



Anaerobic Bioreactor Technology (ABT) for the Treatment of Acid Mine Drainage (AMD)

Karumanchi Bhavya, Sameena Begum and Anupoju Gangagni Rao

Abstract

Acid mine drainage (AMD) is considered as a widely spread environmental problem that affects several countries involved in mining activities. Because of its high acidity as well as high metal(loid)s content generating environmental and health toxicity, AMD poses a threat to the surrounding ecosystems. Generally, when exposed to air and water, sulfide minerals undergo oxidative dissolution, which results in formation of AMD. Treatment of AMD at source is regarded to be an effective option; however, this might not be possible at all the sites. Technologies for treating AMD can be governed through the application of various physical, chemical, and biological processes to defuse acidity and remove metal(loid)s from the liquid streams. However, the physicochemical techniques are intended to achieve process viability and cost-effective when the treatment stream is of high volume and sulfate rich. In contrast to this, biological processes are economical to run and do not require a high concentrations of sulfate in the targeted stream. The present chapter critically reviews the state-of-the-art on available aerobic and anaerobic bioreactor technologies with an emphasis on anaerobic bioreactors for the treatment of AMD. In the remediation of AMD, the anaerobic process is a type of biological remediation that relies on neutralizing acidity and precipitating the metal contaminants by natural microbial consortia preferably the sulfate-reducing bacteria (SRB). However, as

the AMD is associated with low organic matter, a supply of source of an external factor carbon that is required to complete the remediation process. Anaerobic bioreactors, such as membrane bioreactors, continuous stirred tank reactors, bioelectrochemical systems, up-flow sludge blanket reactors, are suitable bioreactor processes for the treatment of AMD wherein the syntrophic activity of both SRBs and other fermentative and few methane forming bacteria takes place. These anaerobic reactors through the application of SRBs are paving its path in the treatment of AMD because of its efficacy and cost-effectiveness. However, adding of external organic substances are required during the treatment of AMD with SRB which could play a pivotal role in determining the cost of the technology. This chapter describes briefly about the aerobic reactors and detailed information on the different types of anaerobic bioreactors available that can be made suitable for AMD treatment. Comparing the passive and active SRB-based alternatives, their substrate choice, and the recent advances in the anaerobic treatment of AMD along with future perspectives as an alternative to conventional techniques are discussed.

Keywords

AMD · Anaerobic digestion · Mining · Sulfide · SRB · Toxic metal

K. Bhavya · S. Begum · A. Gangagni Rao (✉)
Bioengineering and Environmental Sciences (BEES) Group,
Department of Energy and Environmental Engineering (DEEE),
CSIR-Indian Institute of Chemical Technology, Hyderabad
500007, India
e-mail: agrao@iict.res.in; gangagnirao@gmail.com

K. Bhavya · A. Gangagni Rao
Academy of Scientific and Innovative Research (AcSIR),
Ghaziabad 201002, India

1 Acid Mine Drainage (AMD) and Its Sources of Generation

The commercial exploitation of naturally occurring mineral resources via mining activities has tremendously increased in the past few years because of the escalating need for metals and their allied products. The mining sector contributes

significantly to improve the global economy claims the Mining, Minerals, and Sustainable Development Project (MMSD). Around 3500 active mining sites across the world have been reported and the majority of them present waste rock dumps and tailing dams (Tayebi-Khorami et al., 2019). As per the recent estimates, the production of mineral and metal supplies has resulted in 100 billion tons of solid waste generation globally (Tayebi-Khorami et al., 2019).

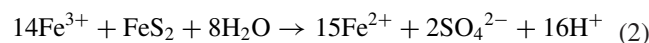
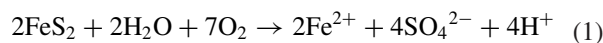
AMD involves the discharge of acidic water and metal conjugates in and around the mining areas (Roy Chowdhury et al., 2015). The seepage and mixing of uncontrolled release of the dissolved metals containing high concentrations of sulfate and acids from abandoned mines and tailing piles into the nearby water bodies and pollutes them. Adverse impacts have been witnessed due to its low pH and high sulfate and metal(loid)s concentration in AMD that are toxic to the aquatic flora and fauna in many of the mining sites across the world (Gontia & Janssen, 2016). Further, long-term exposure of reactive sulfide minerals like the pyrite and pyrrhotite ores to oxygen and water in the lack of adequate neutralizing minerals, results in the weathering of mines and contributes greatly to the formation of AMD (Neculita et al., 2007; Tsukamoto et al., 2004). Acid mine drainage is thus a metal-rich, acidic wastewater, and other toxic substances like sulfuric acid and dissolved iron, generated from a mining site. The process of AMD formation during the mining activities and subsequent natural weathering is shown in Fig. 1. Considering the negative impacts of AMD to the environment, the pollution control bodies have brought up policies for the treatment and storage of mine wastes before releasing into the surroundings.

Additionally, the formation of AMD is prominent in both active as well as abandoned mines in addition to open pit sites. The damage caused in open pits and active sites cannot be evident while they are in fully operational condition, however its long-term operation results in AMD formation. As the water table rises during the constant pumping limits, the atmospheric air enters the mass of the rock leading

to the oxidation of metals and other inorganic salts forming iron sulfate salts which can readily suspend in ground water, thus contaminate both the water as well as the ground. The dissolution of sulfate and iron salts in the water often results in the formation of free sulfuric acid thereby making the water more acidic and scarcely rich in organic materials. Due to the formation of a strong acid in the aqueous stream, the pH of the water drops to lower limits to as low as 2, which is extremely dangerous to any living being (Jong & Parry, 2003; Verburg et al., 2009). Due to its acidity, metal toxicity, sedimentation, and other unfavorable characteristics, AMD once developed can have a significant adverse effect on the environment in the vicinity. Thus, before being released into the environment, AMD must be collected and treated to remove metal ions and ensure that its pH is neutral (Neculita et al., 2007; Roy Chowdhury et al., 2015).

1.1 Characteristics of AMD: Sulfide Minerals and pH Profile

Generating metal ions and sulfate rich waste streams are not just limited to mineral and mining activities, many manufacturing processes such as scrubbing of flue gases, processing of paper and pulp, chemical manufacturing streams release effluents similar to that of AMD (Dhir, 2018; Gontia & Janssen, 2016; Rambabu et al., 2020). Sulfide minerals available in AMD are depicted in Fig. 2a. Sulfide mineral rocks such as marcasite, pyrite, and chalcopyrite weather to form AMD, when they come in contact with O₂ from air and H₂O from rain which is an aqueous geochemical process as shown in equation Eq. (1). The main redox reaction in the AMD formation is shown in equation Eq. (2). Among many sulfide minerals, pyrite (FeS₂) and marcasite (FeS₂) are the most common and, abundantly available in nature (Verburg et al., 2009). Under ideal conditions, neutrophilic and acidophilic sulfur-oxidizing bacteria can speed up this oxidation cycle resulting in the release of metal ions, sulfate ions, and sulfuric acid (Demersa et al., 2015; Kadnikov et al., 2019). The ensuing acidic water vigorously dissolves aluminum oxides, and carbonates of different minerals, contributing to pH buffering process leading to instant dissolution of Al, Ca, and with other substances in AMD (Ighalo et al., 2022; Kim et al., 2002).



AMD can be broadly classified into 3 categories based on its pH, it may be classified as saline drainage, acid mine drainage, or neutral mine drainage as shown in Fig. 2b. The typical drainage characteristics of acidic mine drainage is

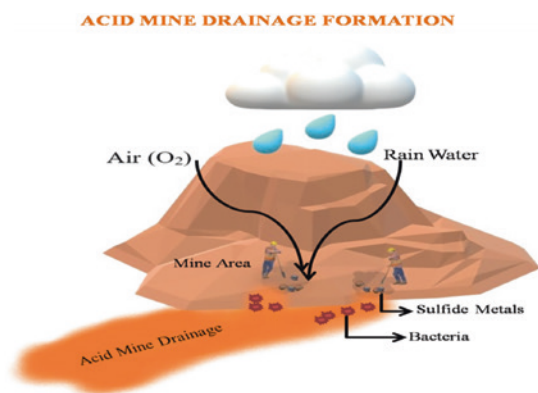
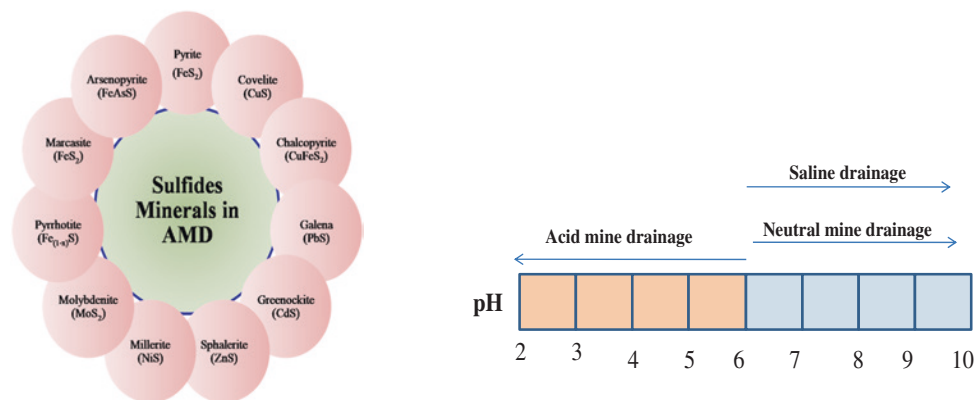


