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An investigation into the Use of Fischer Tropsch wastewater as an organic source in the treatment of acid mine drainage (AMD) using dissimilatory sulfate reduction

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2024
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FACULTY OF Engineering and the Built Environment.

SCHOOL OF CHEMICAL AND METALLURGICAL ENGINEERING.

Research Topic: An Investigation into the Use of Fischer Tropsch Wastewater as an Organic Source in the Treatment of Acid Mine Drainage (AMD) Using Dissimilatory Sulfate Reduction.

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An investigation into the Use of Fischer Tropsch Wastewater as an Organic Source in the Treatment of Acid Mine Drainage (AMD) Using Dissimilatory Sulfate Reduction.

Dedication

My Mother Sthembiso and brother Bevington.

Acknowledgements

Firstly, I would like to thank the University of Witwatersrand Postgrad Merit Award, which made it possible for me to carry out and complete this study. Special thanks go to my supervisors Professor Craig Sheridan and Professor Karl Rumbold whose guidance enabled the completion of this research. My gratitude is extended to the Industrial and Mining Water Research Unit (IMWARU) for creating space to allow for the sharing of ideas and skills.

I thank my employer, The African Leadership Academy, for the funding, time and facilities used throughout the study. My gratitude also goes to my colleagues for the encouragement and support during the study. I would also like to extend my gratitude to Greatman Mpala, Nigel Kandengwa, Zivanai Chabata, Oluwademilade Fayemiwo, Nicole Gwindi, and Pedzisai Sibanda for the assistance rendered during the study.

Research output

Publications

Type	Details	Status
Journal article	Magowo, W.E., Sheridan, C., and Rumbold K., 2020 Bioremediation of acid mine drainage using Fischer Tropsch wastewater as a feedstock for dissimilatory sulfate reduction. *Journal of Water Process Engineering.* **35** (2020) 101229.	Published
Journal article	Magowo, W.E., Sheridan, C., and Rumbold, K. Global co-occurrence of acid mine drainage and organic rich industrial and domestic effluent. Biological sulfate reduction as a co-treatment option. *Journal of Water Process Engineering.* **38** (2020) 101650.	Published
Journal article	Magowo, W.E., Sheridan, C., and Rumbold K. Biological precipitation of dissolved iron from acid mine drainage using Fischer Tropsch wastewater as an electron donor for sulfate reduction. *Minerals Engineering.*	Submitted

Conference output

Best student presentation at the WISA 2020 virtual conference.

Abstract

Acid mine drainage (AMD) and Fischer Tropsch wastewater (FTWW) are two major pollutants associated with coal mining and usage, as such these pollutants are likely to be found in proximity to each other in coal mining regions. AMD is characterized by high sulfate and dissolved ion concentrations with little to negligible organic content, while FTWW has a very high organic content made mainly from alcohols and short chain fatty acids (SCFA). FTWW has very high COD of up to 30 000 mg/L. Sulfate reducing bacteria (SRB) can use organic substrates to reduce sulfate to sulfide in the process generating alkalinity. The hydrogen sulfide reacts with dissolved metals to form metal sulfide precipitates, while the alkalinity attenuates pH. This means SRB can be used to remove organic pollutants from FTWW and dissolved metals and sulfates from AMD. This study sought to use FTWW as the carbon source and electron donor for biological sulfate reduction in a fixed bed bench scale bioreactor treating AMD. Batch and continuous flow reactors including single stage and two stage continuous sulfate-reducing bioreactors were evaluated in this investigation. The reactors were assessed on their ability to remove COD from the FTWW, sulfate and dissolved iron from AMD. Considerable success was observed in batch reactors, with up to 99 % of iron removed from AMD, sulfate removals was at 95 %, while more than 99 % COD was removed from the effluent. Fed batch and continuous reactors were not as successful as the treatment efficiency dropped with time due possibly to the accumulation of inhibitory substances such as hydrogen sulfide and metal sulfide precipitates. The two-stage continuous bioreactor performed better compared to the single stage continuous reactor. All the reactors however maintained the pH above 7.0 against an influent pH of 2.0. Lower temperatures during winter reduced the performance of the bioreactors as the pH of the effluent dropped to below 6.0. There was a a large amount of residual sulfate, iron and COD in the reactors operated in winter than in those operated in summer. Another 2-stage bioreactor system consisting of a sulfate reducing bioreactor connected in series to a sulfate oxidising bioreactor was operated for converting the hydrogen sulfide produced in the sulfidogenic bioreactor to sulfur. Micro aerobic conditions were applied by pumping limiting amounts of oxygen into the sulfur oxidising reactor to allow for the oxidation of sulfide to elemental sulfur. Up to 92 % of sulfate was removed in the silfidogenic reactor with the subsequent production of an average 116mg/L/d of hydrogen sulfide during the operational period. The hydrogen sulfide was converted to sulfur in the oxidising reactor with 97 % sulfur recovery. An average102 mg/L/d sulfur was produced in the sulfur oxidising reactor. The results indicate the potential in using FTWW as a cost-effective electron and carbon source for biological sulfate reduction allowing for the co-treatment of AMD and FTWW.

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List of Abbreviations

ABR	Anaerobic Baffled Reactor
AFR	Anaerobic Filter Reactor
AHR	Anaerobic Hybrid Reactor
ALDs	Anoxic Limestone Drains
AMD	Acid Mine Drainage
APS	Adenosine phosphosulfate
APS	Alkalinity Producing Systems
BTL	Biomass to Liquid
COD	Chemical Oxygen Demand
CSIR	Council for Scientific and Industrial Research
CSTR	Continuous Stirred Tank Reactor
CTL	Coal to Liquid
DSR	Dissimilatory Sulfate Reduction
DWA	Department of Water Affairs
DWs	Diversion Wells
EPA	Environmental Protection Agency
FBR	Fixed Bed Reactor
FT	Fischer Tropsch
FTWW	Fischer Tropsch Wastewater
GDP	Gross Domestic Product
GTL	Gas to Liquid
HTFT	High Temperature Fischer Tropsch
LLBs	Limestone Leach Beds
LTFT	Low Temperature Fischer Tropsch
MA	Methanogenic Archaea
OLDs	Oxic Limestone Drains
PRBs	Permeable Reactive Barriers
RAPs	Reducing Alkalinity Producing Systems

PSS	Primary Sewage Sludge
SA	South Africa
sAMD	Simulated Acid Mine Drainage
SAPS	Successive Alkalinity Producing Systems
SASOL	South Africa Synthetic Oil Liquid
SCFA	Short Chain Fatty Acids
sFTWW	Simulated Fischer Tropsch Wastewater
SLBs	Slag Leach Beds
SOB	Sulfur Oxidising Bacteria
SRB	Sulfate Reducing Bacteria
SRBR	Sulfate Reducing Bioreactor
STP	Standard Pressure and Temperature
UASB	Anaerobic Sludge Bed Reactor
USA	United States of America
VFW	Vertical Flow Wetland
WHO	World Health Organisation

1.0. Introduction

1.1 Background and Motivation

Water is regarded as one of the most important resources in the world. The availability and quality of water is essential for the survival of ecosystems and the sustainability of communities and activities associated thereof. The twenty first century has faced challenges associated with the sufficient supply of high-quality water. Water shortages have been attributed mainly to intermittent rainfall patterns, population growth, which has led to urbanisation and industrialisation, increasing the demand for water usage as well as pollutants from industry and domestic activities (Fayemiwo, 2018). According to the US National Intelligence Council (2009), available freshwater resources will continue to decrease in the coming years due to increasing demand of a rising world population (Figure 1-1). Many areas of the world currently experiencing a shortage of water resources are most likely going to see their water issues worsening, triggering hardships to millions of people.

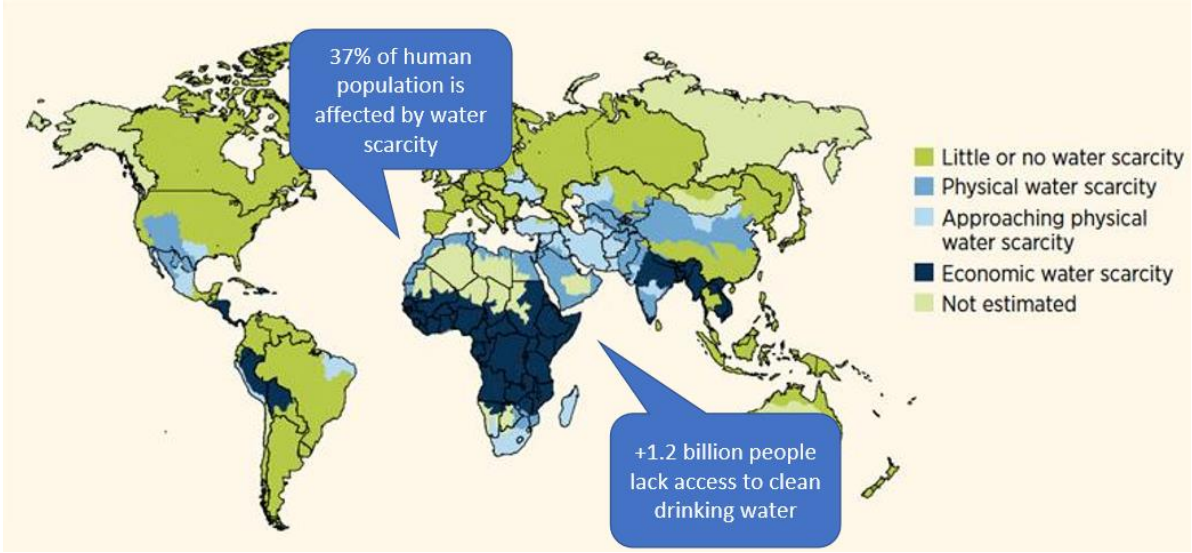


Figure 1-1: Projected Water Scarcity 2025. Source: World Water Programme (2012)

Water pollution is one of the biggest threats to the availability of clean water. According to the US Environmental Protection Agency (EPA) (2015), any activity whereby chemicals or waste is discharged into the environment, either purposefully or fortuitously, has the likelihood of polluting ground water. Most of the contaminants in the ground water migrate with the water, which makes it problematic to decontaminate ground water once polluted. Surface water is easily polluted if waste streams are discharged into streams and rivers with partial or without treatment.

Untreated industrial and domestic wastewater contribute to large amounts of both inorganic and organic pollutants, sometimes including pathogens from faecal contamination (Sasakova *et al*., 2018; Qadir *et al*., 2010; Gardner and Finlayson, 2018). In many rural communities in the developing world, ground or surface water intended for consumption is taken from wells, streams and rivers and is used without further treatment. These communities are therefore potentially exposed to various water borne diseases (Sasakova *et al*., 2018; Wang *et al*., 2012; Qadir *et al*., 2010).

The disposal of sulfate and metal rich wastewaters is one of the biggest threats to the environment (Vasquez *et al*., 2016). The acid and sulfur-rich wastewaters are produced by a diversity of industries (Hussain *et al*., 2014). These industries use sulfuric acid, sulfate rich feedstocks or other reduced sulfur compounds and they include the pulp and paper production, molasses fermentation, tanneries, and seafood processing (Yu *et al*., 2014; Lens *et al*., 1998). Combustion of fossil fuels also contributes to a large quantity of sulfur dioxide into the atmosphere, which can be oxidised to sulfate which is dissolved in raindrops to form acid rain.

The major contributor of sulfate and metal rich effluent however is the mining industry (Johnson and Hallberg, 2005). The activities associated with the exploitation of coal and metals from ores containing sulfidic minerals can lead to the generation of substantial amounts of

sulfate and metal rich wastewater (Oyekola, 2008; Akcil and Kodas, 2006). Sulfidic minerals contain the element sulfur combined with different types of metals including zinc, iron, copper, lead and arsenic. Amongst the various sulfides, iron sulfide (FeS_2) is the most abundant and is usually found in association with both metal and coal deposits (Evangelou, 1995).

The excavation and processing of sulfide containing metal or coal deposits to extract valuable minerals or coal exposes the iron sulfide to water, oxygen, and bacteria. Under the conditions the sulfide is oxidised to sulfate, releasing a low pH, sulfate and metal rich effluent called acid mine drainage (AMD). (Kaksonen *et al*., 2008; Bomberg *et al*., 2015, Vieira *et al*., 2014; van den Berg *et al*., 2016; Moodley, 2017; Hesketh *et al*., 2010).

AMD is generated from the mining operations as well as from waste rock piles and from tailing dams (Greben *et al*, 2009; Macingova and Luptakova, 2012; Akcil and Kodas, 2006). This water usually contains high levels of acids, salts, and dissolved metals (Neculita *et al*., 2010, Macingova and Luptakova, 2012; Balintova *et al*., 2018; Ay *et al*., 2018), resulting in a low pH and often corrosive wastewater. The low pH of AMD results in the dissolution of minerals. This has the effect of releasing toxic metals into the environment (Sangita *et al*., 2010).

The AMD is carried from the source and may be transported for long distances by rainwater or surface drainage and deposited into nearby streams, rivers, lakes, and ground water (Figure

1-2) (Mineo consortium, 2000). AMD polluted water may be virtually unusable and contaminated streams maybe devoid of life (Ay *et al*., 2018).

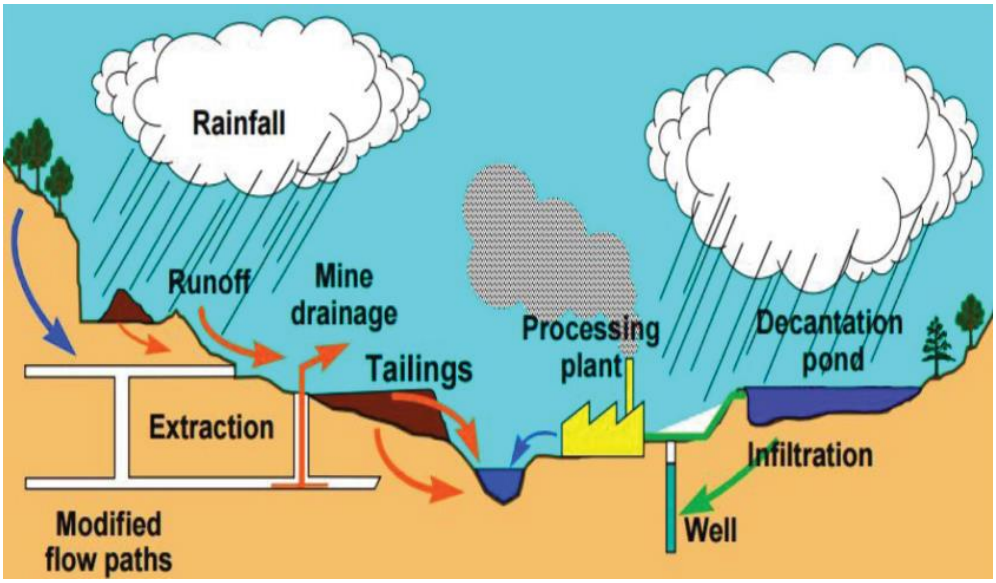


Figure 1-2: Surface and ground water contamination by mining operations.

