Diversity of Acidophilic Bacteria and Archaea and their Roles in Bioremediation of Acid Mine Drainage

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Authors' contributions

Article Information

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ABSTRACT

Aims: To show the potential of acidophilic bacteria and archaea in bioremediation of acid mine drainage.
The Mining industry generates wealth, but its long term adverse effects, which include acid mine drainage (AMD), cannot be overlooked. Acid mine drainage occurs as a result of biological and chemical oxidation of sulphide containing minerals with consequent production of acidic metal rich effluents. AMD is a serious environmental pollution problem in both active and abandoned mines worldwide, resulting in continual contamination of surface and groundwater resources with heavy metals. Acidophilic bacteria and archaea have been known to contribute to the accentuation of this problem by speeding up the reaction time for biological oxidation of sulphide containing mineral

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waste rock. The dominant metal present in AMD is iron with high sulphate content; the iron may be present in either ferrous or ferric form or both depending on the water pH. Reduction of these two important constituents by generating alkalinity through chemical or biological means has been reported to have a significant effect in AMD impacted water. The metabolic activities of the acidophilic bacteria and archaea through ferric iron and sulphate reduction, a natural attenuation process, also help in remediating this pollution problem by generating alkalinity that immobilizes metals thereby reversing the reactions responsible for the genesis of AMD. This article reviews the various groups of the acidophilic prokaryotic microorganisms and their metabolic activities that help in remediating the problem of AMD in gold mines.

1. INTRODUCTION

Acid mine drainage, also known as acid and metalliferrous drainage (AMD) or acid rock drainage (ARD), is the biochemical oxidation of sulphide bearing minerals, which results in the production of acidic water that contains high concentrations of heavy metals sulphate and low pH. Earth disturbances such as construction activities and mining processes in the rocks that contain abundance of sulphide minerals as well as natural rock weathering processes can also contribute to the generation of AMD. This indicates that AMD is the generation of acidic water from sources other than mining. Typically, AMD is characterized by low pH value, high sulphate content and, often times, elevated concentrations of ferric iron and other metals such as copper, zinc, chromium, cadmium and nickel. The chemical composition of AMD varies depending on the kind of sulphide mineral associated with coal and metal ores [1,2]. Acid mine formation is greatly enhanced by the mining process which increases the surface area of the sulphide containing mineral exposed to air and oxygen thereby increasing the rate of acid generation [3]. Bacterial activity is an important factor in acid mine generation because it helps in accelerating the rate of decomposition and oxidation of sulphide minerals.

1.1 Origin of Acid Mine Drainage

Many factors are known to contribute to the development of AMD. Acid mine drainage can be generated as a result of coal and metal mining activity. Some of these sources are: Mined materials like spent ore from heap leach operations, spoil, waste dump or tailings, overburden material, mine structure such as pit walls in surface mining operation and underground workings associated with underground mines and subgrade ore piles. All these are known to contain sulphide minerals like pyrite, which is the most abundant of all sulphide minerals, and others such as galena, covelite, chalcopyrite, realgar and arsenopyrite whose oxidation leads to the formation of AMD [4]. After extraction of ores from underground or open pit, 80-90% of the crushed ore is dumped as tailings waste which contains large amounts (between 10 and 30 kg/ton) of sulphide minerals.

1.2 Biodiversity of Acidophilic Micro-organisms in AMD

Acid mine drainage usually contains a variety of microorganisms. As a result of the characteristics features of the acid mine drainage, prokaryotic microorganisms have been found to be the predominant life forms existing in the environments [5]. These prokaryotes are found in the groups of bacteria and archaea domains with ability to thrive well in the extremely acidic environments. Acidophiles have immense contribution to sulphur and iron biogeochemical cycle [5,6]. Acidophilic microorganisms are a subdivision of the extremophiles which have been gaining a lot of research interest as a result of their ecological and economic importance. The ecology and biodiversity of the acidophilic prokaryotes has been reviewed by Hallberg and Johnson [7]. Acidophiles have been classified using many criteria. On the basis of mineral solubilization, two groups are recognized. The first group is those that accelerate mineral dissolution by an oxidative route (iron and sulphur oxidizers) while the second group uses the reductive route (iron reducers) [8]. Some species (mostly acidophiles) can reduce ferric iron as well as oxidize ferrous iron, depending on the prevailing environmental conditions. The iron and sulphur oxidizers are found in both bacteria and archaea domains and their metabolic activities have been utilized in extraction of gold from refractory ores (biomining) [9]. The bacteria

are found within the Proteobacteria and comprise (*alpha*, *beta* and *gamma* classes), Nitrospirae, Firmicutes, Actinobacteria and Acidobacteria phyla and in the domain archaea within the Crenarchaeota and Euryarchaeota phyla. The alpha proteobacteria, particularly *Acidithiobacillus ferrooxidans, Acidithiobacillus caldus* and *Acidithiobacillus thiooxidans* are the most extensively studied group [10].

