

Bioremediation of acidic mine effluents and the role of sulfidogenic biosystems: a mini-review

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Abstract Acid mine drainage (AMD) is recognised as a serious and global environmental problem. The major producer of these toxic effluents is the mining industry. Owing to the severe effects of these effluents, their prevention and treatment have been a primary focus of research over several decades. The problems have invited the attention of a large group of researchers, governmental bodies, educational and research establishments, mining industries, general public and environmental specialists. A preferable option is to prevent the formation and movement of AMD from its source of origin; however, it is not possible in many locations. It, therefore, becomes essential to collect and treat AMD to which a number of treatment techniques are available. Despite the extreme environmental conditions, several communities of autotrophic and heterotrophic bacteria and archaea are seen to flourish that mainly drive the rate of release of sulfur and toxic metals into the environment. The present review briefly discusses the cause and occurrence of AMD and the microbial diversity observed in such ecosystems. In addition, the bioremediation options are briefly presented with a discussion on the role of sulfidogenic biosystems in the bioremediation of the AMD.

Keywords Acid mine drainage · Bioremediation · Environment · Microbial diversity · Sulfidic biosystem

Introduction

Over several decades, mining activities for extraction of gold, copper, nickel, etc., have significantly increased to meet the growing demand of metals. The mining techniques have also improved over the years and lower grade ores are also been exploited. The wastes generated as a result of such activities can principally be categorized as “waste rock” and “mine tailings” and are considered as a potential threat to the environment. Wastes generated from different mines vary in terms of their properties due to the differences in terms of mineralogy and different mining techniques used. As a result, the impact that these wastes have on the environment also seems to vary. Some threats on the environment can be acidic or alkaline drainage, release of metals, radionuclides, dust and suspended solids [27, 28]. Drainage from such mine wastes is a result of weathering (microbiological, chemical or hydrological) and cause the dissolution of heavy metals, toxic substances such as cyanides and acids. Such toxic effluents offer serious concerns to the waterways and the biodiversity [2, 28]. Acidic overburdens increases the severity of the discharge as no neutralization is immediately available once it starts seepage from the ore/rock body. On the other hand, alkaline discharges (a more common feature seen in the underground mines in contrast to the surface mines) have a lower impact on the environment than the acidic ones. However, higher concentrations of ferrous iron in some alkaline drainage contribute to lower the pH of the effluents by oxidation and hydrolysis thereby making them acidic [1].

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The present review briefly discusses the cause and occurrence of acid mine drainage (AMD) and the microbial communities identified in these systems. In addition, a brief discussion is provided on the bioremediation strategies and the role of sulfidogenic biosystems in the treatment of AMD.

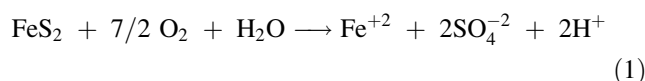
Acid mine drainage: causes and occurrence

Metal-rich acidic effluents are formed by chemical weathering of metal sulfide-bearing rocks, i.e., due to the exposure of the sulfide-bearing material to oxygen and water. These effluents, often referred to as Acid Rock Drainage (ARD), are generally hot because of metal sulfide oxidation which is exothermic in nature. As a result of mining, the metal sulfides are more exposed to air and water which in turn increases the rates of acid generation. At places where these rocks have a lower buffering capacity, extremely acidic and toxic effluents are generated. Such effluents are known as Acid Mine Drainage (AMD) [1, 5]. The sources of AMD and the factors determining the rate of acid generation is summarized in Table 1.