Source: Mineo Consortium.

An assessment by the Council for Scientific and Industrial Research (CSIR) (2013), on some high-risk communities including Khutsong, Coronation and Clarinet in South Africa (SA), established the presence of AMD and elevated levels of toxic heavy metals in the local surface water and vegetables. These communities reported high levels of symptoms and illnesses related to heavy metal exposure as compared to reference communities with low risk of AMD.

AMD pollution tends to lessen the water available for recreation, domestic use, and industrial purposes. AMD also influences plant and animal life; this includes causing stunted growth, lower reproduction rates, deformities, and lesions (Sangita *et al*., 2010), sometimes leading to the elimination of life in contaminated streams (EPA, 2015). AMD has also been shown to affect microbial community dynamics in the surrounding soil and aquatic environments (Gao *et al*., 2019).

AMD is corrosive and may damage infrastructure such as pipes, bridges and other metal and concrete structures. Soil affected by AMD is structurally unstable and highly prone to erosion (RoyChowdhury *et al*., 2015). Once the conditions necessary for the formation of AMD are obtained, the generation of AMD will only stop when the sulfidic minerals are exhausted. This means AMD can continue to be generated even after mining activities have ceased to occur. Proper management of preventing or treating AMD should therefore be practised to prevent the discharge of AMD into natural watercourses.

Studies have been carried out on the treatment of AMD using Dissimilatory Sulfate Reduction (DSR) due to several advantages over the traditional physico-chemical methods of treatment. The advantages of using DSR in the treatment of AMD include low operational and labour costs, permanent removal of both metals and sulfates, greater ability to recover valuable metals and sulfur, and the generation of a less hazardous, low volume sludge (Rambabu *et al*., 2020).

DSR uses sulfate reducing bacteria (SRB) in the presence of an organic/electron donor to reduce sulfate to sulfide. This process also produces hydrogen carbonate. The sulfide reacts with metals forming insoluble metal sulfide precipitates (Bowell, 2004; van den Berg *et al*., 2016). The hydrogen carbonate consumes the protons in AMD, neutralising the acidity thereby increasing the pH of the effluent (Greben *et al*., 2009; Johnson and Hallberg 2005; Lottermoser, 2010; van den Berg *et al.*, 2016; Zagury and Neculita, 2007; Cao *et al*., 2009; Kaksonen *et al*., 2007. The application of DSR for AMD treatment has been limited by, the cost and availability of electron donors, the relatively slow growth of sulfate reducers as well as the management of the sulfide product, which is toxic, corrosive, and malodorous (Rose, 2013; van Hille *et al*., 2015).

Different sources of organic material such as alcohols and organic acids have been successfully tested for their ability to support DSR (Sahinkaya *et al*., 2017; Papiro *et al*., 2013, Bharati and Kumar, 2012). These are expensive, leading researchers to look for cheaper and readily available alternatives such as cellulosic material and compost (Ramla and Sheridan, 2014; Magowo *et al*., 2014).

SRB are not able to use complex organic compounds, therefore SRB rely on cellulose decomposers and fermenters to provide short chain fatty acids and alcohols (Magowo *et al*., 2014), a factor which make the treatment process complex and slow. It is therefore necessary to find cheap substrates, which can easily be used by SRB.

Several organic rich industrial waste streams can be considered as cheap alternatives in the bioremediation of AMD using DSR. Some of these waste materials are situated on or near areas, which produce large amounts of AMD. It is an advantage to use these wastes as they are cheaper, and the cost of transportation is reduced or eliminated as these waste streams are located close to each other.

This study seeks to explore the possibility of using Fischer Tropsch wastewater (FTWW) as a substrate for dissimilatory sulfate reduction. FTWW is generated by the Fischer Tropsch (FT) process, which converts coal and natural gas to synthetic fuels (Van Zyl, 2008; Majone *et al*., 2010). FTWW has high chemical oxygen demand (COD) levels of up to 28000 mg/L. The COD is mainly composed of alcohols and short chain fatty acids (SCFA) which cannot be separated economically from the wastewater (Van Zyl, 2008).

Currently, Sasol is using the activated sludge method to treat FTWW. This method is expensive due to costs incurred due to aeration and agitation and leads to the loss of organic material through oxidation to carbon dioxide and water (Sasol media centre, 2013). These costs can be avoided by using DSR as the process is anaerobic and does not require energy for aeration.

In this study, it is hypothesised that the alcohols and SCFA in FTWW can be used as substrates for the biological removal of sulfates and metals from AMD. The AMD will be combined with the FTWW in the presence of SRB. The FTWW provides low molecular weight carbon compounds which are used as substrates for the DSR process. Dissolved metals in the AMD react with the hydrogen sulfide produced through biological sulfate reduction to form insoluble metal sulfides. The pH of the AMD also increases through biologically generated alkalinity.

A subsequent aerobic step that converts excess hydrogen sulfide to elemental sulfur will be carried out. This step is necessary to remove both the odours associated with, and the corrosive nature of H_2S. COD is removed from FTWW, and sulfates, acidity and metals are removed from AMD. In this way, the feasibility of the joint treatment of the two waste streams will be evaluated.

In South Africa, the Sasol FT process is situated in Secunda, a region that is rich in coal and hence has a high potential to produce large volumes of AMD. There are various CTL plants operating or at different levels of construction in the world. These plants have the potential to generate large amounts of AMD (due to coal mining and associated activities) and FTWW (from the FT process). Given the proximity of FT plants to coal mines, this study seeks to combine these waste streams to remediate both the AMD and the FTWW using DSR. This will significantly lower the costs of treatment since the FTWW is a waste material and the transport costs are significantly reduced due to the proximity of the two wastewater streams.

Chapter 2. Literature Review

2.1 Acid mine drainage an overview

Coal and metal mining activities are major contributors to many economies across the world. This however comes at a cost, as mining is one of the major environmental polluters. The pollution may be land degradation, soil contamination, air pollution or the contamination of water resources (Chepkemoi, 2017).

The contamination of water resources through mining activities is a major environmental concern second only to global warming (Durand 2012; Sheridan *et al*., 2018). Mining activities may result in the discharge of a highly acidic and metal-rich effluent called AMD. AMD is responsible for many ecological disasters as it causes long term damage to water ways and biodiversity (Akcil and Kodas, 2006; Gardner and Finlayson, 2018)

AMD is formed through the interaction between sulfide bearing rocks, water, and air (Dzvairo and Mujuru, 2017). Both chemical and biological processes contribute to the formation of AMD. The rate of AMD formation is dependent on the size and therefore the surface of the rock exposed, climate, hydrology, and the geology of the mining area (Mujuru *et al*., 2016; Jonhson and Hallberg, 2005; Akcil and Kodas 2006; Jamil and Clarke, 2013; Ramla and Sheridan, 2014; Nancucheo and Johnson, 2012). These parameters are different from region to region making the prevention, composition, containment, and treatment of AMD difficult to predict (Mujuru *et al*., 2016; Akcil and Kodas, 2006).

The oxidation of sulfide rocks occurs slowly in nature. Mining activities greatly accelerate the process by exposing the sulfidic rocks to water, oxygen, and bacteria (Sheridan *et al*., 2018; Mujuru *et al*., 2016; Magowo *et al.,* 2014), as a result AMD is prevalent in mostly mining areas (Sangita *et al.,* 2010; Feris and Kotze 2014; Bratkova *et al*., 2018). The exposed sulfide is oxidised to sulfate in chemical reactions that are greatly enhanced by the activities of bacteria (van den Berg *et al*., 2016; Moodley., 2017; Hesketh *et al*., 2010; Hiibel *et al*., 2011).

Iron sulfide (Pyrite) is the major contributor of AMD (Dzvairo and Mujuru, 2017). Under aerobic conditions and in the presence of water, pyrite is oxidised to sulfate resulting in the mobilization of metals and the formation of sulfuric acid leading to the release of a low pH effluent (RoyChowdry *et al*., 2015; Ramla and Sheridan, 2014; Lukovic and Stankovic, 2012; Magowo *et al*., 2014). Pyrite oxidation occurs in several steps, which involve an oxygen-independent reaction and an oxygen dependant reaction (Lukovic and Stankovic, 2012). The overall stoichiometric equation for the formation of AMD is as shown in Equation 1.0.

$FeS_2 + 3.75O_2 + 3.5H_2O \rightarrow Fe(OH)_3 + 2SO_4^{2-} + 4H^+ + energy$ (Equation 1.0)

In nature, this reaction occurs slowly, and the water can buffer both the acidity and soluble metal ions. Mining activities amplify these reactions as they expose large surface areas of sulfidic rocks resulting in the production of large volumes of AMD which overwhelms the natural buffering capacity of natural water streams (Feris and Kotze 2014; Ochieng *et al*., 2010; Jennings *et al*., 2008).

The formation of AMD produces acidity which contributes to solubilising and therefore mobilising metal ions such as iron, zinc, radium, lead, uranium and many more (RoyChowdry *et al*., 2015; Feris and Kotze 2014; Sangita *et al*., 2010). All these oxidation products result in an effluent with a high load of dissolved heavy metals, sulfate, and a very low pH (Sangita *et al*., 2010; Neculita *et al*., 2010; Bratkova *et al*., 2018; Westernsee *et al*., 2018) which results in adverse impact on the environment.

At low pHs, Fe^{3+} precipitates as $Fe(OH)_3$ (Akcil and Kodas, 2006; Sheridan *et al.*, 2018). This produces a yellow-orange coloration of the water termed yellow-boy, which affect the ecosystems' food chains and webs as light is blocked for photosynthetic aquatic organisms (Sheridan *et al*., 2018). The quoting of the stream surfaces by metal hydroxides precipitates reduces habitat (Hogsden and Harding, 2012). The acidity is also not suitable for aquatic life and usually results in streams devoid of life. Dissolved metal ions including uranium, copper, lead mercury, cadmium, and zinc, affect both the marine and terrestrial life as they accumulate, up the food chain. Infrastructure is also threatened, as AMD is often corrosive (Bobbins, 2015).

2.1.1. Mining and AMD

In natural environments, the rate of formation of AMD occurs slowly and the natural buffering capacity of water can easily remove the acidity (Mccathy., 2011). Activities that expose sulfide-containing minerals to oxygen, water and bacteria accelerate the rate of formation of AMD (Kaksonen *et al*., 2008; Bomberg *et al*., 2015; Viera *et al*., 2014 Naidu *et al*., 2018).

Mining and associated activities are the major contributors of AMD, with both abandoned and active mines releasing large amounts of AMD into the environment (Sangita *et al*., 2010; Feris and Kotze 2014; Bratkova *et al*; 2018; Sheridan *et al*., 2018). The processes involved in the extraction of minerals from ores involves digging up the ore which can be underground or open pits, crushing and milling to concentrate the ore (Blowes *et al.*, 2003), smelting to separate the high-grade minerals from the rest of the rock, storage of waste solid material and process water. The fragmentation of the rock material exposes and increases the surface area of the sulfidic

minerals to atmospheric oxygen and water thereby speeding up the rate of AMD production (Naidu *et al*., 2018).

2.1.1.2. Underground workings and open cast mining

The most frequently used methods of mining are underground, strip and opencast mining (Blowes *et al.,* 2003). Opencast or surface mining is applied when extracting minerals, which are located close to the earth surface. Though surface mining is less expensive to operate than underground mining, it has negative environmental impacts such as noise, dust, vibrations, air, and water pollution (CSIR, 2019). Figure 2-1 is a photograph of surface mining for iron ore mining at Kolomela mine in Northen Cape, South Africa (CSIR, 2019). It shows that open cast mines expose large areas of rock and produces substantial waste material due to excavations to remove overburden and waste rock (Fashola *et al*., 2016).

Underground mining is used to access mineral ores that are located deep underground as shown in Figure 2-2 (CSIR, 2019). Underground workings expose rock faces to air and moisture resulting in the oxidation of sulfidic minerals. A fluctuating water table ensures that oxidised material is dissolved, and the sulfidic surface is continuously renewed to undergo further oxidation (Burgers, 2002). The flooding of underground minerals leads to the release of AMD to the surface, contaminating soils, ground, and surface water.



Figure 2-1 Open Cast Mining at Kolomela mine in Northen Cape, South Africa (CSIR, 2019)

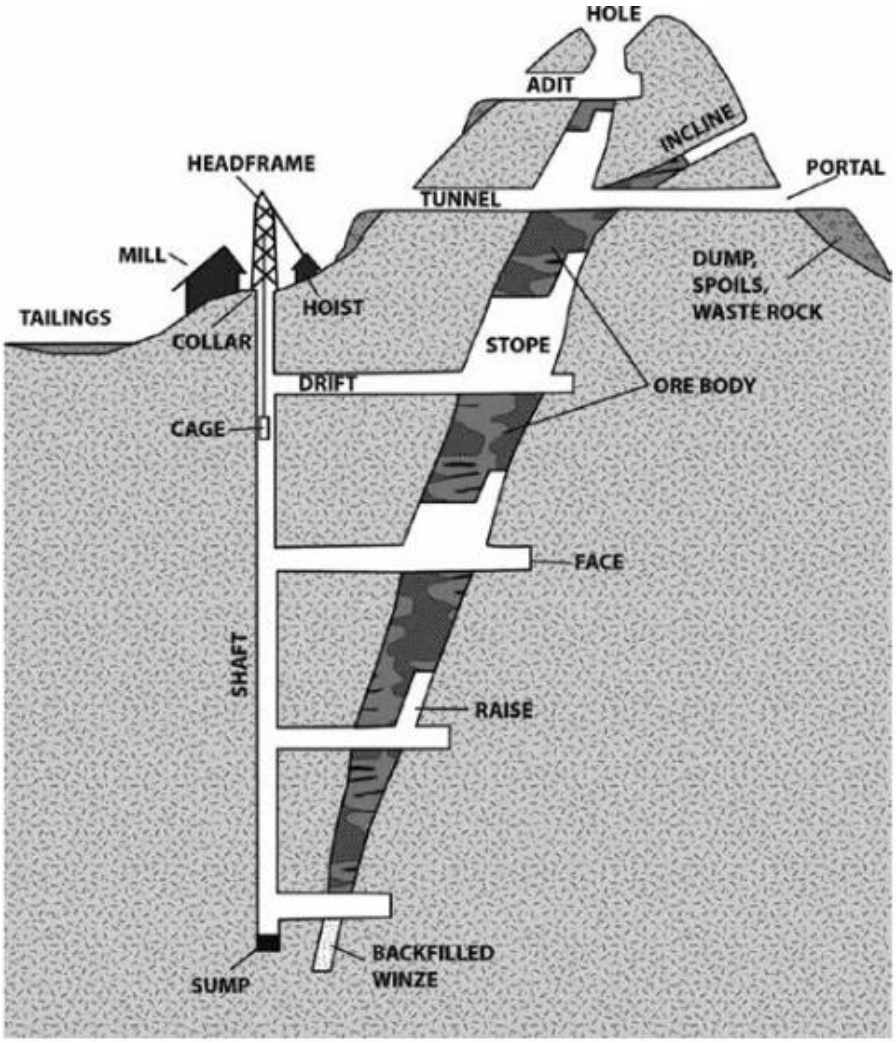


Figure 2-2: Illustration of underground mining

Source: CSIR (2019)

In active mines, the contact time between water, oxygen and sulfide is not usually enough to allow for the formation of AMD because water is continuously pumped out of the mines (Sheridan *et al*., 2018). The problem occurs after the mines are closed and pumping is discontinued resulting in water accumulating in the voids (Durand, 2012). If sufficient oxygen is available, AMD formation is inevitable. In flooded underground mines, the rate of formation of AMD is slow because oxygen is excluded therefore less acid is released in comparison to free draining mines (Pope *et al*., 2010).