The iron oxidizers are able to thrive in the sulphide mineral rich environments as a result of their ability to use ferrous iron as their electron donor, and they continually regenerate the ferric iron which in turn oxidizes the sulphide minerals present in the acidic liquor. The major iron oxidizers are the *At. ferroxidans* and *Leptospirillium ferroxidans* [11]. The genus *Leptospirillium* is the most commonly encountered iron oxidizing organism in mineral leaching environments, due to its high affinity for ferrous iron, tolerance of ferric iron and moderately thermal (>40°C) environments [7]. Others such as *Acidimicrobium ferrooxidans, Sulfolobus metallicus, At. thiooxidans* and *Metallosphaera sedula* also have a central role in the dissolution of sulphide minerals [12]. The number of iron oxidizing cells and their level of activity determines the extent to which these sulphide minerals are oxidized by the microorganisms.

Sulphur compounds released during pyrite oxidation can be oxidized to sulphate by ferric iron, and this tends to make the population density of sulphur oxidizers in AMD to be low due to non-availability of sulphur to support their growth [13]. After the initial oxidation of sulphide by the iron oxidizers, the remaining sulphides are oxidized by neutrophilic sulphur oxidizers such as *T. thioparus and T. novellus*. The pH of the AMD is lowered to 4.0 and 4.5 as a result of the oxidative activities of the bacteria and the initial environmental condition. Acidophilic sulphur-oxidizers such as *Acidithiobacillus* and *Acidiphillum* species then oxidize the remaining sulphur [14]. Ferrous sulphate ($FeSO_4$) is oxidized to ferric sulphate ($Fe_2(SO4)_3$) by iron-oxidizing acidophiles [15,16]. Additional sulphate is produced as a result of further oxidation of FeS_2 and ferric sulphate by iron-oxidizing acidophiles. Elemental sulphur is also oxidized to sulphuric acid (H_2SO_4) by sulphur–oxidizing acidophiles which also lowers the pH. Dissimilatory oxidation of iron and /or reduced sulphur compounds are catalyzed by other groups which could be mixotrophic (assimilate organic and inorganic carbon) or obligatehete

rotrophs. Heterotrophic microorganisms are known to be abundant in AMD where they rely on carbon originating as waste products from the autotrophs, and it has been suggested that these heterotrophs might supply the autotrophs with additional carbon for their growth [17]. Okibe and Johnson [18] observed in their study that dissolution of pyrite was accelerated by consortia containing heterotrophic microbes than those of iron-oxidizers and sulphur-oxidizers. The authors opined that the heterotrophic contribution must be due to the stimulation of carbon flow between the heterotrophs and autotrophs and not as a result of generation of ferric iron or removal of sulphur from the mineral surface which aid in the dissolution of the pyrite.

Other important groups found in AMD are the neutrophilic iron oxidizers such as *Leptothrix ochracea* [19] *Gallionella ferruginea* [20]. With the exception of *Leptospirillium* spp and *Ferrovum (Fv.) myxofaciens, the* majority of the iron oxidizing bacteria also reduces ferric iron to support their growth.

Based on environmental factors required for growth, acidophiles have been subdivided further into various groups, namely:

Temperature: mesophilic, moderate thermophiles and extreme thermophiles.

pH: acidophiles, alkalinophiles, neutrophiles.

Nutritional type: heterotrophs, autotrophs, mixotrophs (Table 1).

The archaea-like *Metallosphaera* spp., *Sulfolobus metalicus* and *Acidianus brierleyi* are the most thermophilic growing at a temperature (65-95°C). They are mostly found inhabiting the most extreme niches on the planet. The mesophiles are the predominant bacterial oxidizers growing at optimum temperature less than 40°C, while the moderate thermophiles grow at optimum temperature of 40-60°C. The mesophilic and moderately thermophilic bacteria are the most extensively studied groups of the acidophilic metal sulphide oxidizing microorganisms. Thermophilic and acidophilic sulphur/iron oxidizers mostly found at a temperature range of 40–60°C are the usual rod-shaped, *Sulfobacillus* species [7,15]. Most life forms which are functional in AMD grow at an optima pH between 2 and 4 or acid-tolerant (pH optimal for growth above that normally encountered in AMD), but can also function in very low pH environments [21].

