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Review

Anaerobic Bioremediation of Acid Mine Drainage Using Sulphate-Reducing Bacteria: Current Status, Challenges, and Future Directions

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Abstract: Biological reduction of sulphates has gradually replaced unit chemical processes for the treatment of acid mine drainage (AMD), which exerts a significant environmental impact due to its elevated acidity and high concentrations of heavy metals. Bioremediation is optimally suited for the treatment of AMD because it is cost-effective and efficient. Anaerobic bioremediation employing sulphate-reducing bacteria (SRB) presents a promising solution by facilitating the reduction of sulphate to sulphide. The formed can precipitate and immobilise heavy metals, assisting them in their removal from contaminated wastewater. This paper examines the current status of SRB-based bioremediation, with an emphasis on recent advances in microbial processes, reactor design, and AMD treatment efficiencies. Reviewed studies showed that SRB-based bioreactors can achieve up to 93.97% of sulphate reduction, with metal recovery rates of 95% for nickel, 98% for iron and copper, and 99% for zinc under optimised conditions. Furthermore, bioreactors that used glycerol and ethanol as a carbon source improved the efficiency of sulphate reduction, achieving a pH neutralisation from 2.8 to 7.5 within 14 days of hydraulic retention time. Despite the promising results achieved so far, several challenges remain. These include the need for optimal environmental conditions, the management of toxic hydrogen sulphide production, and the economic feasibility of large-scale applications. Future directions are proposed to address these challenges, focusing on the genetic engineering of SRB, integration with other treatment technologies, and the development of cost-effective and sustainable bioremediation strategies. Ultimately, this review provides valuable information to improve the efficiency and scalability of SRB-based remediation methods, contributing to more sustainable mining practices and environmental conservation. To ensure relevance and credibility, relevance and regency were used as criteria for the literature search. The literature sourced is directly related to the subject of the review, and the latest research, typically from the last 5 to 10 years, was prioritised.

Keywords: acid mine drainage; bioremediation; sulphate-reducing bacteria

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Academic Editor: Haiming Zhao

Received: 4 February 2025
Revised: 26 March 2025
Accepted: 10 April 2025
Published: 15 April 2025

Citation: Mafane, D.; Ngulube, T.; Mphahlele-Makgwane, M.M. Anaerobic Bioremediation of Acid Mine Drainage Using Sulphate-Reducing Bacteria: Current Status, Challenges, and Future Directions. *Sustainability* **2025**, *17*, 3567. https://doi.org/10.3390/su17083567

1. Introduction

The condition of the environment has a direct impact on the quality of life on Earth. A clean, hygienic, and pollution-free environment is crucial to good health. Pollution, which arises from human activities, industrial processes, or natural causes, significantly affects air, water, and soil [1]. Water pollution, especially acid mine drainage (AMD), severely impacts freshwater ecosystems by lowering pH levels to 2 and increasing heavy metals beyond safe thresholds [2]. For example, in South African coal mining areas, AMD contamination has resulted in sulphate levels exceeding 30,000 mg/L and iron concentrations exceeding 8000 mg/L, exceeding the World Health Organization drinking water standards of 250 mg/L for sulphate and 0.8 mg/L for iron [2]. Despite significant efforts to clean polluted areas, water pollution remains a critical issue that continues to pose health risks. This problem is particularly acute in developing countries, where traditional sources of pollution, such as mining, industrial emissions, inadequate sanitation, poor waste management, contaminated water supplies, and other human activities, are predominant.

In recent years, AMD has emerged as a major global contaminant due to its harmful impact on local ecosystems and organisms [3]. AMD is a great challenge in regions with extensive coal and gold mining activities. It becomes difficult to manage and treat, often involving high costs. The environmental effects of AMD are severe, affecting soil, water resources, and aquatic ecosystems. AMD is typically caused by the weathering of sulphide minerals in abandoned and inactive mining sites, mainly iron sulphides such as pyrite, after being exposed to water, oxygen in the atmosphere, and acidophilic iron oxidising bacteria (acid thiobacillus ferroxidase) [4], as shown in Figure 1.

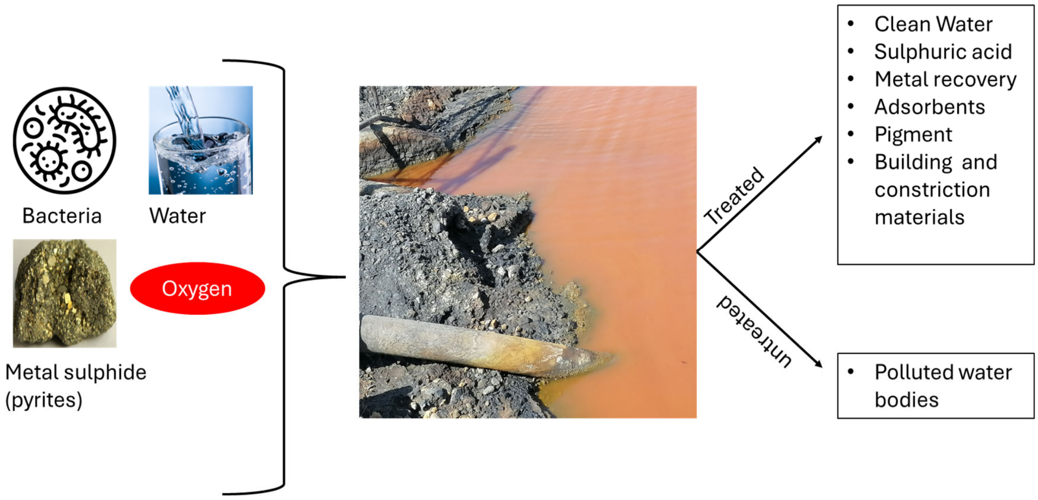

Figure 1. A diagram showing the universal steps in the formation of AMD and its derivatives [4].

This process produces sulphuric acid and an acid solution rich in potentially toxic heavy metals such as iron (Fe), copper (Cu), aluminium (Al), mercury (Hg), manganese (Mn), and lead (Pb) [5]. In addition, the weathering of sulphur-containing minerals leads to acidic streams with high sulphate concentrations. The composition of AMD is influenced by the mineralogy of the extracted ores, the surrounding geology, and the climate conditions [6]. High concentrations of heavy metals in AMD severely threaten the parameters of groundwater and surface water, leading to fatal consequences [5]. AMD has severe negative effects, such as loss of biodiversity, endangering human health, and degrading aquatic species. The toxicity of AMD can cause prolonged environmental damage, highlighting the growing need for effective solutions to remediate both AMD and the affected environments over recent decades [7].

An effective treatment and management system is essential to mitigate the damage caused by AMD and improve ecological sustainability. Economically viable remediation methods, such as lime neutralisation, passivation, in-situ biological remediation, backfilling, sulfidogenic bioreactors, waste-heap covers, adsorption, constructed wetlands, desalination, anoxic limestone drains, and permeable reactive barriers, have been recommended to alleviate the impacts of AMD. Among these, biological treatments have attracted a significant amount of academic attention due to their promising potential for AMD treatment [8]. Biological treatment of AMD, also known as bioremediation, has gained attention over chemical-based treatments because of its reduced labour and operational expenses, easier process design and control, and more effective recovery of sulphate and metals. Essentially, AMD bioremediation utilises bacteria to facilitate the microbial recovery of heavy metals in acid mine wastewater.

Although several reviews have examined the use of SRB bioreactors for AMD treatment, most have focused on general bioremediation approaches or specific reactor configurations without addressing long-term operational challenges, economic feasibility, or large-scale implementation. Existing studies highlight the efficiency of SRB in removing sulphate and heavy metals, but gaps remain in optimising reactor performance under varying environmental conditions, substrate limitations, and microbial community dynamics. Additionally, few reviews explore the integration of SRB bioreactors with other treatment technologies for enhanced sustainability. The primary objective of this review is to also address gaps in the current understanding of anaerobic bioremediation of AMD using SRB. This review critically examines existing methods and technologies that utilise SRB for AMD bioremediation, highlighting their effectiveness, limitations, and technical, environmental, and operational challenges faced. Recent advances in SRB research and technology are analysed, focussing on innovations that improve process efficiency and sustainability.

Additionally, the review explores the integration of SRB with other conventional treatment technologies to achieve regulatory water quality standards and assess the feasibility of recovering valuable minerals from AMD [9]. Economic and environmental sustainability considerations are also discussed. By evaluating SRB-based bioreactors and related advancements, this review seeks to bridge the gaps in the existing literature and contribute to the progression of knowledge in the realm of AMD prevention and treatment technologies.

2. Overview of Acid Mine Drainage

Managing AMD challenges requires a broad understanding of its formation, impacts, and mitigation strategies. Although several natural environmental processes contribute to acid mine wastewater formation, they are mainly the result of human activities. It is a substantial factor when pyrite minerals [iron sulphides (FeS_2)] undergo oxidative breakdown in the presence of water and oxygen [10], as shown in the following Equations (1)–(4).