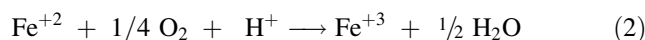
As shown in the table, several physical, chemical and biological factors play an essential role in determining the rate of acid generation. The physical factors such as the permeability of the waste rock dump play an important role. In case where the oxygen input is higher due to better permeability in a dump, the oxidation process is higher with higher temperatures and increased oxygen convection. Microorganisms such as *Acidithiobacillus ferrooxidans* have been well studied for pyrite oxidation; however, it may accelerate the oxidation reactions for the sulfides of Sb, Ga, Mo, As, Cu, Cd, Co, Ni, Pd and Zn [1]. It is important to note that *Acidithiobacillus ferrooxidans*, an iron and sulfur-oxidizing microbe belonging to the γ -proteobacteria group, is the well studied microorganism ([35] and references

therein). *Acidithiobacillus ferrooxidans* along with *Lep-tospirillum ferrooxidans* and *Acidithiobacillus thiooxidans* constitute a remarkably important consortium for metal sulfide oxidation and in particular finds a number of applications for bioleaching of metals, biodesulfurization of coal, etc., [3, 31–33, 36–41]. The chemical activity of biogenic Fe^{+3} also contribute towards acid generation.

It is well known that the predominant and common metal sulfide mineral in most of the rocks is pyrite (FeS_2). Most often these deposits are mined for extraction of metals such as Ag, Au, Cu, Zn and Pd. Such metals are typically seen to be impurities in pyrite. In addition, they also occur in sulfide minerals such as chalcopyrite (CuFeS_2), sphalerite (ZnS) and galena (PbS) which tend to be associated with pyrite [5]. The release of acidic effluents has high concentrations of Fe, Al and Mn, along with lower concentrations of other toxic heavy metals. The first and the most important reaction is the oxidation of the sulfide mineral. Iron is dissolved with the formation of sulfate and protons are generated that contribute to lower the pH (Eq. 1).



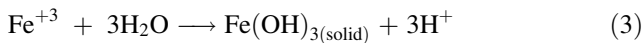
The generation of dissolved ferrous iron from pyrite, sulfate and protons indicates an increase in the total dissolved solids and acidity of the released effluents. The conversion of Fe^{+2} (reference to the generated Fe^{+2} as seen in Eq. 1) is dependent on the oxidizing environment, pH and bacterial activity. The oxidation of ferrous iron is shown in Eq. 2.



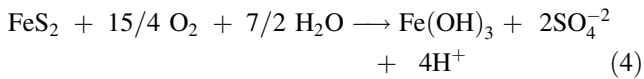
The generated Fe^{+3} (from Eq. 2) remains soluble at $\text{pH} < 2-2.3$; however, at pH values >2.3 up to 3.5, Fe^{+3} tends to precipitate as $\text{Fe}(\text{OH})_3$ along with jarosite formation. The reaction for formation of ferric iron precipitates is shown in Eq. 3.

Table 1 Sources of AMD and the primary factors determining rate of acid generation (Adapted with modifications from [1])

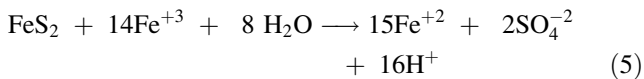
| Sources of AMD | | Factors |
|--|---------------------------------|--|
| Primary | Secondary | |
| Construction rocks (e.g., used in roads, dams, etc.) | Concentrated Load-out | Chemical activation energy |
| Diffuse seepage water from replaced overburdens in rehabilitated areas | Spilled concentrates over roads | Degree of saturation with water |
| Dumped mine rocks | Emergency ponds | Exposed surface area of the metal sulfide |
| Open pit and Underground mine | Stockpiles | pH |
| Pumped or naturally discharged underground water | Fractures in rocks | Temperature |
| Tailings dams | Treatment sludge pounds | Oxygen content in gas phase (if saturation is $< 100\%$) and its concentration in the water phase |
| | | Bacterial activity |
| | | Chemical activity of Fe^{+3} iron |



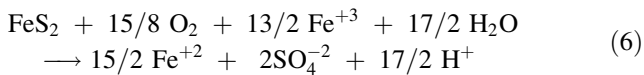
The overall reaction combining the above equations (Eq. 1–3) is shown as Eq. 4 (when Fe^{+3} tend to precipitate).



Ferric iron still remains in solution (i.e., do not precipitate) as shown in Eq. 2, tends to further oxidise the metal sulfide (FeS_2) as per the following equation:



Under such conditions overall reaction for the action of soluble ferric towards further oxidation of additional pyrite (FeS_2) can be combined (Eq. 1–3) and represented as Eq. 6.