The concentration of dissolved organic carbon in the majority of extremely acidic environments has been found to be very low (<20 mg L^{-1}). Thus these environments can be characterized as oligotrophic environments. In abandoned deep mines where light penetration is restricted, the nutrition type that exists will be mainly chemolitho-autotroph, which is the oxidation of ferrous iron and reduced sulphur compounds [5]. The majority of iron and sulphur-oxidizing acidophiles are regarded as autotrophic, but utilization of formic acid as carbon source has also been reported in some of them such as *At. ferroxidans* and they have been found to be responsible for the production of ferric iron and acid [22].

The chemolithotrophs are the first prokaryotes isolated from extremely acidic environments and *At. ferroxidans* was the first iron-oxidizing acidophile to be isolated and characterized [23]. Due to this reason, *At. ferroxidans* has been the most well studied isolate in acidophilic microbiology. Reports abound in the literature on its physiology and biochemistry [16,24,25].

2. PYRITE OXIDATION

The development of acid mine drainage is dependent on six factors, namely (a) abundance of sulphide minerals (b) water content (moist environment) (c) oxygen and ferric iron (oxidant) and pH (hydrogen ion concentration), (d) surface area of the exposed sulphide mineral, (e) activation energy and (f) presence of sulphur and iron oxidizing bacteria (biological activity) [26].

Sub-surface mining often progresses below the water table, so water must be constantly pumped out of the mine in order to prevent flooding. However, when a mine is abandoned, the pumping ceases, and water floods the mine, which results in the accumulation of contaminated water in the environment [27,28]. Introduction of water is the initial step in most acid mine drainage generation. This results in the production of drainage water that is highly polluting because of low acidity which increases mobility and heavy metal content. This acidity occurs as a result of dissolution of the acidic salts that have built up in the pore spaces of the exposed walls and ceilings of underground chambers. Exposing pyrite to oxygen and water leads to an oxidation reaction, where hydrogen, sulphate ions and soluble metal cations are created as shown in the equation below:

$$2FeS_2 + 7O_2 + 2H_2O \rightarrow 2Fe^{2+} + 4SO_4^{2-} + 4H^+ \quad (1)$$
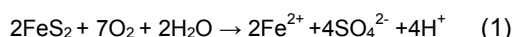
Further oxidation of ferrous iron Fe^{2+} to ferric iron Fe^{3+} occurs as a result of availability of dissolved oxygen in water or in the atmosphere

$$4Fe^{2+} + O_2 + 4H^+ \rightarrow 4Fe^{3+} + 2H_2O \quad (2)$$
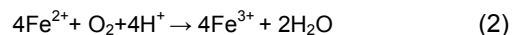
Ferric iron (Fe^{3+}) can also precipitate as ochre (Fe $(OH)_3$), the reddish-orange precipitate often observed in acid mine drainage waters:

$$Fe^{3+} + 3H_2O \rightarrow Fe (OH)_3 + 3H^+ \quad (3)$$
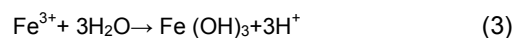
Fe^{3+} that did not precipitate from (2) left in solution from (3) will precipitate additional pyrite as indicated in equation (4):

$$FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+ \quad (4)$$
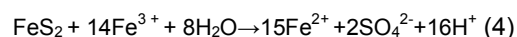
Pyrite oxidation occurs through either direct or indirect pathways and it is very difficult to determine which of the pathways is important in a given situation. The direct pathway involves close proximity of the sulphide bearing mineral with microorganisms such as acidophilic *At. ferroxidans*, *L. ferroxidans* which aids in the oxidation of the sulphide mineral [29-31].

In the indirect pathway, sulphide is reduced to ferrous as a result of its chemical oxidation by ferric iron and the ferric iron is further regenerated by iron-oxidizing microorganisms, leading to continuous oxidation of the sulphide mineral. The resultant effect of this reaction is the production of acidic water with characteristic corrosive patterns [32]. The acidity of the medium which results from generation of the hydrogen ion makes the heavy metals contained therein highly soluble, thereby preventing their precipitation out of the solution. Indirect production can also occur through the reaction of some metal ions such as Fe^{3+} and Al^{3+} with water [33]. Further acidity can also be generated by dissolution of sulphide containing minerals in the anoxic sediment of a constructed wetland or spoil heap as a result of the ferric iron concentrated acidic water infiltrating through them [34].

