN advanced study course. Pécs, Hungary, 23–27 June 2003. Umweltbundesamt, Wien/Vienna, pp 72–100
281. Younger PL, Banwart SA, Hedin RS (2002) Mine water. Hydrology, pollution, remediation. Kluwer Academic Publishers, Dordrecht
282. Yu Y, Lee C, Hwang S (2005) Analysis of community structures in anaerobic processes using a quantitative real-time PCR method. Water Sci Technol 52:85–91
283. Zagury GJ, Kulnieks VI, Neculita CM (2006) Characterisation and reactivity assessment of organic substrates for sulphate-reducing bacteria in acid mine drainage treatment. Chemosphere 64:944–954
284. Zamzow KL, Tsukamoto TK, Miller GC (2006) Waste from biodiesel manufacturing as an inexpensive carbon source for bioreactors treating acid mine drainage. Mine Water Environ 25:163–170
285. Ziemkiewicz P, Skousen J, Brant D, Sterner P, Lovett R (1996) Acid mine drainage treatment with armored limestone in open limestone channels. J Environ Qual 26:1017–1024
286. Ziemkiewicz P, Skousen J, Lovett R (1994) Open limestone channels for treating acid mine drainage: a new look at an old idea. Green Lands 24:36–41

Adv Biochem Eng Biotechnol (2014) 141: 173–209
DOI: 10.1007/10_2013_250

Published Online: 25 December 2013

Biosorption: A Mechanistic Approach

Marios Tsezos

Abstract The ability of microbial cells to sequester solutes selectively from aquatic solutions, via nonmetabolically mediated pathways, has been termed biosorption. The mechanism of biosorption has been shown not to be simple and often specific to the biomass–solute pair. The understanding of the mechanism at play, in each biosorption system, is a prerequisite for the understanding of the stoichiometry, the equilibrium, the kinetics, the selectivity, and the engineering process application potential. Biosorption has been studied mostly for inorganic ionic solutes, but there is also reported work on the biosorption of organic molecules. Reference is also made to the biosorption engineering application issues.

Keywords Bioaccumulation · Biosorption · Competing ions · Kinetics · Mechanism · Metal Ions · Metals · Microorganisms · Modeling · Organics

List of Acronyms

EDS	Energy-Dispersive X-ray Spectroscopy
EPR	Electron Paramagnetic Resonance spectroscopy
FTIR	Fourier Transform Infrared spectroscopy
RBS	Rutherford Backscattering Spectrometry
SEM	Scanning Electron Microscopy
TEM	Transmission Electron Microscopy
XPS	X-Ray Photoelectron Spectroscopy

Contents

M. Tsezos (✉)
National Technical University of Athens (NTUA),
School of Mining and Metallurgical Engineering,
Laboratory of Environmental Science and Engineering,
Heroon Polytechniou 9, 15780 Zografou, Greece
e-mail: tsezos@metal.ntua.gr
URL: http://envlab.metal.ntua.gr

1 Introduction: Definitions

The rapid population increase along with the industrial development of the last several decades gave, among other influences, rise to the concern for environmental protection and sustainability. The traditional environmental pollution control technologies of the biological or physical–chemical type have been successfully refined and applied. Their efficiency, however, may not readily allow compliance with the more recent "zero discharge" principle for environmental pollutants. As a result, the quest for new and more efficient sequestering technologies strengthened the emerging need for new processes that would be able to take over from where the traditional ones ended. In other words, it is desirable to be able to feed the final treated wastewater that flows out of traditional treatment schemes into new installations that would allow the ultimate effluents to have even lower pollutant levels, employing novel treatment operations.

In addition to the environmental protection and within the frame of sustainability, the recovery of resources contained in effluents or natural waters also became of significance. Such examples are the use of seawater, acid mine drainage, and industrial effluents for resource recovery purposes.

All such potential technological developments require underlying processes that are selective, low-cost, efficient, and environmentally friendly. Such a candidate process is the interaction of microbial biomass with ionic species in complex aquatic environments, resulting in their sequestration by the microbial biomass.

This is a phenomenon that had been noticed and reported in basic scientific research not later than the middle of the previous century. The complexing of uranium ions with unknown yeast cells "surface components," both metabolically and not metabolically linked, has been reported as early as 1948 [1]. The accumulation of radionuclides by marine organisms from seawater through processes "likely independent of cell life function" was also reported [2].

Gradually, the interaction of metal ionic species with microbial cells attracted further attention with reports appearing even more frequently in the scientific literature [3–8].

Specific tools for the detailed mechanistic and engineering study of the phenomenon of metal–microorganism interactions were not available. One first systematic study on biosorption adopted and applied the tools that were being used in the study of activated carbon adsorption processes, namely the determination of the sorbent–sorbate equilibrium and kinetics, using sorption equilibrium isotherms and classical batch reactor kinetics studies. At the same time, it was also proposed to use the term biosorption as a hybrid of the term adsorption [9, 10]. This term has since been well accepted and is being used within a variety of contexts.

As first, biosorption was proposed as a term for the phenomenon of the sequestering of metal ions from solutions by inactive (nonliving) microbial biomass, leaving all metabolically mediated processes out [9, 11]. The term was not initially meant to describe the purely chemical interactions of biopolymers, such as chitin or cellulosic polymers, with metal ions. As the reported work on biosorption expanded, the mechanistic significance of cellular biopolymers became evident [11–13]. The boundaries, however, between inactive microbial cells biosorptive uptake and metal uptake by the biopolymers alone or other biomaterials (e.g., agricultural residues) became less distinct in the literature.

The semantics of biosorption became even more cloudy when the study of biomass-based sequestering processes included the study of living cells where the passive physicochemical biosorptive phenomena could couple with metabolically mediated sequestering via, for example, the complexing or precipitation of the sequestered species through metabolic routes. Terms such as bioaccumulation and bioprecipitation have been proposed and are being used in this context [14].

An additional significant line of study, that is, the sequestering of organic molecules by inactive microbial biomass in the general area of organics biosorption, also emerged. Early systematic work on the subject of organics biosorption has suggested and confirmed the ability of nonliving cells to sequester organic molecules via sequestering paths that were shown not to be associated with active biodegradation/biotransformation processes [15–21].

It appears that a clearer generic definition of the term biosorption would be useful. The definition should encompass all the nonmetabolically mediated sequestering processes. Biosorption can be described as: the sequestering of a moiety, such as that of an ion or molecule, by a solid material of biological origin in a way similar to the sorbent–sorbate interactions in physicochemical sorption [11, 14]. All other metabolically mediated sequestering processes could be described by the generalized terms of bioaccumulation and bioprecipitation [22].

An attempt to present a systematic mechanistic approach to biosorption follows.

2 Equilibrium and Kinetics of Biosorption

2.1 Equilibrium Uptake Capacity

The engineering design of processes, at first, requires information on the equilibrium and the kinetics of the process under consideration. In biosorption studies, the parameter of primary interest is the mass of sequestered moiety retained per unit mass of biomaterial, frequently termed as "uptake capacity" (q) and given in units [M/M].

Biosorption has also been shown experimentally to be a potentially reversible equilibrium process, with the experimentally determined overall uptake capacity (q) being a function of the residual equilibrium concentration of the biosorbing species in solution (C_{eq}). Hence, the relationship between q and C_{eq} is commonly described by a curve termed (by borrowing from the adsorption theory) "biosorption isotherm curve" [11, 23–25]. Biological materials are substantially more sensitive to temperature than inorganic adsorbents used in ordinary industrial sorptive processes; hence, the useful temperature range for biosorption applications is substantially narrower. Furthermore, it has not been shown that the observed overall biosorptive uptake capacities are a strong function of solution temperature and, therefore, the term "biosorption isotherms", although it signifies equilibrium data at a given temperature, can be considered as a general descriptive term of lesser mechanistic significance.

Experimental data on the uptake capacity of a very wide variety of biological materials, for mostly metal ionic species, have been reported in the literature including archaea, Gram-positive and -negative bacteria, algae and fungi. Several reviews have been published where uptake capacities are summarized and reported. Instead of again presenting such a summary, several relevant references are provided [21, 23–35].

At times, the reported biosorptive uptake capacities do not make reference to the very important experimental conditions used during their determination, such as the rigorous definition of the biosorbing species, the solution equilibrium pH, the residual sorbate concentration and speciation, or the equilibrium attainment contact time. The missing information can make comparisons among the reported biosorptive uptake capacities difficult, if not impossible.

2.2 Solution pH

The subject of the contact solution pH brings to the foreground the issue of the key parameters that can affect the observed biosorptive uptake capacities. Reported experimental experience has shown that solution pH is a dominant

parameter affecting the observed uptake capacity, especially for metal ionic species [11, 28, 29, 36, 37]. Solution pH affects the chemistry and speciation of both the uptaking biomaterial functional groups as well as the biosorbed metal ionic species identity.

An excellent compilation of the hydrolysis equilibria for most of the elements, including detailed speciation equilibrium parameter values as well as speciation diagrams has been presented by Baes and Mesmer [38], providing much needed information on the sorbate side chemistry.

Hydrolysis reflects the chemical reactions by which a moiety is split by water. Such reactions are the rule for most cations, although the water self-dissociation constant is very small, with the hydroxyl radical present at varying concentrations over the range of pH values. A general hydrolysis reaction would be:

$$x\,M^{n+} + y\,OH^{-} \rightleftarrows M_x(OH)_y^{(xn-y)} \tag{1}$$

This generalized reaction suggests that speciation is affected, at any given pH and metal oxidation state, also by concentration. Hence, the quantification of the resulting speciation, provided that the ionic identities and the relevant stability constants are known, is quite complicated. We must, of course, keep in mind that the interlinked hydrolysis species concentrations are in dynamic equilibrium. Hence, the selective removal of one species by a biosorptive process will trigger the readjustment of solution speciation concentrations for the other species as well, until equilibrium is re-established. The quantification of this task is rather difficult for two main reasons, namely by the fact that hydrolysis products may often be complex and polynuclear, and by the fact that the soluble species presence will be affected by the precipitation or the exsolution of an element through its hydroxides or oxides.

In biosorption processes, we anticipate the interaction of soluble ionic species with the biomaterial. Therefore, the relative abundance, form, and conversion rates of the soluble biosorbing metal hydrolysis species are especially important, as they may define the ionic competition effects that have been observed and reported in biosorption equilibrium studies, as discussed later in the chapter.

Looking at the biomaterial side, the subject becomes more complex. The reason for the complexity is the substantially different chemistry and structure of the cell walls or membranes of the various classes of unicellular organisms, which, of course, affect the functional groups present as well as the three-dimensional physical structure of the cell wall or membrane that forms the mechanically active sequestering subbase, as it has been shown for the cases of uranium, thorium, palladium, aluminum, iron, and so on [11–13, 39, 40].

Several candidate functional groups that are present in biomaterials and may exhibit active mechanistic involvement in biosorptive sequestering have been proposed, including the chitin amine nitrogen, exopolymeric clusters, cellulose, glucans, carboxyls, sulphydryls, and others [12, 14, 27, 41].

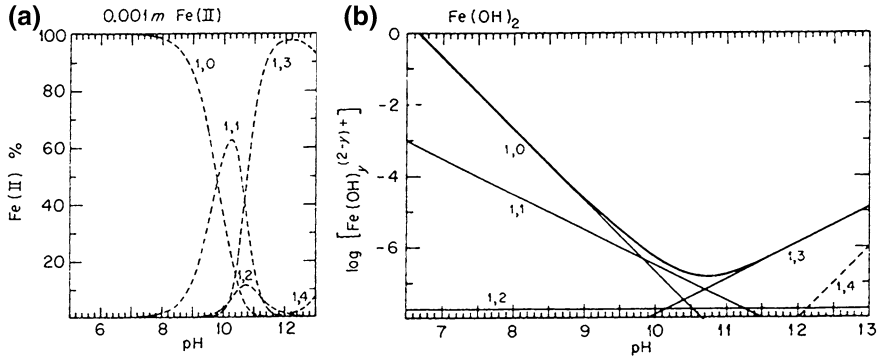

Fig. 1 Distribution of hydrolysis products (x, y) at $I = 1$ m and 25 °C in **a** 10^{-3} m Fe (II) and **b** solution saturated with Fe $(OH)_2$. The *dashed curves* in **a** denote regions supersaturated with respect to Fe $(OH)_2$; the heavier curve in **b** is the total concentration of Fe (II) [38]

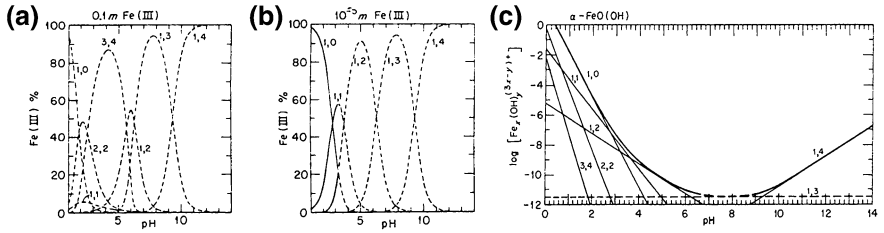

Fig. 2 Distribution of hydrolysis products (x, y) at $I = 1$ m and 25 °C in **a** 0.1 m Fe (III) and **b** 10^{-5} m Fe (III) and **c** solutions saturated with a-FeO(OH). The *dashed curves* in **a** and **b** denote regions supersaturated with respect to a-FeO(OH); the *heavier curve* in **c** is the total concentration of Fe (III) [38]

A typical example of such hydrolysis equilibria, demonstrating the strong effect of the solution pH on speciation and relative concentrations, can be seen in the example diagrams (Figs. 1 and 2) taken from Baes and Mesmer for Fe(II) [38].

The "pH point of zero" biomass charge, which is the pH at which the overall biomaterial surface charge is zero, has been proposed as a tool for the understanding of the pH effect and as useful in assisting biosorptive uptake enhancement [42]. The idea is that, at pH values below the "point zero", "protonation of functional groups" is facilitated on the biomaterial and the biomaterial may exhibit an overall positive charge, thus attracting negatively charged species. Such an approach assumes a rather simplistic electrostatic attraction driving mechanism, which has been shown not to be the only case in biosorption.

The effect of solution pH on equilibrium uptake should rather be interpreted via the above outlined hydrolysis equilibria approach, which is applicable to both the sorbate (biosorbing moiety) and the biomaterial (sorbent) functional groups. Carboxyl-, carbonyl-, amino-, phosphorous-, and sulphur-based moieties have been proposed as active in biosorptive processes. All these, as well as other

functional groups, are sensitive to pH and hydrolysis effects. Their detailed role in a given biosorption equilibrium can only be realistically, and not hypothetically, assessed when the underlying biosorption mechanism has been elucidated at the molecular level.

2.3 Solution Concentrations

The effect of sorbate solution concentration on the observed biosorptive uptake is indirect and quantifiable. This sorbate concentration, in association with the solution pH, dictates the detailed speciation of the biosorbing moiety, as explained previously in Sect. 2.2. This speciation affects the chemical identity of the ionic species present in the biosorption environment as well, thus guiding the biosorptive uptake preference. In addition, the residual equilibrium concentration of the biosorbing species, via the biosorption isotherm relationship discussed in Sect. 2.1, will dictate, in a dynamic interplay, the ultimate biosorptive uptake for the existing conditions, provided that sufficient contact time is given for the underlying reactions and mass transfer processes to reach equilibrium.

On the basis of the understanding that biosorption is an equilibrium process, the initial biomaterial dosage effect on the observed overall biosorptive uptake, for a given initial sorbate concentration in a batch contact environment, is only a matter of a predictable distribution of the biosorbing species between the solution (liquid phase) and the biomaterial (solid phase). This distribution is described by the relevant biosorption isotherm and the biomaterial dosage should not be treated experimentally as a parameter that can independently affect the biosorptive uptakes under study.

Some form of analytical expression for the relationship between the solid and liquid phase equilibrium concentration distribution of the sorbate is, therefore, useful. In other words, a biosorption isotherm model is needed for the above calculations.

2.4 Biosorption Isotherm Modeling

Data reported in the literature for the equilibrium distribution of the biosorbing moiety between the biomaterial phase (solid) and the contact solution (liquid), in the form of a q/C_{eq} diagram, for a given solution pH and isothermal conditions have shown linear as well as nonlinear behaviors. The common generic types of biosorption isotherms, which have been reported, are shown schematically in Fig. 3 and are of the general shapes of concave, convex or sigmoidal. Quasi-linear behaviors may be seen for a narrow range of residual equilibrium solute concentrations [9, 35, 37, 41, 43].

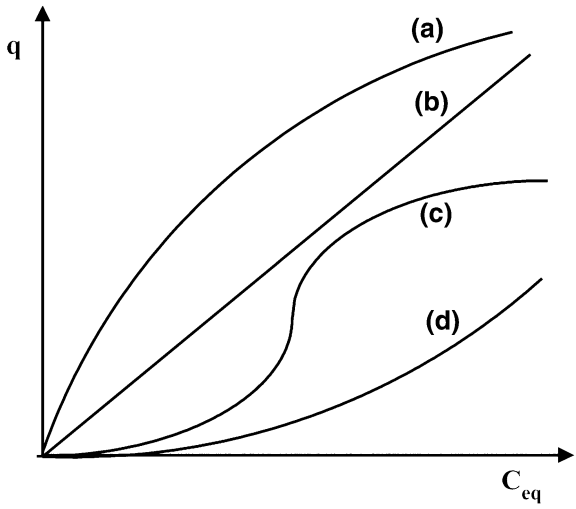

Fig. 3 Common generic types of biosorption isotherms: **a** convex, **b** linear, **c** sigmoidal, and **d** concave

The commonly used biosorptive isotherm models have all been borrowed from the ideal adsorption theory for monolayer or multilayer surface coverage of a sorbent. As such, and in contrast to the ideal adsorption theory of gases, it is clearly understood that they bear no mechanistic significance for the biosorption phenomena. Instead, they should be viewed as simple mathematical empirical correlations of the equilibrium uptake capacity (q) to the equilibrium residual solution solute concentration (C_{eq}).

In the ideal adsorption theory, two or more parameters models have been proposed based on different mechanistic approaches of the ideal adsorption. Of the many adsorption theory isotherm models, the simpler two-parameter models of Langmuir and Freundlich have most often been used in biosorption equilibrium study reports and reference has been made to more complex models [11, 23, 35, 37, 44]. Table 1 summarizes some of the common two- and three-parameter models used in the literature [41].

Looking into the classical adsorption theory, additional models are potentially available in order to try to describe the biosorption equilibrium data. We should, however, keep in mind that, as their mechanistic significance for biosorption is absent, augmenting the number of a model's parameters may simply lead to an easier curve-fitting exercise.

The Langmuir and Freundlich models can be readily linearized. Thus, one can then determine, from their linearized form and the associated experimental data fitting, the model parameter values pertinent to the specific experimental conditions employed [11, 14, 45]. The availability of biosorption equilibrium model parameters is very useful in the modeling of the performance of engineering reactor configurations for the practical applications of biosorption, as shown later in the chapter.

In cases where the biosorbing metal solute concentration increases substantially, one has to be aware and respectful of the pH/solubility/speciation constraints for the

Table 1 Example of common adsorption equilibrium isotherm models also used in biosorption

Two-parameter models	*Isotherm model*	*Model parameters*
Langmuir	$q = \frac{q_m\, b\, C_{eq}}{1+b\, C_{eq}}$	q_m, b
Freundlich	$q = k\, C_{eq}^{1/n}$	k, n
Temkin	$q = a + b\, \log\, C_{eq}$	a, b
Three-parameter models	*Isotherm model*	*Model parameters*
Redlich–Peterson	$q = \frac{k\, C_{eq}}{1+aC_{eq}^{b}}$	k, a, b
Radke–Prausnitz	$\frac{1}{q} = \frac{1}{a\, C_{eq}} + \frac{1}{b\, C_{eq}^{c}}$	a, b, c

adsorbing moiety in question, as already presented in Sect. 2.2. It is possible to experience a steep sigmoidal isotherm shape at the high end of the C_{eq} values, should sorbate exsolution initiate for the reasons explained above.

2.5 Co-Ion Effects

Industrial or natural pregnant solutions, for which biosorption may be applied for selective element sequestering, are almost always complex in chemical composition. So, more than one element, each present in solution via its own hydrolysis equilibria spectrum, will contact the biomaterial functioning as the sequestering phase. It is obvious that ionic competition effects will occur. Such effects can only be elucidated if the underlying biosorption mechanism is understood to a reasonable extent.

Most of the work reported on biosorption ionic competition effects has been based on executing batch biosorption equilibrium isotherm studies, using contacting solutions containing more than one targeted element. The observed uptake values have then been presented in two- or three-parameter graphic presentations, often associated with descriptive, but not mechanistic, mathematical expressions for the observed equilibria [30, 46, 47]. Such results are of reduced application significance as they refer to and are valid primarily for the specific set of contact conditions of the individual report.