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

$$Fe^{2+} + \frac{1}{4}O_2 + H^+ \rightarrow Fe^{3+} + \frac{1}{2}H_2O \quad (2)$$

$$Fe^{3+} + 3H_2O \rightarrow Fe(OH)_3 + 3H^+ \quad (3)$$

$$FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+ \quad (4)$$

Solid pyrite undergoes an oxidation process that results in the formation of dissolved ferrous iron ions (Fe^{2+}) along with two units of hydrogen ions (H^+) and sulphate ions (SO_4^{2-}) dissolved in water. These ferrous ions are further oxidised to form ferric ions (Fe^{3+}) upon exposure to air or dissolved oxygen. The acidity of the aqueous medium is enhanced through the direct reaction of ferric ions with pyrite minerals [10]. Furthermore, the formation of acidophilic bacteria reacts with ions to increase the acidity of the water. The ferric ion produced in Equation (2) precipitates as ferric hydroxide, [$Fe(OH)_3$], at pH values ranging from 2.3 to 3.5, in the presence of water, effectively reducing the concentration of Fe^{3+} and continuously lowering the pH value. Figure 2 illustrates that pyrite oxidation can occur through multiple pathways involving surface interactions with dissolved O_2, Fe^{3+}, and other mineral catalysts [3].

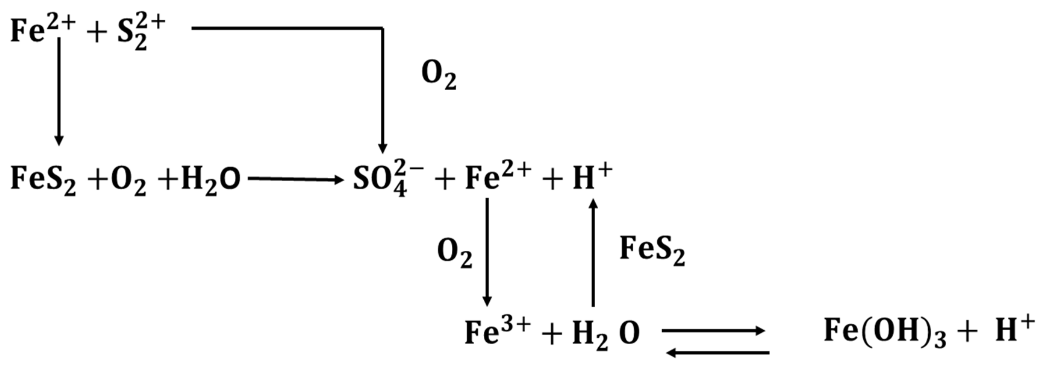

Figure 2. Model for the oxidation of pyrite minerals [4].

The environmental challenges associated with acid mine wastewater stem mainly from its low pH, which ranges from 2 to 4.5, and elevated concentrations of dissolved metals, which are potentially toxic. The extraction and refinement of valuable metals from mining sites generate substantial volumes of wastewater containing various impurities, including oil, grease, anions such as sulphate and hazardous elements such as Cr^{6+}, Hg^{2+}, As^{3+}, and Cd^{2+}. These contaminants have long-term adverse effects on the environment. Consequently, various regulations have been implemented to ensure that mining companies manage the waste they produce responsibly. As such, numerous countries dependent on mining operations face a critical need to treat hazardous mining wastewater, a process that incurs significant costs. Table 1 elucidates the composition of different acid mine effluent samples analysed in the aforementioned studies, providing a comprehensive overview of the chemical makeup of acid mine drainage (AMD) produced by prevalent mines worldwide. According to the findings, AMD close to mining sites exhibits a pH range of 2 to 4 and high sulphate concentrations, ranging from 1000 to 50,000 mg/L. It is evident that the composition of AMD varies considerably according to the source. Iron (Fe) is identified as the most dangerous dissolved metal, with Table 1 indicating its presence in substantial quantities (100–5000 mg/L). When iron interacts with dissolved oxygen in water, it forms precipitates of iron oxide, posing risks to aquatic ecosystems and disrupting the entire food chain. Additionally, the formation of ferric hydroxide precipitates can occur, further reducing the pH of the environment. The presence of heavy metals in the soil adversely affects plant life.

Table 1. The composition of acid mine drainage samples from the aforementioned studies.

pH	Sulphate (mg/L)	Iron (mg/L)	Zinc (mg/L)	Manganese (mg/L)	Aluminium (mg/L)	Copper (mg/L)	Reference
3.22	29.9	4.66	0.401	-	0.28	-	[11]
2.32	471.75	169.22	-	0.016	21.04	-	[12]
2.9	3500	750	-	100	50	10	[9]
2.29	4520	788	0.25	19.4	310	3.42	[13]
2	5800	70	320	5.5	210	-	[14]
2.53	5880	2143	8.71	43.2	3735	17.4	[15]
3	7550	2516.7	-	104.9	257	-	[16]
2.2	10,845	3867	410	120	216	515	[17]
2.3	11,700	744	976	467	251	165	[18]
2.6	13,200	4420	13.1	126	460	0.11	[19]
2.5	24,530	2490	500	6590	-	2670	[20]
2.1	28,980	6120	-	155	506	-	[21]
2.7	29,530	66	55	245	2317	65	[22]
2	30,000	8000	-	75	300	-	[2]
2.2	42,862	3867	410	120	216	515	[23]

Copper (Cu), zinc (Zn), manganese (Mn), and aluminium (Al) are also other vital metals present in AMD. The most common heavy metal that harms aquatic life is Cu [24]. When AMD contaminates groundwater, rivers, and lakes, it causes a number of environmental problems and poses major risks to human health. Numerous investigations have been carried out on the ecotoxicology of AMD [24–27].

Environmental Problem Caused by AMD

Acidity levels of AMD substantially exceed established discharge standards for industrial wastewater, resulting in significant environmental challenges, particularly when combined with other pollutants such as sulphates and heavy metals, as illustrated in Table 1 [28]. Acid mine drainage has a profound impact on ecosystems and food chains and leads to the loss of plant habitat and the extinction of terrestrial and aquatic species. The contaminants, predominantly toxic metals present in AMD, have the potential to induce teratogenic, carcinogenic, and mutagenic effects in living organisms upon exposure, affecting organisms inhabiting diverse environments, including soil, water, and air [27,29]. Exposure to harmful substances present in mining wastewater may disrupt plant metabolic functions, homeostasis, and ionic equilibrium, resulting in toxicological effects. Acidity from AMD causes a decrease in pH in the ecosystem, a higher mortality rates among ichthyofauna and flora and potentially necessitating species migration, if feasible [30]. Moreover, the presence of heavy metals, metalloids, anions, and radionuclides in AMD poses significant threats across various trophic levels through bioaccumulation in organisms and biomagnification within the food web. AMD has been associated with a spectrum of pathological conditions, including dermal lesions, hyperpigmentation, oncogenesis, and upper respiratory, pulmonary, gastrointestinal, and cardiovascular impairments, in addition to neurological damage and multi-organ failure [31]. The environmental impacts of AMD can be categorised into five distinct classes: chemical, physical, biological, ecological, and socio-ecological, as illustrated in Figure 3.

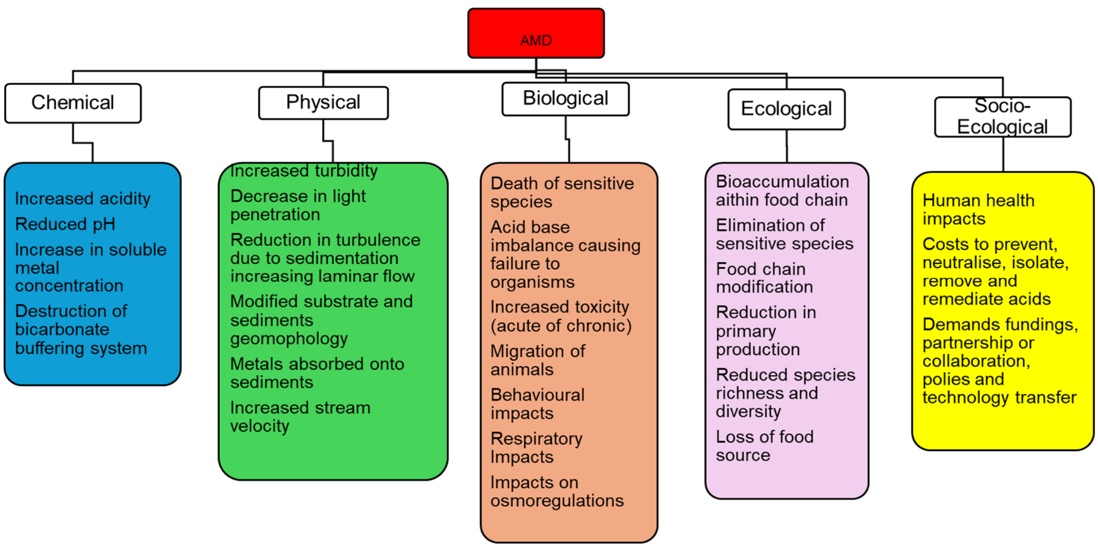

Figure 3. Negative impacts of AMD on the environment [20].