Bacteria play a prominent role in the genesis of acid production because they act by accelerating the rate of decomposition and oxidation of the sulphide minerals [8]. Evangelou and Zhang [35] stated that the major reaction which ensures continuous oxidation of the sulphide mineral is continuous regeneration of ferric iron which is reduced to ferrous upon reaction with pyrite. Hence, the primary oxidant is the ferric iron and not molecular oxygen as initially proposed in the classical equation above.

Pyrite oxidation is a two-phase reaction. The first phase involves ferric iron attack on the sulphide mineral, while the second phase is the reoxidation of ferrous iron to ferric, which is an oxygen dependent reaction. The reduced sulphur compounds produced as intermediates in the reaction are also oxidized to sulphate [35]. Dissolution of the sulphide mineral occurs after its attachment to oxygen and this result in the oxidation of the sulphide moiety which occurs in non-ferrous sulphides such as Cu_2S or of both iron and sulphur in minerals such as pyrite (FeS_2) and pentlantite (FeNiS).

2.1 Disadvantage of Acidophilic Micro-organisms

The oxidation of sulphide minerals can be abiotic, but the rate of oxidation is greatly enhanced by several orders of magnitude by sulphur and iron-oxidizing bacteria and archaea [19]. Acidophilic bacteria and archaea raise the amount of available ferric iron which increases the rate of pyrite oxidation. *At. Ferrooxidans,* for example, uses reduced ferrous iron in AMD areas as an electron donor for energy creation at low pH. Oxidation of sulphur by autotrophic and heterotrophic bacteria and archaea generates sulphuric acid, which if not neutralized by carbonates or other basic minerals present, results in acid generation. Acidophilic bacteria and archaea colonize the concrete surface and its pores, capillaries and micro-cracks and cause biogenic sulphide corrosion of concrete sewer pipes by altering hydrogen sulphide sewage gas into sulphuric acid [36].

2.2 Different Treatment Technologies for AMD

Acid mine drainage (AMD) poses a serious threat to human health, animals and ecological systems. Polluted water becomes acidic and contains elevated concentrations of radionuclides and heavy metals such as Cu^{2+}, Fe^{3+}, Mn^{2+}, Zn^{2+}, Cd^{2+} and Pb^{2+}, which are not biodegradable. These metals accumulate in living organisms (bioaccumulation) and the concentrations increase as they pass from lower trophic levels to higher trophic levels (a phenomenon known as bio magnifications) causing various diseases and disorders [37]. It is, therefore, important to treat acid mine decants effectively before allowing their release into the ecosystem.

A wide variety of technologies are available for the treatment of AMD. They comprise one or more chemical, physical and biological processes. They involve the following: pH control, adsorption/absorption, complexation, chelation, biological remediation, oxidation/reduction, electrochemistry, sedimentation, flocculation/ filtration/settling, ion exchange and crystallization [38].

Two different treatment technologies are utilized for the treatment of acid mine: passive and active treatment.

2.2.1 Passive treatment

Over the years, different types of passive treatment systems have been developed that do not require continuous chemical inputs. A variety of passive treatments have become the most predominant innovative treatments that are applied aside from active treatment. These treatment systems take advantage of naturally-occurring chemical and biological processes to cleanse contaminated mine waters or soil and require minimal upkeep [39]. Treatment involves the use of sulphate-reducing bacteria and/ or lime to neutralize acidity and precipitate metals. Examples of these technologies include wetlands (natural and constructed); anoxic limestone drains (ALD), successive alkalinity-producing systems (SAPS), limestone ponds, and open limestone channels (OLC). In treating metals common to hard rock mining such as Zn, Pb, Cd, As, Mo, Au, and Ag, sulphate reduction by bacteria is usually the principle behind the design of passive treatment with the aim of inducing metal precipitation as sulphides. For metals (Fe, Al, and Mn) common to coal mining, aerobic processes, with or without an alkaline agent are the most commonly applied [40]. The numerous innovative technologies employed are based on the same principles. Permeable Reactive Barriers (PRBs), bio-reactors, and constructed wetland technologies can all utilize alkaline agents and sulphate reducing bacteria to treat mine drainage. With the exception of PRBs that utilize iron in the treatment of uranium, many of these technologies are in-situ applications that manipulate natural processes to treat acidic and/or metal contaminated water. They differ only in construction and water source. PRBs have a subsurface reactive section that groundwater flows through to be treated following its natural course [40].