Alternatively, if the underlying mechanism of biosorptive sequestration is understood better, the ionic competition effects would also be better understood and, then, more effectively integrated into engineering process design. Such designs may employ and handle a much wider range of biosorption contact conditions and environments with improved efficiency.

The case of the ionic competition of aluminum on the biosorption of uranium by *Rhizopus arrhizus* is a good example of this approach [40, 48]. The reported work, making use of the hydrolysis speciation of aluminum and uranium, as a function of solution pH and concentrations, showed that at pH = 4, where solubilities are reduced, the presence of aluminum interferes with uranium biosorption. At the lower solution pH = 2, where the aluminum speciation is different and the

element is present in a much simpler form, the ionic competition is not discernible, as aluminum exhibits low preference for coordination with the chitin amino nitrogen site on *R. arrhizus*.

When the contact solution conditions are such that the aluminum solution concentrations and the solution pH lead the aluminum speciation towards the overall solubility minimum, a metastable polymeric aluminum precipitate forms inside the R. arrhizus three dimensional chitin network. This precipitate subsequently hinders, in a steric way, the access of uranium to the chitin coordination site, thus limiting the observed overall uranium biosorptive uptake [12, 38–40, 46].

The mechanistic understanding of aluminum interference on uranium biosorption was effectively put into use in the pilot scale engineering application of immobilized *R. arrhizus* biomass for the continuous biosorptive selective recovery of uranium from the uranium-bearing bioleaching solutions of a mining site at Elliot Lake in Canada. This application managed the ionic competition effects well and recovered uranium successfully using upflow contact reactors over repeated biosorption–elution cycles [40, 45, 49, 50].

This reported experience showed that biosorption engineering applications, based on the mechanistic understanding of the underlying biosorption processes, lead to the proper handling of the contact solution chemistry and of the associated contacting conditions for the specific biomaterials in use. Approaches of this kind can open up the range of efficient engineering application of biosorption-based technologies.

An attempt to rationalize the ionic competition effects observed among co-ions present in solution during the biosorptive process, making use of a broader conceptual frame, has been made through the use of the concept of Pearson's classification of the elements [32, 51, 52]. The Pearson's approach classifies the elements into three main groups: class A or "hard" ions, class B or "soft ions," and "borderline" ions. This classification is based on the chemical coordination characteristics of the elements. Class A elements tend to form ligands preferably with oxygen as a donor atom and with reported preference sequences [25]. Class B elements tend to coordinate preferentially with ligands of decreasing electronegativity whereas borderline elements are characterized by intermediate coordination behavior. It can be deduced that each class of elements should then exhibit preference for different sites of a biosorbent, depending on the structural chemistry of the site which may preferentially exhibit atoms such as nitrogen, oxygen, sulphur, phosphorous, and so on.

The systematic study of the co-ion competition effects in biosorption, between pairs of elements belonging to the three different Pearson's classes, has been reported using the biosorption of palladium, silver, yttrium, uranium, nickel, and gold. The work showed that significant competition effects can be observed between metals belonging to the same Pearson's class. Ionic competition may not be significant between elements belonging to different classes whereas borderline elements were affected by the presence of co-ions [32, 51]. The work also suggested that better understanding of the ionic competition effects will need reliable information on the biosorptively active loci.

2.6 Reversibility of Biosorption

Conventional gas/solids adsorption is mostly a reversible process; meaning that, when the adsorption driving forces reverse, the adsorbate can leave the adsorbent surface. The work presented thus far on the mechanisms underlying biosorptive separations has shown that, unlike conventional gas/solids adsorption, biosorption is substantially more complex. It involves, among others, coordination, hydrolysis, ion exchange, exsolution, and valence change processes; as a result, the reversibility of biosorption should be expected to be a more complicated issue.

The biosorptive uptake capacity has been shown to be related to the residual solution concentration of the biosorbing moiety in a way that resembles an adsorption isotherm as already described in Sect. 2.1. We have emphasized, however, that such descriptions are deprived of any mechanistic significance and should only be assessed as useful mathematical tools to describe a biosorbent's uptake capacity as a function of the biosorbing entity residual solution concentrations over relatively narrow ranges of concentrations for the reasons explained in Sect. 2.2.

On the basis of the above considerations, the reversibility of biosorption has been examined and it has been shown qualitatively more to resemble the elution stage of an ion exchange process, whereby selected eluents are used to remove the biosorbed entities from the biosorbents. The ion exchange elution methods were shown to be a useful tool for the study of the biosorption reversibility.

The first systematic study of the desorption equilibrium stage of a biosorptive sequestering of metals made use of *R. arrhizus* as the biosorbent and of uranyl nitrate as the adsorbate [53]. The key reason for the selection of this specific pair of biosorbent/biosorbate candidates was the detailed understanding of the underlying mechanism [12]. The study is a good example of a systematic mechanistic approach to the reversibility of biosorption, as it selected a range of potential eluents that could reverse the preference of the biosorbed and chitin cell wall retained uranium species, while damaging as little as possible the fragile biomass structure, which had to be preserved for the ensuing cycles of biosorptive use [49, 53, 54].

This work showed that the reversal of biosorptive uptake resembles the ion exchange elution operations and that the recovery of the biosorbed species is possible. The biomaterial functioning as biosorbent, however, due to the fragility of the cellular structure and under the mildest elution conditions, may not withstand as large a number of elution cycles. The biomaterial functioning as biosorbent was shown, via SEM/EDS observations, to suffer structural damage from even dilute mineral acid eluents, and sulphate ions suggested changes to the functional biosorbing biomolecule crystallinity. This alteration confined the (under normal conditions) motile biosorbed uranium within the altered cell wall chitin network making the elution inefficient [53].

In another related desorption equilibrium study, referring to radium biosorption, the final eluent selection was dictated more on the basis of the need for complete

radium recovery at the highest radium eluent concentration (for reasons of elution solution volume minimization) rather than the biosorbent reversible uptake capacity preservation, hence leading to a substantially different desorption strategy [54].

Parameters such as eluent type, eluent concentration, and solid-to-liquid ratio during the desorption stage have been shown to be significant, with the kinetics of desorption reported to be rapid [53, 54]. The application of this strategy, during the requested pilot scale continuous biosorptive recovery of uranium from Elliott Lake bioleached solutions, was proven to be successful and allowed the use of *R. arrhizus* immobilized inactive microbial biomass in multiple cycles [45, 50, 53].

2.7 Kinetics

In studying the rate of biosorptive processes, one must make a clear differentiation, whenever applicable, between the intrinsic biosorption rate and the overall experimentally observed uptake rates. This is also valid in batch biosorption kinetic experiments where bulk mass transfer rates for the biosorbing entity must be accounted for in the experimental design.

Reported intrinsic biosorption rates are rapid, as has been reported from the relevant studies [11–13, 23]. The overall biosorptive uptake rate, with equilibrium usually attained within the first minutes of contact, is dependent on the mechanism involved and the related overall mass transfer effects [11–13, 23]. There are numerous reports in the literature proposing first-, second-, pseudo first- or pseudo second-order or other rate models, all of which have been borrowed from traditional sorption and chemical process rate studies [35, 41, 42]. There is no shortage of potential kinetic models. What is not readily available are true intrinsic biosorption rates for the initial few minutes of the equilibrium process determined from experimentally well-defined biosorption systems.

Once the intrinsic rate becomes analytically defined, then the tools available for the simulation of mass transfer rates for the removal of a soluble species from solution by a solid biosorbent are applicable and this can then yield the appropriate overall biosorption rate expressions for any system under consideration. The above approach has been successfully applied and has been reported for the simulation of the overall biosorptive uptake rate of uranium by the porous immobilized microbial biomass of *R. arrhizus* in batch or packed-bed reactors [55–59].

Such models focus on the concept of local equilibrium and may provide simulated equilibrium attainment rate curves for batch or column operating systems and require a realistic expression for the description of the intrinsic biosorptive uptake rate as already discussed [55].

Complete models and design equations for common biosorption reactor configurations such as a batch, fixed-bed, or fluidized-bed reactor, become somewhat more complicated as they have to describe a non-steady-state operation because

the biosorbing species concentration, in the solid and liquid phases, is not constant. Advanced numerical analysis can be applied for the solution of the resulting nonlinear differential equations, often not yielding explicit form solutions [55].

3 Mechanistic Understanding Approach

Throughout the previous sections that relate to the technology as well as the equilibrium and kinetics of the biosorptive process, frequent reference was made to underlying mechanisms. Their understanding has emerged as a necessary prerequisite for the meaningful quantification of the parameters affecting and defining a biosorptive process [60].

The literature on biosorption in general has been expanded almost exponentially over the last 20 years or so. An excellent review by G. M. Gadd has summarized the number of papers appearing in the international literature with the topic "biosorption" as listed in the ISI Web of Science for the years 1970–2008 [14, 61].

The earliest papers on biosorption appeared in the late 1970s to the early 1980s, as already presented in Sect. 1 of this chapter, but were few. The corresponding number of citations, as recorded by the ISI Web of Science, also increased almost exponentially, signaling a strong interest and activity on the subject [14].

Of the work reported in the literature a rather small subset has been devoted to the systematic and explicit elucidation of the mechanisms underlying biosorption, in accordance with the definition of the term "biosorption" proposed in Sect. 1.

The key characteristic of the mechanisms underlying biosorption is their diversity, which limits our ability to generalize. The same or different elements may follow different mechanisms of sequestration, depending on the biomaterial acting as the biosequestering solid phase. Palladium, for example, was shown to be retained in the intracellular loci of *Alcaligenes eutrophus* in a complex sequence of coordination and reduction steps, whereas yttrium and silver were mostly retained on the outer cell area [39, 62, 63]. Uranium has been shown to accumulate inside the *R. arrhizus* cell wall, and thorium is retained on the external cell wall region of the same microorganism [11–13, 64].

An attempt is made below to summarize the reported work that has proposed biosorption mechanistic models for specific metal–biomass pairs.

3.1 Uranium and Transuranium Elements

The systematic study of uranium biosorption equilibrium by inactive microbial biomass eventually resulted in the formulation of a detailed molecular base biosorption mechanism for the biosorptive uptake of uranyl ions by an inactive *R. arrhizus* biomass.

The mechanism involves three sequential interlinked steps. The first step involves the formation of a coordination bond between uranium and the nitrogen of the chitin monomer unit. Complexed uranium, subsequently, acts as a nucleation site for the further sorption of uranyl ions inside the three-dimensional network of the chitin polymer (second step). The chitin nitrogen–uranium complex hydrolyzes, releasing the nitrogen site, leading to the precipitation of uranyl hydroxide within the chitin polymeric network (step three). The released chitin nitrogen re-engages in a new coordination bonding with fresh uranyl ions, which subsequently hydrolyzes again accumulating additional uranyl hydroxide within the cell wall. Effectively, this repeating cycle acts in a way similar to an uranyl hydroxide formation pump that takes soluble uranium out of the solution and, via the coordination/hydrolysis steps, immobilizes the uranium in the form of an insoluble hydroxide within the cell wall chitin network. Figure 4 shows a typical micrograph of the fungal cell wall showing the biosorbed uranium species as electron dense layers.

This detailed mechanistic model is potentially applicable to fungal cell walls where a three-dimensional chitin network, with gloucosamine nitrogen available for coordination, is present.

This mechanistic model can also explain experimentally observed ionic competition effects on the biosorptive uptake of uranium by *R. arrhizus*. The complexation of iron and zinc by chitin has been documented in the literature with the stability of chitin–metals complexes following, as for most ligands, the Irving Williams series [13]. The observed and reported suppression of the *R. arrhizus* uranium uptake in the presence of iron and zinc can be understood via their competition for the chitin nitrogen coordination sites. Inasmuch as they limit the formed chitin uranium nucleation sites, they eventually reduce the resulting overall uranium uptake [12].

The use of EPR spectroscopy, in the case of copper–uranium competition, suggested that copper appears to form two separate complexes with chitin, at two possible sites, that may be related to the two main crystalline forms of chitin (chitin α and chitin β). Both copper–chitin complexes involve one chitin nitrogen ligand atom with the remaining being likely oxygen atoms. Thus, copper competes with uranium by limiting the nitrogen coordination sites on the chitin macromolecule available for uranyl coordination, leading eventually to the reduced uranium overall biosorptive uptake for the specific biomass type [65].

Aluminum, on the other hand, as already presented in Sect. 2.5, reduces the overall uranium biosorptive uptake by *R. arrhizus* via a different pathway of interference in accordance with the presented uranium biosorption mechanistic model [49]. Aluminum chemistry and hydrolysis speciation show that aluminum precipitates readily in the pH range of 4 to 5. The aluminum speciation is also linked to its concentration and the anions present in solution, all of which affect the course, composition, texture, and structure of the resulting aluminum precipitates. The Al–N bond is not a preferred bond, therefore the observed reduction in uranium biosorption uptake is unlikely to be the result of aluminum competition with uranyl ions for the chitin nitrogen coordination site. When the contact solution pH is in the mildly acidic region, a metastable polymeric aluminum precipitate settles inside the

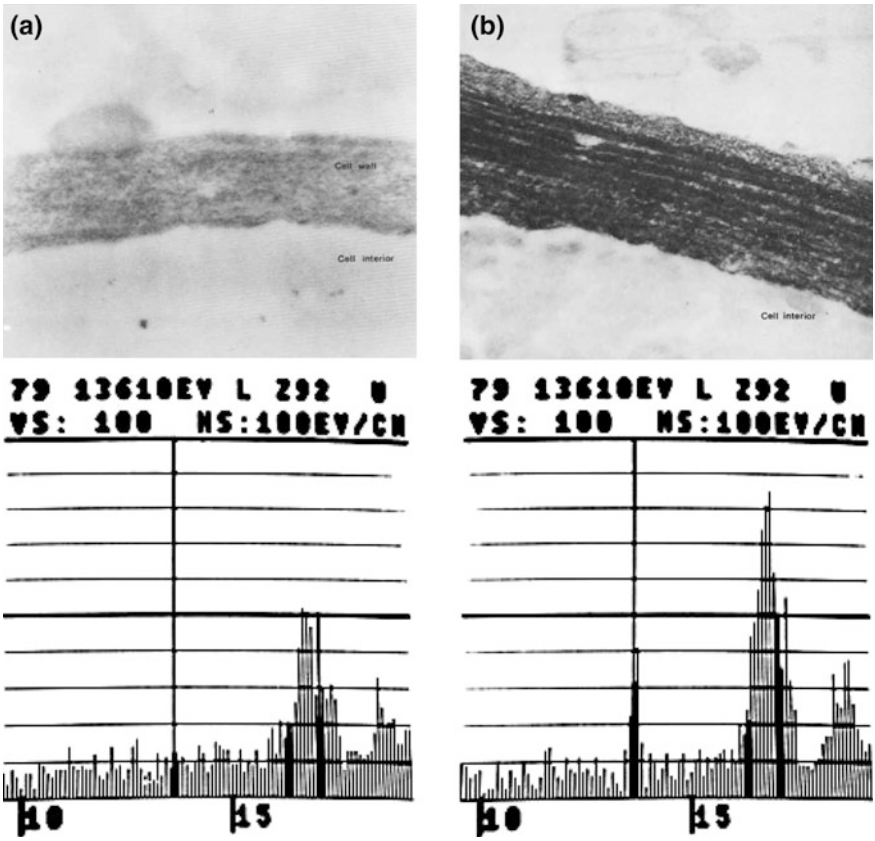

Fig. 4 TEM micrographs (× 80,000) and corresponding X-ray energy dispersion analysis spectra of thin sections of *R. arrhizus* nonliving cells **a** before and **b** after exposure to uranium solutions [12]

chitin cell wall network, limiting the space available for the sorption, hydrolysis, and precipitation of the uranyl ions inside the chitin cell wall, in a competition mechanism that has been described as "steric hindrance" interference [40, 49].

Work reported on the biosorption of uranium by the fungal biomass of *Mucor miehei* also confirmed the significance of solution pH, because it contributes to the definition of the solution ionic speciation as well as to the cell wall chemistry. Within moderate pH, uranyl hydroxides are reported as inducing multilayer uranium sequestering in contrast to a monolayer sequestration in the acidic pH range [66].

As a result of environmental concerns, the biosorption of the isotope Americium 241 by the biomass of *R. arrhizus* and of *S. cerevisiae* has been studied [67, 68]. The cell walls are the biosorptive active sites for both cases. The strong dependence of biosorptive uptake on solution pH was confirmed. Protein or carboxyl cell wall functional groups have been suggested as cell wall complexation

loci, and ion exchange with hydrogen and calcium from the cell walls of *R. arrhizus* and *S. cerevisiae* are also active. The use of Europium (Eu^{3+}) and Neodymium (Nd^{3+}) surrogates for ^{241}Am in the biosorptive studies revealed that Eu and Nd compete efficiently with Am for the biosorption loci. Biosorptive contact solution pH drift monitoring as well as RBS showed that ^{241}Am and the surrogates exchange with biomass cell wall hydrogen and calcium, thus supporting the proposed ion exchange as one of the active biosorption mechanisms [67, 68].

3.2 Thorium

Detailed, molecular basis, mechanistic work on thorium biosorption has also been reported for the biosorption of thorium by *R. arrhizus* [11, 13]. The proposed thorium biosorption mechanism is not the same as the one described above for uranium.

The thorium biosorptive uptake by *R. arrhizus* involves two separate processes. Process A operates via the formation of a coordination complex between thorium and the nitrogen of the chitin cell wall matrix. Chitin, being a strong Lewis base, exhibits a significantly higher sequestering potential for thorium than other cell wall Lewis bases, such as hydroxyl groups of the aminopolysaccharides. The contribution by process A to the experimentally determined overall thorium biosorptive uptake is, however, limited to under 5 % of the total uptake, as specified by the stoichiometry of the thorium–chitin coordination. The dominant contribution to the overall biosorptive thorium uptake is contributed by a second process, which involves the adsorption of hydrolyzed thorium ions by the outer layers of the *R. arrhizus* cell wall [11, 13].

Making use of the above-described two-process thorium biosorption mechanisms, we can then more readily understand possible competition effects on the overall thorium biosorptive uptake of the *R. arrhizus* inactive biomass. Using the same reasoning as described in the previous sections, any competing cation effects, acting via the chitin nitrogen coordination process, will be limited to the chitin nitrogen coordination pathway contribution, which only accounts for less than 5 % of the reported overall thorium biosorptive uptake. Therefore, the chitin nitrogen competition effects, that were shown to be significant in uranium biosorption, will have a much reduced negative consequence on the overall thorium biosorptive uptake of *R. arrhizus*. The dominant effects on the observed overall thorium biosorptive uptake will be realized via the thorium hydrolysis speciation pathway, with the solution pH accounting for the most significant influence.

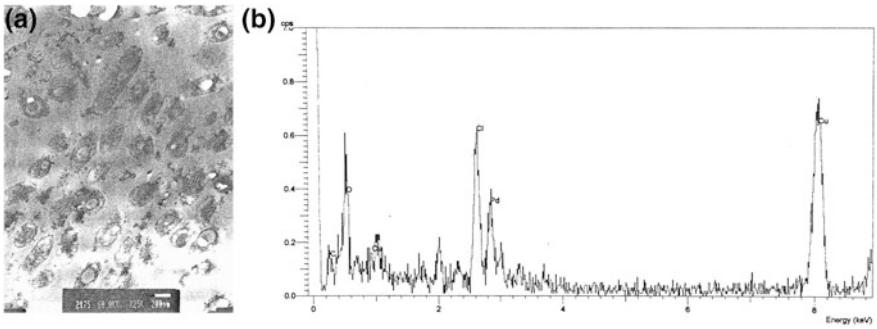

Fig. 5 Palladium biosorption by strain AS 302: **a** TEM micrograph of AS 302 cells after contact with a palladium-containing solution and **b** EDS spectrum inside the cell wall [62]

3.3 Palladium, Gold, Platinum

Palladium is usually present in solution in the form of complex ions that may be anionic or cationic, for example, $Pd(NH_3)_4^{2+}$ or $PdCl_4^{2-}$. Work reported on the mechanism of palladium biosorption has mostly made use of bacterial microbial species such as of the *Alkaligenes*, *Pseudomonas*, and *Arthrobacter* genus. The reported work has suggested that the initial approach of positively charged palladium to the microbial biomass may be facilitated by Coulombic interactions [39].

Subsequently, for the tetra-ammonium palladium ion, a *trans* or *cis* substitution of ammonium by a cellular ligand takes place [39]. These ligands are mainly carboxyl and to a lesser extent amino groups. *Trans* structures are expected to yield a likely stable binding of the palladium to the biomass. Biosorbed palladium is mainly located in intracellular loci of the cells with eventual reduction and formation of elemental palladium, as seen in the micrograph of Fig. 5 [39, 62, 63].