2.1.1.3. Waste Rock

After the extraction of the mineral bearing ores from the earth, there is need to separate the minerals from the waste rock. This is done by first crashing the ore to allow the recovery of minerals of economic value. The high-grade mineral ore is then separated from the low-grade

ore. The low-grade ore is stored in mine dumps, which are commonly found in metal mining areas (Dold, 2014; Blowes *et al*., 2003). The composition of the waste piles varies from mine to mine due to variations in ore deposits and host rock mineralogy (Blowes *et al.*, 2003). Waste rock is generally made of course material, which might still have high concentrations of sulfide (Dold, 2014). Because waste rock materials have large particle size, they have higher permeability for oxygen and water, which allows the oxidation of sulfide to occur. The products of the oxidation process are released in large quantities in the wet period and may result in the contamination of the environment causing instability in the system (Burgess 2002).

The production of acid depends on the mineral composition of the waste rocks. Waste rock, which is rich in carbonates, can keep pH at levels close to neutrality. This allows metals to precipitate reducing the transportation of both metals and acidity into the environment (Villa *et al*., 2008). When the carbonates are exhausted, acidity begins to accumulate, and the pH begins to drop allowing metal ions to be mobilised.

2.1.1.4. Process tailings

The high-grade ore is further processed to obtain the target mineral. The processes involve crushing, grinding, and milling to reduce the size of grain to very fine particles (Blowes *et al*., 2003). The process tailings are stored as dumps. These tailing dumps may reach up to 52 km^2 in area and maybe up to several hundreds of meters high (Dold, 2014). Process tailings have finer particle size, meaning they might contain a higher sulfate content increasing their potential to generate AMD compared to waste rock piles. However, because of the smaller particle size, water and oxygen moves slowly through the dumps, slowing down rate of AMD formation. Tailings, therefore, tend to produce AMD at a much slower rate than waste rock (Burgess, 2002).

2.2 Chemistry of AMD Formation

The oxidation of pyrite within the waste materials and the mine working results in the release and mobilisation of oxidation products (Blowes *et al*., 2003, Kaksonen *et al*., 2008; Bomberg *et al*., 2015, Vieira *et al*., 2014). Though a host of sulfidic minerals contributes to the formation of AMD, the oxidation of iron sulfide is the main process that results in the release of acid into AMD (Akcil and Kodas, 2006; Neculita *et al.*, 2007; Sangita *et al.*, 2010, Herricks, 1982). The process starts when pyrite, oxygen and water come together, and reacting to produce ferrous

iron sulfate and acidity (Equation 1.1) (Sangita *et al*., 2010; Pradhan and Deshmukh, 2008; Jennings *et al*., 2008).

$2FeS_2\ (s) + 7O_2\ (g) + 2H_2O\ (l) \rightarrow 2Fe^{2+} + 4SO_4^{2-} + 4H^+$ (Equation 1.1)

The ferrous iron (Fe^{2+}) is solubilised by the oxidation of sulfide. In the presence of oxygen, the released Fe^{2+} is oxidised to the ferric (Fe^{3+}) state (Equation 1.2). The bacteria, *Thiobacillus ferrooxidans*, hasten this process.

$4Fe^{2+}\ (aq) + O_2\ (g) + 4H^+ \rightarrow 4Fe^{3+}\ (aq) + 2H_2O\ (l).$ (Equation 1.2)

The ferric iron may either precipitate as $Fe(OH)_3$ giving AMD its characteristic yellow colour, or it may further oxidise the iron sulfide to produce more ferrous iron and acidity (Equation 1.3 and 1.4) (Jennings *et al*., 2008; Blowes 2003).

$2Fe^{3+} + 6H_2O \rightarrow 2Fe(OH)_3(s) + 6H^+.$ (Equation 1.3)

$FeS_2\ (s) + 14Fe^{3+}\ (aq) + 8H_2O\ (l) \rightarrow 15Fe^{2+}\ (aq) + 2SO_4^{2-}\ (aq) + 16H^+\ (aq).$ (Equation 1.4)

The oxidation reactions and the precipitation of metals as hydroxides both have the net effect of releasing hydrogen ions, generating acidity, thereby lowering the pH off the effluent, and maintaining the solubility of the ferric ions (Blodau, 2006; Dold, 2014). As more acidity is produced, more ferric ions are released, the ferric ions will then oxidise the ferrous ion and more acid is produced. These reactions allow the cycle to continue until the exposed sulfide material is exhausted (Sams and Beer, 2000).

Other metal sulfides can also be oxidised upon exposure to oxygen and water. The solubilised metals contribute to the acidity as they hydrolyse and precipitate out of solution. Aluminium is the second most abundant metal contributing to the formation of AMD (Equation 1.5) (Espana, 2007; Taylor *et al.*, 2005).

$Al^{3+} + 3H_2O \rightarrow Al(OH)_{3(s)} + 3H^+$ (Equation 1.5)

If there is sufficient acid-consuming material available to neutralise the acid, the produced acidity can be neutralised. Once the alkalinity is consumed, the acid will begin to accumulate (Sams and Beer, 2000; Vyawahre and Rais, 2016), resulting in the release of a metal rich and low pH effluent. The effluent enters the environment through affected waterways and sub-flow systems where it can be transported for distances (Sams and Beer, 2000).

2.2.1 Factors influencing the formation of AMD.

Many factors are involved in the formation of AMD. These are broadly divided into physical and chemical factors (Figure 2-3). The factors include but are not limited to temperature, moisture content, pH and the surface area of exposed sulfide mineral, porosity and permeability of the rock mass, type and quantity of sulfide minerals, texture and morphology, content of acid consuming material, total organic content, concentration of dissolved metals and other solutes as well as biological activity (Otwinowki, 1994; Mendez-Garin *et al*., 2015; Kuyacak, 2002; Akcil and Kodas, 2006).

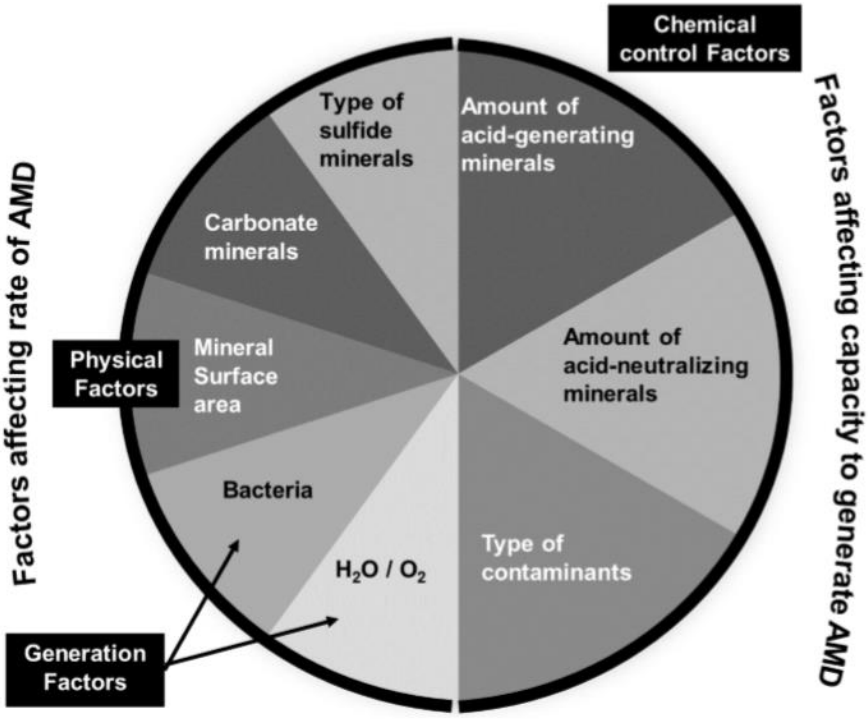


Figure 2-3: Factors affecting the formation of AMD.

Source: Pat-Espadas *et al*. (2018)

2.2.1.1. Biological activity

Biological activity is a result of the interaction between sulfide minerals and bacteria. *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidants* have been identified as some of the main microorganisms that contribute to the formation of AMD (Udayabhanu *et al*., 2010). There are however many other bacteria species involved. According to Ehrlich *et al*. (1991), the microbial mediated oxidation of sulfide mineral takes place from pH 8.5 to 1.9 with several microbial successions occurring as the pH decreases.

The bacteria involved in the reactions leading to the formation of AMD get their energy from the oxidation of reduced sulfur and iron compounds using carbon dioxide as the carbon source (Udayabhanu and Prasad, 2010; Luo *et al*., 2016). The bacteria oxidise ferrous iron to ferric iron. Either the ferric iron generated can be precipitated as ferric hydroxide or it can further oxidise ferrous sulfate forming more ferric ions and increasing the rate of supply of ferric iron,

which is an important oxidant to the surfaces of dissolving sulfide minerals. In this way, the formation of AMD is perpetuated, and the rate is increased (Druschel *et al*., 2004; Udayabhanu and Prasad, 2010).

Singer and Stumm (1970) observed that the rate of the overall pyrite oxidation process in the presents of the bacteria *Acidithiobacillus Ferrooxidans* increases from 3 x 10^{-12} mol L^{-1} S^{-1} to 3 X 10^{-7} mol L^{-1} S^{-1}. These figures were supported by the findings of Espana (2007), who obtained an increase by up to 10^5 over the abiotic rate.

2.2.1.2. Dissolved oxygen content

Pyrite oxidation requires that oxygen is present in sufficient quantities to initiate the reaction. Pyrite can be oxidised directly to produce acidity (Equation 1.6)

$$FeS_2 + H_2O + 3.5\ O_2 \rightarrow Fe^{2+} + 2H^+ + 2SO_4^{2-} \qquad \text{(Equation 1.6)}$$

According to Pozo-Antonio *et al*., (2014), the direct oxidation of pyrite is limited by the oxygen availability, which is usually low due to difficulties in diffusion hence there is insufficient levels of oxygen in ground water to initiate the oxidation of pyrite. The reaction cannot occur when the oxygen concentration is low (Eh < 30mV) (Baker and Banfield, 2003).

2.2.1.3. pH

Though a variety of microorganisms are involved in the formation of AMD at different pH ranges, biological oxidation peaks at around pH of 3.5, thus, as pH decreases more weathering occurs (Gaikwad and Gupta, 2007). In the absence of microbes, pyrite oxidation is controlled by the rate of oxidation of the ferrous ion, which decreases rapidly with decreasing pH. The availability of dissolved oxygen in the presents of pyrite material supports microbial growth, which in turn accelerates the oxidation of ferrous ion by ferric iron, releasing hydrogen ions. The acidity produced reduces the pH to levels below 4 allowing acidophilic microbes to thrive and maintain the solubility of ferric ion (Blowes *et al*., 2003).

The presents of alkaline material such as carbonates and hydroxides in the sulfide material or waste rock can maintain the pH of AMD at high levels as the acidity is neutralised by alkaline dissolution within the waste material (Webber *et al*., 2013; Villa *et al*., 2008). At pH around neutral metals are precipitated as hydroxides, reducing their transportation into the environment (Villa *et al*., 2008). The amount of alkaline material available in the mine settings should be more than the sulfide minerals so that the pH is maintained at or above neutral (Blowes *et al*., 2003).

2.2.1.4. Temperature

Temperature is essential for microbial growth and metabolism. Most organisms have an optimum temperature at which they grow and function best, with most microbial reactions observing the Q_{10} rule where the rate of reaction double for every 10^{o}C increase in temperature up to the optimum temperature. Temperature also affects the abiotic oxidation of pyrite as solubility of reactants increases with temperature. The rate of diffusion of reactants also increases with increases in temperature.

The bacteria involved in the oxidation of pyrite have been reported to work in wide temperature ranges. Some of the bacteria are phycophiles, growing at temperatures below 20^{o}C. *Thiobacillus* species are mainly mesophilic, growing at temperature between 25^{o}C and 35^{o}C. Thermophilic bacteria can grow at temperatures of up to 90^{o}C, examples are *sulfolobus* and *sulfobacillus* species. Interior temperatures in mine waste piles can reach temperatures of up to 80^{o}C due to the exothermic nature of pyrite oxidation, thermophilic bacteria have been found in these conditions (Kuyucak, 2002).

2.2.1.5. Surface area of exposed sulfidic minerals

In natural environments, the rate of formation of AMD occurs at a slow rate, because the amount of exposed sulfide material is limited (Sheridan *et al*., 2018). Mining activities however exposes rock surfaces. During processing, the rocks are fragmented into smaller particles that

have larger surface area which are exposed to water and oxygen thereby increasing the rate of formation of AMD (Afriyie-Debra *et al*., 2010; Sheridan *et al*., 2018).

Waste rocks typically have mean particle diameter above 20 cm while process tailings are less than 0.2mm. Tailings have a large surface area, which enhances the rate of oxidation (Kuyucak, 2002), this however is offset by the low availability of water and oxygen due to limitations in permeability caused by the smaller size of the tailings (Burgess *et al*., 2002).