Acid mine drainage is a major continuing problem due to the irreversible nature of converting inactive ores into large quantities of toxic substances, hazardous waste, and the difficulty of treatment. To prevent and manage AMD, mining operations can employ a variety of methodologies, including capping, layering, and blending. However, once AMD has formed, the variety of alternative management strategies becomes significantly constrained [1]. Due to the acidic nature and elevated concentrations of toxic metals in AMD, the environmental and human health impacts have been extensively documented. It is important for AMD to undergo treatment before it is released into the environment, to attain acceptable standards because it does not comply with the drinking water regulations. Various regulated standards for metal concentrations in drinking water are established by the United States Environmental Protection Agency (US-EPA), the World Health Organisation (WHO), and the South African National Water Standards (SANS) 241, some of which are presented in Table 2.

Table 2. WHO, EPA, and SANS 241 standards for metals in drinking water [32].

Metals	WHO and EPA Limits (mg/L)	SANS 241 (2015) Limits (mg/L)
Fe	0.8	0.4
Cu	1.3–2	2
Mn	0.4	0.1
Al	0.1–0.2	0.3
Zn	0.3	5
SO_4^{2-}	250	250
Ca	30	30
Mg	10	0.05
As	0.01	0.01
Cr	0.05–0.1	0.05
Ni	0.02	0.07
Hg	0.006	0.006

Sulphate is generally considered to be inorganic, non-toxic, and stable; however, ingestion of quantities greater than 1000 mg/L can induce catharsis and dehydration [33]. Research has revealed that sulphate could subsequently have adverse effects. For example, elevated concentrations of sulphate have been shown to facilitate the release of phosphates bound to sediments, which may result in eutrophication. Sulphate-reducing bacteria (SRBs) also generate highly hazardous bioavailable methylmercury [34].

The sudden release of mine wastewater from the abandoned Kromdraai Coal mine site in Mpumalanga, South Africa, resulted in severe environmental impacts. Acidic mine wastewater was discharged into the Wilge and Klein Ollifant rivers in Mpumalanga eMalahleni, thus contaminating the surface water. This significant event transpired in February 2022, resulting in the contamination of more than 58 km of the river's biodiversity. According to the Mpumalanga Parks and Tourism Agency, this incident represented the most severe river ecology disaster in the past four decades, killing 23 native fish species [35]. Fortunately, some of these fish can still be found both upstream and downstream. Experts have projected that the rehabilitation of the impacted riverbanks and the restoration of lost fish species after the incident will likely require a time frame of 15 to 20 years [36]. It is crucial to continuously monitor, treat, and prevent the discharge of mine wastewater into the environment to protect ecosystems and human health from the enduring ramifications of this hazardous phenomenon. Considering the persistent legacy of abandoned mines, comprehensive management strategies are imperative to ameliorate the environmental degradation instigated by AMD.

3. Current Status

3.1. AMD Bioremediation Options

In recent years, scholars have investigated various methodologies and techniques employed for the effective remediation of AMD. There are two primary remediation strategies for AMD treatment, namely active and passive treatments. Numerous technologies have been developed for the treatment of acid mine wastewater, including chemical precipitation, coagulation and flocculation, electrochemical methods, membrane filtration, ion exchange, and adsorption [27]. However, these methods are characterised by substantial energy and chemical requirements. Treatment of wastewater with alkaline substances involves chemical processes such as neutralisation and ion exchange, which induce reactions between sulphur dioxide (SO_2^{2-}) and heavy metals, resulting in the precipitation of metal hydroxides and gypsum. However, these processes may result in secondary contamination and their cost is prohibitive due to suboptimal removal efficiency [37–39]. On the contrary, biological methods demonstrate remarkable advantages, characterised by reduced energy consumption, reduced sludge production, and the potential to recover valuable elements, compared to conventional treatment options, as shown in Table 3 [40].

Table 3. Comparison between SRB-based bioremediation and conventional treatment technologies such as lime neutralisation.

Parameter	SRB-Based Bioremediation	Conventional Treatment
Capital costs	Moderate to High	Low to Moderate
Operational costs	Lower (with passive systems)	Higher (requires continuous chemical dosing)
Metal Recovery Potential	High (metal sulphide)	Low
Energy requirement	Low (with passive bioreactors and bio-electrochemical systems)	Moderate to High
Long-term sustainability	High (self-sustaining systems)	Low (continuous cost of reagents)
Environmental Impact	Low substrates (biodegradable substrates)	High (generates sludge and secondary waste)

In the context of the remediation and transportation of acid mine wastewater treatment, bioremediation techniques can be classified into in-situ and ex-situ categories, as illustrated in Figure 4. In the application of in-situ bioremediation methods, it is imperative to manage contaminated materials directly at the site of pollution. This approach negates the need for excavation, thus maintaining the integrity of the topsoil structure with minimal disturbance. The absence of excavation-related costs makes these techniques potentially more cost-effective compared to ex-situ bioremediation methods [41]. However, a significant challenge lies in the financial implications associated with the design and installation of advanced on-site equipment to increase microbial activities during bioremediation. Examples of in-situ bioremediation methods include bioventing, biosparging, and phytoremediation. These techniques have shown success in the remediation of polluted sites contaminated with hydrocarbons, heavy metals, dyes, and chlorinated solvents [42].

Ex-situ bioremediation entails the removal of contaminated materials from polluted sites, followed by their transfer to an alternate site for further treatment. The selection of these methods is dependent on factors such as financial implications, extent of contamination, nature, and severity of pollutants, as well as the geographical and geological characteristics of the site [43,44]. Examples of ex-situ techniques include bio-piles, bioreactors, and land farming [44,45]. The advantages of ex-situ techniques include their minimal requirement for preliminary site evaluation, which simplifies and reduces the costs associated with the initial stages [46]. Given that pollutants are extracted, problems such as inconsistent pollution due to depth and varied concentrations can be effectively managed by adjusting parameters such as temperature, pH, and mixing. This adaptability allows for better regulation of the biological, chemical, and physical conditions essential for successful remediation [47]. Furthermore, once the contaminated soil is excavated, soil porosity, which can affect pollutant transport during remediation, becomes a lesser concern. However, it is noteworthy that ex-situ bioremediation is not always viable in certain contexts, such as areas beneath structures, concealed urban locations, or operational work sites [39].

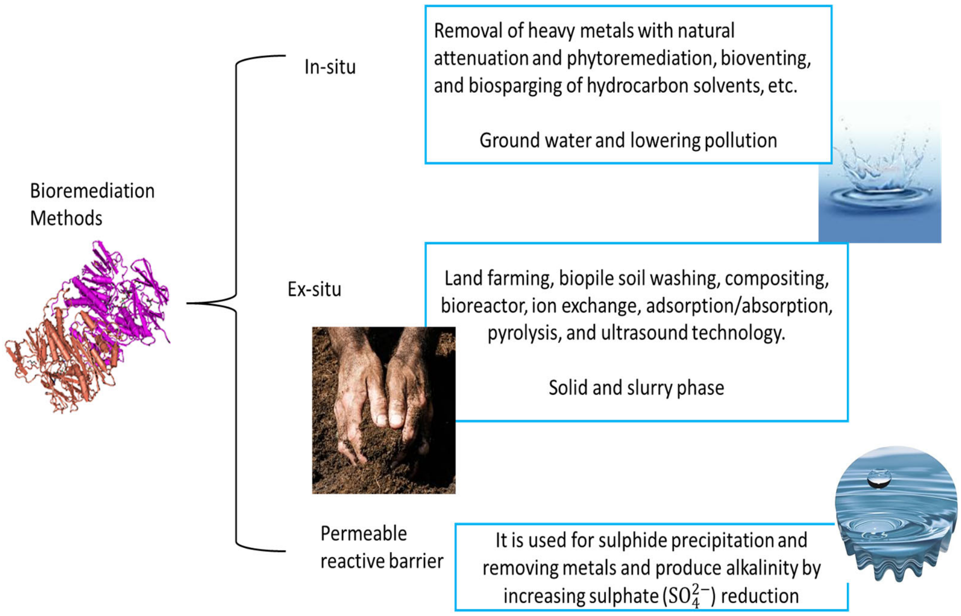

Figure 4. Categories of bioremediation techniques with their examples [39].

Research advances have clarified methodologies into abiotic and biological categories, with active and passive systems identified as subclasses. Instances of passive treatment systems include continuously stirred tank reactors, anaerobic contact processes, and up flow anaerobic filter reactors. In contrast, active treatment systems encompass the injection of substrates into the subsurface and the application of permeable reactive barriers. Biological treatment methods to address AMD have been developed over extensive research periods, concentrating on source control and mitigation strategies. Implementing source control methods poses technical challenges due to the necessity of removing oxygen and water to avert the oxidation of pyritic substances [48,49]. Furthermore, many mining operations ceased operations before the full implications of AMD were understood, making renovations challenging and frequently impractical [50]. Table 4 below compares different bioreactors for treating mine water using SRB, highlighting various approaches with unique advantages and limitations depending on operating conditions and goals (cost-efficiency, scalability, or environmental sustainability).

Table 4. Advantages and disadvantages of various bioremediation bioreactors.