We should note that, although the initial electrostatic approach mechanism seems to be accepted, evidence against it being the principal retention mechanism does exist. A reversal of the electrostatic interaction should be feasible by solution pH adjustment, which could result in reversal of the Coulombic attraction forces towards the biomass surface and that should release retained species back to solution. Reported palladium and platinum desorption attempts, however, did not manage to reverse the biosorption equilibrium by simple pH shifts and had to resort to the use of strong chelating agents, such as thiurea, for the desorption.

In the case of $AuCl_4^-$, a first step of coordination to the reactive carboxyl sites has been proposed, followed again by the reduction of the ion to elemental gold. The loci for the biosorption of gold have been shown to be on and within the bacterial cell walls, with the initial biosorption sites acting as nucleation points for the ensuing reduction of gold and the growth of gold microcrystals, as shown in the micrograph of Fig. 6 [7, 39, 69, 70].

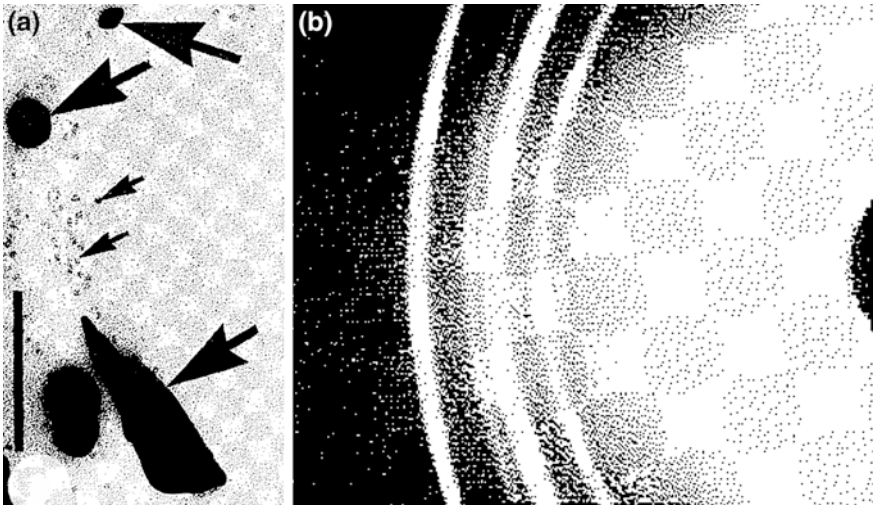

Fig. 6 **a** TEM micrograph of *Bacillus subtilis* cell wall that reacted with Au(III) and is representative of a crystalline-staining reaction. **b** X-ray diffractogram of a preparation of Au(III)-reacted walls, which shows the high crystalline order of the elemental crystals [8]

Given that both the ionic form of palladium and gold are proposed to initially bind to carboxyl sites and then reduce to elemental form, it is then expected that a strong competition effect could be anticipated between $PdCl_4^{2-}$ and $AuCl_4^{2-}$ [39, 51].

The above-proposed mechanism for Pd biosorption can also explain the significant effect of solution pH on the observed biosorptive uptakes. In mildly alkaline pH environments (e.g., pH = 8), the biomass carboxyl groups protonation is not favored, making them available for nucleophilic substitution. In the acidic range (e.g., pH = 3), however, substantially fewer nonprotonated groups should be available, resulting in the observed reduced overall biosorptive uptakes [39].

The initial electrostatic approach, followed by coordination interactions using functional groups containing nitrogen or sulfur atoms, for example, amine and thiol groups, have also been proposed for platinum biosorption with little further specific evidence [32].

Recent work on the biosorption of Au(III) by *B. megaterium* biomass demonstrated that the binding of Au(III) mostly occurs with oxygenous and nitrogenous active groups of polysaccharides and proteins in the cell wall biopolymers. Functional groups suggested as active are the hydroxyls of the saccharides, the peptidic bonds, and so on. Initially bound Au(III) acts as a nucleation site for the subsequent reduction and eventual growth of Au(0) in situ [71]. In gold biosorption of *S. cerevisiae*, the binding of Au(III) to the oxygen of the peptide bond was reported as causing significant rearrangements in polypeptide backbones, such as the occurrence of unbound carboxyl- and nitrogen-based groups, being freed from the intermolecular hydrogen bonding. In short, Au(III) is suggested as

activating the polypeptidic structures that in turn react actively with Au(III) leading to reduction in Au(0), nucleation, and microcrystal growth [71].

An array of spectral and chemical techniques was used to investigate the biosorption of platinum by *Bacillus megaterium*. In the case of Pt(IV), the interaction with the biomass appears to materialize through the interaction with oxygenous and nitrogenous functional groups of the cell wall biopolymers. Again, the hydroxyls of the polysaccharides, carboxylate anions, and carboxyls of amino acids are among the initial binding loci. Bound Pt(IV) is reported as reduced in situ to Pt(0) by cell wall biopolymer-based electron donors. The Pt(IV) initial binding is suggested as resulting in a change of the secondary structure of the cell wall proteins, such as a transformation of polypeptide chains. The protein conformation is suggested as likely to be responsible for the steric stabilization of the Pt(0) microcrystals [72].

3.4 Silver and Rare Earth Elements

Work reported on the elucidation of the biosorption mechanism of other elements, such as silver and elements of the rare earth group, is gradually becoming available. Work on silver biosorption has suggested that silver accumulates on or outside the outer cell wall of the microbial cells examined, with the possibility of reduction of ionic silver to elemental silver [62]. The possible involvement of extracellular polysaccharides in silver biosorption has also been suggested [63].

Molecular level work on Ag^+ biosorption, reported on the biomass of *Bacillus cereus* and of *Lactobacillus* sp., has shown the involvement of an initial chemical binding step followed by redox reactions resulting in elemental silver (Ag(0)) microcrystal generation [73, 74].

The initial interaction of Ag^+ with the biomass is pH sensitive, as expected and already discussed. At pH values above 4, carboxyl groups of the microbial cell walls ionize, facilitating the uptake of Ag^+, via an ion exchange-type mechanism. The carboxylate anions and the hydroxyl groups of the cell wall peptidoglycan layer are suggested as playing the key role in Ag^+ binding by the biomass. Reducing sugars resulting from the biomass polysaccharides have been proposed as the acting electron donors that, subsequent to initial binding of Ag^+, result in the in situ reduction of Ag^+ to Ag^0 [73, 74]. Figure 7 shows the SEM micrograph of Ag^0 nanocrystals formed inside the biomass of *B. cereus* following Ag^+ biosorption, along with the corresponding XPS spectrum [74].

Yttrium, on the other hand, a member of the rare earth element group, was shown to accumulate on the outer cell membrane and on inner specific sites with no further details on the chemistry of the interaction [62].

The subject of rare earth element biosorption has received considerable attention in the recent past, primarily due to the economic significance of the rare earths for important industrial sections. In addition to yttrium, other members of the family have been examined including Eu, Pr, Sm, Dy, La, Tb, Yb, and Ce.

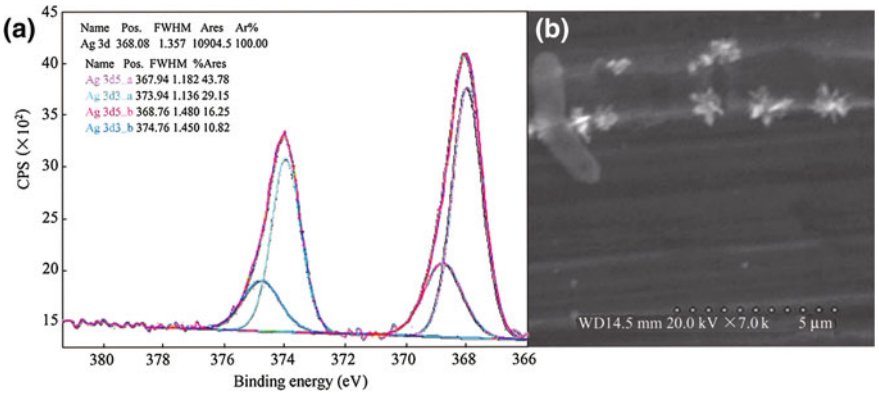

Fig. 7 Photoelectron spectrum of *Bacillus cereus* biomass loaded with Ag^{+} and micrograph of biosorption resulting in Ag^{0} microcrystals [74]

Most of the reported equilibrium data are for nonstrongly alkaline solutions and for several different types of microbial biomass [48, 62, 75–83]. The weakly hydrolyzing rare earth elements, in the nonalkaline pH ranges, exist primarily in the form of simpler cations [38]. The indirect evidence is based on the linear correlation between the meq of biosorbed rare earth solute and the meq of divalent cations, such as Ca^{2+} or Mg^{2+}. These ions are released into the solution from the microbial biomass, following the establishment of the biosorption equilibrium. This fact has led to the advancement of a hypothesis of a primarily ion exchange mechanism underlying rare earth biosorption. Carboxyl groups have been proposed as being active in this ion exchange mechanism hypothesis [78, 79, 81, 83].

Work reported on the biosorption of Tb^{3+} by living *Escherichia coli* cells has shown that the uptake is nonmetabolic and the peptidoglycal layer of Gram-negative cell walls is responsible for the sequestration of Tb^{3+}. The initial approach of Tb^{3+} to the microbial cell is favored by electrostatic attraction by the negatively charged lipopolysaccharides of the outer membrane [82].

Extracellular polysaccharides are produced by many microorganisms with a variety of polymeric structures. Divalent cations, such as Pb^{2+}, Cu^{2+}, Cd^{2+}, or Ca^{2+}, have been shown to exhibit an affinity for such polysaccharides [27]. An egg-box structure representing the gelation of alginates, such as the one induced by calcium presence, may be indicative of a mechanistic operon for the sequestration of elements, such as silver, by selected bacterial cells [27, 62, 63].

Summarizing what was noted above, the reported work on the mechanism of biosorption has shown chemical and physical processes, such as electrostatic attraction, coordination, chelation, adsorption, and hydrolysis, acting alone or together in a sequence of steps, forming biosorption mechanisms. The mechanisms appear to be specific to the metal/microorganism pairs involved and do not favor the proposal of a generalized biosorption mechanism hypothesis.

Successful engineering applications of biosorption should be based on the understanding of the underlying mechanism in each application. This understanding will enable the definition of the parameters that may significantly affect the targeted biosorptive sequestering as well as of the steps that should be taken for the optimization of the efficiency of an engineering process applying biosorption.

3.5 Copper, Cadmium, Lead, Cobalt

The study of the biosorption of several other metals, which belong to different groups of the periodic table, has been reported in the literature, with environmental concerns acting as the main stimulus for the initiation of the reported studies.

Copper biosorption has received considerable attention. A proposed mechanism for the understanding of copper biosorption by *R. arrhizus* has already been presented in Sect. 3.1 and suggested the coordination of copper by the cell wall chitin nitrogen functional groups.

Further work on the chitin nitrogen proposed copper biosorption mechanism has been reported using the biomass of *Ganoderma lucidum*. This work, using among others EPR spectroscopy, confirmed the interaction of a cell wall free radical with the biosequestering of Cu^{2+}. It confirmed that the free radical is not directly involved in copper sequestering. This free radical has been observed in several biosorbents. The cell wall matrix of the biosorbents, which encompasses and traps this free radical, opens up upon initial Cu^{2+} coordination. Then, the exposed cell wall matrix can interact freely with the metal ions in solution, resulting in rapid further sequestration of metals [84].

Copper uptake by *S. cerevisiae* has been suggested as the result of coordination bonding of Cu^{2+} with carboxyl and amino groups of the yeast cell wall [85]. On the other hand, the biomass of *Fucus serratus* and of *Spirulina* sp. have been reported to exchange primarily the Cu^{2+} ions during biosorption with protons resting on surface functional groups, such as carboxylic amine and phenolic moieties. As a result, solution pH emerges as a dominant parameter affecting the biosorption process [86, 87].

Cadmium biosorption by a microbial biomass has been shown to rely primarily both on the coordination of Cd^{2+} to surface active moieties mainly based on oxygen and nitrogen, such as carboxylic and amino groups, as well as on the exchange with structural exchangeable counter-ions of the cell wall [60, 88–92]. This is the case for *Pseudomonas plecoglossicida* with a strong Cd^{2+} uptake capacity, and reported FTIR and SEM analyses confirming that the principal Cd^{2+} biosorption mechanism is the cell wall surface residing chemical complexation with a minor contribution of a proton-based ion exchange [88]. Support for a similar type of Cd^{2+} biosorption mechanism has been reported for the fungus *Auricularia polytricha* with an increased relative importance of the ion exchange contribution [89].

Examining the Cd^{2+} biosorption by the brown macroalgae *Sargassum vulgaris*, it has been observed that Cd^{2+} replaces Ca^{2+} and Mg^{2+} which are cross-linking structural ions of the alginic acid polymers (see Sect. 3.4) of the seaweed cell wall. Cadmium biosorption replaces some of the Ca^{2+} and Mg^{2+} in the cell wall matrix, creating a stronger alginic polymer cross-linking because of the stronger electrostatic and coordinative bonding of cadmium with the cell wall polymer counterparts. In addition to oxygen and nitrogen, carbon, phosphorous, and sulfur atoms have been shown to participate in the cadmium coordination process. Chelation has been reported as the strong contribution to the overall Cd^{2+} biosorptive uptake by *S. vulgaris* [60, 90, 91].

The biosorption of lead, which is an important industrial and environmental pollutant, has received some attention. The biosorptive interaction of Pd^{2+} with *Sargassum vulgaris* has suggested that, similar to the case of cadmium, the chelation of Pb^{2+} with counter-ions from the alginic cell wall network and with protons is active. The substitution of the cell wall cross-linking ions by lead, in the cell wall alginates, has been proposed as producing an even denser structure than that induced by Cd^{2+} biosorption and substitution. Lead, however, has been reported as eventually participating in redox reactions, resulting in metallic lead microprecipitation in the cell wall matrix similar to the cases reported for other elements such as gold and silver [90].

A proposed mechanism of divalent cobalt (Co^{2+}) biosorption by *Pseudomonas halodentrificans* also relies on the ion exchange chemical complexation duo. Stoichiometric monitoring of the Ca^{2+} released during Co^{2+} biosorption suggested that Co^{2+} is exchanging for Ca^{2+} present in the biomass. Upon depletion of exchangeable Ca^{2+}, a further lesser uptake of Co^{2+} follows as a result of chemical coordination of Co^{2+} to the cell wall, weak affinity, and unidentified sites [93].

3.6 Chromium

Chromium is an important industrial metal that exists potentially in several oxidation states with Cr^{3+} and Cr^{6+} as the most common states. Cr^{6+} is a powerful oxidant and this chemical property is important in modulating chromium biosorptive behavior. Cr^{6+} is also characterized by high toxicity which makes Cr^{6+} an important environmental concern [94].

Cr^{6+} interaction with *Termitomyces clypeatus* biomass has suggested that the Cr^{6+} biosorption follows a sequence of two steps. At first, Cr^{6+} (as $Cr_2O_7^{2-}$) is chemically and electrostatically bound by amino, carboxyl, and phosphate groups on the surface of the fungal cell wall, followed by a reduction of Cr^{6+} to Cr^{3+} by cell wall reductive groups, such as hydroxyl and carbonyl moieties. The resulting Cr^{3+} remains bound to the cell wall [95].

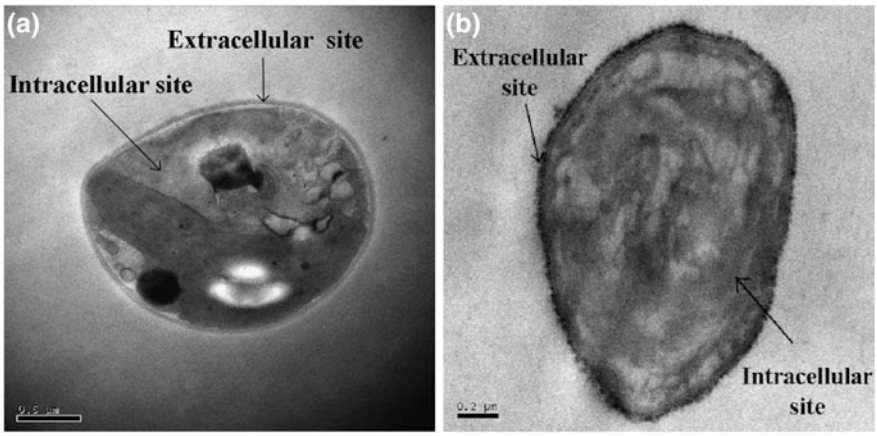

Fig. 8 TEM micrographs of *Chlorella miniata*: **a** Control cell without Cr treatment and **b** algae cell treated with 100 mg/L Cr(III) at pH 4.5 for 24 h [97]

The above two-stage mechanistic hypothesis for Cr^{6+} biosorption was further supported by the work on the biosorption of Cr^{6+} by the cyanobacterium *Synechococcus* sp. and by the microalgae *Chlorella miniata* [96, 97]. Figure 8 presents TEM micrographs of *C. miniata*, before and after Cr(III) biosorption, where electron-dense areas, suggesting the presence of chromium loaded sites, both extracellular and intracellular, are shown [97].

4 Biosorptive Technologies

The application of biosorption, as the base for an industrial selective sequestering process, passes through the selection stage of the appropriate biomass type for the targeted application, including the decision on the use of living or inactive biomass.

The use of inactive (dead) biomass has emerged as the preferred way for the following reasons [98].

(a) Any contact solution toxicity with respect to the involved biomass is not a concern.
(b) The process does not need to satisfy biomass growth condition requirements.
(c) Maintenance of culture purity is not a concern.

Native inactive microbial biomass exhibits a number of undesirable properties, in terms of its direct technological application potential as a sequestering agent. Particle sizes are small, of relatively low density, and of low mechanical strength. Contacting native biomass with large solution volumes containing the targeted metals has been shown to be impractical, with the main difficulty lying in the need

for a rapid yet efficient separation of the pregnant biomass from the biosorption mixture, after contact equilibrium is attained [37, 99]. The above features limit the engineering design choices to biomass contact reactors, such as the well-mixed reactor type, which must be followed by a biomass–solution separation stage that should make use of a classical separation process, such as sedimentation, centrifugation, filtration, and the like.

Improvements on the efficiency of biosorption-based processes could be achieved if the biomaterial is transformed into controlled particulate form, which would then open up the applicability of well-developed reactor types that are already in industrial use in operations such as adsorption and ion exchange. These contact reactors carry substantial industrial application experience and process optimization knowhow [98, 100, 101].

The transformation of native inactive microbial biomass to a particulate form requires the use of an immobilization process that can yield immobilized biomass particles with:

(a) Consistent composition
(b) Controlled size
(c) Limited nonbiosorbing materials content
(d) High porosity
(e) Hydrophilicity
(f) Good mechanical properties.

Whole-cell immobilization methods make use of several different physical mechanisms to achieve immobilization, which rely on approaches including:

(a) Attachment to surface
(b) Entrapment within a porous matrix or a gel
(c) Self-aggregation
(d) Chemically assisted aggregation
(e) Containment behind a barrier.

The surface attachment approach requires an inert medium as support and leads to particles with reduced mass ratio of cells to support (usually less than 20 % w/w; 102).

Earlier attempts, using the aggregation approach on fungal and algal biomass immobilization, proposed the use of agents such as formaldehyde, glutaric dialdehyde, urea, or alkaline solutions as binding-inducing agents, followed by processing and sizing [98, 100, 103–105]. The chemically induced alteration potential on sensitive biomass cellular structures, some of which (e.g., fungal cell walls) have been shown to be the mechanistically important functional groups of the exhibited biosorptive uptake potential, are of concern and have been seen to affect adversely the resulting immobilized biomass biosorptive uptake capacity.

Microbial biomass that naturally aggregates during growth in the form of biomass pellets could be considered as immobilized by self-aggregation. The not so good stability and the low mechanical strength of such pellets may, however, limit their applicability in continuous, industrial-scale biosorption operations. The example of

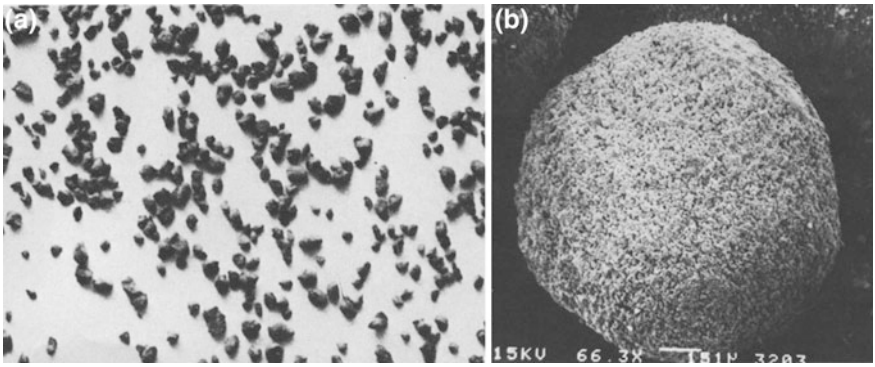

Fig. 9 Electron micrographs of immobilized *Rhizopus arrhizus* biomass particles: **a** General view and **b** magnification of the particle porous structure [45]

R. arrhizus pellets that were naturally grown during the biomass fermentation stage and subsequently were tested for the continuous recovery of uranium ions, in sequential biosorption-elution stages is characteristic [106].