Fig. 1 Acid mine drainage formation due to natural weathering

Fig. 2 The typical mineral characteristics of AMD and its classification based on pH



(a) Sulfide Minerals in AMD

(b) Types of Mine drainages produced by sulfide oxidation in relation with pH (modified from GARD Guide, 2009; <http://www.gardguide.com/>).

having an acidic pH ranging between 2 and 6 with moderate to elevated metal(loid)s and sulfate concentrations. On the other end, the neutral mine drainage has a near neutral to alkaline pH with low to medium concentration of metals, sulfate concentration. The third category of mine drainage is the saline drainage which has a neutral to alkaline pH with low metals and may have moderate levels of sulfate, iron, manganese, and calcium (Nordstrom et al., 2015).

1.2 Microbial Community in AMD

Despite the extreme acidity, toxicity, and high metal concentration, the AMD ecosystem does not limit the microbial diversity (Chen et al., 2016; Mendez-Garcia et al., 2015). Mineral–microbe interactions are critical in AMD ecosystems, as AMD is a prevalent environmental problem. Microbial activity accelerates acid production and may be accountable for the massive amount of AMD produced (Baker & Banfield, 2003).

The underlying mechanisms of microbial sulfide oxidation and the role of microbes in the amount of AMD formation are now well known (Edwards et al., 2000; Panda et al., 2016; Sheoran et al., 2010). Microorganisms are thought to be responsible for around 75% of the total AMD generated (Edwards et al., 2000). Advances in isolation, culturing, 16S rRNA gene sequencing, and molecular methods have enhanced our understanding of microbial diversity in connection to AMD ecosystems over several decades (Kuang et al., 2012). Microorganisms belonging to the phyla *Proteobacteria*, *Nitrospira*, *Actinobacteria*, *Firmicutes*, and *Acidobacteria*, *Aquificae*, and *Candidate division TM7*, to mention a few, are among the major bacterial lineages found in AMD. *Acidithiobacillus ferrooxidans*,

Leptospirillum ferrooxidans, *Ferrovum spp.*, *Acidiphillum*, *Acidocella*, *Acidicaldus*, *Acidomonas*, *Metallibacterium scheffleri*, *Acidithrix ferrooxidans*, *Ferrimicrobium acidiphilum*, *Alicyclobacillus spp.*, and other microorganisms have been found in AMD environments (Das et al., 2009). Microorganisms in AMD that live in such harsh environments are naturally evolved to greater potentially toxic metal concentration as well as having the unique capacity to decrease them to less toxic chemical forms.

1.3 Effects of AMD on the Environment

AMD has a vast array of dissolved minerals and metals due to the low pH which promotes the growth of acidophilic bacteria, which have been known of producing acidic waters as a catalyst from sulfide minerals (Gao et al., 2019; Sánchez Espana et al., 2005) by generating sulfuric acid (H_2SO_4) as it is a strong acid that dissociates into H^+ ions and SO_4^{2-} . The high concentration of metals in AMD easily makes their way into the surrounding soils, contaminating them (Gao et al., 2019). Enzymes and microorganisms are hampered by toxicity in the soil, resulting in a decrease in the biochemical characteristics and quality of the soil (Auld et al., 2013; Ferreira et al., 2021). AMD has the ability to contaminate groundwater, rendering it unsafe for use in agriculture, as well as for other applications and human intake. AMD runoff may seriously impact aquatic life; contaminate water sources and lower pH levels (Ighalo et al., 2022; Kaur et al., 2018). AMD also effects biodiversity, disturbs ecosystems, corrodes infrastructure, and water supplying systems are contaminated often in water scarce areas (Jong & Parry, 2003; Rambabu et al., 2020).

Because of the toxicity of AMD may severely affect the ecosystem, in recent decades, there has been an increase in the demand for efficient remediation methods for AMD and its afflicted environment (Gupta & Sar, 2020). AMD that has not been treated and is released into the environment has been shown to have a number of adverse effects on living things (Amanda & Moersidik, 2019; Ighalo et al., 2022). Rhizosphere functions also get inhibited when there is surge in absorbing metal ions like Al^{3+} when bound to cell membrane (Skousen et al., 2017). Plant root dysfunction and soil acidification is triggered by bacterial and fungal activity inside soils. Therefore, movement and absorption of AMD is affected by many factors like soil condition, presence of metallic ions, capacity of dissolved ions solubility, and related micro flora (Skousen et al., 2019). Increased suspended particles, potentially toxic metals mobilization, lower pH in water bodies, and groundwater pollution are all effects of AMD, as are potentially toxic metals penetration into the food chain and absorption by plants and animals, as well as the deterioration of water resources quality (Silva et al., 2013). Human and animal cells can be affected by toxic metals in water, which lowers the ability of cells to survive (Acharya & Kharel, 2020; Dutta et al., 2020). Acidic drainage has different effects depending on the location, past use of land, climate, the size of mining, geochemistry of excess material, and the composition of mine water. Accumulation of potentially toxic metals in soils and water bodies leads to increase in toxic amount of bio-concentration and bio-accumulation in flora, fauna, and humans through food chain and food web. Environmental risks from AMD are “second only to global warming and ozone depletion (Acharya & Kharel, 2020; Moodley et al., 2018)” according to the US Environmental Protection Agency (EPA).

2 Remediation Strategies

Different methods have been developed for the treatment of AMD through many years of research and broadly categorized into two types namely control at source and mitigation

techniques (Kaksonen & Sahinkaya, 2012; Rambabu et al., 2020). In source control, the basis of the working principle is on preventing the seepage of oxygenated water by removing the O_2 and H_2O to cease the process of oxidation by sulfide minerals; however, these source control strategies are effective and demanding. Retrofitting is not always achievable as most mines ceased operations before the AMD hazards have been discovered (Ma et al., 2001). Mitigation control approach is based on reducing acidity by neutralizing pH of acid mine water and favoring metal precipitates formation (Yilmaz et al., 2019). They are categorized as active and passive systems (Garcia et al., 2001; Muyzer & Stams, 2008) (Table 1). The above-mentioned two treatment techniques are effective in reducing acidity and lowering the concentration of potentially toxic metals (Johnson & Hallberg, 2005; Tsukamoto et al., 2004). However, the advancement in research have modified and re-classified treatment methods into abiotic and biotic methods which are additionally divided into active and passive treatment systems. Biological treatment methods provide many benefits that include the removal of sulfate ions and potentially toxic metals permanently from mine waters, while generating less hazardous water plus the recovery of valuable metals. The aim of these treatment technologies is to decrease the pollutants to permissible limit or to create conditions where they show near neutral or minimal impact on environment which is achieved through biological activity (Kaksonen & Puhakka, 2007; Mendez-Garcia et al., 2015). Sulfate-reducing bacteria (SRB) are mainly used in these biological systems. SRB's are considered as working agents and are therefore, further used in wetlands ecosystems or used as substrate barrier to neutralize pH and reduce metal leachate concentration.