Bioreactor Type	Environmental Conditions	Advantages	Limitations	Reference
Continuous flow bioreactor (Bio I and II)	Operated at 30 °C, pH 2.5, stirred at 40 rpm, nitrogen sparging, uses acidophilic SRB consortium	Higher efficiency (reduced sulphate to 4.7–19 nM from the initial 30 nM) under acidic conditions and stable operations over 302 days of operations.	Requires strict controlled conditions, and there is a moderate energy input for stirring and sparging.	[51]
Modular continuous flow bioreactor	Plackett-Burman bioreactor design, tested for both low and high concentrations of metals and sulphate	Enhanced tolerance for heavy metals (with efficiency of up to 99%), the modular is designed for optimization.	Limited sulphate removal of up to 58.89% compared to metal removal, the modular design is complex.	[52]

Table 4. *Cont.*

Bioreactor Type	Environmental Conditions	Advantages	Limitations	Reference
Passive-field bioreactor	Observed over 6 months with varying flow rates (6–130 L/h)	Effective for arsenic removal varied between 3–97%, stable biofilm formation, and suitable for sludge management.	Efficiency depends on flow rates and physicochemical parameters, and the treatment process is slow.	[3]
Passive bioreactors	Focusses on anaerobic SRB and reactive mixture compositions	Low operational costs, stable sludge formation, and minimal energy consumption.	Large land area required, slower reaction rates, and less effective in high-flow or acidic environment. Highly dependent on the composition of the organic carbon source.	[3]
Active bioreactors	Uses renewable material for greener mitigation solutions	Environmentally sustainable approach, not fully quantified but promising for advancements in the efficiency of sulphate and heavy metal removal.	Requires further research to optimise the use of renewable materials in bioreactors.	[3,53]
Fluidized-bed bioreactor	The product uses carrier materials for the formation of biofilms and recycles the effluent for fluidization	Improved biomass retention, suitable for both mesophilic and thermophilic conditions.	Requires strict control of fluoridation and biofilm formation, potential clogging issues.	[54]
Up-flow anaerobic sludge blanket bioreactor	Requires a granular sludge bed, upward flow of wastewater through the sludge blanket, and	Maintain stable pH for the effluent, can remove nearly 100% Fe, Zn, Co, and Cu, and can be used simultaneously with other treatment technologies.	Requires careful management of granular sludge, potential for washout if overloaded.	[55]

Although using SRB-based bioremediation provides a sustainable method for addressing AMD, it also poses environmental concerns, mainly because of the generation of H_2S. The effect of sulphides on the growth of SRBs depends on their concentration. Increased levels of sulphide promote the transformation of sulphur ions into H_2S. Furthermore, too much sulphide can be harmful, negatively affecting the growth and efficiency of SRB bioreactors [56]. There are two main approaches to the biological treatment of AMD: directly treating AMD or preventing leaching while rehabilitating areas already affected by AMD. Bioreactors and wetlands have been shown to efficiently treat AMD before it is released into the environment, thus preventing leaching [57]. Regarding remediation, methods such as algal and microbial treatments, in addition to the use of wetlands, are the most well-documented strategies for successfully managing AMD-contaminated environments.

3.2. Bioremediation Process Using Sulphate-Reducing Bioreactors

Bioremediation uses biological systems, including plants, animals, and microorganisms, to remediate harmful contaminants from aquatic environments. Microbe-assisted bioremediation has been widely used for the removal of heavy metals from wastewater [58].

Bioremediation entails the utilisation of microbes to degrade contaminants under controlled environmental conditions to a level that is less toxic and acceptable. The bioremediation process is based on the natural microbial capacity to perform the mineralization of organic compounds, which ultimately yields CO_2, H_2O, and biomass [59]. Traditional methods for the removal of heavy metals are often more affordable and can negatively impact aquatic systems. A researcher [60] demonstrated that bioreactors colonised by sulphate-reducing bacteria (SRB) have benefits such as reduced operational costs and minimal maintenance expenditures. Accordingly, microbe-assisted bioremediation and phytoremediation are cost-effective treatment strategies for the elimination of toxic metals. The substrates utilised in anaerobic sulphate reducing bioreactors may include soil, peat moss, mushroom compost, sawdust, manure, hay bales, or other organic carbon-based substances, often combined with limestone. The wastewater permeates the organic subsurface stratum, which is characterised by its density and porosity, resulting in oxygen depletion attributable to elevated biochemical oxygen demand. The insufficient oxygen conditions facilitate the reduction of sulphate to sulphides by bacteria, consequently forming insoluble metal precipitates such as FeS_2. Furthermore, reduction of microbial sulphates produces alkalinity, thus inducing metal precipitation as oxyhydroxides [50,61]. The sorption onto organic materials represents an additional potential methodology for the removal of metals from AMD. The ability to remove metals is constrained by the presence of adequate functional groups within the substrate; nevertheless, alkalinity (resulting from limestone dissolution or reduction of sulphate), the availability of sulphate and the presence of a community of sulphate-reducing bacteria are an essential factor [62].

Biochemical reactors operate by cultivating sulphate-reducing bacteria (SRB) that transform sulphate in mine wastewater into sulphide, which subsequently reacts with metals, resulting in the precipitation of metal sulphides. These metal sulphides become immobilised within the reactor, thereby effectively being removed from the water. Microorganisms generate substantial alkalinity in the form of bicarbonate, reducing the acidity of the reactors [63]. The SRB synthesizes hydrogen sulphide (H_2S) gases from the degradation of sulphate accompanied by various sources. The production of H_2S can be recovered by injecting it back into the precipitating bioreactor, allowing it to interact with heavy metals in the wastewater [63].

Microbes help electrons move from electron-rich substrates to oxidants such as oxygen or sulphate so that metabolic activities can generate energy. The oxidation of the substrate (electron donor) occurs simultaneously with the reduction of sulphate (terminal electron acceptor). SRB utilises the resulting energy for growth and development. This reaction is typically represented as follows:

$$2CH_2O + SO_4^{2-} \rightarrow S^{2-} + 2CO_2 + 2H_2O \tag{5}$$

$$S^{2-} + 2CO_2 + 2H_2O \rightarrow 2HCO_3^- + H_2S \tag{6}$$

CH_2O represents the main organic carbon source. Dissolved inorganic carbon raises pH and promotes precipitation of metal carbonate minerals. The soluble sulphides such as H_2S, HS^-, and S^{2-}. Metal sulphide precipitates form by reacting with the metals. They oxidise low molecular weight substrates (such as hydrogen, acetic acid, lactic acid, ethanol, etc.) to produce CO_2 as a complete oxidation product and acetic acid salt as an incomplete oxidation product [64].

A basin full of substrate is called a sulphate-reducing bioreactor. To regulate the water depth, water is supplied at the top or bottom, travels through the substrate, and gets released at the other end through the discharge pipe that is placed at the bottom or top of the reactor. In addition, a liner is integrated underneath the substrate, as seen in Figure 5.

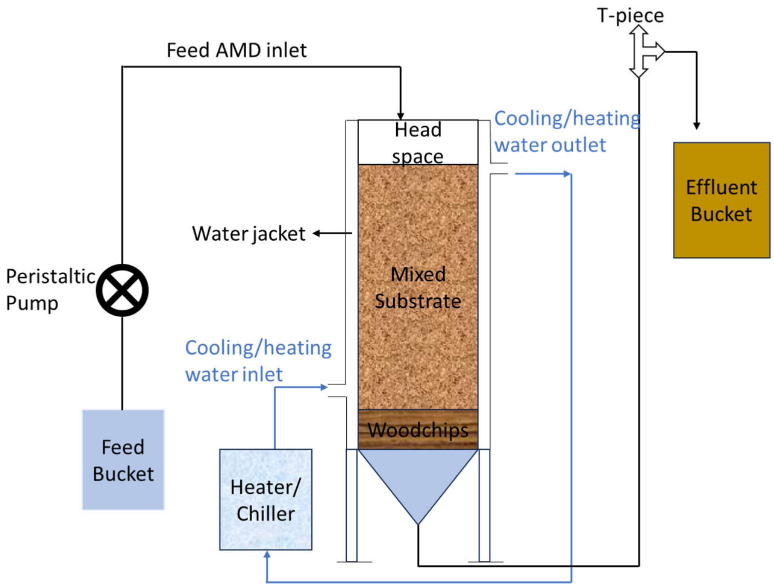

Figure 5. A schematic diagram of the bioreactor filled with substrate [65].

The sulphate-reducing bioreactor consists of a properly designed shallow basin with a subsurface flow of AMD. Chemical reactions in biological sulphate reduction, enabled by Desulfovibrio, Desulfotomaculum, and Desulfobacterium bacteria, have been studied using various bioreactor designs, such as batch bioreactors, sequencing batch bioreactors, continuously stirred tank bioreactors, anaerobic contact processes, anaerobic baffled bioreactors, anaerobic filters or packed bed bioreactors, fluidized-bed bioreactors, gas lift reactors, up-flow anaerobic sludge blanket reactors, anaerobic hybrid reactors, and membrane bioreactors, as mentioned in Table 4.