During that application, the reactor superficial velocity had to be limited because of the phenomenon of biomass natural pellet compression and disintegration difficulties that worsened as a function of the contact solution pH and the reactor operating flow conditions [102, 106].

Calcium alginates, polyacrylamides, and other organic polymers have been proposed as an entrapment agent for biomass immobilization. The use of silica gel matrices (Sol Gel process) have also been proposed for biomass immobilization. The resulting particles, however, are soft and of limited mechanical strength, with significant content of nonbiomass materials and have not received wide attention [104].

Containment behind a barrier or encapsulation is an approach that has found extensive commercial application especially in the medical, food, and pharmaceutical industries. The related literature is very extensive and rapidly expanding, but beyond the scope of this chapter.

A proprietary air suspension method for microbial biomass immobilization, by encapsulation, for the purpose of use in biosorption reactors, has been reported and tested in continuous pilot scale [102, 107]. This approach utilized hydrophilic polymeric membranes, with controlled permeability and with less than 15 % w/w nonbiosorbing material content so as to optimize the resulting immobilized biomass particles' mechanical strength, and permitting favorable solutes mass transfer rates.

This approach made use of polysulphone, cellulose acetate, polyvinyl formal, and several solvents, yielding particles of desirable size and narrow particle size distribution [36, 102, 103, 107]. Typical reported photographs of such immobilized *R. arrhizus* biomass particles are shown in Fig. 9. These particles have been successfully used in packed-bed reactors for the recovery of uranium from bioleach mine waters of an Elliot Lake, Canada, uranium mine, in multiple biosorption–elution cycles [103].

Substantial work has been reported on the study of the engineering parameters that are important in the biosorption of ions using immobilized microbial biomass. Well-developed chemical engineering simulation techniques, used for separation processes such as adsorption or ion exchange, have also found application in the study and simulation of biosorption reactors.

Batch kinetic mass transfer models, describing the transport of soluble species from the bulk solution into the immobilized biomass particles, have directed our attention to the significant immobilized biomass particle design parameters that affect the overall process efficiency [37, 56, 57, 59, 104]. Parameters such as the particle size, the content of nonbiosorbing agents, the intrinsic rate of the underlying biosorption process, the initial solute concentration, the maximum native biomass uptake capacity, and the effective solute diffusivity in the immobilized particle have been shown to be important [37, 56, 57, 59].

A basic approach to a biosorption batch reactor mass transfer kinetic model, for immobilized biomass particles, is the one utilizing a mass balance of the solute across each of the assumed mass transport resistances [57, 102]. A sequence of expressions describes the transport across three assumed barriers: namely, the solution boundary layer around the immobilized biomass particle [Eq. (2)], the transport across the nonbiosorbing immobilizing polymeric membrane [Eq. (3)] and the transport/retention of the solute into the particle core utilizing Fick's law [Eq. (4)], as shown in the summary below:

$$K_f(C_b - C_{b,KR}) = \frac{D_m}{\delta}(C_{M,KR} - C_{M,R}) \tag{2}$$

$$4\pi(KR)^2 \frac{D_m}{\delta}(C_{M,KR} - C_{M,R}) = 4\pi R^2 D_p \lambda \left.\frac{\partial C}{\partial r}\right|_{r=R} \tag{3}$$

$$\lambda \frac{\partial C}{\partial t} + \rho \frac{\partial q}{\partial t} = D_p \lambda \left(\frac{\partial^2 C}{\partial r^2} + 2\frac{\partial C}{\partial r}\right) \tag{4}$$

where

C	solute concentration in biomass core pores
C_M	solute concentration in polymeric membrane
C_b	solute concentration in bulk solution
r	radial variable in biomass core
KR	radius of immobilized biomass particle
q	biosorptive uptake (solute concentration in the contained biomass)
λ	apparent biomass core porosity
D_p	effective solute diffusivity in biomass core pores
ρ	apparent biomass core density
D_m	effective solute diffusivity in the polymeric membrane
δ	polymeric membrane thickness
k_f	external fluid film mass transfer coefficient.

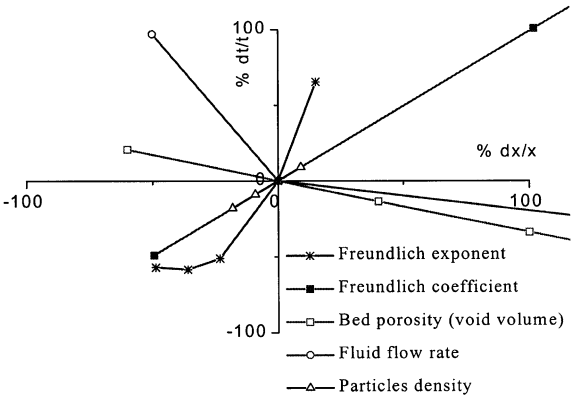

Fig. 10 Sensitivity analysis plot, showing the effect of each parameter and its relative importance on the breakthrough time: abscissa normalized percentage change of parameter and ordinance normalized percentage change on the column breakthrough time [55]

The above model was used in conjunction with detailed, controlled, uranium biosorption kinetic data [102]. Model simulations agreed well with the experimental results, once the model parameter values were optimized using experimental data [57, 102].

The subsequent modeling of the operation of continuous packed-bed biosorption reactors has also been studied using the concept of local equilibrium [55]. The model has successfully predicted breakthrough curves and has demonstrated the significance of the high biosorbent uptake capacity and the sorption intensity, as expressed by the Freundlich isotherm coefficients for the overall efficiency of the process, while also demonstrating the effect of axial dispersion on the shape of the resulting breakthrough curves [55].

The model's sensitivity analysis has provided evidence for the effects that can be seen on the packed-bed operation for selected important parameters as shown in Fig. 10 [55]. The relative importance of the various parameters can be identified by plotting the relative change of the value of interest (i.e., breakthrough time) against the relative change in the value of the parameter under investigation. The percentage relative changes have been calculated by the formula:

$$\%\frac{\mathrm{dx}}{x} = \frac{x_{\text{new}} - x_{\text{base point}}}{x_{\text{base point}}} 100\,\%$$

In the dataset shown in Fig. 10, the origin of the axis corresponds to the base point. The x-axis shows the percentage deviation from the base point of the variable under consideration. In the y-axis, the percentage change of the breakthrough time from the base point breakthrough time is presented. The analysis example shown in Fig. 10 clearly suggests that the most important operating parameter is the sorption intensity and the sorbent uptake capacity, as expressed by the Freundlich exponent and coefficient, respectively [55].

5 Sequestering by Living Cells

Living cells may exhibit both passive physicochemical and metabolically mediated sequestering mechanisms for targeted solutes. As we already discussed in Sect. 1, such solute sequestration should not be classified as a biosorptive separation process.

Living microbial cells have been used mostly in fixed-film reactor configurations, for example, in rotating biodiscs or packed-bed reactors. Such reactor configurations make possible the continuous separation of the functioning biomass from the contact solution, with the exception of the biofilm sloughing-off effects. Provision should be made for the supply of the required biomass nutrients and the protection of the biofilm from contact solution composition-induced toxicity effects.

The interaction of the biomass with the contact solution ionic species often relies on redox reactions that are metabolically mediated and yield insoluble, often elemental microprecipitates [75, 76, 108].

Another significant separation pathway is that of the precipitation of the targeted solutes via metabolically generated precipitation moieties, such as in the form of sulphates, sulphides, or phosphates [108]. The above process falls into the category of what we described as bioprecipitation processes. Bioprecipitation is a separate well-developed and widening area of study, which is beyond the scope of the present work.

6 Biosorption of Organic Molecules

Most of the work on biosorption has been focused on the interaction and sequestering of inorganic ionic species by inactive microbial biomass or biomaterials. The sequestering of organic molecules by inactive microbial biomass can also be regarded as a biosorptive separation process and has also received attention.

Early systematic work with detailed equilibrium sorption isotherms, including temperature effects on the quantification of organic molecule biosorption, focused on the interaction of selected priority pollutants with different inactive microbial biomass types [15–20].

Selected toxic organic molecules including lindane, diazinon, malathion, pentachlorophenol, 2-chlorobiphenyl (of the PCB family), and chloroethanes have been examined for the biosorptive removal from aquatic solutions, as well as the reversibility of this separation. The quantitative equilibrium separation results were successfully and consistently described by the commonly used Freundlich biosorption equilibrium isotherm model, over, at least, three orders of magnitude of residual solute equilibrium concentrations, while always remaining within the respective solubility ranges [15–17, 20].

Table 2 Freundlich model parameter values (activated sludge)

Biosorbed compound	k	$1/n$
Lindane	1.5	1.0
Pentachlorophenol	10.1	0.8
Diazinon	0.4	1.0
Malathion	408.5	0.5

Examples of the respective Freundlich model parameter values for a mixed microbial population biomass (activated sludge) are given in Table 2.

The combined assessment of the biosorption and elution equilibrium results for the above-selected organic molecules assisted in the formation and reporting of preliminary mechanistic understanding hypotheses for their biosorptive sequestering from solution and are summarised in the ensuing sections [16–20].

6.1 Lindane Biosorption

The use of simple or mixed inactive microbial biomass types showed essentially linear biosorptive isotherms, over the extended residual equilibrium concentration range, characterized by a low energy of biosorption.

The low solubility of lindane (ca. 10 mg/L), via the imposed low residual lindane equilibrium concentrations, likely resulted in the observed reduced equilibrium uptake capacities. The observed biosorptive uptakes were fully reversible, thus likely pointing to a primarily physical sorption sequestering mechanism [15–17].

6.2 Diazinon Biosorption

Diazinon is characterized by slightly higher water solubility (ca. 40 mg/L). It has also exhibited almost linear biosorption isotherms for single or mixed microbial biomass types. The biosorption equilibrium was shown to be fully reversible and this, in association with the low energy of sorption, suggested the involvement of a physical sorption mechanism [15–17].

6.3 Malathion Biosorption

Malathion is characterized by higher water solubility (ca. 150 mg/L). It is known to be subjected to decomposition by enzymatic reactions. As a consequence, enzymes associated with the microbial biomass used may induce some decomposition of the malathion molecule while in the contact solution and higher apparent biosorptive uptake results.

Malathion biosorptive uptake equilibrium at lower (5 °C) and higher (20 °C) temperatures have exhibited dissimilar desorption behavior. Biosorption appears reversible at 5 °C, suggesting a rather physical sorption mechanism in effect. At 20 °C, the biosorption of malathion was reported as nonreversible, suggesting the involvement of a chemical reaction in biosorption, which likely resulted in the differentiation of malathion. The two mechanisms of physical sorption and chemical alteration are likely acting in tandem, with their relative contribution to the experimentally observed overall malathion biosorption affected by the contact solution temperature [15–17].

6.4 Humic Acids Biosorption

Humic acids comprise a very complex and structurally difficult to identify class of high molecular weight compounds, often present in natural waters. Reported work has shown that fungal biomass of *Aspergillus niger*, of *A. ustus*, and of *Stachybotrys* sp. are efficient biosorbents for humic acids.

Equilibrium is reversible and the kinetics are rapid, suggesting a likely physical sorption mechanism [77]. The understanding of such interactions is significant for the study of the mobility and fate of humic substances in aquatic environments.

6.5 Chloroethanes Biosorption

The biosorptive separation behavior of 1,1,2-trichloethane (TCE) and 1,1,2,2-tetrachloroethane (TTCE) using different types of microbial biomass have been reported [19, 109]. The reported results have suggested significant differences among the observed biosorptive sequestering capacities of the examined biomass types. An inverse relationship between the water solubility of the organic solute and the observed biosorptive uptake capacity has been found.

The lipid content of the microbial biomass used was shown not to correlate directly with the organic compound uptake whereas the observed biosorptive uptake suggested the operation of competition effects between the two molecules (TCE and TTCE). The single solute biosorptive uptake capacities are reported to be higher than the corresponding multisolute uptake capacities [19, 109].

The above examples on the quantification of the biosorption equilibrium, the kinetics, as well as a mechanistic understanding of the biosorption of organic molecules point to the fact that the experimental techniques that have been developed for the study of inorganic biosorption are suitable for use for the study of the biosorption of organic molecules.

The issues of uptake equilibrium quantification, modeling, reversibility competition effects, and mechanistic understanding are equally important in the

rationalization of the significance of the biosorption of organics in other processes such as the treatment of wastewater and the ultimate fate of organic pollutants in the environment.

6.6 Reactive Dyes Biosorption

Reactive dyes are complex organic molecules of industrial significance in textiles that generate colors. The reactivity of the molecules is commonly pH dependent. Work reported on the biosorption of Reactive Blue, Reactive Orange 16, and Reactive Yellow by *Corynebacterium glutamicum* has suggested successful biosorptive uptake capacities of the order of 150–180 mg/g, strongly dependent on the solution pH, as expected [110]. The effect of pH on the reactivity of the dyes can be seen as an analogue to the pH effect of the speciation of metals via the hydrolysis of ions presented earlier in this chapter. The proposed dye biosorption mechanism is based on the nucleophilic addition and nucleophilic substitution reactions between the reactive dye molecules' vinyl sulfone group and the biomass-based hydroxyl groups [110, 111]. This interaction, which is strongly dependent on the solution pH, results in the binding and retention of the organic molecule onto the microbial cell surface.

7 Conclusions

The work that has accumulated and has been reported over the years on the subject of biosorption has helped us to understand better how the microbial biomass interacts with solutes of an inorganic or organic nature and how such solutes are sequestered and accumulated by the microbial biomass. Instrumental to this understanding is the elucidation of biosorption mechanisms.

Biosorption mechanisms can vary significantly, depending on the characteristics of the microbial biomass and on the chemical identity of the solute, under the contact solution conditions. These differences become even more significant when the biomass is alive, as metabolically mediated pathways may become decisive as far as the observed solute sequestration potential is concerned. This refers to the recommended terminology differentiation in Sect. 1.

The engineering modeling and process simulation tools that have been developed for well-established chemical engineering separation processes, as adsorption or ion exchange, have been shown to describe well the performance of reactor configurations that utilize microbial biomass as the acting agent for the biosorptive sequestering of solutes.

The weak structural stability of the native inactive microbial biomass may not permit the use of the same biomass for many biosorption/elution cycles. The

controlled immobilization of the native inactive microbial biomass can partially alleviate this concern.

Biosorption is an excellent selective sequestering process, especially for applications in solutions that are complex and contain the targeted elements at low concentrations.

In addition, the quantification of the significance and of the effects of biosorptive phenomena, in engineering processes and in environmental issues, as supplementary to or complementary to other co-existing and co-functioning processes, is important and this is valid for both inorganic and organic solutes.

References

1. Rothstein A, Frenkel A, Larrabee C (1948) The relationship of the cell surface to metabolism. III. Certain characteristics of the uranium complex with cell surface groups of yeast. J Cell Comp Physiol 32:261
2. Polikarpov GG (1966) Radioecology of aquatic organisms; the accumulation and biological effect of radioactive substances. Reinhold Book Division, New York
3. Tezuka Y (1969) Cation-dependent flocculation in a *Flavobacterium* species predominant in activated sludge. Appl Microbiol 17:222
4. Chiu YS (1972) Recovery of heavy metals by microbes. Ph.D. thesis. University of Western Ontario, Ontario
5. Paskins-Hurlburt AJ, Tanaka Y, Skoryna SC (1976) Carrageenan and the binding of lead. Bot mar 19:59
6. Jilek R, Fuska J, Nemec P (1978) Biologicky sorbent pro dekontaminaci vod od uranu. Biologia 33:201
7. Beveridge TJ, Murray RG (1976) Uptake and retention of metals by cell walls of *Bacillus subtilis*. J Bacteriol 127:1502
8. Beveridge TJ (1978) The response of cell walls of *Bacillus subtilis* to metals and to electron-microscopic stains. Can J Microbiol 24:89
9. Tsezos M (1980) Biosorption of uranium and thorium. Ph.D. thesis. Department of Chemical Engineering, McGill University, Quebec
10. Weber WJ (1972) Physicochemical processes for water quality control. Wiley-Interscience, New York
11. Tsezos M, Volesky B (1981) Biosorption of uranium and thorium. Biotechnol Bioeng 23:583
12. Tsezos M, Volesky B (1982) The mechanism of uranium biosorption by *Rhizopus arrhizus*. Biotechnol Bioeng 24:385
13. Tsezos M, Volesky B (1982) The mechanism of thorium biosorption by *Rhizopus arrhizus*. Biotechnol Bioeng 24:955
14. White C, Wilkinson SC, Gadd GM (1995) The role of microorganisms in biosorption of toxic metals and radionuclides. Int Biodeterior Biodegradation 35:17
15. Bell JP (1986) Biosorption of hazardous organic pollutants. Ph.D. thesis. Department of Chemical Engineering, McMaster University
16. Bell JP, Tsezos M (1987) Removal of hazardous organic pollutants by adsorption on microbial biomass. Water Sci Technol 19:409
17. Tsezos M, Bell JP (1989) Comparison of the biosorption and desorption of hazardous organic pollutants by live and dead biomass. Water Res 23:561
18. Tsezos M, Bell JP (1988) Significance of biosorption for the hazardous organics removal efficiency of a biological reactor. Water Res 22:391

19. Tsezos M, Seto W (1986) The adsorption of chloroethanes by microbial biomass. Water Res 20:851
20. Tsezos M, Bell JP (1991) A mechanistic study on the fate of malathion following interaction with microbial biomass. Water Res 25:1039
21. Aksu Z (2005) Application of biosorption for the removal of organic pollutants: a review. Process Biochem 40:997
22. Mishra A, Malik A (2012) Recent advances in microbial metal bioaccumulation. Crit Rev Environ Sci Technol 43:1162
23. Tsezos M, Keller DM (1983) Adsorption of radium-226 by biological origin absorbents. Biotechnol Bioeng 25:201
24. Yakubu NA, Dudeney AWL (1986) Biosorption of uranium with *Aspergillus niger*. In: Eccles HH, Hunt S (eds) Immobilisation of ions by biosorption. Ellis Horwood Publishers, London
25. Gadd GM (2009) Biosorption: critical review of scientific rationale, environmental importance and significance for pollution treatment. J Chem Technol Biotechnol 84:13
26. Tsezos M (1990) Biosorption of radioactive species. In: Volesky B (ed) Biosorption of heavy metals. CRC Press, Florida
27. Davis TA, Volesky B, Mucci A (2003) A review of the biochemistry of heavy metal biosorption by brown algae. Water Res 37:4311
28. McKay G, Ho YS, Ng JCY (1999) Biosorption of copper from waste waters: a review. Sep Purif Rev 28:87
29. Mukhopadhyay M, Noronha SB, Suraishkumar GK (2011) A review on experimental studies of biosorption of heavy metals by *Aspergillus niger*. Can J Chem Eng 89:889
30. Sağ Y (2001) Biosorption of heavy metals by fungal biomass and modeling of fungal biosorption: a review. Sep Purif Rev 30:1
31. Das N (2010) Recovery of precious metals through biosorption—a review. Hydrometallurgy 103:180
32. Mack C, Wilhelmi B, Duncan JR, Burgess JE (2007) Biosorption of precious metals. Biotechnol Adv 25:264
33. Vijayaraghavan K, Yun Y-S (2008) Bacterial biosorbents and biosorption. Biotechnol Adv 26:266
34. Monteiro CM, Castro PML, Malcata FX (2012) Metal uptake by microalgae: underlying mechanisms and practical applications. Biotechnol Prog 28:299
35. Dhankhar R, Hooda A (2011) Fungal biosorption—an alternative to meet the challenges of heavy metal pollution in aqueous solutions. Environ Technol 32:467
36. Tsezos M (1986) Adsorption by microbial biomass as a process for removal of ions from process or waste solutions. In: Eccles H, Hont S (eds) Immobilisation of ions by biosorption. Ellis Horwood Publishers, London
37. Tsezos M (1990) Engineering aspects of metal binding by biomass. In: Ehrlich H, Brierley C (eds) Microbial mineral recovery. McGraw Hill, New York
38. Baes CF, Mesmer RE (1976) The hydrolysis of cations. Wiley, New York
39. Remoundaki E, Tsezos M, Hatzikioseyian A, Karakoussis V (1999) Mechanism of palladium biosorption by microbial biomass. The effects of metal ionic speciation and solution co-ions. In: Amils R, Ballester A (eds), Biohydrometallurgy and the Environment toward the Mining of the twenty first century, Proceedings of the international biohydrometallurgy symposium IBS 1999, Elsevier
40. Tsezos M, Georgousis Z, Remoudaki E (1997) Mechanism of aluminum interference on uranium biosorption by *Rhizopus arrhizus*. Biotechnol Bioeng 55:16
41. Wang J, Chen C (2006) Biosorption of heavy metals by *Saccharomyces cerevisiae*: a review. Biotechnol Adv 24:427
42. Farooq U, Kozinski JA, Khan MA, Athar M (2010) Biosorption of heavy metal ions using wheat based biosorbents—a review of the recent literature. Bioresour Technol 101:5043