2.3. Effect of AMD on the ecosystem

The discharge of AMD from closed mines and mine waste have led to the contamination of water resources in many sites in the world. Water sources contaminated with AMD have high concentrations of sulfate ions and dissolved heavy metals as well as high acidity (Sierra-Alvarez *et al*., 2006; Hesketh *et al*., 2010; Reis *et al*., 2014; Jencarova and Luptakova, 2012). The toxic metals dissolved by AMD including manganese, zinc, aluminium, nickel, cobalt, copper, radium, and uranium are characterised by varying degrees of toxicity and radioactivity. Uranium and radium can cause cancer in human beings (Taylor *et al.*, 2005; Kousi *et al*., 2015). The mobilisation of acidity and metals lead to the deterioration of waterways leading to their reduced application for activities such as recreation and irrigation. Some of the receiving streams may have a pH as low as 2.0. This leads to the destruction of vegetation in areas affected by AMD and the decimation of aquatic life (Viera *et al.*, 2014).

Metal precipitates are also found in sediments of AMD polluted water bodies. Stream bottoms are covered with orange or yellow-brown iron oxide or white aluminium oxide precipitates. The physical coating of the stream surfaces destroys habitat and decreases the availability of clean gravel for spawning (Jennings *et al.*, 2008; Sheridan *et al*., 2018), This affects the food chain dynamics in the system leading to lack of biodiversity (Sams and Beer, 2000; Sangita *et*

al., 2010). The AMD may seep into underground water resources leading to the acidification and the contamination of groundwater as well as soils by heavy metals (Poinapen, 2008).

Sulfate, though not especially toxic, has a laxative effect if consumed at concentrations above 600mg/L in drinking water (Luptakova *et al*., 2016). As such, there are no strict guidelines from the World Health Organisation (WHO), however, WHO recommends the authorities be notified if sulfate levels exceed 500mg/L in drinking water. Some of the negative impacts of AMD on the ecosystem are listed in Table 2-1.

Table 2-1: Effects of AMD on aquatic systems

Chemical	**Physical**	**Biological**	**Ecological**
Increased acidity	Increase in stream velocity	Behavioural	Loss of habitat
Reduction in pH	Substrate modification	Affects respiration	Niche loss
Destruction of bicarbonate buffering system	Turbidity	Affects reproduction	Bioaccumulation within food chain
Increase in soluble metal concentration	Sedimentation	Osmoregulation	Loss of food source for prey
Increase in particulate metals	Adsorption of metals onto sediments	Acute and chronic toxicity	Reduces biodiversity
Increase in particulate material	Decrease in light penetration	Acid base balance fails in organisms	Reduction of primary production

Source: Singla *et al.,* (2017).

2.4. Water and AMD situation in South Africa (SA)

South Africa (SA) receives below average rainfalls of less than 500 mm per annum. Within SA the distribution of the rainfall is uneven with the eastern part of the country receiving more rainfall than the Western part (Figure 2-4). Major droughts are occasionally experienced in SA

(WWF, 2016) as exemplified by the 2017-2018 drought experienced in the Cape Town region. Rainwater is very important, as it is the primary source of water resources in SA. Water resources include surface water that flows in rivers and wetlands or is stored in dams and lakes, and ground water that is mainly underground water and is stored in soil, rock pores and crevices and aquifers. Most urban settlements depend on surface water that is pumped into their homes, while rural communities use surface or ground water obtained from wells for their daily consumption (WWF, 2016).

According to Donnenfield *et al*., (2016), because of insufficient rainfall and overexploitation of water resources in SA over the past few years, dam levels have drastically dropped from around 93% full to as low as 48% in November 2016. Dam levels as low as 20% have been reported in the Breede-Gouritz catchment area in Western Cape. Rivers have not been spared either as a report from the Department of Water and Sanitation reported that of the 565 rivers in SA, 105 are categorised as having very low flows, with another 105 categorised low, while a further 88, rivers have moderately low flows (Donnenfield *et al*., 2016).

The quantity and quality of ground and surface water is also threatened by pollution from domestic, industrial, and mining activities. This is exuberated by the increase in the population and hence the economic activities to support the population increase. According to CSIR (2019), 60 % of the South African river ecosystems are threatened by pollution while 25% are critically endangered. This pattern is also seen in wetlands, with 65% of wetlands being threatened while 48% are already considered critically endangered. AMD is cited as the major contributor of water pollution in SA and globally.

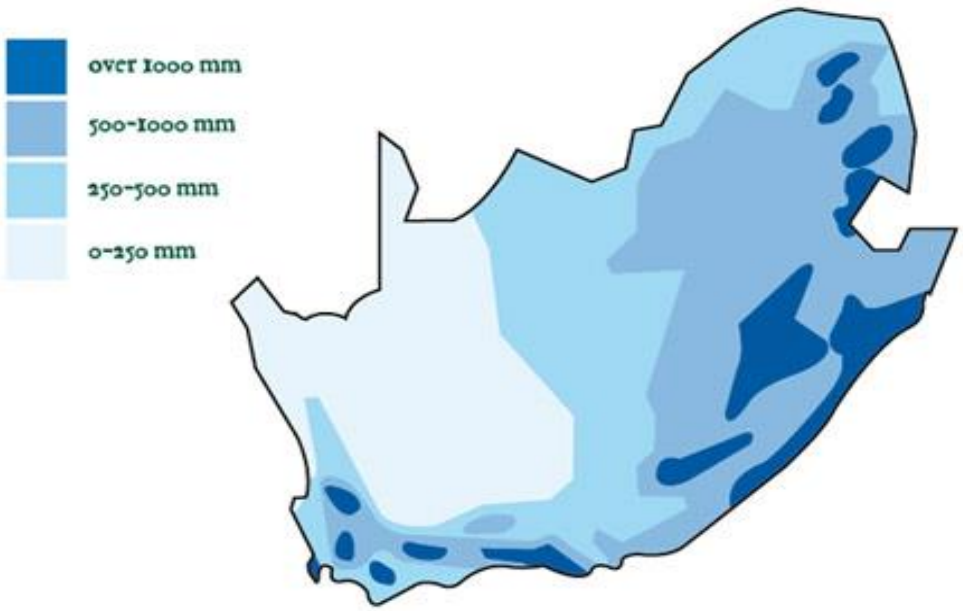


Figure: 2-4: Distribution of annual rainfall patterns in South Africa

Source: Rand water (2018)

2.5. AMD and mining in South Africa.

Mining has been one of the major contributors of the South African economy. According to De Lange *et al*. (2019), mining contributed 464 667 jobs, R312 billion to the gross domestic product (GDP), and R16 billion in tax in the 2017 financial year alone. The legacy of mining in South Africa is not only the abandoned infrastructure, but also the amount of water mining activities consumes as well as millions of litres of wastewater generated through these activities. According to Haggard *et al* (2013), mining is a major consumer of water, utilising 3% of the total water consumed in SA. Mining has been noted as a major contributor in the production of wastewater laden with heavy metals (Matsimoto *et al*., 2016).

As discussed in Section 2.2., AMD formation is a result of the interaction between pyritic rock, oxygen and water that is enhanced by mining activities. Releasing raw AMD from abandoned

mines and tailings is a threat to water and the economic security of SA and the world over. Because of AMD, high levels of sulfate and dissolved metal ions as well as acidity are introduced to ground and receiving water surfaces (Sierra-Alvarez *et al*., 2006; Hesketh *et al*., 2010; Reis *et al*., 2014; Jencarova and Luptakova, 2012).

AMD is responsible for the mobilisation of manganese, zinc, aluminium, nickel, cobalt, copper, radium, and uranium. Radium and uranium are characterised by varying degrees of toxicity and radioactivity (Taylor *et al.*, 2005; Kousi *et al*., 2015). The contamination of potable water by AMD reduces the application of water for both domestic and industrial purposes. According to the CSIR (2018), The Witwatersrand Gold fields, in SA, has the potential to produce about 350 ML/day of AMD, which equates to a tenth of the potable water supplied daily by Rand Water. The produced AMD has the potential to pollute ground and surface water as well as soil.

According to Ochieng *et al*. (2010), over 70% of water used in both urban and rural SA is surface water drawn from rivers, lakes, ponds, and streams. As such, AMD discharges in SA are negatively affecting these freshwater resources.

2.5.1. Gold Mining in South Africa

Gold has contributed to the South African economy since its discovery in 1886 in Johannesburg. Gold was discovered in the Witwatersrand area of South Africa and is still one of the major gold deposits globally. The Chamber of mines of SA put the current gold production to date at more than 2 billion ounces of gold.

The Witwatersrand area is composed of the Western basin, the Central basin, and the Eastern basin. Other gold mining regions contributing to AMD are the Free State gold fields, Klerksdorp-Orkney-Stifontein-Hartsbeesfontein gold fields, the far Western basin and the Evander gold fields (Mjimba *et al*., 2016).

The exploitation of ore bodies led to the proliferation of both ground and surface mining activities, especially in the gold-rich Gauteng region. Because of the large scale, mining operations specifically in the Witwatersrand, mines were so close together that it led to mine shafts being interconnected (Winde and Stoch., 2010). Many of these shafts are so deep that they eventually met the water table resulting in flooding of the mineshafts. In operating mines, it is necessary to pump the water so that mining activities are continued. In closed mines, the pumping has stopped resulting in water accumulating in the underground voids and rising to the surface leading to the contamination of both ground and surface water resources (Bobbins, 2015; McCathy, 2011; Sheridan *et al*., 2018).

2.5.2. Coal mining in South Africa

Coal is one of the major mineral resources in SA according to the South African chamber of mines, SA has 3.5 % of the world coal resources, while the global estimates of recoverable coal reserves were at some 411 321 million tonnes as of 2011 (Pooe and Mathu, 2011).

According to XMP consulting (2013), coal mining in SA commenced in 1870 in the Eastern Cape to provide energy for the Kimberly diamond fields. Mining in Mpumalanga started in 1894 after the discovery of gold deposits in the Witwatersrand (1894) to provide coal for the growing gold and diamond mining industries (McCathy, 2011; XMP consultancy, 2013). The mining of coal is by either opencast (64 %) or underground methods (36 %) (XMP consultancy, 2013). Coal is mainly mined in Mpumalanga, KwaZulu Natal and the Free State (Figure 2-5), with 83 % of the total tonnage coming from Mpumalanga. As with gold mining, some coal host rocks contain pyrite, which is one of the principals in the formation of AMD. The threat of AMD from coalmines is currently from closed coalmines in Witbank. There is still a threat from the active mines if proper management is not done (Mjimba *et al*., 2016). Other mining activities contributing to AMD include the extraction of uranium, platinum, chrome, copper, and phosphate (Figure 2-5) (CSIR, 2013).

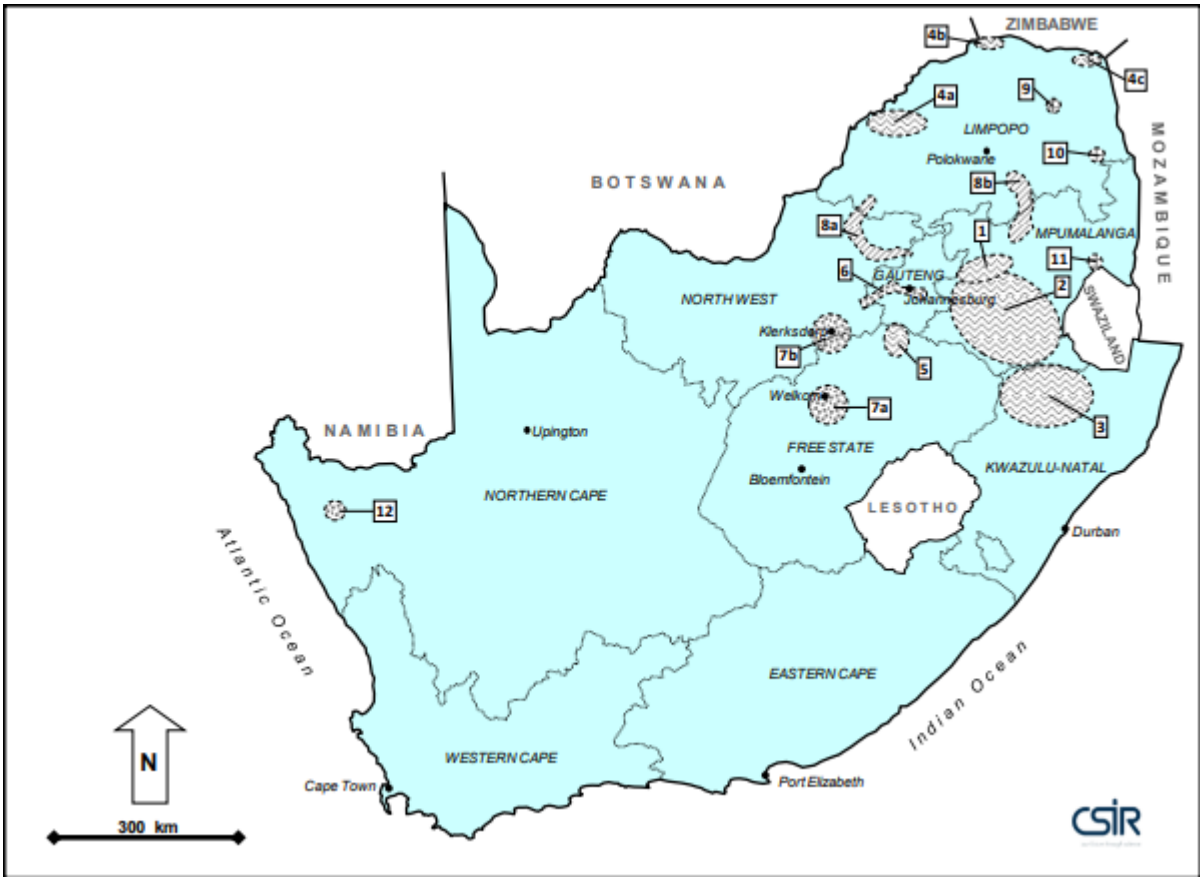


Figure 2-5: Mining areas and minerals particularly susceptible to the formation of AMD: 1 = Coal (Witbank), 2 = Coal, (Mpumalanga), 3 = Coal (Kwazulu Natal) 4 = coal (a) Waterberg, (b) Mapungubwe, (c) Pafuri), 5 = Coal (Free State), 6 = Gold and Uranium (Witwatersrand), 7 = Gold and Uranium ((a) Free State, (b) North West), 8 = Platinum and Chrome ((a) Western Bushveld, (b) Eastern Bushveld), 9 = Gold (Limpopo), 10 = Copper and Phosphate (Phalaborwa), 11 = Gold (Barberton), 12 = Copper (Okiep).

Source: CSIR (2013)

2.5.3. Incidence of AMD contamination in South Africa

AMD is released from most of the abandoned mines in SA. Waste piles are found mostly in mining areas where mining is active or inactive and contributes large volumes of AMD. This wastewater is responsible for the pollution of both ground and surface water. In active mines, the pumping of relatively clean ground water, which has not yet acidified can be done (Fig, 2011). The closure of these mines hence the ceasing of pumping has led to the accumulation of acidified water in the old tunnels. According to Durand (2012), most mines in the Witwatersrand are no longer operating, leaving the responsibility of pumping to three mines

that are still operating. This has led to large volumes of AMD being decanted into water streams (Ochieng *et al*., 2010).