In addition to pyrite, other metal sulfides (e.g., pyrrhotite (FeS) and chalcocite (Cu_2S) may also contribute towards AMD. The resulting acidic products as described in the above equations may get washed away by flowing water over the rocks and encounter an acid-consuming mineral which to some extent can contribute to its neutralization. If, in case, there is no flowing water the acidic products gets accumulated and in due course of any event of water movement, may be simultaneously washed. AMD may form in ground waters of the underground deep mines and is of least importance when a mine is in operation [25]. In addition, acidic metal-rich effluents may also generate from discarded heaps or dumped mine tailings. The strength of these effluents in terms of acidity and toxicity may be more than the effluents generated directly from the mine. As a result of the concentrated nature of acid generating minerals (in case of mine tailings), the effluents are seen to be more aggressive [1, 25]. It is also important to consider that the problem of AMD may continue for many years after the mines are closed or even the heaps and tailings dams are not put to use or abandoned. As a result, it has become very essential to prevent or solve the problem of AMD and come to a decision to adopt or use potential remediation techniques.

Prokaryotic microbial communities in AMD

AMD ecosystems constitute a different environment containing AMD solutions, sediments and biofilms which provide several niches for the AMD microbes. A wide range of microorganisms inhabit or populate in these

ecosystems despite of the higher toxicity, acidity and concentrations of metals [13]. In such environments, mineral–microbe interactions are very important since AMD is a widespread environmental issue. A chemo-autotrophical biosphere is formed which is sustained by the electron donors that are derived from sulfide minerals, phosphates released due to water and rock interactions, CO_2 , O_2 and N_2 from atmospheric air. Microbial action increases the rate of acid generation (as discussed in Sect. “[Acid mine drainage: causes and occurrence](#)”) and may be responsible for the immense AMD that is generated [48]. The underlying mechanisms of mineral–microbe interactions or microbial sulfide oxidation are now well understood [45, 47]. Hence, the detailed pathways and the underlying mechanism are not discussed herewith. Many experiments have been performed to understand the role of microbes towards acceleration in pyrite oxidation and also towards understanding the amount contributed by the microbes towards AMD formation [14, 15, 46, 49]. It is reported that about 75% of the AMD produced is a result of microbial activity [14]. Although, AMD is an acidic metal-rich solution, the physico-chemical properties varies with variation in their origin [21]. A world-wide comparison of the physico-chemical properties of AMD along with the microbial population has been discussed by Hallberg [21] and is presented in Table 2.

Microbial diversity in AMD ecosystems

Over several decades, the advances in isolation, cultivation, 16S rRNA gene and molecular-based techniques have improved our understanding of the microbial diversity in relation to AMD ecosystems [5, 13, 24, 26, 30]. Microorganisms belonging to the group *Proteobacteria*, *Nitrospira*, *Actinobacteria*, *Firmicutes*, *Acidobacteria*, *Aquificae* and Candidate division TM7 are among the primary bacterial lineages detected in AMD. Microorganisms such as *Acidithiobacillus ferrooxidans*, *Leptospirillum ferrooxidans*, *Ferrovum* spp., *Acidiphillum*, *Acidocella*, *Acidicaldus*, *Acidomonas*, *Metallibacterium scheffleri*, *Acidithrix ferrooxidans*, *Ferrimicrobium acidiphilum*, *Alicyclobacillus* spp., etc., have been detected in AMD ecosystems [10]. A list of bacterial and archaeal communities that have been detected and reported in AMD ecosystems is shown in Tables 3 and 4, respectively. In addition to bacteria, fungi and algae have also been detected in AMD ecosystems [12]. The most extensively studied microbes are the acidophiles [13]. The biofilms produced by the acidophiles can vary significantly in terms of morphology. This can be attributed to the changes in environment and its chemistry. At early stages of development, thin biofilms are observed floating at the sub-aerial surfaces and they depend on ferrous iron and RISCs oxidation for growth [18, 43]. Acetate