Table 1. Acidophilic prokaryotic microorganisms

Organisms	Temperature	pH	Nutritional class	References
Iron oxidizers				
L. ferroxidans	Mesophiles	Acidophiles	Autotrophs	[11]
At. ferrivorans	Mesophiles	Acidophiles	Autotrophs	[5]
At. ferroxidans	Mesophiles	Acidophiles	Autotrophs	[11]
Ferrimicrobium acidophilus	Mesophiles	Acidophiles	Autotrophs	[17]
Gallionella ferruginea	Psychrophiles/mesophiles	Acidophiles/Neutrophiles	Autotrophs/ Mixotrophs	[20]
Ferroplasma acidophilum	Mesophiles/ thermophiles	Acidophiles	Autotrophs	[5]
Acidomicrobium ferroxidans	Moderate thermophiles	Acidophiles	Autotrophs/Mixotrophs/Heterotrophs	[8]
Sulfobacillus acidophilus	Moderate thermophiles	Acidophiles	Mixotrophs	[41]
S. thermosulfidooxidans	Moderate thermophiles	Acidophiles	Mixotrophs	[41]
At. prosperous	Mesophiles	Alkalinophiles	Autotrophs	[5]
Metallosphaera sedula	Extreme thermophiles	Acidophiles	Autotrophs	[8]
Leptothrix ochracea	Mesophiles	Neutrophiles	Autotrophs	[19]
Sulphur oxidizers				
At. thioxidans	Mesophiles	Acidophiles	Autotrophs	[42]
At. albertis	Mesophiles	Acidophiles	Autotrophs	[5]
Sulfolobus metallicus	Extreme mesophiles	Acidophiles	Autotrophs	[8]
Hydrogenobacter acidophilus	Moderate thermophiles	Acidophiles	Autotrophs	[5]
Thiobacillus denitrificans	Thermophiles	Neutrophiles	Autotroph	[43]
Iron-reducers				
Acidiphilium spp.	Mesophiles	Acidophiles	Heterophic	[44]
Acidomicrobium ferroxidans	Moderate thermophiles	Acidophiles	Autotrophs/Mixotrophs/Heterotrophs	[8]
Non-mineral degraders				
Acidocella spp.	Mesophiles	Acidophiles	Heterotrophic	[5]
Alicyclobacillus spp.	Thermophiles	Acidophiles	Heterotrophic	[5]
Acidobacterium capsulatum	Mesophiles	Acidophiles	Heterotrophic	[5]

The active and passive technologies of treatment also do not allow potentially valuable metals present in mine waters to be recovered and recycled. New innovative developments in mine water remediation technologies now exist. These developments enhance sequential and/or selective removal of dissolved metals and different pollutants from AMD. This results in recycling of additional valuable elements and immobilization of the poisonous pollutants in targeted forms. It involves combination of the physical-chemical and biological-chemical methods to remove metals and metalloids from AMD. An Example of these technologies is the selective sequential precipitation (SSP) which precipitates metals using solutions of sodium hydroxide (NaOH) and hydrogen sulphide produced by sulphate reducing bacteria. This approach produces metals with a high degree of purity and is environmentally friendly [50].

2.2.2 Active treatment

The most widely used approach for remediating mine impacted waters is to aerate (to oxidize ferrous iron to ferric) and add neutralizing chemicals such as calcium carbonate, calcium hydroxide or anhydrous ammonia to raise the pH. This precipitates metals as hydroxides and carbonates, thus reducing the activity of the iron-oxidizing bacteria [51].These methods have a lot of drawbacks such as high energy and chemical requirements, low efficiency and usually the production of large amounts of sludge, from which separation of precious metals is difficult and high cost and interference by other wastewater constituents occurs [52].