43. Gadd GM, White C (1992) Removal of thorium from simulated acid process streams by fungal biomass: potential for thorium desorption and reuse of biomass and desorbent. J Chem Technol Biotechnol 55:39
44. Rudzinski W, Plazinski W (2010) How does mechanism of biosorption determine the differences between the initial and equilibrium adsorption states? Adsorption 16:351
45. Tsezos M, Baird MHI, Shemilt LW (1987) The use of immobilised biomass to remove and recover radium from Elliot Lake uranium tailing streams. Hydrometallurgy 17:357
46. Tsezos M, Noh SH (1984) Extraction of uranium from sea water using biological origin adsorbents. Can J Chem Eng 62:559
47. Volesky B, Holan ZR (1995) Biosorption of heavy metals. Biotechnol Prog 11:235
48. Andrès Y, Texier AC, Le Cloirec P (2003) Rare earth elements removal by microbial biosorption: a review. Environ Technol 24:1367
49. Tsezos M, Georgousis Z, Remoudaki E (1997) Ionic competition effects in a continuous uranium biosorptive recovery process. J Chem Technol Biotechnol 70:198
50. Tsezos M, McCready RGL, Bell JP (1989) The continuous recovery of uranium from biologically leached solutions using immobilized biomass. Biotechnol Bioeng 34:10
51. Tsezos M, Remoudaki E, Angelatou V (1996) A study of the effects of competing ions on the biosorption of metals. Int Biodeterior Biodegradation 38:19
52. Pearson RG (1963) Hard and soft acids and bases. J Am Chem Soc 85:3533
53. Tsezos M (1984) Recovery of uranium from biological adsorbents—desorption equilibrium. Biotechnol Bioeng 26:973
54. Tsezos M, Baird MHI, Shemilt LW (1987) The elution of radium adsorbed by microbial biomass. Chem Eng J 34:B57
55. Hatzikioseyian A, Tsezos M, Mavituna F (2001) Application of simplified rapid equilibrium models in simulating experimental breakthrough curves from fixed bed biosorption reactors. Hydrometallurgy 59:395
56. Tsezos M, Deutschmann AA (1992) The use of a mathematical model for the study of the important parameters in immobilized biomass biosorption. J Chem Technol Biotechnol 53:1
57. Tsezos M, Noh SH, Baird MHI (1988) A batch reactor mass transfer kinetic model for immobilized biomass biosorption. Biotechnol Bioeng 32:545
58. Tsezos M, McCready RGL, Salley J, Cuif JP (1989) The use of immobilised biomass for the continuous recovery of uranium from Elliot Lake ore. First pilot plant results. In: Salley J, McCready RGL, Wichlacz PL (eds), Biohydrometallurgy, Proceedings of the international symposium on biohydrometallurgy 1989, Canada Centre for Mineral and Energy Technology
59. Tsezos M, Deutschmann AA (1990) An investigation of engineering parameters for the use of immobilized biomass particles in biosorption. J Chem Technol Biotechnol 48:29
60. Fourest E, Roux J-C (1992) Heavy metal biosorption by fungal mycelial by-products: mechanisms and influence of pH. Appl Microbiol Biotechnol 37:399
61. Arief VO, Trilestari K, Sunarso J, Indraswati N, Ismadji S (2008) Recent progress on biosorption of heavy metals from liquids using low cost biosorbents: characterization, biosorption parameters and mechanism studies. Clean: Soil, Air, Water 36:937
62. Tsezos M, Remoudaki E, Angelatou V (1997) Biosorption sites of selected metals using electron microscopy. Comp Biochem Physiol A Physiol 118:481
63. Diels L, Corbisier P, Hooyberghs L, Glombitza F, Hummel A, Tsezos M, Pümpel T, Pernfuss B, Schinner F, Mergeay M (1995) Heavy metal resistance and biosorption in *Alcaligenes Eutrophus* ER 121. In: Cerez CA, Vargas T, Toledo H, Wiertz JV (eds) Biohydrometallurgical processing, Proceedings of the international biohydrometallurgy symposium IBS 1995. University of Chile, Chile
64. Sakamoto N, Kano N, Imaizumi H (2008) Biosorption of uranium and rare earth elements using biomass of algae. Bioinorg Chem Appl 2008(706240):8. doi: 10.1155/2008/706240
65. Tsezos M, Mattar S (1986) A further insight into the mechanism of biosorption of metals, by examining chitin EPR spectra. Talanta 33:225

66. Guibal E, Roulph C, Le Cloirec P (1992) Uranium biosorption by a filamentous fungus *Mucor miehei* pH effect on mechanisms and performances of uptake. Water Res 26:1139
67. Liao J, Liu N, Yang Y, Luo S, Luo Q, An Z, Duan Y, Liu M, Zhao P (2008) Preliminary investigation on biosorption mechanism of ^{241}Am by *Rhizopus arrhizus*. J Radioanal Nucl Chem 277:329
68. Liu N, Liao J, Yang Y, Luo S, Luo Q, An Z, Duan Y, Liu M, Zhao P (2008) Biosorption of ^{241}Am by *Saccharomyces cerevisiae*: preliminary investigation on mechanism. J Radioanal Nucl Chem 275:173
69. Beveridge TJ, Murray RG (1980) Sites of metal deposition in the cell wall of *Bacillus subtilis*. J Bacteriol 141:876
70. Gee AR, Dudeney AWL (1988) Adsorption and crystallisation of gold at biological surfaces. In: Norris PR, Kelly DP (eds), Biohydrometallurgy, Proceedings of the international symposium on biohydrometallurgy 1987, Science and technology letters
71. Lin Z, Ye Y, Li Q, Xu Z, Wang M (2011) A further insight into the biosorption mechanism of Au(III) by infrared spectrometry, in: BMC Biotechnology. BioMed Central, 11:98. doi: 10.1186/1472-6750-11-98, http://www.biomedcentral.com/1472-6750/11/98
72. Lin Z, Xue R, Ye Y, Zheng J, Xu Z (2009) A further insight into the biosorption mechanism of Pt(IV) by infrared spectrometry, in: BMC Biotechnology. BioMed Central, 9:62. doi: 10.1186/1472-6750-9-62, http://www.biomedcentral.com/1472-6750/9/62
73. Lin Z, Zhou C, Wu J, Zhou J, Wang L (2005) A further insight into the mechanism of Ag^+ biosorption by *Lactobacillus* sp. strain A09. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 61:1195
74. Li L, Hu Q, Zeng J, Qi H, Zhuang G (2011) Resistance and biosorption mechanism of silver ions by *Bacillus cereus* biomass. J Environ Sci 23:108
75. Pümpel T, Ebner C, Pernfuss B, Schinner F, Diels L, Keszthelyi Z, Stankovic A, Finlay JA, Macaskie LE, Tsezos M, Wouters H (2001) Treatment of rinsing water from electroless nickel plating with a biologically active moving-bed sand filter. Hydrometallurgy 59:383
76. Diels L, Spaans PH, Van Roy S, Hooyberghs L, Ryngaert A, Wouters H, Walter E, Winters J, Macaskie L, Finlay J, Pernfuss B, Woebking H, Pümpel T, Tsezos M (2003) Heavy metals removal by sand filters inoculated with metal sorbing and precipitating bacteria. Hydrometallurgy 71:235
77. Vukovic M, Domanovac T, Briski F (2008) Removal of humic substances by biosorption. J Environ Sci 20:1423
78. Diniz V, Volesky B (2005) Biosorption of La, Eu and Yb using *Sargassum* biomass. Water Res 39:239
79. Vijayaraghavan K, Sathishkumar M, Balasubramanian R (2010) Biosorption of lanthanum, cerium, europium, and ytterbium by a brown marine alga, *Turbinaria Conoides*. Ind Eng Chem Res 49:4405
80. Zhang L-L, Feng XX, Xu F, Xu S, Cai WM (2005) Biosorption of rare earth metal ion on aerobic granules. J Environ Sci Health A 40:857
81. Philip L, Iyengar L, Venkobachar C (2000) Biosorption of U, La, Pr, Nd, Eu and Dy by *Pseudomonas aeruginosa*. J Ind Microbiol Biotechnol 25:1
82. Achyuthan K, Arango D, Carles E, Cutler C, Meyer L, Brozik S (2009) Luminescent investigations of terbium(III) biosorption as a surrogate for heavy metals and radionuclides. Mol Cell Biochem 327:87
83. Oliveira RC, Jouannin C, Guibal E, Garcia O Jr (2011) Samarium(III) and praseodymium(III) biosorption on *Sargassum* sp.: batch study. Process Biochem 46:736
84. Muraleedharan TR, Venkobachar C (1990) Mechanism of biosorption of copper(II) by *Ganoderma lucidum*. Biotechnol Bioeng 35:320
85. Zhang Y, Liu W, Xu M, Zheng F, Zhao M (2010) Study of the mechanisms of Cu^{2+} biosorption by ethanol/caustic-pretreated baker's yeast biomass. J Hazard Mater 178:1085
86. Ahmady-Asbchin S, Andrès Y, Gérente C, Cloirec PL (2008) Biosorption of Cu(II) from aqueous solution by *Fucus serratus*: surface characterization and sorption mechanisms. Bioresour Technol 99:6150

87. Chojnacka K, Chojnacki A, Górecka H (2005) Biosorption of Cr^{3+}, Cd^{2+} and Cu^{2+} ions by blue–green algae *Spirulina* sp.: kinetics, equilibrium and the mechanism of the process. Chemosphere 59:75
88. Guo J, Zheng X-d, Chen Q-b, Zhang L, Xu X-p (2012) Biosorption of Cd(II) from aqueous solution by *Pseudomonas plecoglossicida*: kinetics and mechanism. Curr Microbiol 65:350
89. Huang H, Cao L, Wan Y, Zhang R, Wang W (2012) Biosorption behavior and mechanism of heavy metals by the fruiting body of jelly fungus (*Auricularia polytricha*) from aqueous solutions. Appl Microbiol Biotechnol 96:829
90. Raize O, Argaman Y, Yannai S (2004) Mechanisms of biosorption of different heavy metals by brown marine macroalgae. Biotechnol Bioeng 87:451
91. Yan G, Viraraghavan T (2008) Mechanism of biosorption of heavy metals by *Mucor rouxii*. Eng Life Sci 8:363
92. Sun F, Wu F, Liao H, Xing B (2011) Biosorption of antimony(V) by freshwater cyanobacteria *Microcystis* biomass: chemical modification and biosorption mechanisms. Chem Eng J 171:1082
93. Ginisty P, Besnainou B, Sahut C, Guezennec J (1998) Biosorption of cobalt by *Pseudomonas halodentrificans*: influence of cell wall treatment by alkali and alkaline-earth metals and ion-exchange mechanisms. Biotechnol Lett 20:1035
94. Li J, Lin Q, Zhang X, Yan Y (2009) Kinetic parameters and mechanisms of the batch biosorption of Cr(VI) and Cr(III) onto *Leersia hexandra Swartz* biomass. J Colloid Interface Sci 333:71
95. Ramrakhiani L, Majumder R, Khowala S (2011) Removal of hexavalent chromium by heat inactivated fungal biomass of *Termitomyces clypeatus*: surface characterization and mechanism of biosorption. Chem Eng J 171:1060
96. Shen L, Xia J-l, He H, Nie Z-y, Qiu G-z (2007) Biosorption mechanism of Cr(VI) onto cells of *Synechococcus* sp. J Cent S Univ Technol 14:157
97. Han X, Wong YS, Tam NF (2006) Surface complexation mechanism and modeling in Cr(III) biosorption by a microalgal isolate. Chlorella miniata J Colloid Interface Sci 303:365
98. Tsezos M (1990) Recovery of metals by biosorption: Fundamentals and technology development. In: Karavaiko GI, Rossi G, Avakyan ZA (eds), Biohydrometallurgy, Proceedings of international seminar on dump and underground bacterial leaching metals from ores, USSR state committee for environment protection, Centre for International Projects, Moscow
99. Brierley JA, Goyak GM, Brierley CL (1986) Considerations for commercial use of natural products for metals recovery. In: Eccles H, Hont S (eds) Immobilisation of ions by biosorption. Ellis Horwood Publishers, London
100. Tsezos M (2001) Biosorption of metals. The experience accumulated and the outlook for technology development. Hydrometallurgy 59:241
101. Tsezos M, Remoundaki E, Hatzikioseyian A (2007) Bioprocessing—principles and applications for metal immobilization from waste-water streams. In: Cox M, Negre P, Yurramendi L (eds) A guide book on the treatment of effluents from the mining/metallurgy. Paper, Plating and Textile Industries, INASMET-Tecnalia, Spain
102. Deutschmann A (1988) The modeling of biosorption kinetics by immobilized biomass. Dissertation for the Degree of Master of Engineering. School of Graduate Studies, McMaster University, Ontario
103. Tsezos M, McCready RGL (1991) The pilot plant testing of the continuous extraction of radionuclides using immobilized biomass. In: Sayler GS, Fox R, Blackburn JW (eds) Environmental biotechnology for waste treatment. Plenum Press, New York
104. Dostálek P (2011) Immobilized biosorbents for bioreactors and commercial biosorbents. In: Kotrba P, Mackova M, Macek T (eds) Microbial biosorption of metals. Springer, Berlin, Heidelberg
105. Veglio F, Beolchini F (1997) Removal of metals by biosorption: a review. Hydrometallurgy 44:301

106. Treen ME (1981) Biosorption of the uranyl ion by *Rhizopus*. Dissertation for the Degree of Master of Engineering. Department of Chemical Engineering, McGill University, Quebec
107. Tsezos M, Noh S (1989) Particle encapsulation technique, United States Patent 4828882. May 9, 1989, p. 5
108. Pümpel T, Macaskie LE, Finlay JA, Diels L, Tsezos M (2003) Nickel removal from nickel plating waste water using a biologically active moving-bed sand filter. Biometals 16:567
109. Seto WCW (1985) Biosorption of chloroethanes by microbial biomass. Dissertation for the Degree of Master of Engineering. School of Graduate Studies, McMaster University, Ontario
110. Won SW, Han MH, Yun Y-S (2008) Different binding mechanisms in biosorption of reactive dyes according to their reactivity. Water Research 42:4847
111. Won SW, Yun HJ, Yun Y-S (2009) Effect of pH on the binding mechanisms in biosorption of reactive orange 16 by *Corynebacterium glutamicum*. J Colloid Interface Sci 331:83

Adv Biochem Eng Biotechnol (2014) 141: 211–235
DOI: 10.1007/10_2013_200

Published Online: 30 May 2013

Microbially Supported Phytoremediation of Heavy Metal Contaminated Soils: Strategies and Applications

René Phieler, Annekatrin Voit and Erika Kothe

Abstract Heavy metal contamination of soil as a result of, for example, mining operations, evokes worldwide concern. The use of selected metal-accumulating plants to clean up heavy metal contaminated sites represents a sustainable and inexpensive method for remediation approaches and, at the same time, avoids destruction of soil function. Within this scenario, phytoremediation is the use of plants (directly or indirectly) to reduce the risks of contaminants in soil to the environment and human health. Microbially assisted bioremediation strategies, such as phytoextraction or phytostabilization, may increase the beneficial aspects and can be viewed as potentially useful methods for application in remediation of low and heterogeneously contaminated soil. The plant–microbe interactions in phytoremediation strategies include mutually beneficial symbiotic associations such as mycorrhiza, plant growth promoting bacteria (PGPB), or endophytic bacteria that are discussed with respect to their impact on phytoremediation approaches.

Keywords Microbially assisted remediation · Phytomining · Phytoremediation

Abbreviations

Cys	Cysteine
Glu	Glutamine
Gly	Glycine
MT	Metallothionein
PC	Phytochelatin
PGPB	Plant growth promoting bacteria
ROS	Reactive oxygen species

R. Phieler · A. Voit · E. Kothe (✉)
Institute of Microbiology—Microbial Communication, Friedrich Schiller University, Neugasse 25, 07743 Jena, Germany
e-mail: erika.kothe@uni-jena.de
URL: www.microbio.uni-jena.de

Contents

1 Introduction

Approximately 1,400,000 sites in Western Europe are highly contaminated with heavy metals [1]. The increasing release of heavy metals and metalloids to the environment due to anthropogenic activities such as mining and smelting operations, burning of fossil fuels, municipal wastes, and agrochemical usage is a serious problem worldwide [2–4]. Soil contamination by metals can lead to loss of soil functions such as buffering, filtering, and transforming capacities, and may lead to contamination of ground and surface waters [5]. Toxic levels of heavy metals in soil are also a potential risk for environmental and human health due to soil-to-plant transfer of metals and their accumulation in animal or human bodies through the food chain [6, 7].

The term heavy metal includes elements with an atomic density greater than 6 g/cm^3 and a specific gravity above five [8]. Some of these metals play an important role as essential elements in biochemical reactions, whereas metals such as Cd, Pb, or As are not essential. The essential metals such as Cu, Fe, Mn, Ni, Zn, Mg, K, and Ca are required in low concentrations as nutrients [9]. They serve as catalysts in biochemical processes or cofactors of many enzymes and are involved in numerous physiological processes. But concentrations exceeding a threshold nevertheless are toxic, as every metal may cause alterations in the conformation structure of nucleic acids and proteins, inhibit enzymes, block functional groups of important molecules, and lead to production of reactive oxygen species (ROS; [10–12]). In contrast to organic pollutants, heavy metals cannot be degraded and their mobility in soil is influenced by soil conditions, metal speciation, and solubility in water. Metal availability in the water phase and hence their bioavailability

for uptake in either microbes or via plants into the food chain depends on pH, redox potential, and cation exchange capacity of soil, as well as adsorption to soil particles and interactions with soil microorganisms [8, 13]. The bioavailability further is strongly influenced by the presence of dissolved organic substances that may form metal complexes [14].

High heavy metal content in soils affects soil fertility as well as plant growth and renders large areas unsuitable for agricultural use. Thus, the remediation of heavy metal polluted soils is of high importance [6, 15]. Here, we discuss microbially assisted phytoremediation and also include an outlook to phytomining.

2 Potential of Phytoremediation Approaches

The remediation of heavy metal contaminated soils is one of the most challenging tasks for environmental engineering and most conventional remediation approaches do not provide satisfactory solutions [16, 17]. Conventional technologies for cleaning metal contaminated sites are mainly ex situ decontamination using physical and chemical methods. These ex situ techniques, such as soil washing, excavation, and thermal treatments, irreversibly affect soil functions, destroy biodiversity, and leave it biologically inactive [18, 19]. Furthermore, these remediation options are often costly, energy intensive, and site destructive [20]. The increasing awareness of public and governmental bodies provides an opportunity for plant-based bioremediation technologies.

The use of green plants to decontaminate heavy metal polluted sites, known as phytoremediation, is an in situ technology with considerable promise for removing metals from areas of low to moderate concentrations [21, 22]. It aims to use metal-accumulating plants to remove, transfer, or stabilize heavy metals at contaminated sites thus reducing the risk to the environment [23].

The idea of using plants that accumulate metals taken up from soil in harvestable biomass was introduced in 1983 and gained public exposure in 1990 [24]. Depending on the contaminants, the level of pollution, and the site conditions, phytoremediation includes five main plant-based technologies (Table 1) with three different mechanisms of action for cleaning up metal contaminated sites: phytoextraction, phytostabilization, and rhizofiltration [5, 25–28]. Among these, phytoextraction and phytostabilization are the most reliable for heavy metal removal from soils [29, 30].

The use of phytoremediation offers the benefits of being in situ, passive and solar-driven technology, and allows for site restoration, applicability to a wide range of sites, promoting future land use, and additionally opens the road to biorecovery of metals [31]. The main costs of phytoremediation are the site monitoring, the preconditioning of the contaminated soil, planting, potentially pest control, and harvest. It also contains the costs for disposal of contaminated biomass, mostly by controlled burning and ash deposition. The estimated costs for removal of contaminants from soils range from $25 to $100 per ton, depending on

Table 1 Phytoremediation types

Process	Description	Contaminant
Phytoextraction	Uptake of pollutants from soil and accumulation in harvestable plant biomass	Inorganic pollutants
Phytostabilization	Reduction of mobility and bioavailability of pollutants by plant roots in the rhizosphere	Inorganic pollutants
Rhizofiltration	Absorption and adsorption of pollutants by plant roots from aquatic environments	Inorganic/organic pollutants
Phytovolatilization	Removal of pollutants from contaminated environment and their release into air	Inorganic/organic pollutants
Phytodegradation	Degradation of pollutants by plants and associated microorganisms	Organic pollutants

site characterization and level of contamination [16, 25]. This cost-effective, green alternative may also be used at sites not readily available to other methods, reduces the exposure to secondary air- or water-borne wastes, and provides a vegetative ground cover for long-term stabilization and erosion prevention [32, 33]. The combination of bioenergy production and the recovery of heavy metals from metal-rich plant ash is possible [34]. Finally, remediation of contaminated sites by using green plants instead of machines or toxic chemicals is more attractive and more acceptable to the public than any other engineering-based approach.