Table 2 AMD from different sites of the world with their physico-chemical characteristics and microbial populations (cells/mL) (Adapted from [21])

| Mine/Type | pH | Eh (mV) | Fe(II), mg/L | Total iron, mg/L | Cu, mg/L | Zn, mg/L | SO ₄ ²⁻ , mg/L | Moderate Fe-oxidisers | Extreme Fe-oxidisers | S oxidisers | Heterotrophic acidophiles |
|----------------------------|---------|---------|-------------------------|-------------------------|----------|----------|--------------------------------------|-----------------------|----------------------|------------------|---------------------------|
| Ynysawred, Wales/coal | 6.2 | +257 | 140 | 160 | ND | ND | 464 | <10 ² | <10 ² | <10 ² | <10 ² |
| Wheal Jane, England/tin | 3.4 | +462 | 250 | 290 | 1 | 132 | 400 | 3 × 10 ⁴ | 1 × 10 ³ | <10 ² | 3 × 10 ² |
| King's mine, Norway/copper | 2.7 | ND | ND | 172 | 16 | 25 | 668 | 1 × 10 ³ | 6 × 10 ⁴ | <50 | 2 × 10 ⁴ |
| Parys mine, Wales/copper | 2.5 | +285 | 650 | 650 | 40 | 60 | 1550 | 1 × 10 ³ | 3 × 10 ³ | <10 ² | 2 × 10 ³ |
| Cantarreras, Spain/copper | 2.7 | +425 | 915 | 1130 | 160 | 24 | 1190 | <10 ² | 1 × 10 ⁵ | <10 ² | <10 ² |
| Richmond mine, USA/copper | 0.5–1.0 | ND | 13–19 × 10 ³ | 13–19 × 10 ³ | 120–650 | 700–2600 | 20–10 × 10 ³ | ND | ND | ND | ND |

ND not determined

productions have been reported as a result of mixed fermentation in biofilms that serves as an electron donor for the heterotrophic sulfate-reducing bacteria (SRB) and Fe reducers. Such an aspect affects the growth of iron and sulfur oxidisers by inhibition of the sulfur and iron oxidation process [16].

Microorganisms inhabiting such extreme environmental conditions are naturally adapted to the toxic effects of higher concentrations of heavy metals and also possess the unique capability to reduce them to less toxic forms.

Bioremediation options

As discussed in Sect. 2.0, the sulfide minerals; water or a humid atmosphere; oxidant such as oxygen from the atmosphere or chemical sources acts as primary ingredients for acid generation. Majorly, the bacterial activity accelerates the rate of generation of acid which can be controlled by the inhibition of their activity. Some of the measures to prevent or minimize the generation of AMD have been briefly discussed by Johnson and Hallberg [25] which are as follows:

- Flooding and sealing of abandoned deep mines;
- Underwater storage of mine tailings;
- Land-based storage (in closed waste heaps);
- Blending of acid-generating and acid-consuming materials;
- Entire solidification of mine tailings;
- Applications of anionic surfactants such as sodium dodecyl sulfate (SDS);
- Microencapsulation technique.

The above-mentioned approaches, although tested, face practical difficulties towards inhibiting the generation of AMD and hence the alternative is to reduce the adverse impact that the polluting water has on the environment. Such an alternative, involves a “migration control” approach which is categorized into two broad processes namely “active” and “passive” systems [25]. A summary of the remediation strategies for treatment of acidic mine effluents is shown in Fig. 1.