Collection and treatment of waters contaminated by AMD is the major step in mitigating and control strategies used in AMD treatment. This approach mainly comprises of collecting all the mine wastes generated by AMD to be treated. The treatment process can be achieved by chemical or biological approach, by bringing the pH to neutral and eliminating metal precipitates and suspended solids. A conventional strategy for treating AMD comprises using alkali

Table 1 Treatment strategies for remediation of AMD

AMD treatment methods			
Active systems		Passive systems	
Abiotic	Biological (Biotic)	Abiotic	Biological (Biotic)
<ul style="list-style-type: none"> • Addition of lime for pH neutralization • Aeration for iron oxidation 	<ul style="list-style-type: none"> • Sulfidogenic bioreactors or anaerobic reactors 	<ul style="list-style-type: none"> • Anoxic limestone channels • Open lime stone channels 	<ul style="list-style-type: none"> • Aerobic wetlands • Anaerobic wetlands • Permeable reactive barriers • Algal bioremediation

to decrease acidity, and neutralizing the pH of the water, and precipitate metals like hydroxides and carbonates. Other different treatment methods include ion exchange process, reverse osmosis and electro dialysis, but they are barely selected because of their high operational and maintenance costs. Biological treatment, which is also known as bioremediation involves SRB to treat waters polluted by AMD. SRB are proficient to generate biogenic H_2S . This is then further used to respond with potentially toxic metals, which results in metal sulfide precipitation (Jamil et al., 2013a, 2013b).

Chemical treatment methods are rapidly being replaced by biological treatment approaches for reducing sulfate. Both active and passive treatment technologies are efficient in treating ground and surface waters contaminated by AMD. But due to high operational costs and intensive manpower requirement for maintaining active treatment technologies, passive methods like constructed wetlands, anaerobic sulfate-reducing bioreactors, anoxic limestone channels, open limestone channels, limestone leach filter beds, and slag drain beds (Roy Chowdhury et al., 2015) are widely opted worldwide over active treatments.

2.1 Active Abiotic Technologies

Active abiotic treatment that involves adding a chemical-neutralizing agent to acidic effluents is the most common technique for treating acidic effluents (Coulton et al., 2003).

AMD's pH will increase if an alkaline substance is added to it.

AMD's pH will increase if an alkaline substance is added to it; in solution many metals will precipitate as hydroxides and carbonates, speeding up the rate of chemical oxidation of ferrous iron, which requires high levels of aeration or the addition of an oxidizing chemical like hydrogen peroxide. This results in a Fe-rich sludge that may also have other metals dissolved in it, such as lime and slaked lime, based on the chemistry of the processed mine water. This approach makes use of a variety of neutralizing substances, including lime (calcium oxide), calcium carbonate, sodium carbonate, sodium hydroxide, and magnesium oxide (Dhir, 2018).

The cost and effectiveness of these resources varies. When calcium-containing neutralizing chemicals are employed, sulfate may be partly removed as gypsum. Although active chemical treatment for AMD can be successful, it does come with certain drawbacks like regular maintenance needs for mechanical systems, use of high quantity chemical reagents, man power required for continuous operation and bulk sludge disposal problems (Dhir, 2018).

2.2 Passive Systems: Biotic and Abiotic

Passive biological treatment systems use natural geochemical processes and microbial activity to enhance the influent water condition, by neutralizing the acidity and reducing the potentially toxic elements loads from mine drainage. Although local abiotic variables like dissolved oxygen concentration and water quality may change over the application time potentially affecting rate of (bio)chemical reactions; these systems require minimum management and maintenance (Gazea & Kontopoulos, 1996; Kaksonen & Puhakka, 2007). pH, temperature, salinity, metal concentrations, and other factors all have a part in determining the efficacy of various AMD treatment methods (Ali et al., 2019a, 2019b).

Chemical, physical, and biological techniques are used to treat AMD in these passive treatment systems. The pH is maintained, sulfate and metal concentrations are reduced, and salinity is controlled using this treatment (Tsukamoto et al., 2004). In passive treatment system, microbes play a crucial role. These treatment methods are best for treating low-acidity mine streams. The benefit of a passive system is that it has a high rate of metal removal while requiring less maintenance and consuming less energy compared to active treatment systems (Neculita et al., 2007). The disadvantage is that it requires a lot of foot print area which may be limited in some cases. The following are some examples of passive bioremediation systems: Limestone ponds, Open limestone channels, Anoxic limestone drains (ALD), Aerobic wetlands, Anaerobic wetlands/compost reactors, Permeable reactive barriers (PRB), and Packed bed iron-oxidation bioreactors.

2.2.1 Injection of an Organic Substrate

Infusing rich organic material into mine shafts or boreholes that reach the depths of AMD sites is one of the techniques for in situ remediation. These organic substrates, which supply energy to SRB underground in AMD, might be ammonium phosphate added with organic substrate to stimulate the microbial activity or acetate-bearing compounds (Sahinkaya, 2009). According to the reported literature, mine water running through an area rich in organic substrate shows significant removal efficiency for Al, Cd, Co, and Zn, as well as a pH increase. During high flow rate seasons, however, this impact may be mitigated and precipitated metals could be resolubilized (Skinner & Schutte, 2006).

2.2.2 Permeable Reactive Barriers (PRB)

One more in situ remediation approach is the permeable reactive barrier (PRB), which involves installing a reactive medium perpendicular to the plume of polluted water,

which frequently necessitates correct flow trajectory calculation. AMD will passively move across the reactive barrier due to the natural hydraulic gradient, undergoing neutralization and metal precipitation. Furthermore, reactive barriers frequently depend on natural flow to carry AMD through specified treatment zones, resulting in substantially longer processing times and less tractability. System efficiency can be harmed by diminishing the substrates and the mineral precipitation blockage (Jeen & Mattson, 2016).

2.2.3 Anoxic Ponds

To decrease dissolved oxygen and ferric ions (Fe^{3+}), anoxic ponds can be utilized upstream of more delicate treatment systems. Plastic liner put behind a gravel layer works as a gas barrier, preventing metal and acidic stream leakage. While collecting CO_2 , apparatus is meant to reduce ambient oxygen intake. This method will generally improve the pH and high metal concentration in the effluent (Skousen et al., 2017).

2.2.4 Wetlands

Wetlands are the most prevalent treatment method, and they've been identified as a cost-effective AMD treatment option (Skinner & Schutte, 2006). Due to the combined impacts of physical, chemical, and biological processes that determine output water quality, wetlands are very complex ecosystems. Aerobic and anaerobic wetlands are the types of wetlands. Rich organic substrates, limestone, and SRB inoculum are submerged in the anaerobic wetland to improve the acidity of metalliferous waters and allow for the reduction of iron and sulfate compounds, whereas aerobic wetlands target on net alkaline waters. Planting vegetation on submerged substrate is a broad topic with a range of potential outcomes. For underlying microbial populations, surface vegetation is advised as a basis of cover and energy (Kaksonen & Sahinkaya, 2012). Surface plants have also been shown to negatively affect SRB performance (Gazea & Kontopoulos, 1996). Alongside these concerns, wetland remediation possibly is not appropriate in arid or semi-arid climatic conditions. Sediments with metal sulfides might be once again oxidized and dissolved, and re-acidified in the treatment area as water levels rise and fall over the seasons (Kaksonen & Puhakka, 2007).

bioreactors, up-flow anaerobic sludge blanket (UASB) reactors (Kaksonen & Puhakka, 2007). Few of the bioreactors along with their configuration, merits and demerits are described in Table 2.

3.1 Aerobic Bioreactor Technology for the Treatment of AMD

3.1.1 Algal-Based Bioreactors

Algal bioreactors are an attractive bioremediation technique because of its cost-effectiveness and high metal removal efficiency and sulfates. Algal bioremediation is a new and appealing biological method for AMD treatment. A variety of algal strains having been examined for the bioremediation of acidic streams, including *Anabaena*, *Chlamydomonas*, *Chlorella*, *Cladophora*, *Oscillatoria*, *Phaeodactylum*, *Scenedesmus*, *Spirulina* sp., and others (Dean et al., 2019). These algal strains behave as “hyper-sorbents” and “hyper-accumulators” for numerous metals and elements, exhibiting exceptional selectivity. In addition, the metabolisms of algal biomass generate high alkalinity, which helps to neutralize the acidic character of the drain stream and facilitates metal precipitation. However, because the efficiency of algal treatment method is highly influenced by the pH, oxygen level, and temperature of acidic streams. Therefore, the bioremediation using algae is constantly employed in combination with various treatment strategies. Recent studies reported that the use of macro algae as possible bioindicators for pollution detection and dissemination (Rambabu et al., 2020). Challenges and potential of algae-based bioreactors are algae can be easily grown from oxidation pond, high-rate algal ponds and mining lakes, reduces CO_2 in air and can be made into biofuels like ethanol, biohydrogen, biochar, and many value-added bio products like, antioxidants, vitamins, antimicrobial drugs. As it is an emerging technology more research must be done to know the reliability of fuels produced by algal bioreactors. Algae have some drawbacks, including their unpredictable responses to complex, changing environments, and crucial environmental factors like solar radiation, the availability of nutrients, temperature, and ecological succession are difficult to understand and must be continuously monitored for the process to be successful.