3.3. Effectiveness of Current Bioremediation Methods Using Sulphate-Reducing Bioreactors

Dynamic experiments revealed that SRB methods facilitated significantly higher sulphate removal rates of up to 93.97% and heavy metals such as copper and zinc [66]. The successful application of passive treatment systems, such as biological sulphate reactors, demonstrates both high efficiency and eco-friendliness in the removal of contaminants from acid mine wastewater. Ref. [63] has proven that anaerobic up flow reactors are effective, as they recovered 100% sulphate, 95% nickel, and 63% copper at an operation time of 0–178 days, pH = 7, a temperature of 25 °C, and 500 mL of mine wastewater. Ref. [67] used the biogenic dissolved sulphide produced in an FBR to gradually precipitate Cu, Zn, Ni, and Fe from leaching liquors produced by bioleaching of slag. Cu and Zn precipitated preferentially with 98% and 99% efficiency at pH 2.8 and 3.9, respectively, while Ni and Fe co-precipitated with efficiencies of approximately 63% and 28% at pH 6.5 [62]. Another work by [68] showed the use of rice bran which contained Zn (15 mg/L), Cu (5 mg/L), Cd (0.06 mg/L), and SO_4^{2-} (155 mg/L), as an electron donor and carbon source for semi-passive sulfidogenic for AMD treatment. Metal recovery was almost 99%, because effluent Zn, Cu, and Cd concentrations were <0.33 mg/L, <0.08 mg/L, and <0.005 mg/L, respectively. This demonstrates that AMD can be effectively treated using conventional, bioremediation, and combined techniques. More studies showing the effectiveness of anaerobic reactors in the treatment of acid mine wastewater are shown in Table 5.

Table 5. The effectiveness of various SRB-based bioremediation technologies for the treatment of acid mine wastewater.

Treatment Technology	Constant Variable	Electron Donor	Effectiveness of Technology	Reference
Biological sulphate-reducing column	Temperature = 30 °C, HRT = 14 days and pH = 5.5	Sodium lactate	Sulphate reduction of 79.04%; 64.78%; and 50.27% using chicken dairy manure and sawdust as organic substrates, respectively. 5% of sulphide precipitation and over 95% of heavy metals.	[69]
Sulfidogenic fixed-bed column bioreactor	Temperature = 30 °C, HRT = 7 days and pH = 4.5	Ethanol	Recovery of Sb at 97.8%; As 98.2%; and Fe(ii) was recovered at 85%.	[70]
Sulphate-Reducing Bacteria Cu/Fe Reactor	Temperature = 37–45 °C; pH = 1, HRT = 48 h COD rate of 27.4 mg COD/(Lh) and nitrate rate of 17.4 mg N/(Lh)	Sodium lactate	High heavy metal removal efficiency such as Cu^{2+} at 98.17; Zn^{2+} 99.67; and Cu at 7.5–10% pH increase rate = 5–9.	[71]
Biochemical Passive Bioreactor	Temperature = 35 °C, pH = 4, and HRT = 2 days	Mushroom compost, limestone, and cow manure	Sulphate recovery of greater than 60% and metal ions such as $Fe^{2+}$and Zn^{2+} of around 95%.	[72]
Sulphate-Reducing Wetland Bioreactors	pH = 6.5 HRT of 6 days Lignocellulosic waste as a substrate	Lactate	Sulphate reduction of 60.7%, with COD removal of 70.6% and recovery of valuable metals such as Fe 99.6%; Zn 99.4%; Mn 9.3%; Cd 99.9% and Cu 94.5%. pH increase rate = 6.5–7.7.	[21]
Downflow Structure Bed Bioreactor	The temperature was kept at 30 °C, pH = 4, and HRT = 19 h	Sugar (sugarcane)	Sulphate removal rate of 55–91%, 80% removal for Co, Ni and Zn; Cu 73%, Fe 70%, and Mn at 60%. pH increase rate = 6.7–7.5.	[73]
Fluidized bed bioreactor	Temperature = 20 °C, pH = 7–8, HRT = 10 h, and COD/sulphate rate = 2.5 − 1.7	Glycerol	Sulphate removal rate of 80–92%, COD removal rate of 58%, and recovery of metals such as Ni, Mn, and Cu at a rate of 90%.	[74]
Sulphate Reducing Bioreactor	Temperature = 30 °C, pH = 5, and HRT = 6 h	Iron sulphate	Metal iron recovery such as Zn(ii) 88.5%; Pb(ii) 92.6%; Cu(ii) 76.0%; Mn(ii) 62.2%; Fe(iii) 56.9; Cd (ii) 78.7% and Ni(ii) 62.5. pH increase rate = 6.5.	[75]

According to current studies, bioremediation may be one of the most effective approaches to managing environmental pollution caused by AMD [76]. Ref. [77] discovered an acid-resistant sulphate-reducing bacterium known as S_4 in the mud of the Vietnamese AMD storage tank. This bacterium showed tremendous promise for the remediation of metals (Fe, Zn, and Cu) in solution and sulphate [78]. A strain of SRB was isolated from a fumarole in Iceland, in a mixed solution containing 0.75 g/L iron, 0.20 g/L zinc, and 0.080 g/L copper, and demonstrated outstanding potential for reducing sulphates. Ref. [79] showed that peanut shells treated with an alkaline solution as a carbon source and had a good removal effect of SO_4^{2-} in solution and SO_4^{2-} biological reduction load of 140,61 mg/g. According to [80], SRB effectively removed Pd(ii), Cd(ii), and Ca(ii) from solution, while sediments such as Pbs and CdS remained persistent in wastewater.

They showed that metals from AMD could be efficiently removed by using bagasse as an electron donor for SRB, with a removal rate of 80%, 73%, and 60% for Zn, Cu, and Mn, respectively, and 55–99% reduction of SO_4^{2-}. Similarly, SRB demonstrated a significant capacity to remove Pb(ii) from the solution. Hundred percent of Pb(ii) was removed by SRB at concentrations of 10–50 mg/L. Pollutant leaked from the tailings could be immobilised by SRB treatment. The capacity of the SRB to remediate pollutants decreased as more tailings were added to each layer. SRB demonstrated the greatest impact on tailing sand treatment when 1 cm of tailing sand was applied to each layer. After treatment, the leachate of 1 cm tailing sand showed immobilization rates of SO_4^{2-}, Fe(iii), Mn(ii), Zn(ii), Cu(ii), and total Cr of 95.44%, 100%, 90.88%, 100%, 96.20%, 86.23%, and 93.34%, respectively [81]. These studies make bioremediation technologies more attractive for the treatment of acid mine wastewater.

Although bioremediation has proven viable for treating contaminated sites, it still has some drawbacks. For example, a biological mechanism requires the presence of active species that are suitable for the growth of microorganisms and can be extremely sensitive [82]. Another drawback is that when bioremediation is compared to other treatment options, it takes longer time. It can be slower with little or no nutrient alteration [83]. The sustainability of AMD prevention and treatment techniques refers to their ability to continuously prevent or treat AMD over time, taking into account the cost and possible secondary environmental and human health effects. However, the effectiveness of these remediation procedures is affected by a number of various parameters such as the type and quantity of heavy metals, duration of treatment, nutrient or substrate, ratio of addition of nutrients, and environmental or operating conditions (such as temperature, pressure, and pH), as shown in Table 4.

3.4. Factors That Affect the Capacity of the Bioreactor That Reduces Sulphate

3.4.1. pH

pH is one of the key factors influencing metal adsorption in the biomass of microalgal. The pH dependence of metal intake is tightly linked to the acid-base characteristics of different functional groups on the surface of microalgal cells. Based on the pH needed for growth, SRB can be separated into neutrophils and eosinophils [84]. Mining wastewater is often acidic with a pH < 3, with a high concentration of metal ions. Therefore, there will be competition between hydrogen and metal ions. The pH of 6.8–7.2 is ideal for the growth and operation of SRBs, while a pH less than 5 is not suitable for SRB activity and sulphate reduction [85].

Neutrophophilic SRB functions best in a pH range of 7–7.8. The sulphate reduction rate is achieved in the pH range between 7.0 and 7.5. When the pH is greater than 9 or lower than 5, inhibition is seen and when the pH is less than 2, there is no activity. At pH levels between 2 and 4, eosinophilic SRB can survive and even thrive [86]. The relationships between various pH levels and SRB activity are shown in Figure 6. The pH is raised by the generation of HCO_3, and lowered by the presence of H_2S and acetate. pH variations substantially impact on SRB activity, thereby affecting sulphate consumption and the synthesis of acetate and H_2S. Thus, the elimination of heavy metals in the SRB is significantly impacted by pH regulation [87].

An alkaline substrate is added to influent water to treat acid mine wastewater using an anaerobic sulphate reduction reactor to adjust the pH level to 7. Alkaline materials cause metal precipitation prior to SRB enhancement [88]. Metal precipitation is caused when sulphide minerals produced by SRB combine with heavy metals present in acid mine wastewater, and metals such as Fe, Cu, Zn, and Ni cannot precipitate well at pH 7, but can precipitate fully and efficiently at pH more than 9.5 [84]. According to the solubility (ksp) of the metal sulphides, Cu is first eliminated, then Zn, Ni, Fe, and finally Mn. The elimination of Mn has been demonstrated to depend on a pH level greater than 8 [89].