However, some serious limitations of phytoremediation need to be considered. One of the greatest disadvantages is the time needed. Phytoremediation is generally slower than the more established, conventional soil remediation techniques such as excavation, incineration, or pump-and-treat systems. Several factors, including life cycles of plants, ordinary growing seasons, metal resistance of the crop used, as well as the level of contamination are influencing site cleanup [35]. In addition, site characteristics such as soil properties, mixed contamination, or climate may exert a strong influence. In addition, the use of plants does not allow a total removal of pollutants, because the lower the concentration of a respective pollutant, the slower the uptake becomes. This biological method is also limited in applicability to surface soils and limited by the bioavailability of the contaminant. Especially for cleaning up metal-contaminated sites, the solubility and bioavailability are of utmost importance [5, 36], and it requires further validation under field conditions in long-term experiments [6]. The use of chelating agents in order to enhance solubility of metals in soil, selection of adapted plant species, or addition of required nutrients or soil amendments might provide strategies to overcome disadvantages [16, 37].

Taken together, the success of the applied technology depends on two major components: choice of plants and soil conditions. Some plant species are well known to accumulate high metal loads in their biomass. Such metallophytes, however, often specifically concentrate one element, indicating limitations in remediation of sites with multiple contaminants. As a result, it is logical to consider crop plants as well that also have been evaluated for metal uptake in some cases. Decisive soil conditions such as homogeneous distribution of pollutants,

contamination with only one specific element, a good bioavailability of this contaminant, pH values between 4 and 7, and a good water-holding capacity of the contaminated soil are promising requirements [38].

There is one additional potential measure that has been underestimated thus far. The use of microbes with phytoremediation approaches might exert a positive influence on plant growth and soil function, which needs to be evaluated to the full before a final decision on feasibility can be made. This positive influence can lower the toxicity of metals in the plant or in the soil, increase the bioavailability of metals to achieve better uptake, reduce the wash-out with percolation water thus reducing the risk for ground and surface waters, or aid plant growth. The increase in biomass even may compensate for lower uptake per gram dry matter of harvested plant biomass. Thus, we discuss mechanisms of microbes, both bacteria and fungi, which are considered to be relevant for phytoremediation.

3 Plant-Based Methods for Bioremediation

3.1 Phytoextraction

Phytoextraction, also called phytoaccumulation, aims at removing inorganic pollutants, especially heavy metals, metalloids, and radionuclides, from contaminated subsurfaces through uptake by plants and accumulation in harvestable plant biomass [19]. Contributing factors for a successful extraction by plants are the extent and bioavailability of contaminants in soil and a plant's ability to tolerate and accumulate pollutants in high concentrations. For a successful metal extraction, the ideal plant should have some important characteristics: (1) rapid growth and high biomass production; (2) high tolerance to pollution and high accumulation of contaminants in aboveground biomass; (3) high root-to-shoot transfer of elements with a low binding capacity to root cell walls; (4) high bioconcentration factor and biological absorption coefficient (also referred to as BCF and BAC, respectively) higher than 1; (5) extended, well-branched, and deep root system; (6) native or easily adapting to the contaminated environment; and (7) simple agricultural management in the field [5, 38]. Unfortunately, even plant species suitable for phytoextraction do not combine all these required characteristics and poor soil conditions such as drought, moisture, and low fertility affect metal extraction. Suitable plants for phytoextraction are metal-accumulating crop species, especially within *Brassicaceae* and *Poaceae*, as well as highly productive tree species such as willow and poplar.

Most metal-tolerant plants are metal excluders. They restrict the transport and entry of metals into their aerial parts over a wide range of metal concentrations in the soil, but still contain high metal concentrations in their roots. Plants that actively accumulate metals in their upper plant tissues and generally reflect metal concentration in contaminated soil are called metal indicators [1, 2]. Some plant

Table 2 Main characteristics of continuous versus induced phytoextraction

Continuous phytoextraction	Chelate-assisted phytoextraction
Hyperaccumulator plants	Excluder, non-hyperaccumulator plants
Slow growth rates, low biomass production	High growth rates, high biomass production
Natural metal hyperaccumulation	Enhanced metal uptake by synthetic or natural chelators
Suitable for highly polluted soils	Suitable for low to moderate polluted soils
Most hyperaccumulators are metal specific	Multi-metal uptake
No environmental risk regarding leaching of metal chelates	Risk of percolation of anthropogenic metal chelates

species are able to accumulate specific metals to significant levels in their aboveground biomass. These hyperaccumulators can be used for a continuous phytoextraction because they accumulate metals at concentrations of more than 0.1 % or greater of their dry weight (Table 2; [39]). More than 400 species in 45 different botanical families can be classified as hyperaccumulator plants. Well-known plant families that contain species of hyperaccumulators are, for example, *Brassicaceae*, *Euphorbiaceae* and *Poaceae*. The hyperaccumulator plants, including *Thlaspi*, *Brassica*, *Apocynum*, *Paspalum*, and *Alyssum* [18], however, often are rather small with a low biomass production. There are, on the other hand, also some trees and shrubs that can accumulate elevated levels of specific metals without symptoms of phytotoxicity [5, 40].

In general, the feasibility of metal extraction from contaminated soil by plants is limited by the time required for cleanup, target metals in soil, depth of contamination, and suitable plant characteristics [41]. Thus, phytoremediation technique is applicable to decontaminate low to moderate metal-contaminated surface soils [38]. The effects of soil microbes discussed below may offer several beneficial traits.

3.2 Phytostabilization

During phytostabilization, metals are converted into inert immobilized species by absorption, adsorption, accumulation, precipitation, and physical stabilization within the root zone. The established vegetation cover provides the rhizosphere wherein metals precipitate. In this way, the plant action prevents metal leaching into groundwater [19]. Phytostabilization does not remove contaminants from soil, but aims at reducing the risk of further environmental degradation [42]. Desirable characteristics of plants selected for phytostabilization at a particular site include: (1) tolerance to high concentrations of metals of concern, (2) fast growth rates to establish ground cover and ability to develop an extended and abundant root system, (3) high retention capacity of contaminants in roots or rhizosphere to immobilize these contaminants and to prevent their spreading through the food chain, (4) low translocation of metals from root to shoots, (5) a high

bioconcentration factor, (6) relatively high transpiration rates to effectively dewater the soil, (7) low sustainment requirements and simple agricultural management, and (8) long-living and indigenous origin [43, 44]. For instance, suitable plants for phytostabilization are native species of perennial grasses, which are highly metal tolerant and adapted to local soil conditions. Additionally, a wide range of metal-tolerant shrubs and trees can be used for restoration of metal-contaminated sites [33, 45]. Typically, applied amendments are phosphate fertilizers, composted organic matter, liming agents, clay minerals, iron oxides, biosolids, or by-products from industrial processes [46]. The addition of soil amendments offers better starting conditions for the plants and may improve soil fertility [42, 47]. Here, microbially supported approaches may be used to substitute for amendments, at least partially.

3.3 Rhizofiltration

Rhizofiltration refers to using hydroponically cultivated roots or seedlings of terrestrial plants to absorb, concentrate, or precipitate metal pollutants from aqueous waste streams [48]. Mechanisms involved in metal removal by plant roots include extracellular precipitation, cell wall precipitation and surface adsorption, as well as intracellular uptake followed by compartmentalization and deposition within the vacuole [16, 19]. Suitable plants for rhizofiltration should combine the characteristics of: (1) high metal tolerance and high accumulation rates of target metals, (2) high translocation rates of metals, (3) high root biomass and large surface area, (4) easy handling and low maintenance costs, and (5) minimal secondary waste production. Fast-growing crop species including Indian mustard, sunflower, wild cabbage, tobacco, rye, and corn have an intrinsic ability to absorb and precipitate various heavy metals such as Pb, Mn, Cd, Ni, Cr, Cu, and Zn from aqueous solutions [16, 48]. At the same time, certain sunflower breeds seem to be promising candidates for rhizofiltration of radionuclides such as U, ^{137}Cs, and ^{90}Sr [27, 48]. Rhizofiltration seems to be most adaptable for large water volumes with a low level of contamination. The use of plant roots or seedlings provides an efficient and inexpensive solution to remove toxic metals from polluted waters and thus prevent hazardous risks to human health [16]. Removal of radionuclides from wastewaters may be particularly effective in combination with beneficial microorganisms [49].

4 Rhizosphere Interactions

Soil bacteria and mycorrhizal fungi have an impact on metal bioavailability and can either enhance or repress metal transfer from soil into harvestable plant biomass [39]. These interactions with direct contact or diffusion based interaction can

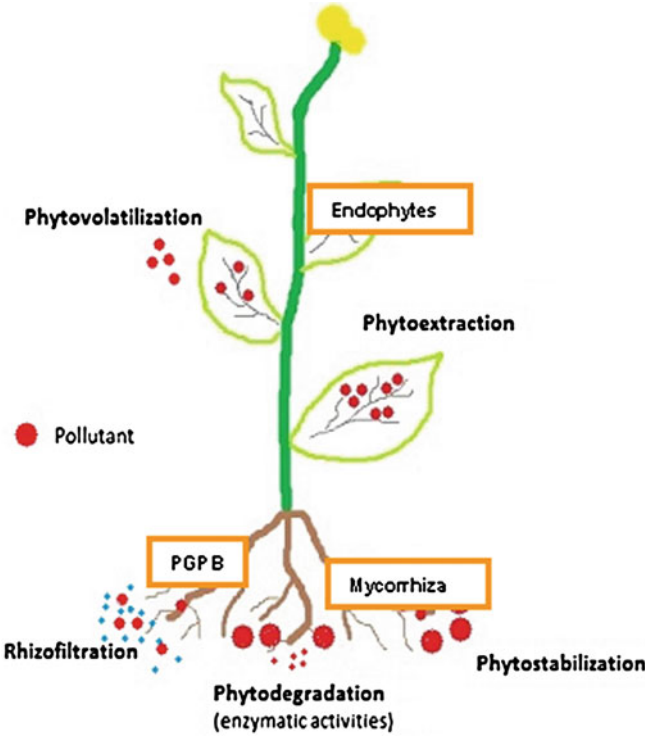

Fig. 1 Microbe–plant interactions in phytoremediation technologies (modified after [36])

be put into context with the general phytoremediation strategies (Fig. 1). A third possible microbe–plant interaction with impact on phytoremediation is the endophytic life style of bacteria or fungi within healthy plant tissues.

Specifically the mutually beneficial mycorrhizal symbiosis positively affects plant growth, biomass production, nutrient uptake, and production of growth-promoting hormones [50]. Within chemically assisted or induced phytoextraction, soil microbes may substitute the effects otherwise provided by application of chemical amendments to enhance the solubility and availability of metals in the soil [16, 51]. The secretion of natural chelating molecules such as phytosiderophores by plants [52] or metal reductases by roots [53] specifically can be complemented with soil bacteria that increase metal bioavailability and metal tolerance in plants [5].

But also alteration of physicochemical properties in the rhizosphere (e.g., pH and Eh) are prone to be dependent on microbial activity. Humic acids are formed by microbial degradation of dead organic matter. They become soluble at higher pH and make soil metals available for plants due to their characteristic carboxyl and phenol groups [54]. Furthermore, humic acids can reduce the mobility of various heavy metals in soil and limit percolation of solubilized metals into groundwater [55].

Phytohormones such as auxins have various positive effects on plants. For instance, auxins are involved in several cellular and physiological processes within the plant and can promote plant growth. Auxins are known to enhance both biomass production and root extension even in low concentrations, which may improve phytoextraction efficiency of metals by plants [5, 56]. Rhizosphere bacteria or fungi are well known to be able to synthesize auxins.

Most metal-accumulating plants are able to develop an extended root system with a high surface area to obtain essential nutrients for growth [16]. At the same time, they also have the ability to accumulate and tolerate elevated levels of nonessential metals. Usually, heavy metals in soil exist as ions and are taken up by plant roots via membrane transporter proteins. This active transport—against a chemical gradient—requires metabolic energy and allows for accumulation above the diffusion-driven adsorption to the apoplastic root surface. For most elements, numerous transporters exist in plants, each with specific properties with respect to transport rate, substrate affinity, and specificity [4, 57]. The storage of chelated metals in the vacuole or apoplast facilitates root sequestration [36].

The two major, heavy metal-binding compounds within plants are phytochelatins and metallothioneins [58]. Metallothioneins (MTs) are small cysteine-rich proteins found in animals and, more recently, in higher plants and bacteria. Typical for metallothioneins are their low molecular weight between 6–7 kDa and their high cysteine content necessary for coordination of specific metal ions in metal–thiolate clusters [59]. These low molecular weight metal-binding proteins are divided into three different classes, with class I being composed of animal MTs. MTs found in plants fall into class II, with wheat Ec protein and a number of MTs in different *Arabidopsis* ecotypes being described [2, 60, 61]. Within bacteria, metallohistins with high cysteine plus histidin contents can additionally be shown [62].

Phytochelatins (PCs) are a family of peptides that were first identified in yeast. They had been included into class III MTs. Whereas MTs are gene-encoded, PCs are enzymatically synthesized and induced by metals in most autotrophic plants, yeast, and some fungi. They are composed of only three amino acids, glutamine (Glu), cysteine (Cys), and glycine (Gly), and are structurally related to the tripeptide glutathione. The structure of these peptides is $(\gamma\text{-Glu-Cys})_nX$, where X is Gly, γ-alanine, serine, or glutamate, and n is in the range of 2–5, depending on the organism [63]. Biosynthesis of PCs can be induced by many metals, including Cd, Ni, Cu, Zn, Hg, Ag, Pb, and As, where Cd seems to be the strongest inducer [64]. PCs may play a role in the detoxification of metal ions by forming PC–metal complexes and thus regulate availability of metal ions in cells in order to prevent metal toxicity [65, 66].

4.1 Plant Growth Promoting Bacteria

Overviews of already published studies on benefits from plant–microbe interactions and their possible applications are given elsewhere [67–70]. The term *plant*

growth promoting bacteria (PGPB, or PGP rhizobacteria, PGPR) was introduced for those bacteria contributing to plant growth in both ways, directly or indirectly [71]. PGPB colonize root surfaces or thrive in the rhizosphere and affect plant development by nitrogen fixation, phosphate solubilization, or the production of phytohormones [69, 70], mostly auxins. Auxins are appropriate for phytoremediation, inasmuch as stimulation of germination, enhanced resistance to biotic or abiotic stress, and plant growth are controlled [69, 72].

Additionally, some PGPB are able to produce siderophores, iron chelators with a high Fe^{3+} affinity [73]. By binding to other metals, siderophores were found to promote plant growth on metalliferrous soils [74, 75]. Heavy metal resistant streptomycetes, Gram-positive aerobic soil bacteria, could be shown to enhance growth of *Vigna unguiculata* by siderophore production and nickel sequestration [76, 77] or preventing excess cadmium uptake in *Helianthus annuus* [78].

Enzymes, osmolytes, biosurfactants, nitric oxide, organic acids, and antibiotics produced by PGPB may contribute to the positive effects on plant performance [69]. However, additional studies should be undertaken to evaluate the sustainability and competition between PGPB and other soil microorganisms that need to be considered [80, 79].

4.2 Endophytes

Infection of plants without causing symptoms, in a harmless and mutualistic symbiosis, is performed by endophytic bacteria or fungi [81, 82]. Natural or genetically engineered endophytic bacteria were successfully used for phytoremediation studies [83–86]. These bacteria can improve a plants' capability of resisting pathogens, heavy metals, and herbivores. Additionally, enhancement in plant growth and supply with fixed nitrogen contribute to plant performance [87, 79]. In contrast to PGPB, endophytes live within healthy plant tissue, where stress and competition with other microbes are easier to overcome [79, 80]. Because fungal or bacterial cell walls are able to sequester substantial quantities of metals, an increase in metal loads of aboveground harvestable biomass seems possible.

One major point that needs consideration with respect to endophytes is that often, any organism isolated from surface-sterilized plant tissue is considered to be an endophyte. However, looking at the definition this is not true. It is very difficult to show that no contamination was remaining in the case of surface sterilization. Hence, it is mandatory that endophytes are reinfected, again growing without causing disease symptoms in the same compartment as before. Only if *in planta* growth can be re-established, should an organism be called an endophyte. However, once this is established, endophytes may well exert a positive effect, on metal sequestration. A mutually beneficial symbiosis may be assumed, however, here the correct denomination would be endophyte unless the beneficial traits to both partners have been clearly established (see [88, 89] and citations therein).

4.3 Mycorrhiza

Mycorrhizal interactions are mutually beneficial symbioses of higher plant roots and fungi [90]. In the environment, almost every plant undergoes mycorrhizal interaction with one or more fungal partners [91]. The plants profit from nutrient and water supplied through the fungus, which enhances plant growth and resistance against diseases [92]. In return, the plant supplies the fungus with glucose, sucrose, and other carbohydrates [90].

The two most common types of mycorrhiza are ecto- and endomycorrhiza, differentiated for lack or occurrence of root cell invasion by the fungus, respectively [91–92]. In both mycorrhiza types, the main fungal cell wall components chitin, cellulose derivatives, and melanin are able to bind and sequester heavy metals [94].

Ectomycorrhiza is ubiquitous in almost 10 % of plant families, especially ligneous plants, which form this root symbiosis with thousands of fungal species within over 200 genera [93, 95]. From the soil mycelium, which can transport nutrients towards the root from several hundred meters distance, ectomycorrhizal fungi form an outer mantle of hyphae covering the short root tips and develop to grow between the root rhizodermis cells, the Hartig' net [91]. Mainly, the benefit for phytoremediation is prevention of heavy metal toxicity [94]. For instance, accumulation of heavy metals has been found in cell wall layers, extramatrical hyphae, and the fungal mantle [94, 96, 97]. In pot experiments with copper and lead contaminated soil, *Betula pendula* has been shown to be protected from heavy metal stress due to colonization with ectomycorrhizal fungi. Although the mycorrhization rate decreased with higher heavy metal concentrations, the content of extracted copper and lead in *B. pendula* leaves was higher as compared to non-inoculated plants. Specifically young seedlings are found to profit from protection against metal stress [98]. A combined inoculation with ectomycorrhizal fungi and *Bacillus cereus* strains showed enhanced plant growth promotion for *Salix viminales* in contaminated soils and enhanced metallothionein production in the plant. Thus, a dual inoculation may be feasible for phytoextraction and phytostabilization [99].

Arbuscular mycorrhiza, the main type of endomycorrha, has been extensively investigated for phytoremediation [6, 90, 92, 94, 100]. Here, Glomeromycota fungi penetrate the root cortical cells [90]. The fungi are obligate biotrophs, not able to grow in the absence of green hosts for more than a few days, due to their inability to absorb carbohydrates [92, 100]. Different species, mostly of the genus *Glomus*, have been isolated from heavy metal contaminated soils. In plants inoculated with these isolates, heavy metals were found to be either more highly concentrated in plants, or were reduced due to mycorrhization [94]. Hence, there seems to be specific plant–fungal associations that need to be carefully combined and tested before field trials are performed, in order to establish a successful promotion of either phytoextraction or phytostabilization.

5 Metal Exclusion from Plant Uptake

The physiological properties of soil microbes not only allow for enhanced plant growth. It has been shown that specifically Gram-positive bacteria dominate at metalliferous sites [101]. One specific example featuring a field trial is the remediation effort at a former uranium mining site in Germany. Because field trials are still rare, these are of specific importance (Ebena and Kothe, [102]).

Different metal-resistance mechanisms of these bacteria may be useful for different remediation actions [103]. One useful property is that microbial biomass may, just like plant roots, immobilize metals in soil [104]. Bacterial and fungal cell walls have been investigated for metal sequestration from the water phase (e.g., [105]). Actively growing cells in soil are preferable over dead biomass, often used in (laboratory experiment) reports. The living microbes, in this case, need to be resistant against the prevailing metals in concentrations observed in the soil that is to be remediated (e.g., [106]).

In addition to the chemical properties of microbial cell walls, biomineralization (see, e.g., [107]) has been reported with heavy metal resistant soil bacteria, specifically streptomycetes. This group of soil bacteria has proven to be able to combine different mechanisms for heavy metal resistance [108], among them induction of metallothioneins and metallohistins [62]. Making use of different properties of metal-resistant soil bacteria thus may provide new approaches to phytoremediation. A thorough understanding of molecular mechanisms would aid such experimental approaches [3].