An active system generally refers to the continuous application of alkaline materials in order to neutralize the acidic mine effluents and precipitates the metals. On the other hand, the passive system refers to the use of natural and constructed wetland ecosystems and has the advantage of low maintenance than the active system. Initially, the passive systems may be expensive or impractical to setup and may require a certain amount of maintenance costs. Broadly, the remediation approaches can be divided as (1) abiotic and (2) biological strategies which can be further subdivided as “active” and “passive” technologies based

Table 3 Representative bacteria detected in AMD ecosystems with their metabolic characteristics (Source: [10])

| Bacteria (arranged alphabetically) | Iron | | Sulfur | | Nitrogen | | Carbon |
|---|-----------|-----------|-----------|-----------|----------|-----------|----------|
| | Oxidation | Reduction | Oxidation | Reduction | Fixation | Reduction | Fixation |
| <i>Acidithiobacillus caldus</i> DSM 22753 | EC | EC | Y | EC | EC | Y | Y |
| <i>Acidithiobacillus caldus</i> ATCC 51756 | EC | EC | Y | EC | EC | Y | Y |
| <i>Acidithiobacillus ferrivorans</i> DSM 22755 | Y | EC | Y | EC | EC | EC | Y |
| <i>Acidithiobacillus ferrooxidans</i> ATCC 23270 | Y | Y | Y | EC | Y | EC | Y |
| <i>Acidithiobacillus ferrooxidans</i> ATCC 53993 | Y | Y | Y | EC | Y | EC | Y |
| <i>Acidithiobacillus thiooxidans</i> DSM 17318 | EC | EC | Y | EC | EC | Y | Y |
| <i>Acidithiobacillus ferriphilus</i> DSM 100412 | Y | Y | Y | EC | EC | EC | Y |
| <i>Acidithiobacillus ferridurans</i> ATCC 33020 | Y | Y | Y | EC | EC | EC | Y |
| <i>Acidithrix ferrooxidans</i> DSM 28176 | Y | Y | EC | EC | EC | EC | EC |
| <i>Acidibacter ferrireducens</i> DSM 28176 | EC | Y | EC | EC | EC | EC | EC |
| <i>Acidiphilium cryptum</i> JF-5 | EC | Y | Y | EC | EC | Y | EC |
| <i>Acidiphilium angustum</i> ATCC 35903 | EC | Y | Y | EC | EC | Y | EC |
| <i>Acidiphilium multivoram</i> AIU301 | EC | Y | Y | EC | EC | Y | EC |
| “ <i>Ferroplasma myxofaciens</i> ” P3G | Y | EC | EC | EC | Y | EC | Y |
| “ <i>Ferroplasma</i> ” sp. FKB7 | G | EC | EC | EC | EC | EC | G |
| <i>Thiomonas delicata</i> DSM 17897 | EC | EC | Y | EC | EC | Y | Y |
| <i>Desulfomonile tiedjei</i> DSM 6799 | EC | EC | EC | Y | EC | EC | Y |
| <i>Desulfovibrio longus</i> DSM 6739 | EC | EC | EC | Y | EC | EC | Y |
| <i>Leptospirillum ferriphilum</i> DSM 17947 | Y | EC | EC | EC | EC | EC | Y |
| “ <i>Leptospirillum ferrodiazotrophum</i> ” | Y | EC | EC | EC | Y | EC | Y |
| <i>Leptospirillum ferrooxidans</i> ATCC 29047 | Y | EC | EC | EC | Y | EC | Y |
| <i>Leptospirillum</i> group IV UBA BS | G | EC | EC | EC | G | EC | G |
| “ <i>Leptospirillum rubarum</i> ” | G | EC | EC | EC | EC | EC | G |
| <i>Alicyclobacillus disulfodooxidans</i> ATCC 51911 | EC | EC | Y | EC | EC | EC | Y |
| <i>Sulfobacillus acidophilus</i> DSM 10332 | Y | EC | Y | EC | EC | EC | Y |
| <i>Sulfobacillus thermosulfidooxidans</i> DSM 9293 | Y | EC | Y | EC | EC | EC | Y |
| <i>Desulfosporosinus</i> sp. aSRB1 (FK) | EC | EC | EC | G | G | G | EC |
| <i>Desulfosporosinus</i> sp. aSRB2 (FK) | EC | EC | EC | G | G | G | EC |
| <i>Acidimicrobium ferrooxidans</i> DSM 10331 | Y | EC | EC | EC | EC | EC | EC |
| <i>Ferrimicrobium acidiphilum</i> DSM 19497 | Y | EC | EC | EC | EC | EC | EC |
| <i>Acidobacterium capsulatum</i> ATCC 51196 | EC | Y | EC | EC | EC | EC | EC |
| <i>Hydrogenobaculum acidophilum</i> DSM 11251 | EC | EC | Y | EC | EC | EC | G |
| <i>Metallibacterium scheffleri</i> DSM 24874 | EC | Y | EC | EC | EC | EC | EC |