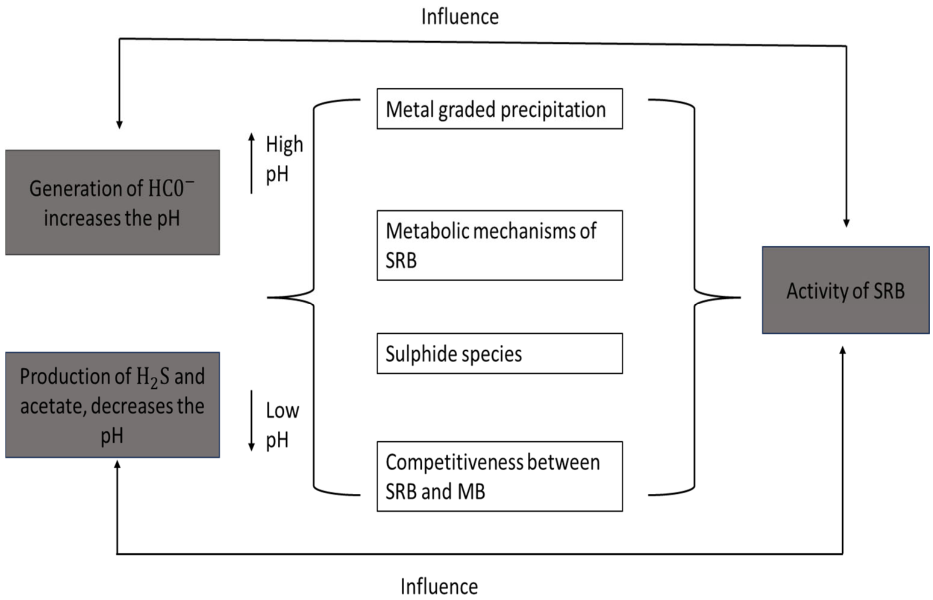

Figure 6. The influence of pH levels on SRB activity [21].

Numerous investigations demonstrated that a strong acidic atmosphere would reduce the ability of the sulphate reduction system to operate. According to a study by [90], an anaerobic packed-bed bioreactor showed satisfactory performance with an influent pH of AMD of 2.8 and high metal concentration (Fe 463 mg/L, Mn 79 mg/L, Cu 76 mg/L, Zn 118 mg/L, and Cd 58 mg/L). The pH of the effluent ranged from 7.8 to 8.3 with a recovery of more than 99.9% for Fe, Cu, Cd, and Zn and a recovery of 42.1–99.3% for Mn. Ref. [91] also highlighted that SRB reached a decrease rate of approximately 553–1053 $molm^3/day$ at a pH of 4.0 to 6.0. Furthermore, SRB has shown to be less efficient in producing alkalinity below a pH of 3, but it can tolerate ethanol feed at a low pH of 2.5. A lower pH increases the concentration of free H_2S, which can impact the development and activity of SRB. The configuration and diameter of the biomass aggregates are influenced by pH, and a high pH > 8.0 promotes SRB over methanogens.

When pH increases, the isolated SRB is highly dependent on biosorption and bioprecipitation. The impact of biosorption and bioprecipitation decreases when the pH is higher than the optimum range. Additionally, changes in pH can negatively affect various microbial metabolic processes, such as homeostasis and dissociation of electron donors [89]. Therefore, controlling the pH is crucial to utilise electron donors since it can impact how the treatment goes and how well electron donors operate energetically. Substrates such as glycerol, alcohols, hydrogen, and sugars are reportedly more effective for fermentation at low pH levels. Reactors operate at low pH and provide several benefits in addition to metal recovery. They also reduce costs by limiting the use of AMD neutralising agents (lime and limestone, ammonium and magnesium hydroxide, and soda ash). Since the generated sulphide is primarily in the gaseous phase at low pH, it is easier to separate it from the effluent using established processes, such as elemental sulphur oxidation with oxygen or subsequent metal precipitation. Furthermore, because methanogens are more sensitive to low pH, SRB will be able to outcompete them successfully. Therefore, the process is overall more efficient because less of the expensive electron donor is wasted in other processes (acetogenesis, methanogenesis) than in the reduction of sulphates [92].

3.4.2. Substrate

The substrate used in sulphate-reducing bacteria-based bioreactors is crucial in treating AMD. Typically, SRB uses basic carbon compounds such as organic acids and alcohol as electron donors for sulphate reduction. The growth and activity of SRB are significantly influenced by the type and availability of organic carbon sources, such as lactate, acetate, ethanol, and glucose, which serve as energy sources for sulphate reduction. The presence of inorganic electron donors such as hydrogen (H_2), nitrogen gas, and sulphide enhances the activity of SRB. Many studies have shown that the overall activity and growth of SRB in the long run are influenced by organic substances (organic waste and nitrogen sources) [40,93,94].

The substrate concentration and composition affect the pH, temperature, and metal toxicity levels which can affect the performance of SRB to reduce sulphate. The reactive mixture that is typically the most efficient contains two different sources of carbon in cellulosic materials such as wood chips, sawdust, as well as organic waste (leaves, manure, and compost) [95]. Furthermore, the use of cellulosic waste as a carbon source combined with supporting substances such as gravel, sand, and sediment in the bioreactor has been shown to improve the circulation of the sulphate-rich AMD that is being treated [96]. Figure 7 shows the microbial processes that play a vital role in SRB activity. The figure shows that cellulose is fermented by bacteria and other cellulose degraders, gradually breaking down into simpler organic compounds. A reasonable degradability rate of the cellulose material can determine a higher sulphate reduction rate in the mine wastewater.

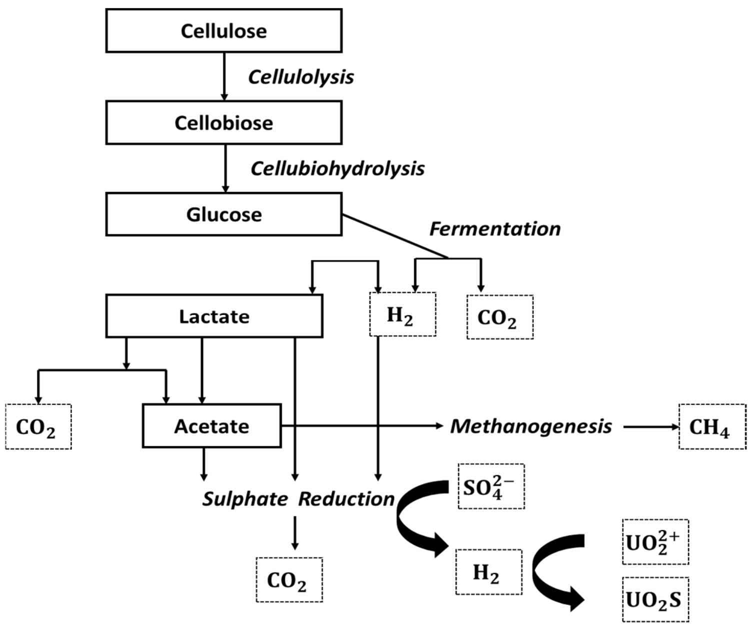

Figure 7. Microbial processes that affect sulphate reduction in the organic carbon substrate [97].

The carbon supply can be handled more effectively by a single or a combination of several liquid substrates. The liquid substrate utilises SRB more easily than the solid substrate consisting of organic compounds that enable acidic mine wastewater treatment to have higher reaction rates. A higher reaction rate allows smaller reactors to operate effectively, especially when site space limits the bioreactor size. To reduce operational costs, it is best to use local organic waste. Numerous research has used organic waste as carbon sources for the growth of SRBs, such as sawdust, cornhusks, rice bran and straw, wood chips, and animal manure with positive results [3,64,68,97–99]. These solid organic materials also provide nutrients, metal adsorption, and microbial attachment, potentially increasing the effectiveness of sulphate removal [100].

3.4.3. Sulphide Concentration and the Ratio of COD to Sulphate

Sulphide (S^{2-}) is a byproduct of SRB metabolism, and its concentration can significantly affect SRB activity and growth. High concentrations of sulphide are toxic to SRBs and other microorganisms. Sulphide toxicity can inhibit SRB activity by disrupting cell membrane function, enzyme activities, and overall cellular metabolism. Free hydrogen sulphide is typically the most toxic form, followed by bisulfide and sulphide. Furthermore, due to its low sulphur value, sulphide concentration had a lower capacity, which decreased some metals and increased toxicity [101]. The findings of a study by [102]; indicate that the ratio of COD to SO_4^{2-} is influenced by the performance of SO_4^{2-} reduction rate at a COD of 1.0 and at a ratio $\geq$0.67, the organic matter gets eliminated completely. The oxidation of sulphate to sulphide needs disbanded oxygen concentrations lower than 0.1 mg/L^{-1} in the sulphide-oxidizing reactor.