3.1.2 Biochemical Reactor (BCR) System

A BCR is an engineered treatment system comprises of three different reactive zones: oxidative zone, transitional zone, and sulfide zone, as well as a free water zone close to the media as shown in Fig. 3 that uses an organic substrate to promote microbial and chemical processes in

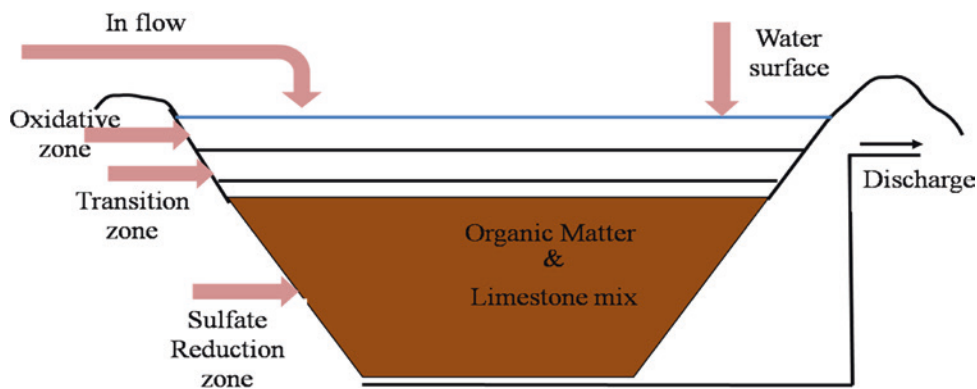
3 Bioreactor Applications in AMD Treatment: Focus on Anaerobic Technologies

A number of reactor configurations have been described in the literature for the biological reduction of sulfate, includes batch reactors, biochemical reactors, sequential batch reactors, anaerobic membrane bioreactors, membrane

Table. 2 Summary of aerobic and anaerobic reactors used in AMD treatment along with their advantages and disadvantages

Reactor type	Advantage	Disadvantage
Continuous stirred tank reactor (CSTR)	Quick, dependable, and constant equilibrium conditions	Inadequate biomass retention
Anaerobic contact process (ACP)	Superior than CSTR in terms of biomass retention	Sludge and flocks are broken down by biomass circulation
Anaerobic filter reactor (AFR)	Minimal shear forces More time for sludge retention Down flow gravitational mechanism	Rise in pressure gradient
Fluidized-bed reactor (FBR)	Adequate surface area for SRB growth Substantial biomass retention Very slight pressure gradients Recycle flow results in lower influent concentrations	Carrier fluidization requires energy Shear force-induced biomass detaching Less biomass capacity is available than in a UASB reactor
Algal based bioreactors	Algae can be easily grown Valuable byproducts can be obtained	Algae is not stable and is influenced by environmental factors
Membrane bioreactor (MBR)	Easy to operate and has a higher nitrogen removal rate	Fouling of membranes which may lead to membrane permeability loss
Biochemical reactor system (BCR)	Require low energy, and may have low maintenance	Space may restrict the effective design of a BCR
Anaerobic membrane bioreactor (AnMBR)	Highs solids retention, rejection of high molecular weight organics and less energy consumption	Membrane fouling and effluent nutrient control difficulty
Up-flow anaerobic sludge blanket reactor (UASB)	No flow channeling Sludge is not compacted Zero clogs formed Potential for high treatment rates	Flushing out biomass

Fig. 3 Schematic diagram representing a biochemical reactor system (Kittrell, 2014)



acid mine water to reduce metal concentrations, acidity, and sulfate. BCRs can be designed in a variety of ways. Pre- and post-treatment units can be used to carry out each process (bioprocesses, chemical reactions, and solid separation) in a separate tank, or they can all be carried out in one unit by the supply of organic materials such as wood chips or manure. Limestone is frequently used with organic substances to provide buffering capacity and substrate permeability (ITRC, 2013). The pH causes the development of metal sulfide solids as alkalinity is added

to or created in the BCR chemically. Many metals solubility is reduced when pH rises, and the metals solidify upon precipitation, which are confined in the solid substrate or caught in the downstream sedimentation cells. Sulfate must now be moved to the sulfide state under reducing circumstances. The BCR contains SRBs, cellulose degraders, and fermenters biologically. SRBs rely on cellulose degraders, such as Bacteroids and Clostridium, to breakdown the substrate, which is often a complex carbohydrate, into simpler carbon molecules (Neculita et al.,

2007). Cellulose degraders are able to thrive in both aerobic and anaerobic environments. Fermentative anaerobes will predominate in a BCR for sulfate reduction. The transitional zone is anoxic to slightly anaerobic, with iron oxidation and organic matter degradation due to increased microbial activity. The sulfide zone is anaerobic and extremely reduced, with a high level of microbial activity. Crushed gravel and perforated pipes are commonly used in drainage systems. BCR may use local materials for the substrate, lowering the initial material cost and simplifying the construction process. BCRs are simple to use and maintain, and they don't require any electricity. It has been demonstrated that a BCR can function for years without the need to replace or replenish the organic substrate, which is particularly advantageous given that they are frequently found in isolated locations with restricted access. BCRs require low energy, and may have low maintenance if properly designed. BCRs, on the other hand, might be troublesome for treating AMD since they frequently need pre- and post-treatment and hence are not stand-alone systems. Organics and nutrients may be discharged, and there may be an increase in biological oxygen demand and color in the effluent, causing it to fail to fulfill water quality criteria on a regular basis. Space may restrict the effective design of a BCR. Over time, it will be necessary to replace the organic substrate, and the BCRs permeability will alter (Kittrell, 2014). A BCR can be used at various mining sites like metals and coal mining and can also work in remote sites with limited infrastructure and extreme conditions. A BCR can be applied at variable pH, sulfate, and metals concentration.

3.1.3 Membrane Bioreactor (MBR)

MBRs, which combine biological treatment (Bioreactor) with a micro or ultra-filtration membrane, allow for process acceleration while also producing a consistent, high-quality effluent. To separate treated water from active biomass, MBR's biological reactor technology may be used with both aerobic and anaerobic suspended growth bioreactors. Membrane technology is regarded as the most modern AMD treatment option due to its low chemical requirement, lack of sludge formation, and small scale of operations (Al-Zoubi et al., 2010). pH, feed concentration, permeate flow, and temperature are other factors that affect how well metal and salt are rejected by the membrane-based AMD treatment (Rambabu et al., 2020). The benefits of MBR could be potential reuse of effluent water and it has smaller bioprocess footprint and low sludge yield. A MBR, as shown in Fig. 4, is easy to operate and has a higher nitrogen removal rate than any other treatment methods. Potential limitation could be fouling of membranes which may lead to membrane permeability loss. MBR is replacing traditional clarifiers and can be used for AMD treatment. It

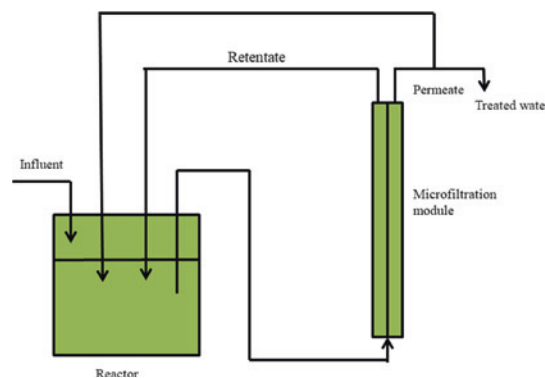


Fig. 4 Schematic diagram of MBR (Barreiros et al., 1998a, 1998b)

possesses high biological oxygen demand (BOD), chemical oxygen demand (COD), and ammoniacal nitrogen removal efficiencies.

3.2 Anaerobic Bioreactor Technology for the Treatment of AMD

Treatment of AMD through anaerobic process for the removal of contaminants is a potential approach as it has a potential to combine SO_4^{2-} , metals, acidity removal in a single reactor with significantly low production of waste sludge in addition to the bioenergy recovery when an external source of organic material is added in excess. Sulfate reduction and potentially toxic metal removal in passive and active systems have been studied over the years, numerous bioreactor types and reactor designs have been used. There are several examples, including the anaerobic contact process (ACP), anaerobic filter (AFR), hybrid reactors, continuous stirred tank reactors (CSTRs), up flow anaerobic sludge blanket reactor (UASB), off-line sulfidogenic bioreactors, and fixed bed reactors (FBRs) (Bartzas et al., 2006; Cruz Viggì et al., 2010; Ñancucheo & Johnson, 2012). Sulfidogenic bioreactors are the active biological systems specially designed for the treatment of sulfate rich wastewaters that have a benefit over passive biological remediation in terms of performance and control, absorption of potentially toxic metals, and reduction in sulfate contents in the treated waters (Bai et al., 2013; Becerra, 2010). Pre-treatment of AMD using chemical neutralization, precipitation, and permeable reactive barrier prior to anaerobic treatment enhances the overall process performance.