Fig (2011) reported outflows of up to 20 million litres of AMD per day, contaminating surface watercourses of both the Wonderfontein and Tweelopiespruit, resulting in the deposition of sulfates and toxic metals in the affected areas. In some areas, sulfates have been measured at around 5000 mg/L, levels that are way above the recommended regulatory concern of 600 mg/L. These figures have been collaborated by McCarthy (2011) who reported sulfate concentrations above 200mg/l in the Middelburg and Witbank dam mainly from coalmines. McCarthy (2010) also reported the rising of water at a rate of 15.2 meters a month in the central basin in Gauteng Johannesburg. This wastewater had sulfate concentration more than 3500 mg/L and very low pH of between 2 and 3 with high levels of dissolved heavy metals.

McCarthy and Humphries (2013) reported the sudden drop in the water quality of Carolina dam in Mpumalanga after a heavy rainstorm. The pH of the water dropped to 3.7 accompanied by elevated levels of aluminium, manganese and sulfate rendering the water toxic and unsuitable to drink. There were reports of fish death and the discoloration of the water to a dark green colour. The pollution was traced to coalmines up stream of the dam. The dam remained polluted for seven months with government providing alternative potable water to the town of Carolina with a population of 23 000 people.

In the Gauteng province, the Western, Eastern and the Central basin are reported to contribute a large volume of the 200 ML/day (Table 2-2) of mining effluent which are discharged annually in the water bodies of the Gauteng region which accounts for sulfate loads of approximately 73 000 tonnes/annum (Ramla, 2012; Greben *et al.,* 2009). Mpumalanga contributes 12 000 tonnes/annum, resulting in the acidification and salination of regional water systems (Greben *et al*., 2009).

Table 2-2: Approximate volumes of AMD decanted to the surface in different regions.

Region	Indicative AMD volume (ML/day
Mpumalanga coal fields	200
KwaZulu Natal coal mining	10
Okiep copper district	Not significant
Wits eastern basin	120
Wits central basin	90
Wits western basin	60
Wits far west	160
Kosh	50
Evander	40

Source: (CSIR, 2019).

Both radioactive and heavy metals that are very toxic are mobilised by AMD (Hesketh *et al*., 2010; Reis *et al*., 2014; Jencarova and Luptakova, 2012). According to Sheridan *et al*., (2018), the ore body found in Johannesburg (South Africa) is uraniferous and thus AMD results in the mobilisation of uranium into the aquatic resources. Tutu *et al*. (2008), found as much as 70 mg/L of uranium in some open and accessible surface water. Robinson lake was declared a radiation area because of high levels of uranium of up to 16 mg/l as compared against a background concentration in water of 0.0004 mg/l. Tweelopiespruit river has also been declared as acutely toxic (Fig 2011). The national nuclear regulator has also classified the Lancaster Dam on the West Rand as a "Radiological hotspot". According to the Mail and Guardian (2010), the Lancaster dam is devoid of life.

Due to the flooding of mines in the Witwatersrand, springs which had dried up because of the pumping of water from the mine voids started flowing again when pumping was stopped. The

spring water was however contaminated with AMD, with high concentrations of toxic and radioactive material and heavy metals (Durand, 2012).

Iron, being the most abundant of the metals in AMD, can be oxidised and precipitated as a yellow coating on the riverbeds and banks threatening habitat for aquatic microbiota (Figure 2-6). The coating of riverbeds by metal hydroxide precipitates diminishes the availability of clean habitats, thereby affecting the aquatic food chains and webs (Jennings *et al*., 2008; Sams and Beer, 2000; Sangita *et al*., 2010). The formation of AMD requires oxygen thus removing dissolved oxygen from the aquatic systems leading to the death of organisms that depend on the dissolved oxygen. Some of the effects of AMD are shown in Figures 2-6 to Figure 2-9.

The outflow of AMD may also cause localised flooding in low-lying areas (Feris and Kotze, 2014). Some of these factors are aggravated in areas where there is little rainfall, South Africa in particular. Because of the low rainfall, the natural dilution effect of rainwater is decreased resulting in increasing salination of water resources (Ferris and Kotze, 2014). The acidity of the AMD results in the conversion of dissolved carbonates and bicarbonates into carbon dioxide and water, reducing the buffering capacity, hence the pH control system in the water.



Figure 2-6: Ferric hydroxide precipitates coating the riverbed yellow.

Source: CSIR (2013)



Figure 2-7: Fish kills are typical of mining spills.

Source: Motsi (2010)



Figure 2-8: Robinson Lake (Randfontein), source of the Tweelospruit showing absence of plant growth.

Source: Durand (2012)



Figure 2-9: Sulfate salts coating rocks and soils on the banks of the Tweelopiespruit River

Source: Durand (2012)

2.5.4. Effects of AMD on health.

AMD is threatening and/or has already had a negative impact on socio economic activities such as its presence in borehole, irrigation and drinking water. Stream and borehole water samples in the Witwatersrand were found with sulfate concentrations of up to 2934 mg/L and 2080 mg/L respectively. In Witbank the stream sulfate levels were 2352 mg/L and borehole water was at 3522 mg/L. Iron and manganese were also found in borehole water in the Witwatersrand at concentrations of 197 mg/L and 18.9 mg/L respectively, levels which are above the recommended limits for drinking water (Gonah *et al*., 2016).

The drinking of water contaminated with AMD, or the consumption of contaminated animal and plant products can lead to the bioaccumulation of metals in the food chain. This may lead to genetic, reproductive, circulatory, renal, and immunological diseases, leading to organ failure, cancers and ultimately death (Fig 2011; Ochieng *et al*., 2010; Udayabanu and Prasad, 2010, Sangita *et al*., 2010). The poor are most affected by the toxicity of AMD since they may depend on natural water for their daily consumption (Ferris and Kotze, 2014), with skin problems having been reported because of these activities.

2.5.5. AMD and Human settlement in South Africa.

Large areas of land are required for the mining as well as dumping sites for mining wastes. Due to this requirement, mining activities compete with other land uses such as human settlement, agriculture, fisheries, and tourism (Dold, 2014). Many informal settlements in Gauteng, SA, are built on or around mine dumps or mineshafts where toxic water flows and toxic dust and radioactive material contaminate the atmosphere (Siphuma, 2012). Humans can be affected directly by breathing contaminated air, consuming contaminated water and soil or indirectly by consuming affected crops, animal products such as milk and meat (CSIR, 2013).

Although the South African authorities have put up guidelines which prohibit human settlements within a radius of a 500m buffer zone from mining waste dumps (Davies, 2018),

the Gauteng province authorities report of 2011, estimates that 1.6 million people were living in townships near to or in one of the 400 zones marked as being affected by mine wastes, exposing these communities to radiation from high levels of uranium (Davies, 2018).

As of 2009, the Auditor General's report listed 5906 abandoned mines across South Africa (Auditor General Report, 2009). The highest density of these abandoned mines (Figure 2-10) is found in the Gauteng region which has the highest population density, resulting in many people including children (Figure 2-11) being exposed to the dangers associated with AMD (CSIR, 2019).

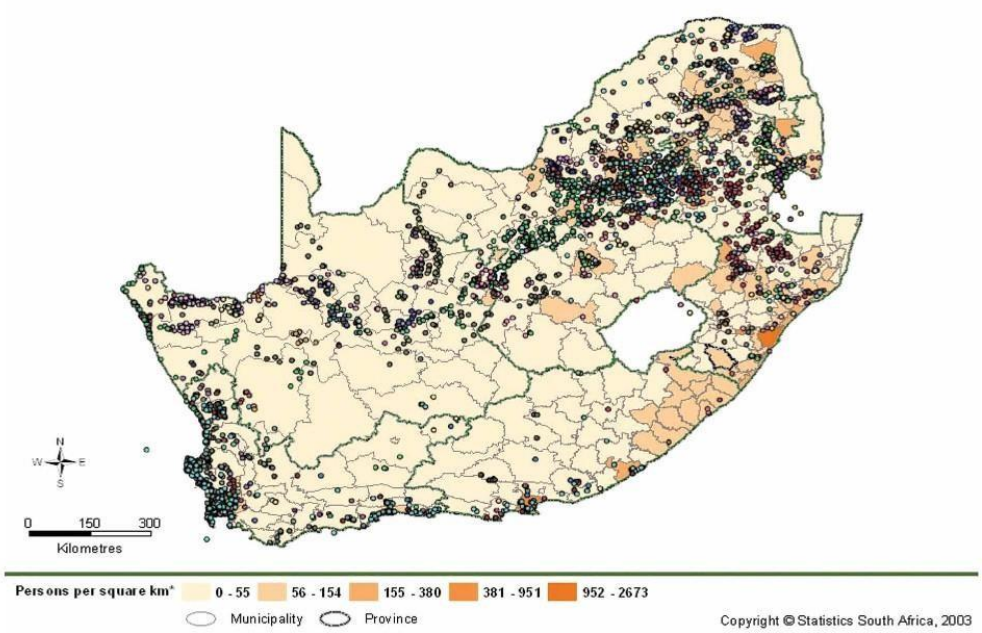


Figure 2-10: The distribution of abandoned mines in relationship to population density in South Africa.

Source: CSIR (2014)



Figure 2-11: Schoolchildren play in AMD outside a school in Davidsonville in South Africa

Source: Jansson (2015)

2.5.6. Effect of AMD on soil quality.

Soils can be affected by overflow and seepage of AMD from mining voids. The biggest contributor to soil contamination is seepage from local mining wastes. These include tailing and waste rock dumps, open cast excavations, water storage facilities and quarries (Bobbins, 2015; Ochieng *et al*., 2010). Bobbins (2015) discusses that approximately 370 of these sites have been identified in Gauteng. These sites are also a source of air pollution as they release radio- active material and dust into the air. Soil may also be polluted from contaminated irrigation water.

Most soils impacted by AMD have a low pH ranging from 2-6 which is strongly acidic to acidic. Mapanda *et al*., (2007) reported soil pH as low as 2.8 depending on the distance from the site of AMD generation. The contaminated soils contained high levels of sulfates as well as heavy metals like zinc, copper, cobalt and many more (Bobbins, 2015).

Most metals inhibit the growth of plants. Copper and zinc in concentration ranges between 1.5-10 millimoles per litre inhibit germination in barley, rice, and wheat. Some metals affect cellular metabolism, thus reducing some important plant processes, such as cell division, water uptake and transportation of nutrients. All these effects lead to the decrease of the yield and quality of crops (Pat-Espadas *et al*., 2018).

High levels of heavy metals in soils affect the soil microbial dynamics by interfering with microbial metabolism (Gao *et al.*, 2019; Fashola *et al*., 2016). Microorganisms are responsible for most of the natural nutrient cycles in the ecosystem. This has a big effect on nutrient absorption by plants in the environment (Fashola *et al*., 2016).

Animal products such as meat and milk have been found to contain high levels of metals from animals grazing in AMD affected soils. Some settlements and mining communities are located on radioactive land amid abandoned or active mines. In a study by Matenga and Gumbo (2016)

on the social impact to communities residing in the Khutsong area in the West Rand, findings showed that communities were exposed to radiation and dust from contaminated soils. There is a high rate of cancer, and other associated diseases, water sources are contaminated, and people need to be relocated because of the threat of sinkholes.

2.5.7. Acid mine drainage and the economy

One of the areas of great economic concern is the impact caused by AMD on agricultural activities. AMD contamination renders the available water resources unsuitable for irrigation. Soils affected with AMD are unavailable for both crop farming and animal rearing reducing the availability of food sources (Ochieng *et al*., 2010). Besides reducing the availability of fresh water due to contamination, AMD also affects infrastructure. The corrosive nature of AMD damages mine infrastructure as well as steel structures such as bridges in affected water streams (Sangita *et al*., 2010; Feris and Kotze 2015; Sheridan *et al.*, 2018).

In urban areas AMD, threatens the flooding of underground structures as well as dissolving cement structures due to the acidity of the water (Feris and Kotze, 2014). This is the case in the Johannesburg CBD where the build infrastructure is threatened. Recreational facilities may also be rendered obsolete due to contamination by AMD reducing outdoor recreational activities that may subsequently lead to a decline in tourism activities directly affecting on the economy of the country (Ochieng *et al*., 2010).

A case in point is the Cradle of Humankind and the nearby Krugersdorp nature reserve. The cradle of Humankind is rich in archaeological and paleontological resources and has been listed as a world heritage site. The caves are mainly dolomite and are susceptible to the acid in the AMD. The Cradle of Humankind has been a great resource to tourism, education, and hospitality in South Africa (Donnenfeld *et al*., 2016).

The quality of water in the Loskop dam, which is part of a nature reserve, has led to the death of aquatic life including fish, turtles, and crocodiles. This has led to a decline in tourism activities in the area (Munnik *et al*., 2010). Fish deaths have also been reported in the Koelenhof dam that is also close to the Cradle's Wonder cave (Rudzainimbilu, 2011). Destruction of aquatic life has also been witnessed in Wonderfontein spruit, Tweelopiespruit, Tudor dam and Robinson Lake. South Africa's National parks department has also reported fish kills in the Kruger national park. According to Munnik *et al*., (2010) agricultural has also been affected in the area as export markets were lost due to water quality concerns.

2.5.8 Global Incidents of AMD.

AMD is not only confined to SA, but extremely low pH AMD (pH 0.52) was recorded at Duke mine in Mazowe found in Zimbabwe (Davies, 2018). In the United States, in the Forest Service land, an estimated 8000-16000 km of stream is impacted by AMD, in Appalachia, estimates vary from 7000-13 000 km while in Pennsylvania 8925 km of stream are affected (Bott *et al*., 2012).

In China, Li *et al.,* (2009) found high concentrations of Cu (17 times), Pb (7 times), Zn (5 times and Cd (2.5 times) in soils contaminated with AMD in comparison to unpolluted soils. Wang *et al*., (2016) reported the reduction in microbial distribution and diversity with sulfate reducing bacteria dominating in AMD irrigated soils in the Guangdong province of China.

AMD has also been reported in Portugal with several closed mines having accumulated large volumes of AMD. A typical example is the Sao Domingo mine whose AMD contain 3000 mg/L sulfate, 500 mg/L iron, 75 mg/L copper and 140 mg/L zinc, with a pH of 2 (Martins *et al*., 2008)

2.6. Current strategies for mitigating AMD in South Africa

South Africa has made some progress towards the closure of mines and the management of mine water by developing and implementing legislation to regulate environmental management

and mine closures (Department of mineral resources, 2009). Polluters are now being held responsible for the rehabilitation and remediation of water pollution, including historic pollution under current legislation (Feris and Kotze, 2014).