A review by [64] showed that the ratio of COD to sulphate in the environment significantly affects the metabolism of SRBs. COD represents the amount of organic matter available for microbial metabolism, while sulphate is the electron acceptor in SRB metabolism [40]. A high ratio of COD or sulphate indicates an abundance of organic substrates relative to sulphate [103]. Under these conditions, SRBs have sufficient electron donors (from COD) to efficiently reduce sulphate to sulphide. SRBs may outcompete other anaerobic microorganisms, such as methanogens, when the COD and sulphate ratio is high because they can utilise both organic matter (COD) and sulphate. A COD ratio ranging between 0.67 and 2.0 g is often considered optimal for effective sulphate reduction. Within this range, SRBs can effectively reduce sulphate without excessive competition for substrates. The COD or sulphate ratio, which influences the competition between SRB and other microbes, indicates how well heavy metals and sulphate gets removed. Because they share a common carbon source for both their evolution. Sulphate received 100% of the electron when the ratio of COD or sulphate is less than the theoretical value of 0.67. The opposition of SRB for the common electron donors with other microorganisms such as methanogenic archaea increases when the ratio is >0.67 g [64].

Ref. [104] documented that there is an efficient reduction in sulphate and total COD when the bioreactor performance is exhibited in a COD or sulphate range of $\geq$2. The results showed a greater removal of total COD at 73.5–80.3% and of sulphate at 82.6%. It was also proven that at a lower COD or sulphate range < 2 methanogenesis would be suppressed through electron competition and sulphate inhibition, thereby deteriorating conversion [105].

3.4.4. Metal Concentrations

The most significant obstacle to the growth and activity of SRB in practical technical applications is the inhibition of metal ions and oxidising agents. Heavy metals and SRB are commonly recognised to negatively impact microorganisms in anaerobic environments. Heavy metals typically have an inhibiting effect because they are difficult to biodegrade [106]. SRB relies on specific enzymes such as sulphate acetyltransferase, APS reductase, sulphite reductase, hydrogenase, format dehydrogenase, and acetyl-CoA synthetase for the reduction of sulphate to sulphide. Heavy metals can cause competition by binding to enzymes and interact causing inhibition [107]. High concentrations of metals like Ca^{2+} and Mg^{2+} may increase the dominance of SRB. The toxicity of heavy metals such as Zn, Fe, Cu, and Mg may have an impact on the growth and performance of SRB. The concentration of heavy metal ions in SRB ranged from Cu 2–50 mg/L, Zn 13–40 mg/L, Pb 75–125 mg/L, Cd 4–54 mg/L, 10–20 mg/L, Cr 60 mg/L, and Hg 74 mg/L [8]. Although, depending on the SRB strain used, this concentration range corresponds to variation.

A high concentration of heavy metals can cause death by inhibiting the growth and performance of SRB (i.e., reducing its capacity to remove sulphate). Therefore, this shows that understanding the mechanisms of heavy metal toxicity and the adaptive strategy of SRB is important to improve bioremediation processes. Implementing pretreatment steps, optimising reactor conditions, selecting resistant SRB strains, and supporting biofilm formation are essential strategies to alleviate the impact of heavy metals and enhance the effectiveness of SRB-based bioremediation of AMD. Metals improve sulphate reduction metabolism by decreasing microbial communities' inhibition of sulphide. Metal precipitates feed on dissolved sulphide during accumulation, causing an imbalance in the chemical equilibrium of the sulphate reduction to sulphide and altering the thermodynamics of the reactors to favour product formation [108,109].

3.4.5. Hydraulic Retention Time

Hydraulic retention time (HRT) is the measurement of how long it typically takes for a compound to remain in a bioreactor. The formula HRT = V/h illustrates it; where v is the total volume of the bioreactor and h is the quantity of chemical (substrate) inside the bioreactor and HRT measured in mL/day or mL/hour. When using a direct organic substrate, it takes roughly 3 to 5 days for sulphate metals to precipitate. But an indirect carbon source allows for double-timing; for example, microorganisms can precipitate metals in AMD over a period of 7–10 days [110]. The primary factor influencing maximum biomass production is SRT. SRT > HRT does not improve productivity at light inadequate levels, while SRT < HRT allows for a reduction in water and nutrient intake [111].

HRT has an impact on reactor efficiency, as it affects both microbial activity and the mix of reactants. To keep an SRB bioreactor running smoothly, it is essential to maintain a balance between breaking down organic matter and reducing sulphate. This balance is influenced by factors such as HRT, microbe metabolic processes, and the structure of the microbial community [109]. Ref. [109] demonstrated that increasing the sulphate loading rate (SLR) to 2.25 g $SO_4^{2-}1^{-}d^{-1}$ and decreasing the HRT from 24 to 16 h improved the sulphate removal to 92.1% with 80% reduction in the chemical oxygen demand (COD). However, when the HRT and SRL were adjusted to 14 h and 2.6 g $SO_4^{2-}1^{-}d^{-1}$, respectively, the sulphate reduction decreased from 80%. Regardless of the circumstances, sulphate reduction oxidised organic materials by more than 50%. However, ethanol treatment for AMD was more efficient when HRT was at 16 h. This shows that decreasing retention time negatively affects the process of sulphate-reducing bacteria-based bioreactors. Therefore, when HRT is too short, the contact time between AMD and SRB within the bioreactor is insufficient, resulting in incomplete reduction of sulphate, resulting in suboptimal removal of metals and sulphate from the effluent [112].

A study by ref. [113] showed that enriching SRB improves the performance of sulphate reduction. The synergy between SRB Proteobacteria and facultative Bacilli is demonstrated by the microbial community. The study also found that after 7 days of continuous operation, there was an 85% decrease in cadmium. An optimal HRT supports the formation of metal sulphides, which are less soluble and can be easily removed from the water. To remove toxic metals such as Fe, Cu, and Zn from AMD, this precipitation is essential.

3.4.6. Temperature

SRBs are well known to be mesophilic and can operate in the temperature range of 20–45 °C [40]. Within this range, SRBs reproduce more quickly, raising overall biomass and enhancing the sulphate reduction process. The suitable temperature for moderately temperature resistant SRB should be kept between 40 and 60 °C. Thermophilic SRBs have an optimal growth temperature of between 65 and 70 °C, and only in marine hydrothermal environments can thermophilic SRBs grow at 80 °C [114]. To effectively break down complex organic substrates into simpler molecules, other anaerobic bacteria must be active. In addition, methanogens prefer mesophilic environments to thrive in and are sensitive to low temperatures. Therefore, it is anticipated that seasonal variation in biogenic H_2S production will occur.

Temperature influences the efficiency with which SRB can utilise organic carbon sources, such as lactate, acetate, or ethanol, commonly used in AMD treatment systems. Higher temperatures enhance substrate uptake and conversion into energy and cellular components. However, if the temperature is too low, substrate metabolism slows down, reducing the efficiency of sulphate reduction. The reduction of sulphate to H_2S is the core metabolic activity of SRB. This process is highly temperature-dependent. At lower temperatures, the reduction rate is slower, which may lead to incomplete metal precipitation and less effective AMD treatment.

4. Challenges, Future Perspective, and Research Potential

4.1. Challenges Faced in the Bioremediation of Acid Mine Wastewater

Currently, one of the predominant challenges is the absence of a unified, reliable, and effective methodology for the treatment of AMD. This challenge has garnered significant interest from scholars worldwide who are in pursuit of practical and efficient strategies for managing and controlling AMD. The primary objective of AMD treatment is to minimise the production of acidic wastewater. As stated by [64], bioremediation is an environmentally sustainable approach that has demonstrated potential in the management of pollutants such as SO_4^{2-} and heavy metals. However, several challenges and problems must be resolved due to the complexity of the response of microorganisms and the wide range of toxicants in mine wastewater. Every day, the mining industries discharge an increasing amount of SO_4^{2-} and its by-product into water supplies. Wastewater contains a variety of toxins, some of which can occasionally be too toxic even for highly tolerant SRBs to tolerate for the bacterial community. SO_4^{2-} molecules form connections with other harmful substances, which makes it challenging for SRB to eliminate them from wastewater.

Large-scale bioremediation is not yet feasible, and industries are still hesitant to employ it. To ensure successful treatment, bioremediation techniques require metabolically active microbial species, ideal growth conditions, and chemically appropriate nutrients. It is difficult to transfer the mechanism from a pilot scale to a larger-scale treatment purpose. This type of technique takes longer to evaluate with other treatment options.

Various bioremediation techniques have been shown to be successful and economical in cleaning up places contaminated with different types of toxins (such as sulphates and heavy metals). Given the significant role of microbes in bioremediation, any bioremediation technique can benefit from understanding the variability, quantity, and community structure of these organisms in contaminated sites. Still, other environmental conditions that could hinder microbial activity must be optimised. For bioremediation techniques to be successful, they require metabolically active microbial species, optimal growth requirements, and chemically suitable nutrients. Therefore, investigating the principles of SRB metabolism is essential to increase SRB activity. Furthermore, various environmental factors can impact the metabolic activity of SRB, including changing the ideal environment, synthesis and improvement of inhibitors, and synergistic/antagonistic interactions.