3.2.1 AMD Treatment in Anaerobic Bioreactors: Mechanism

The solid organic substrate matrix comes into contact with the AMD water moving horizontally or vertically through the reactor (Dhir, 2018; Nordwick et al., 2006) where the

complex organic carbon compounds in the AMD are metabolized by the SRB. (Lu et al., 2011).

Microorganisms help in shifting the alkalinity generating processes and in the reduction of complexity of AMD by decreasing the metals and sulfate dissolved concentrations. An acidophilic heterotrophic bacterium which is present in acid mine water plays a key role in AMD treatment. By oxidizing ferrous ions, acidophilic heterotrophic bacteria catalyze the dissimilatory reduction of sulfate to sulfide. When a strong acid is transformed into hydrogen sulfide, alkalinity forms. Under anaerobic circumstances, heterotrophic bacteria such as *Pseudomonas*, *Clostridium*, and *Desulfovibrio* reduce Mn and Fe by using them as final electron acceptors. Ammonification and denitrification are biologically mediated processes that can help neutralize the AMD. Bacterial species such as *Pseudomonas*, *Paracoccus*, *Flavobacterium*, *Alcaligenes*, and *Bacillus spp.* support this process. SRBs such as *Desulfovibrio spp.*, use acidic mine water as an electron donor to produce bicarbonate and convert sulfate to sulfide when organic carbon nutrition sources are present. Reduced sulfate forms sulfides, which increase the quantity of bicarbonate that causes alkalinity, produce insoluble metal complexes (Sand et al., 2001) as shown in Eq. (3)



Metals in high concentrations are eliminated as hydroxides as a result of precipitation or co-precipitation (Jong & Parry, 2003).

The overall AMD treatment in anaerobic sulfate-reducing bioreactors is shown in Fig. 5. Metals are effectively removed by precipitation when the pH is increased. Metals such as Cu, Zn, Cd, Pb, Ag, and Fe mostly precipitate as metal sulfides as a result of hydrogen sulfide generated during sulfate reduction.

3.2.2 Anaerobic Sequencing Batch Reactor (ASBR)

Anaerobic sequencing batch reactors (ASBRs) are high-throughput anaerobic treatment systems that follow a cyclic process that includes feed, reaction, settling, and decantation (Fig. 6). The initial stage is to introduce the wastewater into the reactor, which is continually mixed with the contents. The amount of substrate supplied is determined by several criteria, such as the target hydraulic retention time (HRT), organic loading rate, and predicted settling characteristics. Because of its improved biological solids retention and process control, ASBRs can be utilized as an alternative to continuous stirred tank reactors for wastewater treatment, resulting in better effluent quality. Furthermore, by properly controlling the cycle duration and discharge operation of batch reactions, effluent regulations may be more readily met when the influencing elements are at adequate levels (Akil & Jayanthi, 2012). In the bioreactor with a sequential design, SRB is the biological agent and the potential pollutants that can be reduced by ASBR are manganese, calcium, magnesium, and other potentially toxic metals. In a contact time of 172 days at a pH 4.5 the removal efficiency of sulfate can be reached to 84.7, 80% of manganese removal, and calcium by 50% and magnesium by 38%. These elements could be precipitated in the form of carbonate or hydroxide in the bioreactor leading to the increased pH of the reactor contents. The system's ability to remove potentially harmful metals is enhanced by the addition of tailing leachate. An ASBR designed for treatment of AMD was evaluated by measuring its capacity to hold the metals concentrations by studying COD and sulfate removal kinetics as reported by Martins Costa et al. (2019) where biological sludge was used for treatment of AMD with high metals concentration.

The microbial population was dominated by *Desulfovibrionaceae sp.* (Gomez et al., 2021; Ighalo et al.,

Fig. 5 Anaerobic sulfate-reducing bioreactors

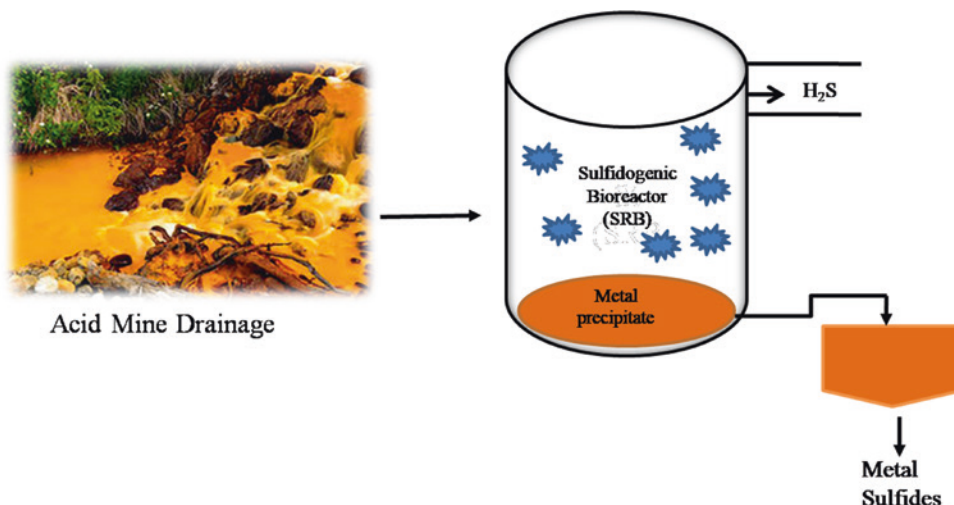
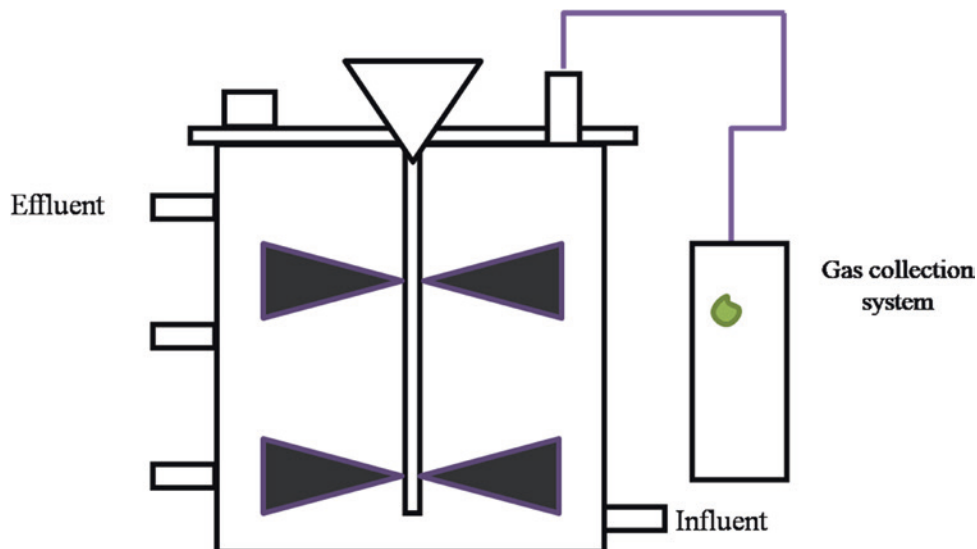


Fig. 6 Schematic diagram of ASBR (Park et al., 2012)



2022). An ASBR requires less space and this system has minimal footprint with high nutrient removal capabilities but one of the potential limitations is the continuous monitoring and maintenance required for the system's steady operation.