One of the main strategies of preventing the formation of AMD has been to rapidly pump water from the mine voids thereby excluding one of the principal reactants in the formation of AMD. Removing the water rapidly reduces the contact time between the water and sulfide material therefore preventing AMD formation. Feris and Kotze (2014) discuss the pumping of water from three priority basins to maintain water levels to at least below the relevant critical levels.

Strategies to reduce the ingress of water into underground workings and abandoned mines have also been implemented. This reduces the volume of water, which need to be pumped and treated to levels that are more acceptable and consequently reduce the operational costs of AMD management (Feris and Kotze, 2014).

Other methods of preventing the formation of AMD are based on isolating one or more of three main elements that are required to initiate the formation of AMD: oxygen, water and *Theobacillus ferrooxidans* bacteria (Pozo-Antonio *et al*., 2014; Kuyucak, 1999; RoyChowdhury *et al*., 2015; Johnson and Hallberg 2005; Kuyucak 2002; Jennings *et al*., 2008). Controlling these factors aim to eliminate the oxidation of sulfide minerals (Kuyucak, 1999). This may be done by removing the source of AMD (pyritic material) (Hester *et al*., 1984; Jennings *et al*., 2008), surface water diversion (Pozo-Antonio *et al*., 2014; Kuyukak, 1999; RoyChowdhury *et al*., 2015), compaction of mine waste (Pozo-Antonio *et al*., 2014), flooding and sealing underground mines and underwater storage of mine tailings (Poinapen, 2008; Johnson and Hallberg, 2005; Hill and Bates, 1979, RoyChowdhury *et al*., 2015), sealing waste rock heaps with clay or plastic covers (Poinapen, 2008; Johnson and Hallberg, 2005, Kuyukak, 1999; Hill and Bates, 1979; RoyChowdhury *et al*., 2015), addition of alkaline chemicals for

acid neutralization (Poinapen, 2008; RoyChowdhury *et al*., 2015) and adding microbial inhibitors as well as adding acidophilic protozoa that feeds on iron oxidising bacteria (Poinapen, 2008; RoyChowdhury *et al.*, 2015). In cases where AMD has already been formed, the only available option is to collect and treat the AMD.

2.6.1. Treatment of AMD

AMD treatment is required to meet recommended standards for potable water before being discharged into water bodies (Figure 2-12) (CSIR, 2019). Treatment of AMD removes the dissolved metals and salts as well as improving the pH. Treatment of AMD is potentially a costing part of mining operations and long-term liability if not managed correctly (Herricks, 1982). According to Taylor *et al*., (2005), the treatment of AMD is required for:

a) Meeting the criteria for the discharge of effluents into surface or ground water.
b) Recycling of water.
c) Preventing damage to infrastructure.

There are numerous available options to treat AMD, which can be classified as chemical and biological, both aim at increasing pH, neutralising acidity, and removal of metals (Herricks, 1982). These systems may be further classified into active systems which require continuous input of resources, including equipment, energy, labour, technical expertise, and maintenance (Trumm, 2010; Sangita *et al*., 2010), and passive systems which do not require much attention after start-up (Genty *et al*., 2011; Taylor *et al*., 2005). Passive treatment systems use Sulfate reducing bacteria (SRB), or limestone or both (Ford, 2003).

In most cases active treatment processes are used at operating facilities while passive systems are preferred for facilities that are no longer in operation, but are continuing to generate AMD (Skousen, 1998; Brown *et al*., 2002). Active systems have some advantages over the passive treatment systems in that they can effectively remove contaminants from AMD, they can also be built to produce effluents with a specific chemistry, and they do not require large areas of

land (Trumm, 2010). However, the major drawback in the application of active treatment systems for the remediation of AMD is the cost involved in their construction, operation, and maintenance (Trumm, 2010; Servi *et al*., 2017).

Technical factors such as the acidity levels, the flow rate and the types and concentration of metals in the water are also considered before the treatment method is chosen (Skousen *et al*., 1998). Previous researchers have put forward various models that can be used to select the best method of treatment. Combinations of active and passive treatment units can also be implemented.

Parameter	Gold drainage	Coal drainage	Neutral drainage	DWS	WHO
pH	2.5 – 3.5	2 – 3	6.5 – 7	6.5 – 9.5	6.5 – 9.5
Acidity	200	15 000	0	-	-
Alkalinity	0	0	100	-	-
Fe	2 000	8 000	0	0.1	0.1
Al	200	500	0	0.1	0.1
Mn	100	200	25	0.5	0.1
SO_4^{2-}	6 000	80 000	5 000	500	500
Mg	200	400	900	-	-
Ca	800	600	600	-	-

Figure 2-12: Chemical compositions of AMD against water quality standards
Source: CSIR (2019)

2.6.1.1. Active chemical treatment systems

Active treatment systems involve routine addition of chemicals and expertise to carry out regular maintenance schedules (Taylor *et al*., 2005; Pat-Espadas *et al*., 2018) as they require equipment such as tanks, mixers, and pumps as well as power (Skousen *et al*., 1998; Servi *et al*., 2017). Active treatment is mostly done through the addition of naturally occurring or manufactured inorganic alkaline chemicals (Johnson and Hallberg, 2005, Herricks, 1982; Taylor *et al*., 2005; Viera *et al*., 2014). These inorganic alkaline chemicals neutralise the acidity in AMD thereby increasing the pH. The increase in pH promotes the precipitation of metal

hydroxide complexes thus reducing the dissolved metal concentration in the effluent (Equation 1.7) (Taylor *et al*., 2005; Johnson and Hallberg, 2005; Seervi *et al*., 2017, Zingel, 2015; McCarthy, 2011).

Alkaline chemicals which have been applied in the treatment of AMD include calcium hydroxide (Ca(OH)), calcium oxide (CaO), sodium hydroxide (NaOH), Sodium carbonate (Na_2CO_3), ammonia (NH_3), and Magnesium oxide/hydroxide ($MgO/Mg(OH)_2$) (Yadav and Jamal, 2016; RoyChowdhury *et al*., 2015). Each of these chemicals may only be applied in the treatment of AMD with specific characteristics under different operational conditions. The costs of reagents, equipment labour and energy are important considerations in the long-term sustainability of the treatment process. The characteristics of the wastewater, including the acidity levels, flow rate, the types, and concentrations of metals in the water, and the rate and degree of chemical treatment, need to be taken into consideration before deciding which reagent is the most appropriate to use (RoyChowdhury *et al*., 2015).

Mineral carbonates such as limestone and dolomite have been used for the treatment of AMD. The addition of limestone precipitates sulfate by the formation of gypsum ($CaSO_4.2H_2O$) from calcium and dissolved sulfate (Equation 1.8 and 1.9) (Viera *et al*., 2014; Kuyucak 2002)

$$Ca(OH)_2 + Me^{2+} + H_2SO_4 \rightarrow Me(OH)_2(s) + CaSO_4(S) \quad \text{(Equation 1.7)}$$

$$CaCO_3 + H_2SO_4 + H_2O \rightarrow CaSO_4.2H_2O + CO_2 \quad \text{(Equation 1.8)}$$

$$Ca(OH)_2 + H_2SO_4 \rightarrow CaSO_4.2H_2O \quad \text{(Equation 1.9)}$$

Limestone is the least expensive of the chemicals used in the treatment of AMD. Limestone is also the safest and easiest to handle but it is not suitable especially for highly acidic iron rich waters due to armouring with ferric hydroxide coatings (Hammerstrom *et al.,* 2003, Poinapen 2008, RoyChowdhury *et al*., 2015).

To achieve complete removal of both acidity and dissolved metals in AMD, excess limestone needs to be added. This result in an effluent with a high pH value. Acid addition maybe required to adjust the pH to meet the limitations of the effluent pH (Mend report 3.21.1 (b), 1995).

A major problem associated with chemical treatment is the formation of sludge resulting from the precipitation process since it requires a suitable place of disposal and does not allow metal recovery (Viera *et al*., 2014; Jong and Perry, 2003; Kitot *et al*., 2010; Hussain and Qazi, 2016; Bratkova *et al*., 2011). The sludge, which has a high volume, still contain large volumes of water and a low solid content of between 0.5 % and 5 % with large quantities of toxic metals (Herricks, 1982; Sukati *et al*., 2018).

The challenge around sludge management can be minimized by using the high-density sludge (HDS) process to treat AMD. In the HDS process, the low-density sludge from chemical neutralization is recycled and mixed with limestone or lime. This promotes the precipitation of more solids by providing surfaces for heterogeneous nucleation. The HDS solids range from 15 % to 70 % compared to 5 % for sludges from conventional chemical treatment methods (Sukati *et al*., 2018; Dinu *et al*., 2014). The HDS substantially reduce the sludge volume by increasing sludge density. The sludge settles faster therefore, a small clarifier is required (Damar-Fakir *et al*., 2017). The HDS technology has been successfully used to treat AMD at the Grootvlei mine (Eastern Basin) in SA. The HDS process has its shortfalls including increased salinity and promoting high concentrations of sulfate in water. A desalination step is therefore required to remove some soluble salts from the treated mining water, increasing treatment costs (Lourenco *et al*., 2021). The continuous use of chemicals, energy and labour makes chemical treatment unattractive especially in remote areas.

Chemicals such as ammonia and sodium hydroxide need to be carefully handled, as they are hazardous. The use of excessive ammonia can lead to nitrification and eutrophication in

receiving water bodies (RoyChowdhury *et al.*, 2015). Some of the advantages and disadvantages of the seven chemicals used mostly in active neutralisation are listed in Table 2-3. Sulfide reagents such as sodium sulfide, hydrogen sulfide and calcium sulfide can also be used to precipitate metals as sulfides. Metal sulfides sludges are less dense than metal hydroxide sludge. Metal sulfides are stable over a large pH range. Chemical sulfide reagents are not commonly used due to cost and the need to filter the suspended metal sulfides precipitate to obtain clean water (Kuyucak, 2002). Biologically produced sulfide is a cheap source of hydrogen sulfide and will be explored later in section 2.6.3.

Table 2-3: Advantages and disadvantages of some of the chemical used in active chemical neutralisation.

Chemical	**Maximum pH attainable**	**Advantages**	**Disadvantages**
Sodium carbonate (Na_2CO_3)	11.6	Effective in removing metals and produces low quantities of sludge	Need careful handling as they may pose a threat to health and safety, poor sludge settling rates.
Calcium hydroxide ($Ca(OH)_2$)	12.4-12.5	Removes most of the metals through precipitation and produces low quantities of sludge	Need careful handling as they may pose a threat to health and safety, poor maintenance can result in plugged dispensing and complete failure
Calcium oxide (CaO)	12.4-12.5	Removes most of the metals through precipitation, widely available, cheaper than most of the chemicals, very effective	Health and safety issues, reagent saturation can lower efficiency, possible armouring of pebbles, poor maintenance can result in plugged dispensing mechanisms and complete failure, must be watertight or will hydrate and form calcium hydroxide and plug the dispensing mechanism.
Ammonia (NH_3 or NH_4OH)	9.2	Removes most of the metals, produces low quantities of sludge	Need careful handling as they may pose a threat to health and safety, poor sludge settling rates, may cause nitrification and eutrophication, threatening aquatic life
Sodium hydroxide (NaOH)	14	Removes most of the metals, produces low sludge volumes	Need careful handling as they may pose a threat to health and safety. Is the most expensive of all the chemicals.

Magnesium oxide or hydroxide (MgO or $Mg(OH_2)$)	9-9.5	Removes most of the metals, Produces low quantities of sludge, low cost	Need careful handling as they may pose a threat to health and safety. Is the most expensive of all the chemicals.
Limestone ($CaCO_3$)	6-7.5	The cheapest of all the chemicals available, Safe to use	Not very efficient in removing metals, susceptible to armouring.

Source: Skousen *et al.* (1998).

2.6.1.2. Physico-chemical treatment of AMD

Physico-chemical treatment techniques processes used in the treatment of AMD include electro chemical treatments, reverse osmosis, filtration, ion exchange and adsorption (Roman, 2004; Taylor *et al.,* 2005).

2.6.1.2.1. Ion exchange

Ion exchange is one of the most effective treatment techniques for low metal content AMD. The treatment process uses ion exchange resins that contain functional groups to remove metals as well as hardness from AMD (Gaikwad and Gupta, 2007). The ion exchange resins maybe synthetic or natural with specific active groups which maybe anionic, cationic, or chelating depending on the structure of the active group. Inorganic ion exchange resins or zeolites are preferred because they are inexpensive and are less selective than polymeric resins (Mend report 3.21.1 (b), 1995).

In ion exchange, an ion from the solution is exchanged for a similarly charged ion attached to the resin (Gaikwad and Gupta, 2007; Yadav and Jamal, 2016). Ion exchange can lead to the recovery of valuable metals. Ion exchange can be applied together with lime neutralisation either to reduce the metal concentration prior to neutralisation, or to remove metals remaining in the effluent after neutralisation (Mend report 3.21.1 (b), 1995).

The ion exchange technique is however, limited by the need to frequently regenerate the ion exchange resins as replacing with new resins is very costly. Acid is mainly used to regenerate the resin (Saha and Sinha, 2018). The quantity of acid required for the regeneration of the resin after the treatment of AMD in an ion exchange plant with capacity of 2.5ML/day was estimated by Feng *et al*. (2000) at 2.5 kg/m^3 acid at a cost of US$0.40/$m^3$ (Saha and Sinha, 2018), making this option impractical to implement. The solution used to regenerate the resins is rich in heavy metals and appropriate disposal solutions should be practiced (Gaikwad, 2010).

2.6.1.2.2. Reverse osmosis

Reverse osmosis uses semi-permeable membranes to remove dissolved metal ions from AMD. The membranes are mainly made from cellulose acetate. Pressure is applied to the AMD and forced through the membrane. The membrane only allows the passage of water molecules while preventing metal ions and other contaminants to pass through (Seervi *et al*., 2017; Yadav and Jamal, 2016). The Emalahleni treatment plant in SA uses reverse osmosis to treat AMD. This method is very efficient as the water is treated to drinking standards; however, the cost of treatment is very high (McCathy, 2011). Reverse osmosis is applied to AMD with low metal concentrations. AMD with high concentration of iron and other metals require some pre-treatments prior to treatment using reverse osmosis. Pre-treatment may include lime dosing and aeration for neutralisation of acidic water and the removal of metals. This is followed by filtration using either sand and cartridge filters, or by ultrafiltration before reverse osmosis (Dama-Fakir *et al*., 2017). The concentrated acid brine solution left after the treatment process is rich in sulfates and iron and will need to be treated before disposal, increasing the cost of treatment (Saha and Sinha, 2018).