4.2. Potential and Technologically Innovative Solutions to Consider

Emerging technologies, such as nanotechnology and genetic engineering, are revolutionising the application of sulphate-reducing bacteria (SRB) in the treatment of AMD, offering promising solutions for enhanced bioremediation efficiency and sustainability. Nanotechnology applications such as nanoparticle-enhanced bioreactors, metal nanoparticles (for example, iron oxide, titanium dioxide, biochar-based nanocomposites, and zero-valent iron) improve electron transfer, enhancing SRB metabolic activity, and sulphate reduction rates [115]. Nanoparticle-based adsorbents have been widely used for the removal of hazardous pollutants in industrial effluents, which facilitate better microbial activity by providing a stable surface for biofilm formation. Zero-valent iron (ZVI) has been widely studied for its ability to reduce sulphate and immobilise heavy metals through redox reductions. Compared to other remediation methods, this approach provides an overall reduction in contaminant levels; however, it is still under research with limited field application [115]. Genetic and metabolic engineering of SRB has significant potential to improve bioremediation efficiency, but they remain underexplored. Recent advancements have focused on the modification of SRB to enhance their sulphate reduction capabilities, increase their tolerance to extreme environmental conditions, and strengthen their resistance to heavy metal toxicity. For example, research by [116,117] has effectively incorporated genes encoding stress response proteins, allowing SRB to thrive in highly acidic and metal-saturated environments using the CRISPR-Cas9 system. Future investigations should explore synthetic biology approaches (CRISPR-Cas9) to enhance SRB metabolic pathways, making them appropriate for extensive AMD treatment applications.

The economic feasibility of SRB-based AMD treatment depends on capital costs, operating expenses, substrate availability, efficiency, and long-term sustainability. Key economic factors to consider for effective SRB-based bioreactor operations.

4.2.1. Capital Investments

The initial capital investment to implement the SRB-based AMD treatment system is an important factor that influences the economic feasibility. The costs initially include bioreactor construction, depending on reactor type (i.e., passive, active, and hybrid), specialised reactor tanks, piping systems, controlled units for maintaining anaerobic conditions, installation of pH, sulphate, and metal sensors, and computational models. Passive systems, such as constructed wetlands and bioreactors, have lower initial costs but require large areas. The size and design of the bioreactor significantly affect capital costs. Active bioreactors such as fluoridised-bed and up flow anaerobic sludge blanket reactors are costlier, as they require higher initial investments but are more efficient [118]. SRB bioreactors have a higher upfront cost compared to conventional chemical treatments such as lime neutralisation, but their long-term benefits in reducing chemical reagents, dependency, and sludge disposal costs make them an attractive investment [119].

4.2.2. Operational and Maintenance Costs

Operational and maintenance costs include energy consumption, substrate supply, system monitoring and replacements, and periodic maintenance. One of the primary operational costs in the availability and cost of organic substrates such as ethanol, lactate, molasses, glycerol, and organic waste are essential for the metabolism of SRB [120]. These traditional substrates can be expensive; therefore, researchers can explore the use of agricultural wastes, brewery effluents, and industrial by-products as cost-effective alternatives. The use of skilled personnel for system operation, microbial health monitoring and troubleshooting, and AI-driven monitoring systems can help reduce labour costs over time. Maintenance includes periodic removal of sludge, biofilm management, and the treatment of potential filter clogging problems in bioreactor systems [121]. Strategies to reduce costs include optimising HRT, utilising renewable energy sources such as solar powered aerations, and implementing automated control systems to minimise manual intervention.

4.2.3. Treatment Efficiency and Scalability

Precipitated AMD metals such as Zn, Cu, and Ni can be recovered and sold, therefore offsetting treatment costs by generating revenue [122]. Hybrid treatment approaches in which SRB-based bioreactors are integrated with constructed wetlands, electrochemical precipitates, and nanotechnology can enhance efficiency and scalability. Passive SRB systems enriched with natural organic substrates can last 10+ years with minimal intervention, thereby improving cost-effectiveness. The use of low-energy systems such as microbial fuel cells (MFCs) can be studied as it improves economic feasibility by generating electricity while simultaneously treating AMD [123].

4.3. Future Perspectives and Research Potential

Prospective researchers are encouraged to concentrate on the development of analytical models that aim to map microbial characteristics and understand the enzymes responsible for inducing the behaviour and functionality of SRB, with the goal of creating a treatment system that is tailored to the specific minerals present in AMD. It is imperative to determine the optimal conditions for SRB activities, including parameters such as temperature, pH, and other environmental factors. Research could also be directed towards the modification of SRB-based bioremediation techniques to accommodate climate variability, ranging from tropical to arid regions, which may influence SRB activity. This requires a thorough understanding of how regional elements such as water availability, temperature, and substrate accessibility impact SRB efficiency. The adoption of efficient reactors and novel electron donor sources that are both applicable and environmentally benign is recommended. Furthermore, the potential effects of SO_4^{2-} on human health, as well as on animals and, in particular, aquatic animal health, should remain a focal point of forthcoming research endeavours. As waste treatment advances towards zero emission, the recovery of heavy metals from sludge emerges as a crucial objective. As such, the exploration of potential applications and strategies for recovering metals generated during sulphate reduction is justified. The future outlook for the anaerobic bioremediation of AMD through the use of SRB appears highly promising, with numerous opportunities for innovation in genetic engineering, bioreactor design, metal recovery, and practical applications. By tackling the fundamental challenges and exploring the research opportunities mentioned above, SRB-based bioremediation could become a crucial tool for sustainable environmental management.

5. Conclusions

Acid mine drainage is a significant source of environmental pollution; therefore, the primary objective is to inhibit the formation or migration of AMD from its origin. The anaerobic bioremediation of AMD employing sulphate-reducing bacteria (SRB) has shown promising results in reducing both acidity and heavy metal concentrations. The SRB-mediated mechanisms depend on their ability to transform sulphate (SO_4^{2-}) into hydrogen sulphide (H_2S) and sulphide ions (S^{2-}) under anaerobic conditions, precipitating heavy metals as insoluble sulphides, thus increasing the environmental pH. This method presents several advantages over conventional chemical treatments, including cost-effectiveness, sustainability, and the generation of fewer secondary pollutants. Key findings of this review include the effectiveness of SRB in diminishing sulphate and precipitating metals such as iron (Fe), zinc (Zn), copper (Cu), aluminium (Al), manganese (Mn), and others. SRBs prosper in environments rich in organic carbon, and the application of organic substrates such as ethanol, glycerol, lactate, acetate, glucose, and cellulose has been essential for their metabolic efficiency and performance. The review established that these bacteria could effectively reduce sulphate concentrations and neutralise the acidic environment of AMD, making it less harmful to the surrounding organisms. However, the efficiency of bioremediation is subject to several factors, including substrate, temperature, pH, organic matter availability, hydraulic retention time (HRT), and the concentrations of toxic heavy metals. Elevated concentrations of specific metals can inhibit bacterial proliferation, thus constraining the overall efficacy of the bioremediation process. Furthermore, the anaerobic conditions necessary for SRB activity can be challenging to sustain in open environments, necessitating controlled and meticulously managed bioreactors. The long-term viability of SRB-based bioremediation systems also warrants further exploration. Despite promising initial studies, the persistence of SRB activity and its adaptability to fluctuating environmental conditions are still being examined. Furthermore, the potential clogging and biofilm formation in bioreactors could decrease the efficiency of the system over time. According to the literature, bioremediation based on sulphate-reducing bacteria has received significant attention from researchers. AMD can be controlled and managed using both active and passive biological strategies, and SRB presents a dual aspect regarding environmental pollution control. Although the reduction of sulphate to hydrogen sulphide and sulphide ions entails certain risks, the judicious application of SRB aids in pollution management. Consequently, more research is required to address the gaps within bioreactor technologies. Challenges persist with regard to the scale-up and long-term application of this technology. In conclusion, while SRB-facilitated bioremediation offers notable advantages, its large-scale practical application requires resolving technical challenges and improving the resilience of the system. Future research must focus on improving bioreactor design, optimising the electron donor, and developing integrated treatment systems that improve environmental management, which could contribute to more sustainable AMD treatment solutions that benefit both industrial wastewater management and ecological conservation. Integrating genetically modified SRB with existing bioreactors could significantly improve AMD remediation efficiency, reduce operation cost, and address long-term sustainable challenges.

Author Contributions: The conceptualisation, manuscript writing, and preparation were done by D.M.; T.N. was responsible for the conceptual framework and editing of the review paper, and M.M.M.-M. was responsible for editing and proofreading the review. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Energy and Water Sector Education Training Authority (EWSETA), LE00026749.

Institutional Review Board Statement: Not Applicable.

Informed Consent Statement: Not Applicable.

Data Availability Statement: Data are contained within the document.

Acknowledgments: The authors wish to express their gratitude to the University of Limpopo (UL) and the Bureau de Recherche géologiques et Minières (BRGM) for their administrative and technical support.

Conflicts of Interest: The authors declare no conflict of interest.

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