3.2.3 Up-Flow Anaerobic Sludge Blanket Reactor (UASB)

Up-flow anaerobic sludge blanket reactors (UASB) are often used to treat domestic sewage and industrial wastewater (Fig. 7). Three-phase separation in the vertical tank mechanism distinguishes this reactor by its simplicity. The basic idea behind the UASB concept is to create circumstances that allow a substantial volume of biological sludge to be retained in the reactor's interior without the requirement for an inert support. These conditions may be obtained by using a three-phase separator, which is linked to various system operating factors such as slow outflow rates and the generation and maintenance of good sedimentation characteristic granules. As a result, the HRT of the reactor may be separated from the sludge biomass retention time (Rodriguez et al., 2012). UASB reactor includes 2 zones, a reactor zone and a settling zone. Granular sludge, which has great mechanical strength and superior settling qualities and is resistant to toxic shocks, is what sets UASB apart from other anaerobic reactors and with high methanogenic properties. UASB is the most commonly used in industrial wastewater treatment and is highly efficient with less space requirement and less energy consumption with less sludge production. It is associated with less operating costs as well as efficient in achieving 65–75% of COD removal. Challenges with UASB would be low pathogen and nutrient removal, odor problems and long start up. Application of UASB for AMD treatment was reported by Leal-Gutierrez et al. (2021) for converting sulfate into sulfide and to

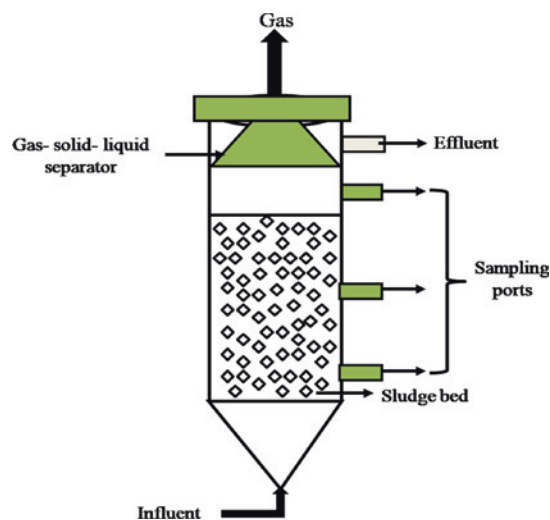


Fig. 7 Schematic diagram of UASBR

determine the effect of pH and COD: SO_4^{2-} ratio. It was concluded that UASB system achieved 69% of sulfate to sulfide bioconversion.

3.2.4 Anaerobic Membrane Bioreactor (AnMBR)

An anaerobic membrane bioreactor (AnMBR) is the application of membrane filtration process for the treatment of wastewater without exposing it to air/oxygen (Fig. 8). AnMBR consists of 2 parts, a sludge bed and the supernatant in which a hollow fiber membrane will be submerged as they work on a similar principle of aerobic and membrane reactors, but they leverage the advantage of the benefits of anaerobic degradation. When compared to their aerobic equivalents, AnMBR can treat wastewater without aeration, produce biogas for energy purposes and substantially less biosolids (Uman et al., 2021). Many of the

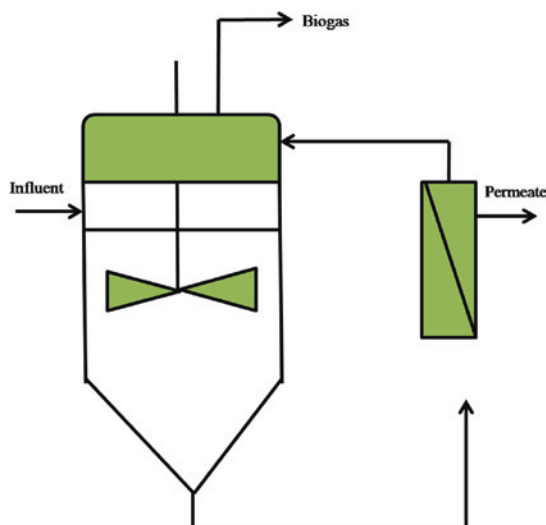


Fig. 8 Schematic diagram of AnMBR (Chang, 2014)

standards may be satisfied by AnMBR. However, issues including membrane fouling, dissolved methane recovery, and management of effluent nutrients must be addressed. Membrane fouling is a primary issue among them, since research suggests that present fouling mitigation strategies constitute a major energy demand for AnMBR (Gong et al., 2019). Low energy consumption, high solids retention, and rejection of high molecular weight organics are the few benefits of AnMBR. The drawbacks of this system include membrane fouling and the challenge of achieving efficient membrane scouring. AnMBR is appropriate for the treatment of both industrial and municipal wastewaters. AnMBR for the treatment of AMD was evaluated by Sahinkaya et al. (2019) where sulfate and COD concentrations of 1500 and 1000 mg L⁻¹ at pH4 were maintained. High COD and sulfate removal efficiency of 95% was reported even at low COD/sulfate ratio. Over 99% of iron, copper, zinc, nickel was removed because of metal sulfide precipitation.

3.2.5 Bioelectrochemical Treatment System (BES)

Bioelectrochemical systems (BES) have emerged as an intriguing technology in terms of wastewater treatment and energy consumption in recent years. BES is based on the metabolic processes of exoelectrogenic microorganisms that can catalyze electrochemical reactions on electrode surfaces of electrochemical cells (Ren, 2013). Bioelectrochemical systems have been built in a variety of configurations which has a cathode and anode chambers with anion exchange membrane separating the two chambers. Exoelectrogenic bacteria oxidize the substrate in the anode, and the electrons are released to the electrode. The electrons are used in the cathode to carry out a reduction process, such as converting O₂ to H₂O, protons to hydrogen (H₂) gas, or reducing other chemical compounds to less refractory forms. In this

regard, the possibility of recovering dispersed metal ions in their elemental form by reducing them cathodically in their oxidized elemental form that can be retrieved is quite interesting (Kim et al., 2015). Electrical energy may be acquired from the electrical circuit in a microbial fuel cell (MFC) based on the thermodynamic energy balance, however electrical energy must be provided by a power source in a microbial electrolysis cell (MEC) (Ghangrekar & Chatterjee, 2017). BES has many advantages similar to that of microbial fuel cells (MFC); microbial electrical cells (MEC) as they are primarily meant for waste water treatment for pollutants removal with simultaneous power generation. AMD can also be treated using BES; however, an external organic carbon source is required as the AMD is deprived of organic material and rich in inorganic compounds. Example of metal recovery from AMD using BES was reported by Lefebvre et al. (2022) where iron was removed from AMD by increasing the pH. For the treatment of AMD with high ferrous iron content, a proton exchange membrane MFC was effective (Fe³⁺ is reduced to Fe²⁺).

3.2.6 Anaerobic Sulfate-Reducing Bioreactors: Active Biotic Systems

Anaerobic digestion (AD) is one of the promising biological processes for the treatment and stabilization of solid and liquid wastes. The wastewaters rich in organic material are amenable for AD, however, treatment of AMD via AD process could also prove beneficial as the anaerobic reactors contain mixed microbial consortia which also include SRBs, methanogens, acidogens, and so on. As the environmental conditions required for the growth of SRBs and methanogens are same, SRBs and methanogens compete for organic matter but SRBs convert sulfate to sulfide whereas methanogens convert the organic matter to biogas which is a mixture of methane and carbon dioxide. The predominance of SRBs in anaerobic reactors is high when the wastewaters rich in sulfates are treated. Therefore, anaerobic sulfates reducing bioreactors is a promising approach for AMD remediation with a potential to combine SO₄²⁻, metals in a single reactor with significantly low waste sludge generation in addition to the bioenergy recovery. SRBs are used in the biological process of anaerobic treatment for sulfate-rich effluents like AMD. Because they are heterotrophic bacteria, SRB need organic matter to serve as electron donors for sulfate reduction. Electron donors may be from complex carbon molecules (Skinner & Schutte, 2006). Potentially toxic metals create insoluble compounds with biogenic sulfides, causing them to precipitate and to be removed as sulfides (Panda et al., 2016). SRB can thrive in pH ranges of 5–9 and shows high activity in this range therefore the AMD pH shall be corrected before being treated in anaerobic processes. In these reactors, a thick

layer of organic-rich materials combined with limestone forms the foundation of anaerobic sulfate-reducing bioreactors. Under the organic layer, a thin coating of limestone is used to provide extra alkalinity while also supporting the underlying drainage channels. The AMD is released into the drainage system after passing through the organic layer and limestone bed vertically. SRBs feed on the organic layer and convert SO_4^{2-} to H_2S and oxidize organic matter (CH_2O) to bicarbonate ions (HCO_3^-) (Li et al., 2018) as shown in Eq. (4). The energy generated in this process is used by sulfate-reducing bacteria to grow and develop.



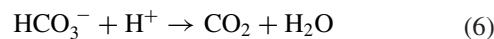
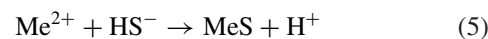
The bicarbonates (HCO_3^-) generated subsequently react with hydrogen (H^+) ions to form CO_2 and water (H_2O). As a result of the consumption of H^+ ions, the pH of AMD water rises. Metal sulfides, oxides, hydroxides, and carbonates begin to precipitate at high pH levels. Metal sulfide precipitation is the most prevalent type in anaerobic sulfate-reducing system (Waybrant et al., 2002). Sulfate-reducing bioreactors therefore aid in the reduction of acidity, metal toxicity, and sulfate content in AMD water, and also enhance overall water quality.