The metabolic information is based on the data obtained through cultivation dependent approaches and/or predicted through omics approaches Y yes, G having genes but experiment not confirmed, EC experiments confirmed with features, N/A no information available

on the nature of the treatment (Fig. 1). The abiotic approach uses both the technologies, i.e., the “active systems” involving aeration and lime addition and “passive systems” involving anoxic lime stone drains. The details of these processes have been discussed by Johnson and Hallberg [25]. Similarly, the biological treatment approaches have also been classified as “active systems” and “passive systems”. The passive bioremediation strategies that are adopted are summarized in Table 5. On the other hand, the active biological system uses offline sulfidogenic

bioreactors and the brief details are discussed in Sect. “[Bioremediation options](#)”.

Sulfidogenic bioreactors: active biosystems in treatment of AMD

Sulfate reduction using sulfate-reducing bacteria (SRB) has now been successfully applied to remove metals and sulfate from AMD. There has been a demand for the use of

Table 4 Representative Archaea detected in AMD ecosystems with their metabolic characteristics (Source: [10])

| Archaea (arranged alphabetically) | Iron | | Sulfur | | Nitrogen | | Carbon |
|---|-----------|-----------|-----------|-----------|----------|-----------|----------|
| | Oxidation | Reduction | Oxidation | Reduction | Fixation | Reduction | Fixation |
| <i>Acidanus brierleyi</i> DSM 1651 | Y | EC | Y | EC | EC | EC | Y |
| <i>Acidiplasma aeolicum</i> DSM 18409 | Y | Y | EC | EC | EC | EC | EC |
| <i>Acidiplasma cupricumulans</i> DSM 16551 | Y | Y | EC | EC | EC | EC | Y |
| “ <i>Ferroplasma acidarmanus</i> ” fer1 | Y | Y | EC | EC | EC | EC | EC |
| “ <i>Ferroplasma</i> sp. Type II” | G | G | EC | EC | EC | EC | EC |
| <i>Ferroplasma acidphilum</i> | Y | Y | EC | EC | EC | EC | EC |
| <i>Metalosphaera sedula</i> DSM 535 | Y | EC | Y | EC | EC | EC | Y |
| <i>Picrophilus torridus</i> DSM 9790 | EC | EC | EC | EC | EC | EC | EC |
| <i>Picrophilus oshimae</i> DSM 9489 | EC | EC | EC | EC | EC | EC | EC |
| <i>Sulfolobus acidocaldarius</i> DSM 639 | EC | Y | Y | EC | EC | EC | EC |
| <i>Sulfolobus solfataricus</i> P2 | EC | EC | EC | EC | EC | EC | G |
| <i>Sulfolobus tokodaii</i> JCM 10545 | Y | EC | Y | EC | EC | EC | G |
| <i>Stygiolobus azoricus</i> DSM 6296 | EC | EC | EC | Y | EC | EC | EC |
| <i>Thermoplasma acidophilum</i> DSM 1728 | EC | EC | EC | Y | EC | EC | EC |
| <i>Thermoplasma volcanium</i> GSS1 | EC | EC | EC | Y | EC | EC | EC |
| <i>Thermogymnomonas acidicola</i> JCM 13583 | EC | EC | EC | EC | EC | EC | EC |
| “ <i>Thermoplasmatales archaeon</i> A-plasma” | NIA | NIA | NIA | NIA | NIA | NIA | NIA |
| “ <i>Thermoplasmatales archaeon</i> C-plasma” | NIA | NIA | NIA | NIA | NIA | NIA | NIA |
| “ <i>Thermoplasmatales archaeon</i> D-plasma” | NIA | NIA | NIA | NIA | NIA | NIA | NIA |
| “ <i>Thermoplasmatales archaeon</i> E-plasma” | NIA | NIA | NIA | NIA | NIA | NIA | NIA |
| “ <i>Thermoplasmatales archaeon</i> I-plasma” | NIA | NIA | NIA | NIA | NIA | NIA | NIA |
| <i>Cuniculiplasma divulgatum</i> JCM 30642 | NIA | NIA | NIA | NIA | NIA | NIA | NIA |
| Candidatus Micrarchaeum acidiphilum ARMAN-2 | NIA | NIA | NIA | NIA | NIA | NIA | NIA |
| Candidatus Parvarchaeum acidiphilum ARMAN-4 | NIA | NIA | NIA | NIA | NIA | NIA | NIA |
| Candidatus Parvarchaeum acidiphilum ARMAN-5 | NIA | NIA | NIA | NIA | NIA | NIA | NIA |