2.3 AMD Treatment Mechanism by Acidophilic Microorganisms

The dominant metal present in AMD is iron, with elevated amounts of sulphate as a result of microbial oxidation of sulphide containing minerals. The iron may be present in either ferrous or ferric form, or both, depending on the water pH. Reduction of these two important constituents by generating alkalinity will have a significant effect in AMD impacted water [4,8]

It is a well-known fact that acidophilic bacteria and archaea help in accelerating the problem of AMD by significantly speeding up the reaction time. Production of acid by ferric iron can take as long as 15 years, but the presence of iron-oxidizing bacteria can shorten this reaction time to 8 minutes [49]. Acidophilic microorganisms

with the ability to generate acid from reduction of ferric iron have been reported [44,47]. The acidity generated results in solubilization of the heavy metals present in the rocks. Ferric iron is almost insoluble at neutral pH, whereas in acidic solutions (pH<2.5) its solubility is greatly increased.

The principle behind the biotic and abiotic remediation strategies is to generate alkalinity to immobilize metals, thereby reversing the reactions responsible for the genesis of AMD [53]. Microbiological processes that help in remediating AMD pollution problems are reductive precipitation, chelation, sulphate reduction and metal sulphide precipitation [54]. The heavy metals contained in AMD are non-biodegradable; as a result, their remediation can only be achieved by removing them from the solution. This occurs by precipitation of heavy metals out of solution as insoluble metal sulphides or precipitation of ferric iron as a result of change in their redox state (transformation) [55]. Since ferric iron is known to be the major oxidant responsible for continuous oxidation of the sulphide minerals, and is usually insoluble at neutral pH, removing it from the solution will go a long way in preventing further oxidation of the sulphide minerals.

The acidophilic chemolithotrophic prokaryotes are known for their accelerated oxidative dissolution of pyrite and other sulphide minerals in AMD using inorganic carbon. Other groups known as heterotrophic acidophiles also catalyse the dissimilatory reduction of iron and sulphur using organic carbon as electron donor and carbon source, thereby reversing the reactions involved in AMD formation.

3. FERRIC IRON REDUCTION

The rate of dissolution of most sulphide containing minerals is largely dependent on the availability of ferric iron, which has been shown to be the major oxidant of these minerals in the environment [26,35]. Acid mine contaminated environments, especially those associated with metal mining, contain high concentration of ferric iron whose solubility is known to be greatly enhanced at acidic pH. Most prokaryotic acidophilic bacteria and archaea use this electron sinks to oxidize organic matters in subsurface environments with high loads of organic matter [21].

The ability to reduce ferric iron to ferrous has been reported in aerobic mesophilic chemoautotrophs (*At. ferroxidans* and *At. thioxidans*) mesophilic heterotrophs (*Acidiphilium* spp. and *Ferromicrobium acidophilium*) [17,44,56] and moderate thermophiles (iron-oxidizing *Sulfobacillus* spp., *Acidomicrobium ferroxidans* and heterotrophic *Alicyclobacillus*-like isolates) [41]. With the exception of *At. thioxidans*, ferric iron reduction has been coupled with growth in all these mentioned bacteria[42]. Ferric iron reduction is coupled with the oxidation of many organic compounds in highly acidic heterotrophs. The majority of the *Acidiphilium* species can reduce and solubilize a wide range of ferric iron containing minerals like $Fe(OH)_3$ and jarosite $(XFe_3(SO4)_2(OH)_6$. Anaerobic dissolution of ferric iron containing minerals: goethite, jarosite and iron hydroxide formed as a result of iron oxidation has been reported in *S. acidophilus* which also oxidizes sulphur compounds using ferric iron. Ferric iron reduction is also common in the Gram-positive mixotrophic iron–oxidizers [41]. Although many neutrophilic microorganisms are known to have ability to reduce ferric iron, the ability to couple organic matter oxidation exclusively to ferric iron reduction in order to conserve energy to support growth is lacking in the majority of them [57].

To remove soluble iron from AMD, the ferrous iron must first be oxidized to ferric. This will enhance the formation of ferric minerals such as schwertmannite and ferrihydrite, which can then be easily precipitated out of solution by the ferric iron reducers. This reduction results in mobilization of iron as well as other metals that may be associated with ferric iron deposit. It is also an alkali generating reaction of high importance in passive treatment in wetland [34].

The ability to reduce ferric iron has been reported to be affected by dissolved oxygen concentration in some of these microorganisms. In a study by Johnson and Bridge [58] to determine the effect of culture condition on the growth of two acidophilic heterotrophic ferric iron reducers *A. acidophilum* and *Acidiphilum* SJH in fermenters, it was discovered that growth of the *A. acidophilum* was affected by dissolved oxygen concentration whereas for *Acidiphilum* SJH the reverse was the case. Also, the expression of the iron reductase system was found to be inducible in the *A. acidophilum* because it was synthesized in the presence of very low concentration of dissolved oxygen while for *Acidiphilum* SJH it was constitutive because it was able to reduce ferric iron irrespective of dissolved oxygen during

growth. It has also been shown in the work of [59] that the iron reductase system can be both constitutive and inducible in some ferric iron reducers as seen in the case of dissimilatory iron reducer *Shewanella putrifeciens* (strain 200). Dissolved oxygen concentration was shown to have effect on the inducible system because ferric iron reduction was high under limited oxygen supply.