6 Metal Translocation into Plant Biomass

The chelation of metals in the root cells is followed by xylem loading and translocation into the shoot which involves two main processes: (1) movement from root symplast into xylem apoplast, and (2) enhanced volume flux through the xylem. The transport from root endodermis into the root xylem is achieved by membrane transporter proteins. The process of xylem loading with metals is energized by a negative membrane potential generated by proton pumping ATP-ases [109, 110]. In the xylem, metals are chelated by organic acids (e.g., histidine, citrate, and malate), nicotianamine, thiol-rich peptides (e.g., glutathione, phytochelatins), or cysteine-rich metallothioneins [16, 111]. This complexation prevents metal immobilization in the xylem and enables movement into the shoot. Unfortunately, for most metals, it is still unclear which transporter proteins are involved in their export to the root xylem and to which chelators they are bound during transfer to above-ground parts.

Epidermal and subepidermal tissues, including leaf trichomes, are sites of metal sequestration in plant tissues. Leaf epidermal cells are preferred compartments, because they allow for removal with leaf litter [112, 113]. Metal-tolerant plants are

able to control and change the solution concentration of free metal ions in their cellular compartments and thus are able to survive at highly contaminated sites.

7 Potentials for Phytomining

The obvious technique for phytomining is to use hyperaccumulator plants for removal of metal ions from the growth substrate. Worldwide, about 450 plant species in different taxa, ranging from annual herbs to perennials have been identified as hyperaccumulators. Approximately two-thirds of these species are known to hyperaccumulate Ni [1]. Only 30 plant species are known to accumulate Cd, Co, Cu, or Zn in large amounts, and there are no known Pb hyperaccumulators yet [114, 115]. Hyperaccumulators show an exceptionally high metal tolerance, efficient root-to-shoot translocation, and high uptake rates of metals to achieve this remarkable accumulation of toxic soil metals. Their hypertolerance to certain metals may result from vacuolar compartmentalization and metal chelation [26]. Boyd et al. [116] have demonstrated that high concentrations of Ni in leaves of the hyperaccumulator plant *Thlaspi montanum* var. *montanum* can protect plants against herbivores and pathogens [116, 117].

The use of hyperaccumulators to remove heavy metals from contaminated soil was first suggested by (Chaney [118]) and 10 years later by McGrath et al. [119]. The concept of phytomining involves the recovery of marketable amounts of metals from incineration ashes. The first studies on Ni phytomining were carried out by Nicks and Chambers in 1994, by using the Californian hyperaccumulator *Streptanthus polygaloides* to extract Ni from serpentine soils. The Ni concentration in this soil was about 0.35 %, well below the economic concentration for direct mining [120, 121]. The second field trials in phytomining for nickel were carried out in Tuscany, Italy, by Robinson et al. [122] using the Ni hyperaccumulator *Alyssum bertolonii*. They could show that *A. bertolonii* can be used to phytomine Ni commercially, and that the use of fertilizers can increase Ni content in plants [122]. The third recorded phytomining field trial for Ni used the high-biomass Ni hyperaccumulator *Berkheya coddii*, an asteraceous perennial plant that can grow to a height of about 2 m. Under controlled field conditions, a dry biomass of 22 t/ha could be obtained after moderate fertilization within one growth period, the highest reported yield [122].

Suitable plants for phytomining should have the characteristics of: (1) high biomass production, (2) easy to grow from seeds, (3) perennial, (4) hardy and adapted to local climatic conditions, and (5) resistance to herbivores and pathogens [123]. Several strategies might be useful to make phytomining a viable technique for the recovery of metals from contaminated plant biomass. These include the use of high-biomass hyperaccumulators with a high metal content, or the use of fertilizers to increase plant biomass and metal yields, where a high metal yield is to

be preferred over high biomass. The use of microbially aided phytoaccumulation has not been explored this far.

Other strategies discussed include amendment with chelating agents, such as EDTA/EGTA, or bioengineering of hyperaccumulators to increase biomass [124]. Phytomining with high-biomass hyperaccumulators would offer the possibility of exploiting ores or metalliferrous soils that are uneconomic for conventional mining techniques. The extracted metals are essentially free of sulfur; their smelting requires less energy than sulfidic ores [34] and does not contribute to acid rain. They often contain more than one metal and have a lower density than conventional ores, and thus require comparatively small space for storage [34]. This green and emerging technology could provide an alternative to open-cast mining of low-grade ores, but its commercialization depends on the metal content of the harvested biomass and the world price of the target metal. At the same time, the economic feasibility of phytomining is limited by its low efficiency with respect to land use and time. Research is required to overcome these potential limitations to make phytomining a successful commercial technique in recovering metals from contaminated soil by plants [12, 125]. These approaches might be even further stimulated by considering endophytic bacteria and fungi (for reviews see [126–128]). However, thus far this route to enhance the phytomining potential of hyperaccumulating plants has not been pursued.

8 Case Study

For a proof-of-principle, a case study is included here. This is within the former uranium mining area in Eastern Thuringia and Western Saxony, Germany, where mining during German Democratic Republic times produced 210,000 tons of uranium for the USSR weapon industry. The mining operations were stopped in 1990 with the reunification of Germany, and remediation of the vast area was started [129]. The size of the mining-related contamination made a multiple-step approach necessary. The mine was closed, the shafts and galleys sealed to prevent easy flow of mine water to the surface, and flooded. The heaps were removed into the former open-pit mine in a structured way by putting in the most acid-generating, sulfidic material at the lowest point and the most neutralizing at the top. The flooding of the mine re-establishes anaerobic conditions preventing further oxidation of the material and thus limiting the future production of acid mine drainage. The former heap sites were recontoured using allochthonous material. In only a few cases heaps were retained and covered. Tailings were stabilized and prepared for dry cover. Finally, acid mine drainage influenced waters have been removed to water treatment plants. All in all, the size of the operation was tremendous and the sum of €6.5 billion was needed to come to this technical solution, performed by the WISMUT GmbH [129]. However, this huge effort still leaves environmental problems unsolved, as could be expected, given the size of the operation.

Fig. 2 Test field site at the time of adding different soil amendments before establishing the site in 2004, and in 2010 after seven planting seasons

One of these remaining areas with problematic environmental influences is the former leaching heap Gessen near Ronneburg, Thuringia, Germany. Here, low-grade ore had been leached resulting in significant problems with acid mine drainage waters influencing the heap base at points where an initial loam base had leaked. At the time of removing the heap material, the base material was removed to a depth of approximately 3–6 m and replaced by a cover of 40–100 cm of new material [130–132]. The area was sown with a mixture of grasses and clover. However, the acidic and heavy metal rich water by capillary rise led to metal contamination of the surface substrate, and plant growth has been limited in this area., The Friedrich Schiller University in Jena established a test field site in this area in 2004 (Fig. 2) where the feasibility of phytoremediation is tested [133]. The setting clearly covers the above-mentioned preconditions for phytoremediation, namely a spatially heterogeneous, comparatively low contamination of a vast area, where geotechnical and engineering solutions are not (or not further) feasible [134].

Several lysimeters were installed to monitor input into groundwater, and sunflowers were sown for five years, inoculated with soil bacteria isolated from the site, and mycorrhizal fungi. In addition, it was tested whether a soil amendment could enhance plant growth ([135, 136]; see also Fig. 2). Thus, 5 cm of topsoil or 5 cm of compost were plowed in with the upper 30 cm of substrate. It was not meant as a cover in which plants could grow, but rather a moderate addition of nutrients to the nutrient-deprived substrate. At the same time, an inoculation was achieved with the compost. This was supposed to be important because we had seen only limited numbers of bacteria in the deprived soil material at the site. Indeed, 9 years after the addition of the amendments, an effect is still observed, even if the added nutrients have already been consumed a long time ago, and the mixed material is acidic and now also metal contaminated [104]. However, establishing a microbial community able to survive the harsh conditions led to soil formation and the beginning processes of pedogenesis enhance plant growth (Fig. 2). Hence, the initial concept of first increasing soil microbiology to see a secondary positive effect on plant performance has proven to be successful.

Had the initial purpose been to neutralize the soil by addition of calcareous material, the prevailing soil microbiology would have been even further diminished. This has been known for a long time and has been seen, for example, after forest soil neutralization as an acid rain counter-measure. Indeed, the loss of trees, at least initially, is strongly enhanced, mainly because the ectomycorrhizal fungi stabilizing this ecosystem are adapted to lower pH and cannot survive the sudden pH increase. The loss of their symbionts is even more detrimental to the trees as compared to the slow decrease resulting from acid rain (see, e.g., [137]).

A similar situation adhering to the same principle of destroying soil microbiology would have been observed had we chosen to add fertilizer to our plots. In this case, the plants would likely not have responded as strongly to the microbial community. Although fertilization is a short-term effect, enhancing microbial activity leads to a longer lasting improvement of soil functions associated with soil microbiology and hence seems preferable, even if associated with a lower initial biomass production. This effect was tested at the field site using different plants and monitoring soil respiration throughout all different planting regimes (Fig. 3). Indeed, soil respiration was influenced by a change of planting regime, as has also been observed with agricultural soils.

The application of microbes, certainly, depends on the sustainability of the added microbes within the autochthonous community. Thus, isolation of indigenous strains, cultivation, and re-application seem advisable, rather than providing a one-for-all “cure strain”. This concept has been tested at the test field site and indeed, a sustainable effect could be observed [138].

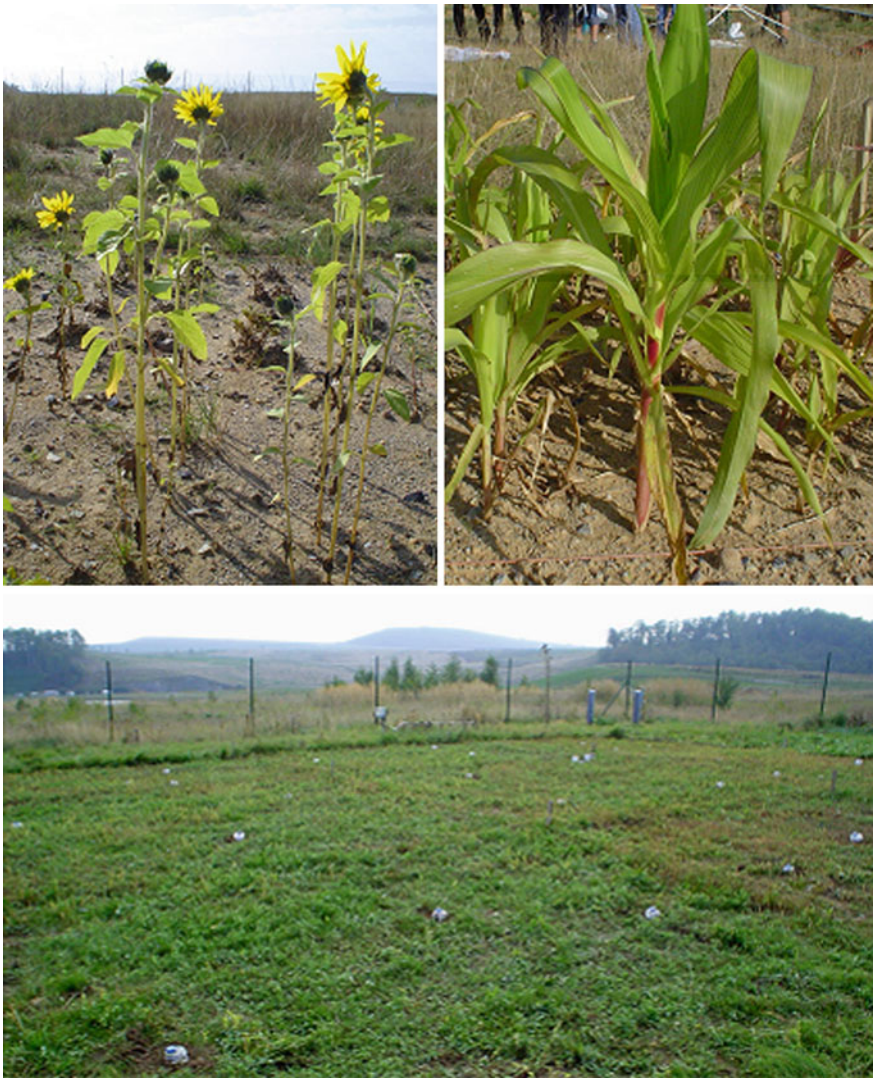

Fig. 3 Planting regimes at the test field site resulted in limited biomass production of sunflower (*Helianthus annuus*) and corn (*Zea mays*). Soil respiration (*lower image*) was obtained in situ

9 Conclusions

The increased metal mining and industrial use of metals lead to both local high contamination and vast areas of heterogeneous, low to medium metal contamination. Whereas for local, highly contaminated areas, geotechnology approaches provide good solutions, the remediation of large landscapes with lower, but still detrimental metal content in the soil may well be prone to undergo biotechnological

remediation. Here, we discussed the potential of phytoremediation assisted by microbial processes for such use.

The results of our review show that microbes may support different tasks in bioremediation applications, from phytostabilization to phytomining. Phytostabilization does not promise to remove the contaminant, but instead provides a solution for establishing a ground cover with the help of soil microbes. The microbes may immobilize metals such that neither uptake into food chains nor excess plant toxicity occurs. Thus, the beneficial effects of a ground cover, with enhanced evapotranspiration and protection from wind and water erosion can be provided. Such a revegetation on usually nutrient-deprived soils strongly benefits from the plant growth promotion of either rhizobacteria or mycorrhizal fungal associations with plant roots. At the same time, sequestration by soil microbes or biomineralization limits contamination of groundwater needed for the drinking water supply in many places.

Another application of phytostabilization is the use of contaminated land for farming, not for production of food or human consumption crops, but for production of bioenergy plants. This allows for sustainable energy production without direct competition with agriculture for food crops. However, to be used in bioethanol or bioenergy production, metal loads of harvested plant biomass needs to be below legislation thresholds.

Soil bacteria, endophytes, and mycorrhizal fungi may, on the other hand, also help in tipping the balance in favor of phytoextraction. The mobilizing activities of PGPB or soil fungi may be especially helpful in achieving high metal uptake into plant-harvestable biomass. Here, specifically the excretion of chelators and acidification potential of physiologically active soil microbes may be useful for geobiotechnology. Even in phytomining, these activities are worth considering. Here, the metal sequestration within plant tissue apoplast at bacterial or fungal cell walls and the compartmentalization, for example, in fungal vacuoles, might further increase both metal tolerance and metal accumulation of plants.

All in all, microbially assisted phytoremediation is only beginning to be explored and field trials are especially urgently needed to evaluate the feasibility and stability of geobiotechnological approaches in metal bioremediation.

Acknowledgments The authors would like to acknowledge financial support by BMBF, DFG-Gk1257, JSMC and MikroInter.

References

1. McGrath SP, Zhao FJ, Lombi E (2001) Plant and rhizosphere processes involved in phytoremediation of metal-contaminated soils. Plant Soil 232:207–214
2. Memon AR, Schröder P (2009) Implications of metal accumulation mechanisms to phytoremediation. Env Sci Pollut Res 16:162–175
3. Haferburg G, Kothe E (2010) Metallomics: lessons for metalliferous soil remediation. Appl Microbiol Biotechnol 87:1271–1280

4. Hassan Z, Aarts MGM (2011) Opportunities and feasibilities for biotechnological improvement of Zn, Cd or Ni tolerance and accumulation in plants. Env Exp Bot 72:53–63
5. Vamerali T, Bandiera M, Mosca G (2010) Field crops for phytoremediation of metal-contaminated land. A review. Env Chem Lett 8:1–17
6. Khan AG (2005) Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation. J Trace Elem Med Biol 18:355–364
7. Sheoran V, Sheoran AS, Poonia P (2011) Role of hyperaccumulators in phytoextraction of metals from contaminated mining sites: A review. Crit Rev Env Sci Technol 41:168–214
8. Gardea-Torresdey JL, Peralta-Videa JR, de la Rosa G, Parsons JG (2005) Phytoremediation of heavy metals and study of the metal coordination by X-ray absorption spectroscopy. Coord Chem Rev 249:1797–1810
9. Nies DH (1999) Microbial heavy-metal resistance. Appl Microbiol Biotechnol 51:730–750
10. Bruins MR, Kapil S, Oehme FW (2000) Microbial resistance to metals in the environment. Ecotox Env Safety 45:198–207
11. Schmidt A, Haferburg G, Sineriz M, Merten D, Büchel G, Kothe E (2005) Heavy metal resistance mechanisms in actinobacteria for survival in AMD contaminated soils. Chem Erde Geochem 65:131–144
12. Rascio N, Navari-Izzo F (2011) Heavy metal hyperaccumulating plants: how and why do they do it? and what makes them so interesting? Plant Sci 180:169–181
13. Salt DE, Smith RD, Raskin I (1998) Phytoremediation. Ann Rev Plant Physiol Plant Mol Biol 49:643–668
14. Kordel W, Dassenakis M, Lintelmann J, Padberg S (1997) The importance of natural organic material for environmental processes in waters and soils. Pure Appl Chem 69:1571–1600
15. Yanez L, Ortiz D, Calderon J, Batres L, Carrizales L, Mejia J, Martinez L, Garcia-Nieto E, Diaz-Barriga F (2002) Overview of human health and chemical mixtures: problems facing developing countries. Env Health Perspect 110:901–909
16. Salt DE, Blaylock M, Kumar N, Dushenkov V, Ensley BD, Chet I, Raskin I (1995) Phytoremediation—a novel strategy for the removal of toxic metals from the environment using plants. Bio-Technol 13:468–474
17. Mulligan CN, Yong RN, Gibbs BF (2001) Remediation technologies for metal-contaminated soils and groundwater: an evaluation. Eng Geol 60:193–207
18. Arthur EL, Rice PJ, Anderson TA, Baladi SM, Henderson KLD, Coats JR (2005) Phytoremediation—an overview. Critical Rev Plant Sci 24:109–122
19. Padmavathiamma PK, Li LY (2007) Phytoremediation technology: hyper-accumulation metals in plants. Water Air Soil Pollut 184:105–126
20. Mulligan CN, Yong RN, Gibbs BF (1999) On the use of biosurfactants for the removal of heavy metals from oil-contaminated soil. Env Progress 18:50–54
21. Cunningham SD, Berti WR, Huang JW (1995) Phytoremediation of contaminated soils. Trends Biotechnol 13:393–397
22. Cunningham SD, Shann JR, Crowley DE, Anderson TA (1997) Phytoremediation of contaminated water and soil. Am Chem Soc 664:2–17
23. Raskin I, Salt D, Krämer U, Schulman R (1998) Phytoremediation: green and clean. Acta Horticult (ISHS) 457:329–332
24. Raskin I, Kumar PBAN, Dushenkov S, Salt DE (1994) Bioconcentration of heavy metals by plants. Curr Opin Biotechnol 5:285–290
25. Cunningham SD, Anderson TA, Schwab AP, Hsu FC (1996) Phytoremediation of soils contaminated with organic pollutants. Adv Agron 56:55–114
26. Chaney RL, Malik M, Li YM, Brown SL, Brewer EP, Angle JS, Baker AJM (1997) Phytoremediation of soil metals. Curr Opin Biotechnol 8:279–284
27. Dushenkov S, Kapulnik Y, Blaylock M, Sorochisky B, Raskin I, Ensley B (1997) Phytoremediation: a novel approach to an old problem. Studies Env Sci 66:563–572
28. Dushenkov S, Vasudev D, Kapulnik Y, Gleba D, Fleisher D, Ting KC, Ensley B (1997) Removal of uranium from water using terrestrial plants. Env Sci Technol 31:3468–3474