2.6.1.2.3. Adsorption

This technique uses sorbents to adsorb ions. Activated carbon is commonly used, though natural and synthetic zeolites, clay, natural clinker, fly ash, zero valent iron, iron, agricultural

waste, microbial biomass activated sludge and lignite may be used (Saha and Sinha, 2018). Adsorption allows for metal recovery, however, there is need to frequently replace the sorbent.

The need for frequent regeneration of reagents adds more cost to the process, as a result successful implementation and treatment is not achieved (Ramla, 2012; Munawar and Riwandi, 2010).

2.6.2. Passive chemical treatment of AMD

Passive neutralisation is based on the ability of calcite to dissolve in water. AMD can be channelled through a bed of crushed limestone, placed into a cell or a drain. Crushed limestone can also be added directly to impacted streams. The calcite dissolution consumes acidity (Equation 2.0) and introduces buffering capacity in the form of bicarbonate ions into AMD. (Lottermoser, 2010; Kuyucak, 2002).

$$CaCO_3 + H^+ \rightarrow Ca^{2+} + HCO_3^- \qquad \text{(Equation 2.0)}$$

Passive chemical treatment systems are usually constructed with enough neutralising material to last a life span; such that once the treatment process has started, no additional inputs are required. The AMD should be given sufficient resident time to ensure effective neutralisation (Skousen *et al*., 1998). Several passive chemical treatment options are available; the most common ones are illustrated and described in sections 2.6.2.1. - 2.6.2.5.

2.6.2.1. Anoxic limestone drains

Anoxic limestone drains (ALDs) consist of shallow trenches which are filled with crushed limestone and covered with plastic or impermeable soil or sediment (Figure 2-13) (Lottermoser, 2010; Zipper *et al.*, 2011). Faulkner and Skousen (1994) recommended to use a mixture of larger particle size (8 cm to 25 cm) and finer particles (2 cm to 4 cm) of limestone to increase hydraulic conductivity and reduce potential plugging of ALDs.

ALDs add alkali to AMD while they maintain the iron in its reduced form to avoid precipitation of ferric oxides/hydroxides on the limestone that would severely reduce the effectiveness of the neutralising agent (Taylor *et al*., 2005; Johnson and Hallberg, 2005, Lottermoser, 2010). Within the drain the partial pressure of CO_2 is increased, accelerating the rate of limestone dissolution thereby increasing alkalinity (Johnson and Hallberg, 2005; Kuyucak, 2002). Elevated amounts of oxygen concentrations within the influent results in ferrous ion being oxidised to ferric iron, which can precipitate as oxides or hydroxides causing armouring thereby reducing the efficiency of the system (Ford, 2003).

The primary function of ALDs is to raise the pH to 6-8 and to optimise the addition of bicarbonate alkalinity (Servi *et al*., 2017). Metals can be removed from the effluent by installing aerobic ponds at the outflow end of the ALD. In these ponds, oxidation reactions occur which facilitate the removal of metals as oxide or hydroxide precipitates.

ALDs should generate enough alkalinity to consume the acidity generated when metals are precipitated in the aerobic pond or wetland (Taylor *et al.*, 2005). ALDs require minimum attention once constructed, however certain waters are not suitable for ALDs. Water with elevated dissolved Fe^{2+}, aluminium and high oxygen concentration will cause armouring (Zipper *et al*., 2011; Lottermoser, 2010). Such waters require modified limestone beds or oxic ponds at the inflow of the ALDs to induce reducing conditions and convert Fe^{2+} to Fe^{3+}.

(Lottermoser, 2010).

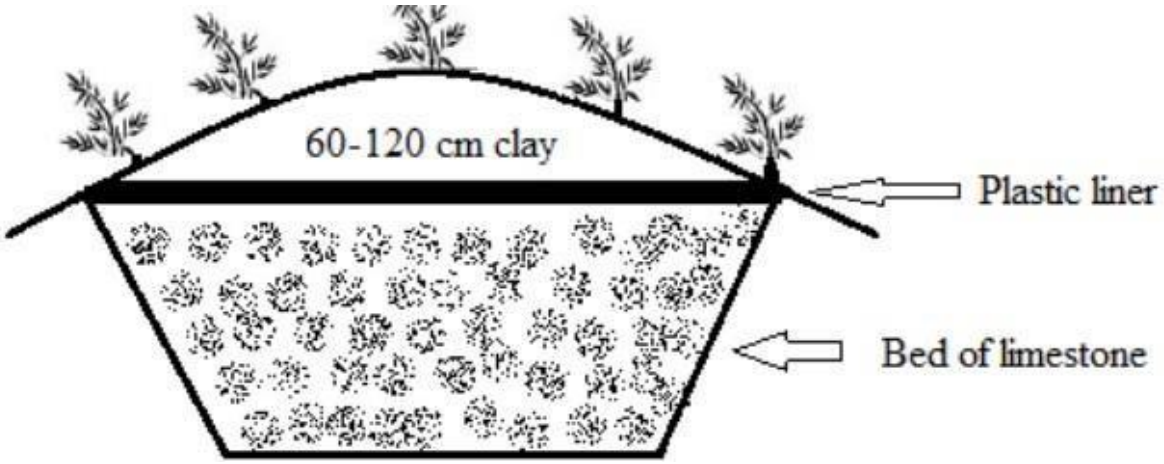


Figure 2-13: A schematic cross section through an anoxic limestone drain

Source: Servi *et al*. (2017).

2.6.2.2. Oxic limestone drains

Oxic limestone drains (OLDs) are open channels containing course limestone aggregates (Figure 2-14) (Zipper *et al*., 2011). They encourage precipitation of metals as hydroxides and/or oxides as AMD flows through the limestone. The metal hydroxide precipitates reduce the efficiency of the OLDs due to armouring of the neutralising material; large amounts of limestone are therefore required to offset the effects of armouring (Taylor *et al*., 2005). The function of OLDs is to raise pH of water, remove acidity and decrease the concentration of dissolved metals (Servi *et al*., 2017). In the presents of oxygen, limestone dissolution may be increased by the active generation of acidity (hydrogen ions) by ferric or aluminium hydrolysis (Wartzlaf *et al.*, 2004.) OLDs are cheap and easy to construct (Lottermoser, 2010).



Figure 2-14: A schematic cross section through an oxic limestone drain

Source: Servi *et al*. (2017)

2.6.2.3. Limestone leach beds (LLB).

Limestone leach beds (LLBs) are ponds designed to intercept water with little or no alkalinity and low concentration of dissolved metals (RoyChowdhury *et al*., 2015). LLBs are filled with limestone cobbles. AMD flows horizontally or vertically through the cobbles, dissolving the limestone, which neutralises the acidity. LLBs allows for at least 12 hours of resident time in the system (Skousen *et al*., 1998; RoyChowdhury *et al*., 2015). LLBs are simple to construct and have a high level of reliability, however, they tend to armour if iron concentration is high (Skousen *et al*., 1998). It is therefore necessary to replenish the limestone after a certain period of operation.

2.6.2.4. Slag leaching beds.

Steel slag leach beds like LLB treat AMD by neutralising acidity and precipitating metals. The SLBs are shallow ponds filled with steel slags. Steel slags are by products in the manufacturing of steel. The slags are composed of basic compounds mainly lime and magnesium. According to Piatak *et al.* (2017), most slags produce pastes with alkaline pH values between 10 and 13 upon their dissolution in water and have a neutralisation potential between 400 kg and 830 kg $CaCO_3$/t. This neutralisation potential is applied in improving the pH as well as precipitating metals from AMD. Simmons *et al.* (2001) proved that the metals contained in the slags are

bound in insoluble forms that remain stable under a wide range of pH. This means that the slag will have a minimal contribution to the dissolved metal ion content of the AMD. Their results also indicated that most of the leaching of the slag occur during the early stages of treatment, hence, the need to continually replenish the alkalinity by adding more slag material. Kruse *et al*. (2012) concluded that the efficiency of the SLBs is dependent on the distribution of alkalinity with SLBs generating hydroxide, carbonate and bicarbonate alkalinity performing better than those having carbonate dominated alkalinity.

2.6.2.5. Diversion wells (DWs)

DWs are in-ground cylindrical and vertical tanks constructed from metal or concrete (Skousen *et al*., 2018; Servi *et al*., 2017). They are usually 1.5m-1.8m in diameter and 2m-2.5m in depth and were initially constructed to treat acidity created by acid rain in Norway and Sweden (Skousen *et al*., 2017; Servi *et al*., 2017). DWs are filled with crushed limestone with average gravel size of between 0.8 inches and 1.2 inches (Skousen *et al*., 1998; Yadav and Jamal, 2016; Schmidt and Sharpe, 2002). The wells are filled to about two thirds with limestone gravel with more than 85% calcium content (Schmidt and Sharpe, 2002). DWs receive contaminated water from upstream main source (Figure 2-15). Water enters the well through a vertical pipe positioned in the centre of the well. The water flows upwards and fluidises the limestone bed. As the limestone dissolves, alkalinity is added to the water as it flows upwards towards the exit pipe (Natalie and Younger, 2005; Yadav and Jamal, 2016; Skousen *et al*, 1998).

Some residual limestone is carried along and will continue to neutralise the acid in the AMD. The force of the water also prevents armouring of the limestone bed. To improve the efficiency of treatment especially in large flows, DWs can be installed in series. The limestone should also be frequently replenished for maximum efficiency (USGS, 2010). In studies carried out at Swatara creek and on Lorbery Creek AMD discharges, Cravota (2010) reported decreases in net acidity, improved pH in the neutral ranges from acidic pH and smaller concentrations of

both aluminium and iron in AMD treated using DWs. Arnold (1991) obtained pH increases from 4.5 to 6.5, with corresponding decrease in acidity using DWs for AMD treatment. Similar results were obtained at Galt site; Western Virginia were pH increases from 3.5 to 5.5 were obtained with corresponding decrease in acidity from 278 mg/L to 86 mg/L as $CaCO_3$. Dissolved iron and aluminium concentration were reduced from 15 mg/L to 2 mg/L and 25 mg/L to 11 mg/L respectively (Faulkner and Skousen, 1995).

For treatment to be effective, the flow rate of the influent water should be low, otherwise multiple DWs are required to improve the effectiveness of treatment. Major drawbacks for using DWs is the need for weekly or bi-weekly maintainance, including replacing the used limestone with new batches of limestone and also clearing some debris brought in by the inflowing water. The areas where treatment is occuring also need to be accessible for limestone deliveries (Schmidt and Sharpe, 2002).

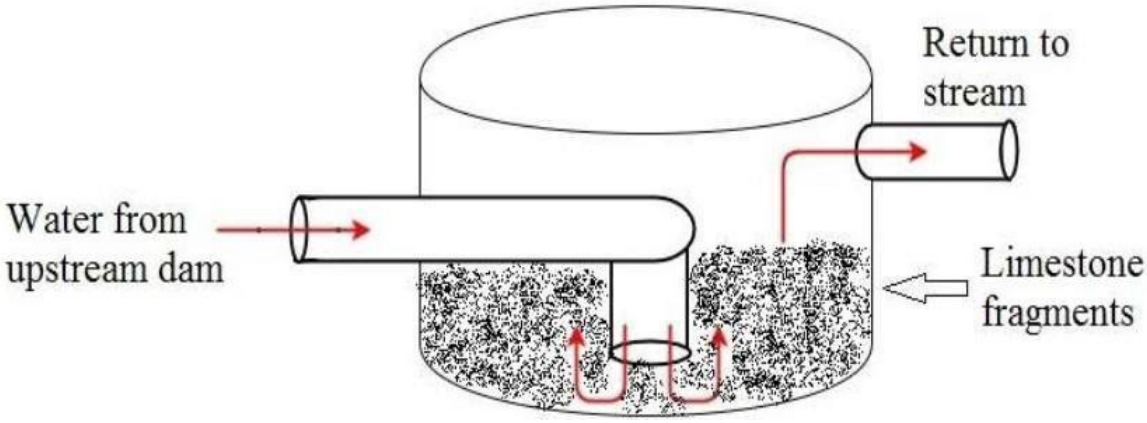


Figure 2-15: A schematic representation of a diversion well. Source: Servi *et al*. (2017).

2.6.3. Biological treatment systems.

Biological treatment systems use microorganisms to detoxify or remove pollutants. They rely on microbial enzymes to remove contaminants (Philp *et al*., 2005). Biological treatment processes are considered as both active and passive (Roman *et al*., 2004). These are differentiated based on the operational and management controls required, with passive systems running without continuous energy and operator inputs. A passive system utilises naturally available resources such as topography, microbial metabolic processes, and chemical energy. A passive system runs with very little maintenance. Passive biological treatment systems require the availability of substrates, which can be used by the participating microorganisms (Taylor *et al*., 2005).

Active biological treatment systems require continuous addition of chemicals, uses energy for pumping and aeration, and require regular operation and maintenance (Taylor *et al*., 2005; Sangita *et al.*, 2010). Though active treatment systems are very effective in the removal of contaminants from AMD, they may be limited by high capital, operational and maintenance costs. (Johnson and Hallberg, 2005).

Biological treatment systems or bioremediation relies on microbial processes to remove acidity, dissolved metals, and other environmental contaminants into less toxic forms. The basis of bioremediation of AMD derives from the abilities of microorganisms to generate alkalinity thereby essentially reversing the reactions responsible for AMD genesis (Johnson and Hallberg, 2005). Alkalinity is produced in the form of bicarbonate. The bicarbonate acts as buffer to neutralise any hydrogen ions (Lottermoser, 2010). Biological systems for AMD treatment use biosorption, biomineralization, bioreduction and alkalinity generation (Kilbon Inc, 1996; Kuyucak, 2002).

The bioremediation of AMD relies on the ability of sulfate reducing bacteria (SRB) to use organic carbon sources as electron donors in the reduction of sulfate to sulfide (Greben *et al.*,

2009; Nancucheo and Johnson, 2012; Krayzelova *et al*., 2015; Philip *et al.,* 2005; Sarti and Zaiat, 2011). The organic source can be any carbon material such as manure, sawdust, compost, grass or biosolids (Neculita *et al*., 2007; Zagury and Neculita, 2007).

Generally, SRB can metabolize short chain organic carbon molecules such as methanol, ethanol, and lactate. These substrates are expensive and can make the treatment of AMD using SRB unsustainable over the long term. Alternative substrates, which are cheaper and able to promote and sustain biological sulfate reduction, can be obtained from organics such as wastes from agriculture, domestic and industrial processing (Zagury and Neculita, 2007). These wastes have been assessed for their potential to promote and sustain sulfate reduction with various levels of success (Neculita *et al.*, 2007; Van den Berg *et al*., 2016).