The metabolic information is based on the data obtained through cultivation dependent approaches and/or predicted through omics approaches
 Y yes, G having genes but experiment not confirmed, EC experiments confirmed with features, NIA no information available

Fig. 1 Strategies for remediation of acidic mine waters (Source: [25])

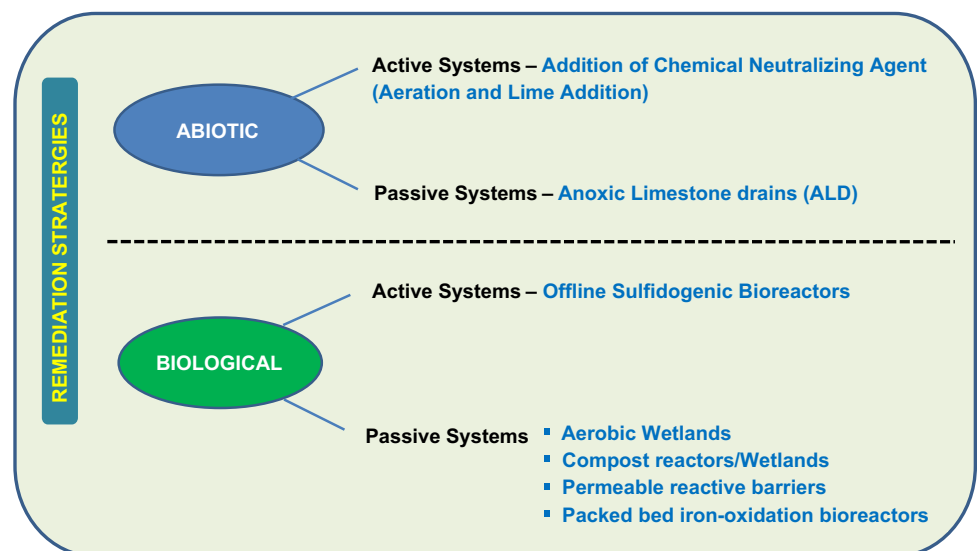
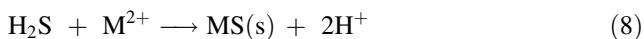
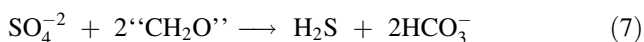


Table 5 Passive bioremediation strategies used to treat AMD (Source: [25])

| Bioremediation strategy | Treatment |
|--|---|
| Aerobic wetlands | Mine waters that are net alkaline |
| Anaerobic wetlands/compost bioreactors | Acidic mine water that are metal rich. E.g., AMD from abandoned mines |
| Composite aerobic and anaerobic wetlands | Full-scale treatment of AMD |
| Permeable Reactive barriers (PRBs) | Wide range of polluted ground waters. PRBs installed to remediate AMD operate on the same principle as compost bioreactors |
| Iron-oxidizing bioreactors | AMD treatment in packed bed bioreactors |