Maintenance of suitable biochemical environment favors the remediation process by the SRBs resulting in the precipitation of dissolved metals and their immobilization as sulfides. Sulfate, anaerobic conditions, and the availability of organic carbon all contribute to this type of environment. Most metals may be successfully removed from mine waters if such conditions are created within a reactive barrier or field-bioreactor (Santos et al., 2015). Sulfate-reducing passive bioreactors have recently gained a lot of interest as a viable technology for AMD treatment. They have several benefits, including high metal removal at low pH, stable sludge, cheap operating costs, and low energy usage. The intended method of pollutant removal is sulfide precipitation; however, in passive bioreactors, several other processes, such as sulfate-reducing passive bioreactors depend on the activity of an anaerobic micro flora, including SRB, which is primarily controlled by the reactive mixture composition, their efficiency is occasionally limited by the adsorption and precipitation of metal carbonates and hydroxides. The source of organic carbon is the most important component in the mixture. (Nordwick et al., 2006).

SRB use organic carbon to reduce sulfate while also producing biogenic hydrogen sulfide (H_2S) and alkalinity.

This causes heavy metal accumulation in AMD, as well as a rise in pH and alkalinity. As described in the equations below, biogenic H_2S reacts with metallic ions (Me^{2+}) present in AMD to create metal sulfides (MeS), whereas hydroxide ions (HCO_3^-) combine with protons (H^+) to

neutralize acidic waters (Kaksonen & Puhakka, 2007) Eqs. (5, 6)



A reduction in sulfate content and potentially toxic metals, as well as an improvement in pH and alkalinity, are predicted in the resultant sulfate-reducing environment.

Metabolism of SRB for Sulfate Reduction Dissimilatory Sulfate Reduction Pathway

There are two types of biological sulfur reduction: assimilatory and dissimilatory. The bacteria, algae, fungi, and plants all take up the SO_4^{2-} ion in the assimilatory reduction process, where it is lowered and fixed in sulfur-containing amino acids like cysteine and methionine inside cells. The dissimilatory reduction happens in two steps. The first involves using sulfate as the final acceptor in the electron transport system under anaerobic circumstances, converting sulfate to sulfide. The second stage is the oxidation of sulfide to elemental sulfur with the help of sulfur-oxidizing bacteria (SOB), which may be used as a fertilizer or a substrate in bioleaching processes (Janssen et al., 1999). SRB treatment for AMD has a low operating cost. The conversion of sulfate ions to sulfide by SRB under anaerobic conditions is used in this approach (Costa et al., 2020).

The most prominent way of sulfate reduction is via the dissimilatory sulfate reduction pathway. This is a metabolic pathway occurring in sulfur reducing bacteria (SRB). The most stable form of sulfur, which is the sulfate, is first reduced to sulfite followed by reduction to sulfide. The microbial cell initially uptakes the sulfate containing compounds. The ATP present inside the cell activates the sulfate and converts it into an intermediate product called Adenosine-5'-phosphosulphate (APS) along with release of two inorganic phosphates. This first step is catalyzed by sulfate adenylyl transferase (Sat) enzyme. The second step is the conversion of APS into sulfite with adenosine monophosphate (AMP) as the byproduct with APS reductase enzyme (AprBA) being the catalyst. The third step is the most crucial step of the pathway where the sulfur atom present in sulfite forms a complex trisulfide bond with reduced DsrC protein. DsrC is a protein with a highly conserved C-terminal arm containing two cysteines which are separated by 11 amino acid residues. This DsrC acts as a substrate for the reaction catalyzing heterodimer protein complex, DsrAB. The C-terminal arm of DsrC inserts itself into a cleft between DsrA and DsrB proteins which is near to the substrate binding site of DsrAB complex. Cysteine of DsrC in their reduced sulphhydryl (R-SH) form will get

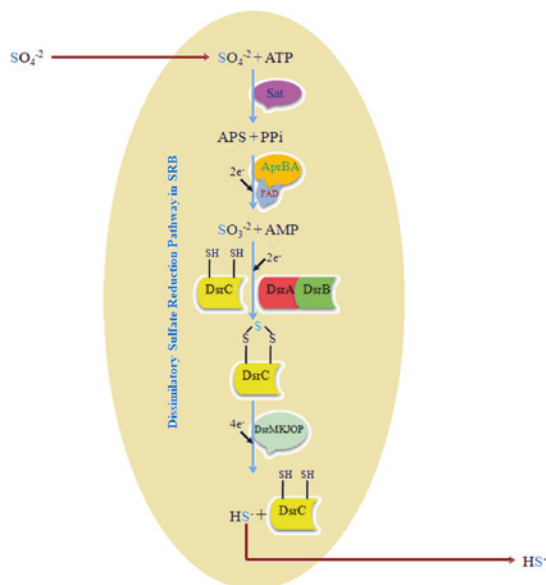
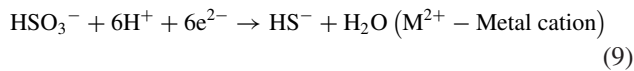
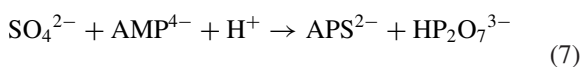


Fig. 9 Dissimilatory sulfate reduction pathway

oxidized and binds with the S atom of sulfite forming a trisulfide-protein complex (Fig. 9). In the fourth and final step, the trisulfide is reduced to sulfide and released from the cell with the DsrC protein being restored. The final step is catalyzed by a membrane protein complex called DsrMKJOP. The whole reduction process requires eight electrons out of which two are needed during APS reduction, another two required for trisulfide-protein complex formation, and last four in the final step of sulfite to sulfide conversion (Santos et al., 2015). The reactions required for sulfate reduction by SRB metabolism are listed below (Eqs. 7, 8, 9) (Xingyu et al., 2013).



3.3 Critical Performance Indicators of Anaerobic Technologies

3.3.1 PH

pH is one of the important process performance indicators that shift the reaction pathway from one to another with slight changes. The anaerobic treatment of wastewaters

is carried out at neutral or weak acidic environments. The optimal pH range of 5 to 6 is required for sulfate reduction and at this range optimum H_2S generation occurs (Broco et al., 2005).

3.3.2 Organic Substrates for Treatment of AMD: Direct versus Indirect Substrate

The major limitation of the biochemical reaction by the SRB is the accessibility of carbon sources. The carbon supply in AMD water is limited, requiring extra or external carbon sources for treatment to be successful (Kolmert et al., 2000). Sulfate reduction is a high-energy intensive process that necessitates a large volume of high-energy reductant (Martins et al., 2009). As a result, the effectiveness and cost viability of bioremediation technology will be influenced by the carbon sources used. The composition of organic material needs to be studied since it influences the efficacy of SRB eco-technology. While functioning as a readily available carbon source, a substrate must be able to establish a proper low redox environment.

Microbial communities are more resilient and sustainable when made up of a variety of readily biodegradable materials and organic carbon sources (Neculita et al., 2007; Sheoran et al., 2010). A crucial component in the development of the substrate for sulfate-reducing bioreactors is organic material. Such products may be purchased for a lower price or for no price at all as they are frequently regarded as waste items. The only expense may be incurred during the transportation to treatment site (Gusek, 2004).

SRB prefers simple organic substrates as a food source, which can be provided directly or indirectly. Maple wood chips, sphagnum peat moss, leaf compost, conifer compost, chicken manure, and conifer sawdust are all examples of indirect organic substrates that can be used (Jamil et al., 2013a, 2013b).

Direct organic substrate sources that do not need to be degraded before being consumed by SRB are alcohols, organic acids, and sugars. In the meantime, indirect organic sources such as organic compost, wood or paper waste, and food manufacturing byproducts must be further degraded in order to generate the required output. Indirect substrate will be more suitable because mining operations are located far from urban areas. In long-term conditions, indirect substrate will be more suited than direct organic substrate since mining sites are located far from metropolitan areas. Even while basic substrates have the benefit of allowing SRB to utilize energy sources rapidly and directly, they are quickly depleted. Indirectly, substrates must be supplied into the system on a continual basis, raising operating, and maintenance costs (Hiibel et al., 2011; Jamil et al., 2013a, 2013b). Effective reactive mixes have an organic carbon source

(different organic/cellulosic wastes), a bacterial source or SRB inoculum (river sediment/animal manure), a solid porous medium (gravel/sand), a nitrogen source (urea), and a neutralizer (limestone) (Dhir, 2018). The reactive mixture's composition predominantly controls the activity of SRB, determines the effectiveness of passive bioreactors. Microbial communities are more likely to be long-lived and sustainable if they are made up of a combination of rapidly degradable materials and different organic carbon sources (Jamil et al., 2013a, 2013b; Nordwick et al., 2006).