29. Kumar PBAN, Dushenkov V, Motto H, Raskin I (1995) Phytoextraction: the use of plants to remove heavy metals from soils. Env Sci Technol 29:1232–1238
30. Burken JG, Schnoor JL (1997) Uptake and metabolism of atrazine by poplar trees. Env Sci Technol 31:1399–1406
31. Baker AJM, McGrath SP, Sidoli CMD, Reeves RD (1994) The possibility of in situ heavy metal decontamination of polluted soils using crops of metal-accumulating plants. Resour Conserv Recycl 11:41–49
32. Vangronsveld J, Colpaert JV, van Tichelen KK (1996) Reclamation of a bare industrial area contaminated by non-ferrous metals: physico-chemical and biological evaluation of the durability of soil treatment and revegetation. Env Pollut 94:131–140
33. Pulford ID, Watson C (2003) Phytoremediation of heavy metal-contaminated land by trees—a review. Env Int 29:529–540
34. Anderson CWN, Brooks RR, Chiarucci A, LaCoste CJ, Leblanc M, Robinson BH, Simcock R, Stewart RB (1999) Phytomining for nickel, thallium and gold. J Geochem Expl 67:407–415
35. Boyd V (1996) Pint-sized plants pack a punch in fight against heavy metals. Env Prot 7:38–39
36. Pilon-Smits E (2005) Phytoremediation. Ann Rev Plant Biol 56:15–39
37. Huang JWW, Chen JJ, Berti WR, Cunningham SD (1997) Phytoremediation of lead-contaminated soils: role of synthetic chelates in lead phytoextraction. Env Sci Technol 31:800–805
38. Ernst WHO (2005) Phytoextraction of mine wastes—options and impossibilities. Chem Erde Geochem 65:29–42
39. Garbisu C, Alkorta I (2001) Phytoextraction: a cost-effective plant-based technology for the removal of metals from the environment. Bioresource Technol 77:229–236
40. Boyd RS, Jaffre T, Odom JW (1999) Variation in nickel content in the nickel-hyperaccumulating shrub *Psychotria douarrei* (Rubiaceae) from New Caledonia. Biotropica 31:403–410
41. van Nevel L, Mertens J, Oorts K, Verheyen K (2007) Phytoextraction of metals from soils: how far from practice? Env Pollut 150:34–40
42. Mendez MO, Maier RM (2008) Phytostabilization of mine tailings in arid and semiarid environments—an emerging remediation technology. Env Health Perspect 116:278–283
43. Kucharski R, Sas-Nowosielska A, Malkowski E, Japenga J, Kuperberg JM, Pogrzeba M, Krzyzak J (2005) The use of indigenous plant species and calcium phosphate for the stabilization of highly metal-polluted sites in southern Poland. Plant Soil 273:291–305
44. Yoon J, Cao XD, Zhou QX, Ma LQ (2006) Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. Sci Total Env 368:456–464
45. Geebelen W, Adriano DC, van der Lelie D, Mench M, Carleer R, Clijsters H, Vangronsveld J (2003) Selected bioavailability assays to test the efficacy of amendment-induced immobilization of lead in soils. Plant Soil 249:217–228
46. Vangronsveld J, Herzig R, Weyens N, Boulet J, Adriaensen K, Ruttens A, Thewys T, Vassilev A, Meers E, Nehnevajova E, van der Lelie D, Mench M (2009) Phytoremediation of contaminated soils and groundwater: lessons from the field. Env Sci Pollut Res 16:765–794
47. Pichtel JR, Dick WA, Sutton P (1994) Comparison of amendments and management practices for long-term reclamation of abandoned mine lands. J Env Qual 23:766–772
48. Dushenkov S, Kumar PBAN, Motto H, Raskin I (1995) Rhizofiltration: The use of plants to remove heavy metals from aqueous streams. Env Sci Technol 29:1239–1245
49. Macaskie LE (1991) The application of biotechnology to the treatment of wastes produced from the nuclear fuel cycle: Biodegradation and bioaccumulation as a means of treating radionuclide-containing streams. Crit Rev Biotechnol 11:41–112
50. Leyval C, Turnau K, Haselwandter K (1997) Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. Mycorrhiza 7:139–153

51. Kaschl A, Romheld V, Chen Y (2002) Cadmium binding by fractions of dissolved organic matter and humic substances from municipal solid waste compost. J Env Qual 31:1885–1892
52. Raskin I, Smith RD, Salt DE (1997) Phytoremediation of metals: using plants to remove pollutants from the environment. Curr Opin Biotechnol 8:221–226
53. Welch RM, Norvell WA, Schäfer SC, Shaff JE, Kochian LV (1993) Induction of iron(III) and copper(II) reduction in pea (*Pisum sativum* L.) roots by Fe and Cu status—does the root-cell plasmalemma Fe(III)-chelate reductase perform a general role in regulating cation uptake. Planta 190:555–561
54. Lagier T, Feuillade G, Matejka G (2000) Interactions between copper and organic macromolecules: determination of conditional complexation constants. Agronomie 20:537–546
55. Halim M, Conte P, Piccolo A (2003) Potential availability of heavy metals to phytoextraction from contaminated soils induced by exogenous humic substances. Chemosphere 52:265–275
56. Zhou J-M, Dang Z, Chen N-C, Xu S-G, Xie Z-Y (2007) Enhanced phytoextraction of heavy metal contaminated soil by chelating agents and auxin indole-3-acetic acid. Huan Jing Ke Xue 28:2085–2088
57. Abedin MJ, Feldmann J, Meharg AA (2002) Uptake kinetics of arsenic species in rice plants. Plant Physiol 128:1120–1128
58. Lasat MM (2002) Phytoextraction of toxic metals: a review of biological mechanisms. J Env Qual 31:109–120
59. Kagi JH, Kojima Y (1979) Nomenclature of metallothionein: a proposal. Experientia Suppl 34:141–142
60. Lane B, Kajioka R, Kennedy T (1987) The wheat-germ Ec protein is a zinc-containing metallothionein. Biochem Cell Biol-Biochim Biol Cellulaire 65:1001–1005
61. Murphy A, Zhou JM, Goldsbrough PB, Taiz L (1997) Purification and immunological identification of metallothioneins 1 and 2 from *Arabidopsis thaliana*. Plant Physiol 113:1293–1301
62. Schmidt A, Hagen M, Schütze E, Schmidt A, Kothe E (2010) *In silico* prediction of potential metallothioneins and metallohistins in actinobacteria. J Basic Microbiol 50:562–569
63. Rauser WE (1995) Phytochelatins and related peptides (structure, biosynthesis, and function). Plant Physiol 109:1141–1149
64. Grill E, Löffler S, Winnacker E-L, Zenk MH (1989) Phytochelatins, the heavy-metal-binding peptides of plants, are synthesized from glutathione by a specific γ-glutamylcysteine dipeptidyl transpeptidase (phytochelatin synthase). Proc Nat Acad Sci U S A 86:6838–6842
65. Thumann J, Grill E, Winnacker E-L, Zenk MH (1991) Reactivation of metal-requiring apoenzymes by phytochelatin-metal complexes. FEBS Lett 284:66–69
66. Yang X-E, Jin X-F, Feng Y, Islam E (2005) Molecular mechanisms and genetic basis of heavy metal tolerance/hyperaccumulation in plants. J Integr Plant Biol 47:1025–1035
67. Gray EJ, Smith DL (2005) Intracellular and extracellular PGPR: commonalities and distinctions in the plant-bacterium signaling processes. Soil Biol Biochem 37:395–412
68. Dimkpa CO, Merten D, Svatos A, Büchel G, Kothe E (2009) Siderophores mediate reduced and increased uptake of cadmium by *Streptomyces tendae* F4 and sunflower (*Helianthus annuus*), respectively. J Appl Microbiol 107:1687–1696
69. Ma Y, Prasad MN, Rajkumar M, Freitas H (2011) Plant growth promoting rhizobacteria and endophytes accelerate phytoremediation of metalliferous soils. Biotechnol Adv 29:248–258
70. Beneduzi A, Ambrosini A, Passaglia LMP (2012) Plant growth-promoting rhizobacteria (PGPR): Their potential as antagonists and biocontrol agents. Gen Mol Biol 35:1044–1051
71. Kloepper JW, Schroth MN (1979) Plant-growth promoting rhizobacteria—evidence that the mode of action involves root microflora interactions. Phytopathology 69:1020–1024
72. Teale WD, Paponov IA, Palme K (2006) Auxin in action: signalling, transport and the control of plant growth and development. Nat Rev Mol Cell Biol 7:847–859
73. Neilands JB (1984) Methodology of siderophores. Struct Bonding 58:1–24

74. Kloepper JW, Leong J, Teintze M, Schroth MN (1980) Enhanced plant-growth by siderophores produced by plant growth-promoting rhizobacteria. Nature 286:885–886
75. Schalk IJ, Hannauer M, Braud A (2011) New roles for bacterial siderophores in metal transport and tolerance. Environ Microbiol 13:2844–2854
76. Dimkpa C, Svatos A, Merten D, Büchel G, Kothe E (2008) Hydroxamate siderophores produced by *Streptomyces acidiscabies* E13 bind nickel and promote growth in cowpea (*Vigna unguiculata* L.) under nickel stress. Can J Microbiol 54:163–172
77. Dimkpa CO, Svatos A, Dabrowska P, Schmidt A, Boland W, Kothe E (2008) Involvement of siderophores in the reduction of metal-induced inhibition of auxin synthesis in *Streptomyces spp*. Chemosphere 74:19–25
78. Dimkpa C, Weinand T, Asch F (2009) Plant-rhizobacteria interactions alleviate abiotic stress conditions. Plant Cell Env 32:1682–1694
79. Doty SL (2008) Enhancing phytoremediation through the use of transgenics and endophytes. New Phytol 179:318–333
80. Rajkumar M, Ae N, Freitas H (2009) Endophytic bacteria and their potential to enhance heavy metal phytoextraction. Chemosphere 77:153–160
81. Misaghi IJ, Donndelinger CR (1990) Endophytic bacteria in symptom-free cotton plants. Phytopathology 80:808–811
82. Lodewyckx C, Vangronsveld J, Porteous F, Moore ERB, Taghavi S, Mezgeay M, van der Lelie D (2002) Endophytic bacteria and their potential applications. Crit Rev Plant Sci 21:583–606
83. Barac T, Taghavi S, Borremans B, Provoost A, Oeyen L, Colpaert JV, Vangronsveld J, van der Lelie D (2004) Engineered endophytic bacteria improve phytoremediation of water-soluble, volatile, organic pollutants. Nature Biotechnol 22:583–588
84. Germaine KJ, Keogh E, Ryan D, Dowling DN (2009) Bacterial endophyte-mediated naphthalene phytoprotection and phytoremediation. FEMS Microbiol Lett 296:226–234
85. Germaine KJ, Liu X, Cabellos GG, Hogan JP, Ryan D, Dowling DN (2006) Bacterial endophyte-enhanced phytoremediation of the organochlorine herbicide 2,4-dichlorophenoxyacetic acid. FEMS Microbiol Ecol 57:302–310
86. Weyens N, CroesS Dupae J, Newman L, van der Lelie D, Carleer R, Vangronsveld J (2010) Endophytic bacteria improve phytoremediation of Ni and TCE co-contamination. Env Pollut 158:2422–2427
87. Selosse MA, Baudoin E, Vandenkoornhuyse P (2004) Symbiotic microorganisms, a key for ecological success and protection of plants. Crit Rev Biol 327:639–648
88. Kothe E, Dudeja SS (2011) Editorial. J Basic Microbiol 51:4
89. Dudeja SS, Giri R, Saini R, Suneja-Madan P, Kothe E (2012) Interaction of endophytic microbes with legumes. J Basic Microbiol 52:248–260
90. Harrison MJ (2005) Signaling in the arbuscular mycorrhizal symbiosis. Ann Rev Microbiol 59:19–42
91. Smith SE, Read DJ (1997) Mycorrhizal symbiosis. Academic Press, San Diego
92. Bonfante P, Genre A (2010) Mechanisms underlying beneficial plant-fungus interactions in mycorrhizal symbiosis. Nature Comm 1:48
93. Rinaldi AC, Comandini O, Kuyper TW (2008) Ectomycorrhizal fungal diversity: separating the wheat from the chaff. Fungal Div 33:1–45
94. Khan AG, Kuek C, Chaudhry TM, Khoo CS, Hayes WJ (2000) Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. Chemosphere 41:197–207
95. Wang B, Qiu YL (2006) Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza 16:299–363
96. Wilkins DA (1991) The influence of sheathing (ecto-) mycorrhizas of trees on the uptake and toxicity of metals. Agricult Ecosyst Env 35:245–260
97. Turnau K, Kottke I, Dexheimer J, Botton B (1994) Element distribution in mycelium of *Pisolithus arrhizus* treated with cadmium dust. Annals Bot 74:137–142

98. Bojarczuk K, Kieliszewska-Rokicka B (2010) Effect of ectomycorrhiza on Cu and Pb accumulation in leaves and roots of silver birch (*Betula pendula* Roth.) seedlings grown in metal-contaminated soil. Water Air Soil Pollut 207:227–240
99. Hrynkiewicz K, Dabrowska G, Baum C, Niedojadlo K, Leinweber P (2012) Interactive and single effects of ectomycorrhiza formation and *Bacillus cereus* on metallothionein MT1 expression and phytoextraction of Cd and Zn by willows. Water Air Soil Pollut 223:957–968
100. Bonfante P (2010) Plant–fungal interactions in mycorrhizas., Encyclopedia of Life SciencesWiley, London
101. Narula N, Reinicke M, Haferburg G, Kothe E, Behl RK (2012) Plant-microbe interaction in heavy metal-contaminated soils. In: Kothe E, Varma A (eds) Bio-geo interactions in heavy metal-contaminated soils. Springer, Heidelberg, pp 143–162
102. Ebenå G, Kothe E (2006) Bioleaching and microbial mineral degradation. In: Varma A (ed) Microbes: health and environment, vol 3. IK International Publishing, New Delhi, pp 389–405
103. Haferburg G, Kothe E (2012) Biogeosciences in heavy metal-contaminated soils. In: Kothe E, Varma A (eds) Bio-geo interactions in heavy metal-contaminated soils. Springer, Heidelberg, pp 17–34
104. Schindler F, Gube M, Kothe E (2012) Bioremediation and heavy metal uptake: microbial appraoches at field scale. In: Koth E, Varma A (eds) Bio-geo interactions in heavy metal-contaminated soils. Springer, Heidelberg, pp 365–384
105. Merten D, Geletneky J, Bergmann H, Haferburg G, Kothe E, Büchel G (2005) Rare earth element patterns: a tool for remediation of acid mine drainage. Chem Erde Geochem 65S1:97–114
106. Schmidt A, Haferburg G, Schmidt A, Lischke U, Merten D, Gherghel F, Büchel G, Kothe E (2009) Heavy metal resistance to the extreme: *Streptomyces* strains from a former uranium mining area. Chem Erde Geochem 69:35–44
107. Haferburg G, Klöß G, Schmitz W, Kothe E (2008) "Ni-struvite"—a new biomineral formed by a nickel resistant *Streptomyces acidiscabies*. Chemosphere 72:517–523
108. Kothe E, Dimkpa CO, Haferburg G, Schmidt A, Schmidt A, Schütze E (2010) Streptomycete heavy metal resistance: extracellular and intracellular mechanisms. In: Sherameti I, Varma A (eds) Soil heavy metals. Springer, Heidelberg, pp 225–236
109. Pitman M (1972) Uptake and transport of ions in barley seedlings II. Evidence for two active stages in transport to the shoot. Aus J Biol Sci 25:243–258
110. Boer AH, Prins HBA, Zanstra PE (1983) Bi-phasic composition of trans-root electrical potential in roots of *Plantago* species: involvement of spatially separated electrogenic pumps. Planta 157:259–266
111. Stephan UW, Schmidke I, Stephan VW, Scholz G (1996) The nicotianamine molecule is made-to-measure for complexation of metal micronutrients in plants. Biometals 9:84–90
112. Vögeli-Lange R, Wagner GJ (1990) Subcellular localization of cadmium and cadmium-binding peptides in tobacco leaves: implication of a transport function for cadmium-binding peptides. Plant Physiol 92:1086–1093
113. Kupper H, Zhao FJ, McGrath SP (1999) Cellular compartmentation of zinc in leaves of the hyperaccumulator *Thlaspi caerulescens*. Plant Physiol 119:305–311
114. Baker AJM, Brooks RR (1989) Terrestrial higher plants which hyperaccumulate metallic elements—a review of their distribution, ecology and phytochemistry. Biorecovery 1:81–126
115. Prasad MNV (2005) Nickelophilous plants and their significance in phytotechnologies. Braz J Plant Physiol 17:113–128
116. Boyd RS, Shaw JJ, Martens SN (1994) Nickel hyperaccumulation defends *Streptanthus polygaloides* (Brassicaceae) against pathogens. Am J Bot 81:294–300
117. Boyd RS, Martens SN (1994) Nickel hyperaccumulated by *Thlaspi montanum* var. montanum is acutely toxic to an insect herbivore. Oikos 70:21–25

118. Chaney RL (1983) Plant uptake of inorganic waste. In: Parr JE, Marsh PB, Kla JM (eds) Land treatment of hazardous waste. Noyes Data Corp, Park Ridge, pp 50–76
119. McGrath SP, Sidoli CMD, Baker AJM, Reeves RD (1993) The potential for the use of metal-accumulating plants for the in situ decontamination of metal-polluted soils. In: Eijsackers HJP, Hamers T (eds) Integrated soil and sediment research : a basis for proper prediction. Kluwer, Dordrecht, The Netherlands, pp 673–676
120. Nicks L, Chambers MF (1998) A pioneering study of the potential of phytomining for nickel. In: Brooks RR (ed) Plants that hyperaccumulate heavy metals. CAB International, Wallingford, pp 312–325
121. Nicks L, Chambers MF (1995) Farming for metals. Mining Environ Meeting 199:15–18
122. Robinson BH, Brooks RR, Howes AW, Kirkman JH, Gregg PEH (1997) The potential of the high-biomass nickel hyperaccumulator *Berkheya coddii* for phytoremediation and phytomining. J Geochem Expl 60:115–126
123. Brooks RR, Robinson BH, Howes AW, Chiarucci A (2001) An evaluation of *Berkheya coddii* Roessler and *Alyssum bertolonii* Desv. for phytoremediation and phytomining of nickel. S Afr J Sci 97:558–560
124. Brooks RR, Robinson BH (1998) The potential use of hyperaccumulators and other plants for phytomining. In: Brooks RR (ed) Plants that hyperaccumulate heavy metals: their role in phytoremediation, microbiology, archaeology, mineral exploration and phytomining. CAB International, Wallingford, pp 327–356
125. Robinson BH, Banuelos G, Conesa HM, Evangelou MWH, Schulin R (2009) The phytomanagement of trace elements in soil. Critical Rev Plant Sci 28:240–266
126. Newman LA, Reynolds CM (2005) Bacteria and phytoremediation: new uses for endophytic bacteria in plants. Trends Biotechnol 23:6–8
127. Mastretta C, Barac T, Vangronsveld J, Newman L, Taghavi S, van der Lelie D (2006) Endophytic bacteria and their potential application to improve the phytoremediation of contaminated environments. Biotechnol Genet Eng Rev 23:175–207
128. Ryan RP, Germaine K, Franks A, Ryan DJ, Dowling DN (2008) Bacterial endophytes: recent developments and applications. FEMS Microbiol Lett 278:1–9
129. Gatzweiler R, Jakubick AT, Meyer J, Paul M, Schreyer J (2003) Flooding of the WISMUT mines - learning by doing or applying a comprehensive systematic approach ? In: Merkel B, Planer-Friedrich B, Wolkersdorfer C (eds) Uranium in the aquatic environment. Springer, Heildelberg, pp 745–754
130. Grawunder A, Lonschinski M, Merten D, Büchel G (2009) Distribution and bonding of residual contamination in glacial sediments at the former uranium mining leaching heap of Gessen/Thuringia, Germany. Chem Erde - Geochem 69S2:5–19
131. Lonschinski M, Knöller K, Merten D, Büchel G (2011) Flow dynamics of groundwater and soil water in the former heap Gessenhalde at the uranium mining area of Ronneburg: a stable isotope approach. Hydrol Process 25:861–872
132. Senitz S, Gaupp R, Büchel G (2009) Hochaufgelöste dreidimensionale Rekonstruktion eines quartären Talaquifers in einem Teilbereich des Gessentals bei Ronneburg (ehemaliges ostthüringisches Uranerzbergbaurevier) durch die Kombination von Geländedaten und geostatistischer Schätzung. Zeitschr dtsch Ges Geowiss 160:367–389
133. Kothe E, Bergmann H, Büchel G (2005) Molecular mechanisms in bio-geo-interactions: from a case study to general mechanisms. Chem Erde Geochem 65S1:7–27
134. Willscher S, Mirgorodsky D, Jablonski L, Ollivier D, Merten D, Büchel G, Wittig J, Werner P (2013) Field scale phytoremediation experiments on a heavy metal and uranium contaminated site, and further utilization of the plant residues. Hydrometallurgy F:46–53
135. Büchel G, Bergmann H, Ebenå G, Kothe E (2005) Geomicrobiology in remediation of mine waste. Chem Erde 65S1:1–5
136. Mirgorodsky D, Ollivier D, Merten D, Bergmann H, Büchel G, Willscher S, Wittig J, Jablonski L, Werner P (2010) Radiation protection of radioactively contaminated large areas by phytoremediation and subsequent utilization of the contaminated plant residues. J Nucl Power 55:774–778

137. Demchik MC, Sharpe WE (2001) Forest floor plant response to lime and fertilizer before and after partial cutting of a northern red oak stand on an extremely acidic soil in Pennsylvania, USA. Forest Evol Manag 144:239–244
138. Reinicke M, Schindler G, Roth M, Kothe E (2013) Multi-metal bioremediation by microbial assisted phytoremediation. In: Actinobacteria. Application in bioremediation and production of industrial enzymes (Amorosos MJ, Benimeli CS, Cuozzo SA, Eds.). CRC, Boca Raton, USA, pp 87–105.

Adv Biochem Eng Biotechnol (2014) 141: 237–241
DOI: 10.1007/978-3-642-54710-2

Index