3.1 Sulphate Reducing Bacteria (Srb)

Microbial dissolution of sulphide containing minerals results in high concentration of sulphate in acid mine drainage. Sulphate reducers are a morphologically diverse group of bacteria with varying nutritional requirements. They are obligate anaerobes that use sulphate or other sulphur compounds as an electron acceptor for the dissimilation of organic compounds [33]. These bacteria are a major part of the total microbial community because they play a vital role in the biogeochemical cycle of carbon and sulphur which helps in regulation of sulphate in the environment [47,60]. SRB are usually found in the anaerobic regions of marine, estuarine and mine waste water sediments as well as saline ponds due to the high sulphate content [33,47]. The sulphate-reducing bacteria include the following genera: *Desulfovibrio, Desulfomicrobium, Desulfobulbus, Desulfosarcina, Desulfobacter* and *Desulfotomaculum* [61,62]. These groups of heterotrophic acidophilic bacteria use sulphate as a terminal electron acceptor, in the process releasing hydrogen sulphide as a result of sulphate reduction. Soluble heavy metals (e.g. copper, zinc, iron, chromium, cadmium) present in the solution react with this biologically produced hydrogen sulphide to form insoluble precipitates of the heavy metals as shown in the equations below:

$$2H^+ + SO_4^{2-} + 2C \text{ (org)} \Leftrightarrow H_2S + 2CO_2 \qquad (5)$$

$$M^{2+} + H_2S \Leftrightarrow MS (\downarrow) + 2H^+ \qquad (6)$$
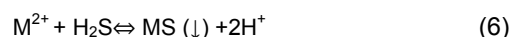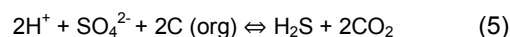
M^{2+} represents the dissolved metal

The acidity of the system is reduced as a result of carbon metabolism and inherent ability of the bacteria to reduce the sulphate. These fundamental properties make SRB useful in mitigating AMD [61,62] and this natural technology has been considered the most promising approach of removing sulphate, acidity and heavy metals from AMD [4,63]. The ability of

the SRB to achieve the proposed sulphate standard of 500 ppm as well as 250 ppm required for drinking water has been reported [64] as compared with the conventional chemical method that can only reduce it to 1500 mg/L.

Other advantages over chemical mitigation methods such as production of more compact sludge which settles faster and is less subject to dissolution, selective precipitation of metal, high efficiency and low cost have been reported [65,66].

Efficient sulphide production using SRB can be achieved by addition of a complementary carbon source because AMD is deficient in carbon electron donors. Therefore, choosing an appropriate carbon source is very crucial in ensuring long time usage, high efficiency, and economic viability of the system. Three factors are usually considered in selecting this carbon source, namely, availability of the carbon source, its degradability which enhances its capacity to allow complete sulphate reduction by the SRB and its cost per unit of sulphate converted [67,68].

Despite the reported success of SRB in the treatment of AMD, the sensitivities of these bacteria to acidity and heavy metals is the major setback in using them, hence there is a need for the addition of a remediation reagent to improve the living conditions so as to enhance their activity [69]. The optimum pH of growth for SRB is between 7.0 and 7.5 and different heavy metals toxicity levels to SRB have been reported [70,71]. Several efforts have been made to address this problem [69,72-75].

In the past, it was generally believed that sulphate reducing bacteria can only thrive between pH 6 and 8 [76,77] but lately some other reports have punctured such notions, with sulphate reduction at a pH as low as 2.7 and 3.8 being observed in their studies [78,79]. But there are a lot of studies in support of the earlier claim [61,80,81].

It has now been proven that microbial sulphate reduction with efficient heavy metals recovery can proceed in AMD impacted environment (Table 2).