3.3.3 Hydraulic Retention Time

For direct organic substrate, a hydraulic retention time (HRT) of 3–5 days is necessary for the precipitation of sulfide metals, whereas indirect organic substrate requires an HRT of 7–10 days for adequate microbial development (Gonçalves et al., 2007).

3.3.4 Temperature

The effect of temperature on SRB growth and sulfate reduction kinetics is significant. SRB can live in both mesophilic and thermophilic environments (Tassé et al., 2003). The ability of sulfate-reducing bacteria to degrade organic complex substrate to a simple form is also influenced by the activity of other anaerobic bacteria. Methanogens, on the other hand, are sensitive to low temperatures, requiring mesophilic environments to develop.

SRBs are found to thrive at temperatures ranging from 0 to 80 °C. Despite the fact that SRB are active in arctic habitats (at temperatures below 5 °C). Low temperatures reduce the effectiveness of passive AMD treatment by lowering the biogeochemical activity. A study reported by Ben Ali et al. (2019a, 2019b) for the treatment of AMD in arctic conditions in their review, for example preliminary laboratory testing of a synthetic AMD in PBR columns at 4 °C versus 25 °C revealed a direct and substantial influence on how a temperature has an impact on microbial activity drop, as well as Cd, Zn, and sulfate removal (Kawaja et al., 2006).

3.3.5 Solid Support

Sand, gravel, and glass beds (Choudhary & Sheoran, 2011) are used as solid supports for SRB and may have beneficial impacts on bacterial growth because of their large pore size, low surface area, and big volume, as well as enhancing metal precipitation. To prevent clogging in the bioreactor, it is desirable to use a solid support with a high pore size, low surface area, and a large volume of solids (Jamil et al., 2013a, 2013b).

3.3.6 Inhibitory Effect

In AMD, high amounts of metallic ions including iron, zinc, copper, and manganese can prevent SRB from growing. 2–50 mg Cu/L, 13–40 mg Zn/L, 75–125 mg Pb/L, 4–54 mg

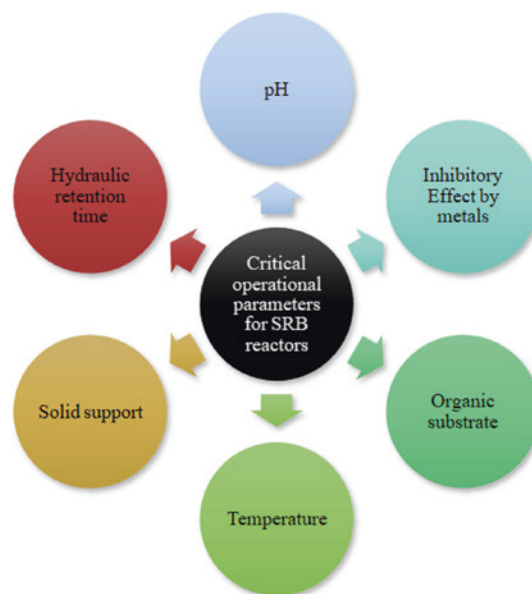


Fig. 10 Sulfidogenic bioreactor parameters

Cd/L, 10–20 mg Ni/L, 60 mg Cr/L, 74 mg Hg/L are the lethal ranges for SRB populations (Tang et al., 2009). These ranges may change depending on the species of SRB that are available (Fig. 10).

4 Present State of Art and Future Perspective

Due to variety of federal and state laws, commercial and government entities are required to develop various AMD treatments or control technologies (Skinner & Schutte, 2006). AMD pollutes the ecosystem, thus avoiding AMD development or migration from its source is generally thought to be the best solution. According to research, bioremediation of AMD using sulfate-reducing bacteria has caught the attention of numerous researchers. Previously, research topics on sulfidogenic bioreactors were focused on substrates with liquids, such as lactate and ethanol, solid substrate materials, on the other hand, having the capacity to be an effective supply of substrate for systems using sulfate-reducing bacteria. The way that SRB activity occurs when a solid substrate material is used, on the other hand, is poorly understood. The factor that restricts the rate of sulfate reduction by SRB is the breakdown of complex organic matter. The system design and location of the biological treatment plant, the profitability of metal recovery, the choice of substrate, and the discharge criteria are only a few of the variables that affect a biological treatment plant's overall operating costs. Finding suitable low-cost substrate substitutes, such as organic solid waste and food waste byproducts, may boost the implementation of SRB

technology (Jamil et al., 2013a, 2013b). As biological treatment of AMD can be divided into two separate components—treatment of AMD and prevention of leaching and remediating the AMD contaminated environment. The soil and water bodies are the most commonly polluted by AMD. Bioreactors and other types of wetlands being reported as having succeeded to treat AMD prior to being released into the surroundings or to minimize leaching. Algal remediation, microbiological remediation, and wetland remediation are the three most commonly reported strategies for effectively treating AMD polluted aquatic environments (Ighalo et al., 2022).

Because there is no single dependable approach for treating AMD, researchers from all over the world have been working to develop effective and beneficial strategies for dealing with acidic mine effluents. According to recent AMD research, biological treatment technologies are particularly promising since they avoid the issues of high operational costs and sludge disposal that chemical treatment systems have. For a number of acidic effluents with various concentrations and other aqueous characteristics, bioremediation procedures are very simple and viable to execute. According to recent research along with anaerobic bioreactors permeable reactive barriers, microbial and algae-based bioremediation and wetlands has uncovered new avenues and possibilities for AMD treatment (Ighalo et al., 2022). Developing prediction models for mapping algorithms that correlate microbiological characteristics with the chemical composition of mining sites, as well as the creation of mineral-specific AMD treatment methods, are two areas of future research scope. Bio-sorbed or precipitated metal sulfides can be used to successfully recover metal, is also an area of research that can be explored using contemporary biological techniques (Rambabu et al., 2020).

5 Conclusion

AMD, as highlighted in this chapter, is a global problem that harms the state of environment and subsequently, health of humans. Conventional methods of treating AMD may not always provide the desired degree of sulfate reduction, and waste disposal needs extra landfill area. As a result, more efficient and sustainable methods must be developed in order to recycle and utilize the trash created. Among the available methods, this evaluation emphasized the treatment of AMD with SRB, a low-cost, highly efficient option showing potential for resource recovery. The SRB converts sulfate ions to hydrogen sulfide by dissimilatory metabolism, which combines with metallic ions to produce metal sulfide precipitation. Because of their efficacy and cost-effectiveness, anaerobic bioreactors are a potential approach for treating AMD contaminated water. There

are a number of bioreactors that uses Anaerobic Bioreactor Technology that can be employed to treat AMD which are discussed in this chapter. The novel reactor configurations in this chapter would be a combination of membrane technology and anaerobic process—Anaerobic membrane bioreactor (AnMBR); Bioelectrochemical systems (BES). However, the effectiveness of the system is determined by the SRB's activity, which in turn determined by the reactive mixture and organic carbon supply available. These anaerobic bioreactors are capable of operating at pH 5.0 and temperatures ranging from 2 to 68 °C. These characteristics make this a method that can be used on a large scale, even at pilot scale. In addition to the reactive mixture's composition and the presence of SRB, the anaerobic bioreactor's overall productivity and long-term operation are also influenced by the reactor's design, Eh, hydraulic retention time, and COD/sulfate ratios. The use of Anaerobic Bioreactor Technology to control AMD has been widely developed to limit the negative consequences. Transportation expenses for liming materials and; the size and terrain of the accessible area; sludge disposal or waste stream generation if poorly managed; as well as labor and maintenance costs, are all important considerations. Importantly, the elements described above should be examined as a function of one another rather than being analyzed separately. As can be observed from current trends, optimizing highly efficient bioreactors uses significantly less space, allowing for a reduction in land requirements. The emphasis should be on improving overall process design that incorporates life cycle evaluation. Furthermore, AMD remediation may also be viewed as a way to capitalize on the extraction of renewable raw materials, including metal recovery via bio-treatment techniques, may yield a strong economic benefit along with waste treatment.

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