sulfate-reducing bacteria (SRB) in environmental remediation programs and interest in their use is also increasing. Castro et al. [9] indicated that SRB represent a diverse group of prokaryotes and are classified under four different taxonomic groups namely (1) gram-negative mesophilic SRB, (2) gram-positive spore forming SRB, (3) bacterial thermophilic SRB and (4) archaeal thermophilic SRB. The details of these microorganisms with their important features have also been discussed. Recently, Rabus et al. [42] have extensively discussed on the types of sulfate-reducing bacteria and archaea along with their physiology, biochemistry, molecular biology and genetic aspects. In general, SRB are heterotrophic bacteria and require organic material as carbon, (e.g., organic acids and alcohols) to serve as electron donors for sulfate reduction. Hydrogen may also substitute as an electron donor and its use has also been reported to be advantageous [25]. The sulfidogens reduce sulfate (terminal electron acceptor) to hydrogen sulfide (Eq. 7) that form metal sulfide precipitates (Eq. 8) under anaerobic conditions [22].



Over the years, several types of bioreactors with different reactor designs have been used to study sulfate reduction and removal of heavy metals in passive and active systems. Examples include the anaerobic contact process, anaerobic filter, hybrid reactors, continuous stirred tank reactors (CSTRs), upflow anaerobic sludge blanket reactor (UASB), off-line sulfidogenic bioreactors, fixed bed reactors (FBR) and permeable reactive barriers (PRBs) [1, 6, 11, 17, 19, 20, 29, 34, 44–50]. Sulfidogenic bioreactors are engineered active biosystems with advantages over passive biological remediation in terms of more predictable performance and control, selectivity of heavy metals and lowered sulfate concentrations in processed

waters [4, 7]. However, construction and operational costs are factors to be considered in such systems [23, 25]. Off-line sulfidogenic reactors are made to optimize the production of hydrogen sulfide. The sulfidogens in these bioreactors are rather sensitive to modest acidity and hence these systems are engineered in order to protect these microbes from direct exposure to AMD.

“Biosulfide” and “Thiopaq” processes have been described as two technologies using offline sulfidogenic bioreactors ([25] and references therein). In case of the biosulfide system, two independent components, i.e., a biological and a chemical unit operate. In the biological circuit, hydrogen sulfide is generated and the raw AMD is allowed to come in contact with the hydrogen sulfide in the chemical circuit. Sulfate source is provided to mixed consortia of SRB in the biological circuit by some of the processed AMD that enters the circuit. Additional alkali (that generated beyond the action of SRB) is required for the systems optimum performance; hence, in such events it is added chemically. On the other hand, the Thiopaq process uses two different microbial populations and processes that makes it different from the Biosulfide process. In this process, firstly SRB converts sulfate to sulfide followed by precipitation of metal sulfides and secondly, sulfur-oxidizing bacteria (SOB) is used to convert excess hydrogen sulfide to elemental sulfur. The use of sulfidogenic systems in AMD bioremediation is increasing and novel sulfidogenic bioreactor systems are also been proposed [51].

Future outlook and concluding remarks

The problems of discharge of acidic mine effluents have invited the attention of researchers to find out novel approaches for its treatment. The environmental impact of AMD can be reduced primarily by preventing acid generation. Since, pyrite has been identified as the main cause of water pollution generated from abandoned mines; management activities particularly prioritize reducing the toxic impact of these effluents on the environment. Particular care has to be taken to avoid rain water contact with pyrite. A secondary control measure can be preventing the migration of the effluents while collection and treatment are the tertiary control measures [1]. However, the choice of remediation depends upon a number of factors. Cost of a remediation system, environmental factors and the sustainability of the remediation system are important issues to be considered. Lime addition (chemical processing) has been a treatment method where huge volumes of discharged acidic mine waters have been treated. Due to the high recurrent costs of lime addition and sludge disposal, mining industries are getting more attracted towards biological treatment systems [25]. With the application of

advanced molecular biology tools and engineered biological systems, it has now become easy to understand numerous interactions and processes of large scale ecosystems [8]. Development of advanced molecular approaches has shed more light on the microbial diversity and their functions in AMD ecosystems. These, in turn, have significantly contributed towards providing more clues for biotechnological applications of these microorganisms and in the future many novel approaches for treatment of AMD are expected to emerge.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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