Sulphate reduction in acidic environments such as acidic mine tailings, lakes and rivers, wetlands and bioreactors has been documented. Denaturing gradient gel electrophoresis revealed the presence of SRB in Rio Tinto river in Spain

with pH as low as 2.3 [45,61,82].Acidophilic sulphate reducing bacteria with ability to selectively immobilize different transition metals has been reported [83]. The isolation of acidophilic sulphate reducers remains an interesting and, due to recent successes, a promising field of research. There is hope in using acidotolerant sulphate reducing bacteria for bioremediation purposes.

The significant contribution of iron and sulphur oxidizing bacteria in the genesis of AMD has been confirmed by many researchers [9,15,16], but the microbial diversity in the AMD sites is yet to be well characterized. [84]. The rate of dissolution of sulphide mineral is a function of the population of iron oxidizing cells present and their level of activity in a given environment. Information about the population of iron-oxidizers is important in deducing the microbial impact of AMD [2] so that appropriate remediation approach (es) can be taken.

Hallberg and Johnson [15] isolated eight acidophilic moderate iron oxidizers from two abandoned mines in the United States and a pilot-scale constructed wetland at one of the sites with pH 3-6. Analysis of the 16S rRNA gene sequences of these isolates showed that they were previously undescribed residents of the acidic waters. Three of the isolates showed greater than 99% genetic relatedness and have 97% gene identity to a clone deposited in the public data base [85]. The closest recognized strain was *Frauteria aurantia,* a neutrophilic acetogenic iron-oxidizer with 93% gene identity, two of the other three sets were found to have 99.6% gene identity and 97.5% to the third isolate. When their gene sequences were compared to the gene sequences in the data bases, *Thiomonas thermosulfata*, a neutrophilic thiosulphate-oxidizer was found to be the closest relative with 96% genetic relatedness. The remaining two isolates had 99.7% gene relatedness to *Propionibacterium acnes,* an unknown anaerobic microbe to inhabit acidic waters.

Auld, et al. [84], in a study using direct sequencing of the 16S rRNA, also isolated three previously unidentified genera in AMD: *Legionella*, a neutrophilic heterotroph, *Alicyclobacillus pohliae,* a Gram positive, aerobic, acidophilic bacterium and *Halomonas ventosae*, a Gram negative, high salt tolerant, halophilicproteo bacteria, from AMD tailing ponds.

Table 2. Microbial sulphate reduction and heavy metals recovery of SRB in acidic environments

System	Initial pH	Final pH	% of heavy metals removed			% of Sulphate removed	References
			Cu	Fe	Zn		
Bioreactor	2.75	6.20	99	9	86	61	[45]
Column + mining soil	2.3	8.0	>90	>90	>90	90	[46]
Fumarole	-	-	97,	100	96	91	[47]
Reactor	2.7	4.3	99.9	99.9	99.9	90	[48]
Reactor	3.7	5.0	99.9	-	99.9	-	[49]

An enormous diversity of ferrous iron oxidizing prokaryotes exists in the acidic environment [7] with different affinities to the prevailing environmental conditions. Variation in environmental conditions such as pH, temperature and oxygen contents exist in acid mine drainage worldwide. This lead to great differences in physiological properties of the acidophilic microorganisms that can be found in these environments. This metabolic variation can be exploited in remediation strategies of various AMD which is the principle behind the emerging strategies of using these acidophiles for oxidation and precipitation of iron from acid mines of different water chemistry [55]. Since environmental factors are not uniform in the mine waters. It will be appropriate to use different consortia to speed up iron oxidation rate in different situations.

3. CONCLUSION

Though acidophilic microorganisms are known to play a prominent role in the genesis of AMD, various advantages have been derived from their metabolic activities which have been harnessed in biomining and bioleaching processes in the mining industries [9].

The diversities of this group of microorganisms are being shaped by geographical conditions prevailing in the various environments which are not uniform. Due to this reason, there is need for more studies on the bacteria diversity, function and the factors affecting the distribution of these microorganisms in the acid mine environments. This will help in designing the appropriate bioremediation strategy for the contaminated sites.

Knowledge of these as well as the various metabolic processes and interactions that exist among these microorganisms will help in identifying the various groups with potential to ameliorate the problem of acid mines. More groups with unknown potentials are being detected every day, and the discovery of acidophilic anaerobic sulphate reducing bacteria has greatly helped in the recovery of metals from polluted AMD.

Further work will be structured on the metabolic pathways involved in the biomineralization of heavy metals by the acidophilic bacteria and archaea. To facilitate the processes involve in the utilization of these microbes in bioremediation, requires optimization of conditions involved.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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