SRB consumes the organic substrate in a process called dissimilatory sulfate reduction (DSR). In DSR sulfate is reduced to hydrogen sulfide and alkalinity is generated by the oxidation of organic matter to produce hydrogen carbonate. The hydrogen carbonate helps in improving the pH of the effluent by neutralising the acidity (Equation 2.1) (Greben *et al*., 2009; Johnson and Hallberg 2005; Lottermoser, 2010; van den Berg *et al.,* 2016; Neculita *et al*, 2007; Cao *et al*., 2009 Greben *et al*., 2009; Kaksonen *et al*., 2007; Church *et al*, 2007; Sheridan *et al.*, 2013; Watzlaf et al., 2004). The hydrogen sulfide produced reacts with metals to form insoluble metal sulfides (Equation 2.3) (Philip *et al.,* 2005; van den Brand *et al*., 2015; Martins *et al*., 2008; Jong and Perry, 2003; Sheng *et al*., 2017; Costa *et al*., 2009; Velasco *et al.*, 2008; Jencanova and Luptakova, 2012; Colipai *et al*., 2018; Liamleam and Annachhatre, 2007). Based on their substrate utilization, SRB can be divided into two metabolic groups which include complete oxidizers such as the genera *Desulfomonas, Desulfobacter, Desulfosarcina* and *Desulfoacium* which can oxidise the substrate to carbon dioxide, while the other group which include the genera *Desulfovibrio, Desulfomicrobium,*

Desulfobolotus and others can only oxidise the substrate to acetate (Brahamacharimayum *et al*., 2019).

$SO_4^{2-} + 2CH_2O \rightarrow H_2S + 2HCO_3^-$ (Equation 2.1)

(Where CH_2O represent organic matter).

When sulfide is purged from the system or precipitated as metal sulfides, the buffer system in the AMD is modified and pH can return to near neutrality (Herricks, 1982). The Bicarbonate and the sulfide ions formed during sulfate reduction equilibrates between CO_2, HCO^{3-}, CO_3^2, H_2S, HS^- and S^{2-} which buffers the solution pH around neutral to slightly alkaline (Kousi *et al*., 2015). The bicarbonate ion dominates at the optimal pH range for sulfate reduction. Metals are precipitated as insoluble sulfides and oxides (Equation 2.3 and Equation 2.4) (Cohen, 2006; Herricks, 1982). Metal sulfides precipitates are more stable than metal hydroxides that are sensitive to pH changes (Liamleam and Annachhatre, 2007).

$HCO_3^- + H^+ \rightarrow CO_{2\,(g)} + H_2O$ (Equation 2.2)

$H_2S\,(aq) + M^{2+} \rightarrow MS\,(s) + 2H^+$ (Equation 2.3)

$Al^{3+} + 3H_2O \rightarrow Al(OH)_3\,(s) + 3H^+$ (Equation 2.4)

(More bicarbonate is produced in equation 2.1 to neutralize all the acidity produced in Equation 2.3 and Equation 2.4 resulting in a pH increase (Church *et al*., 2007, Gilbert *et al*., 2002)).

In most cases, bioremediation of AMD using SRB has occurred in designed systems such as anaerobic wetlands where oxidation and reduction reactions are augmented by special organic substrates and limestone. Wetlands can be designed and constructed to provide aerobic or anaerobic conditions to support the microbial and abiotic reactions (Kilborn Inc, 1996). In a few cases, substrates are incorporated into spoils to aid in in-situ treatment of water by use of

indigenous microorganisms (URS, 2003). According to Kilborn Inc, 1996, the reactions, which occur in wetlands, include:

i. Exchange of metals by organic rich substrate.
ii. Sulfate reduction and precipitation of iron and other sulfides.
iii. Metal uptake by living plants.
iv. Filtering of suspended and colloidal material.
v. Adsorption or exchange of metals on algae.

Biological sulfate removal is limited by:

1) Substrate utilisation in the presence of other anaerobic bacteria. In the presence of sulfate, acetogens and methanogens compete with SRB for available substrates (INAP, 2003).
2) Toxicity induced by H_2S and dissolved metal. SRB are generally less sensitive to H_2S (g) than other anaerobic bacteria. The toxicity may be attributed to the non-ionised state of H_2S (g) which allows it to cross the membrane more readily (INAP, 2003).

2.6.3.1. Passive biological treatment systems.

Passive biological treatment systems use a simple flow design with AMD feed over a solid reactive mixture acting as a source of carbon for SRB and physical support for microbial attachment and metal sulfide precipitation (Neculita *et al*., 2007). Efficient reactive mixtures contain an organic source (several organic and cellulosic wastes), a bacterial source or SRB inoculum (river sediment/animal manure), a solid porous medium (gravel/sand), a nitrogen source (urea) and a neutralising agent (limestone) (Sheridan *et al*., 2013; Neculita *et al*., 2007).

The efficiency of the passive bioreactors depends on the activity of SRB which is mainly controlled by the reactive mixture (Neculita *et al*., 2007), which is the most important component of a sulfate reducing bioreactor (Neculita *et al*., 2006). It has been shown that a

mixture of several wastes performs better at remediating AMD than a single source (Gilbert *et al*., 2002). Moreover, the most efficient mixtures usually contain relatively easily biodegradable sources (animal manure or sludge) and recalcitrant ones (sawdust, hay, alfalfa, or wood chips) (Neculita *et al*., 2007). Waybrant *et al*., (1998) obtained 100% sulfate removal from wastewater containing 3300mg/L sulfate using a mixture of sludge, leaf mulch, wood chips and sawdust. Similar results were obtained by Christensen *et al*., (1996) using a mixture containing vegetal compost and cow manure. In both cases, metal removal was reported above 95% (Gilbert *et al*., 2002).

Passive SRB-based treatment systems offer solutions with relatively low operating costs and minimal maintenance requirements; thus, they can be operated in remote areas, however, they may require large areas of land and require longer process time for the treatment process to be successfully implemented (Kefeni *et al*., 2017). Passive biological treatment systems are difficult to control and predict because of seasonal variations in rainfall and temperature. Difficulties in metal recovery are also encountered in the passive treatment of AMD (Kaksonen and Sahinkaya, 2012). For a successful long-term passive treatment process, there is need to periodically remove the possible toxic sludges formed in passive treatment systems (Johnson and Hallberg, 2005).

The most common used type of passive biological treatment systems is aerobic and anaerobic wetlands.

Aerobic wetlands are usually shallow (Figure 2-16) (< 30cm depth) (Zipper *et al*., 2011; RoyChowdhury *et al*., 2015), they are used when incoming water is alkaline (Lukovic and Stankovic, 2012). Their primary function is to precipitate metals from water. They collect water and provide enough residence time to allow metal oxidation and hydrolysis thereby causing metal precipitation. (Kilborn inc, 1996, Lukovic and Stankovic, 2012, Skousen and

Ziemkiewiz, 2005). The reactions in aerobic wetlands generate net acidity which requires sufficient alkalinity to neutralise (Equations 2.5 and 2.6) (Lukovic and Stankovic, 2012). The incorporation of limestone drains may be required to amend the net acid generating reactions. Disadvantages of using aerobic wetlands are that they are not able to remove sulfate and are less effective at high metal concentration (Roychowdry *et al*., 2015).

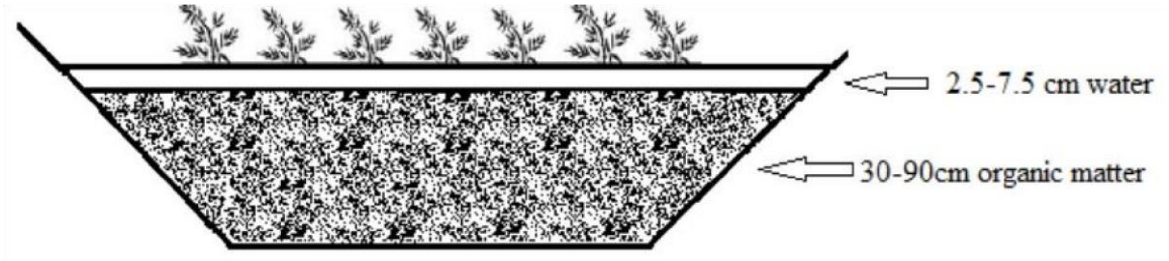


Figure 2-16: A schematic cross section through an aerobic wetland
Source: Servi *et al*., (2017).

Anaerobic wetlands are deeper than aerobic wetlands and are designed for the treatment of acidic water (Zipper *et al*., 2011; Ford, 2003; Lukovic and Stankovic, 2012). In some cases, they are constructed underground to prevent oxygen from entering the treatment system (Roychodhury, 2015; Sheridan *et al*., 2018). Anaerobic wetlands utilize organic rich substrates to generate anaerobic conditions, which prevents the quoting of the limestone with metal precipitates (Metesh *et al*., 1998; Schmidt and Sharpe, 2002). Spent mushroom compost or a mixture of manure and straw or a mixture of peat and sawdust can be used as the organic source (Skousen *et al*., 1998; RoyChowdhury *et al*., 2015). These organics serve as long term substrates for SRB due to their slow bio-degradation rates (RoyChowdhury *et al*., 2015). Anaerobic wetlands contain limestone (Figure 2-17) for acid neutralisation. Limestone dissolution and biogenic alkalinity helps to raise pH and precipitate metals as hydroxides and carbonates, while biogenic H_2S reacts with metals to form insoluble metal sulfide precipitates

(Skousen and Ziemkiewicz, 2005, Lukovic and Stankovic, 2012).

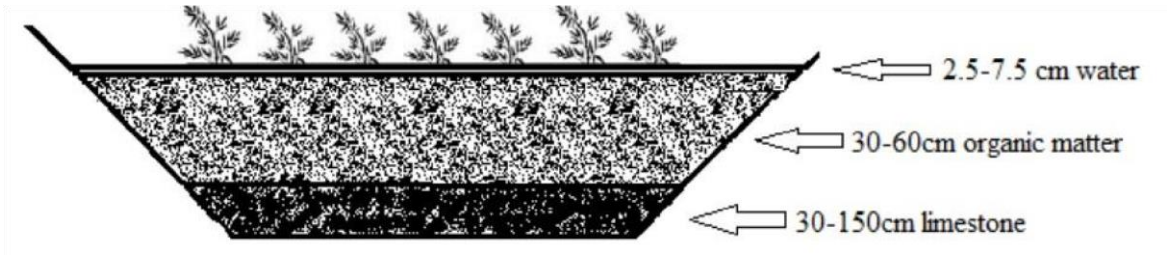


Figure 2-17: A schematic cross section through an anaerobic wetland

Source: Servi *et al*., (2017)

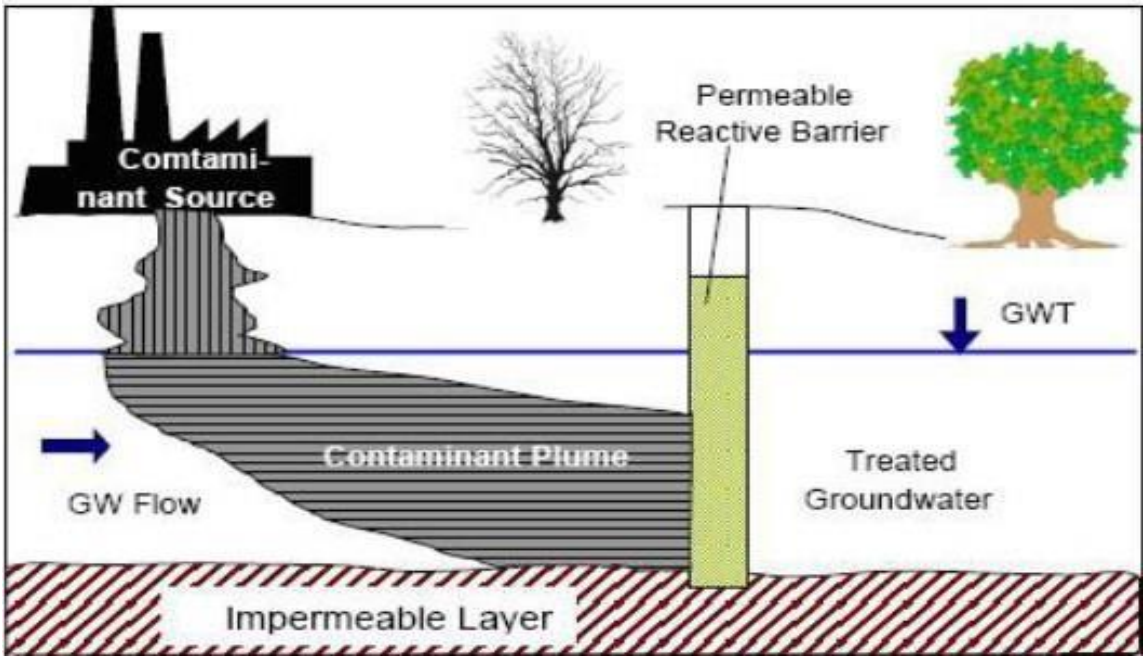


Figure 2-18: Representation of a PRB. Source Shabalala *et al*., 2004.

2.6.4. Active biological treatment technologies.

Like passive biological treatment technologies, active bioreactors rely on SRB to remove metals from AMD and to generate alkalinity. In the presents of an organic source, the bacteria convert sulfate to sulfide, bicarbonate is also produced in this reaction. The sulfide precipitates metals, which can be recovered and processed, while the bicarbonate neutralises the acidity in AMD hence increasing the pH (Johnson *et al*., 2004; Johnson and Nacucheo, 2012).

In active biological treatment systems, conditions for microbial activities are optimised to maximise efficiency. The inputs are added in continuously and there is a need for intensive management (Nancucheo and Johnson, 2012). In comparison with passive biological treatment systems, active bioreactors are more compact (can be operated in small spaces) and offer more consistent performance and control (Kaksonen and Sahinkaya, 2012). The treatment processes may be implemented in one- or two-stage systems where the sulfate reduction and the metal precipitation can occur simultaneously in one reactor or sulfate reduction and metal precipitation can occur in separate reactors (Kousi *et al*., 2015, Nevalto *et al*., 2010), the single stage process is cheaper to operate compared to a multistage treatment system. However, metal sulfide precipitates and hydrogen sulfide may accumulate in the reactor thereby inhibiting the microbial consortium in the reactor. This may lead to a decrease in the efficiency of the treatment process with time (Nevalto *et al*., 2010).

The reduction of sulfate can be achieved with freely suspended bacterial cells or immobilised cells. Freely suspended cells can be washed out easily therefore, they require a high residence time. Immobilised cell reactors can be operated at a high flow rate, as the cells will be attached to a matrix. The biofilm formed in the immobilised cell bioreactors protects the cells from extreme conditions such as low pH, High metal concentrations and variations in temperature (Baskaran, 2005, Bekmezci *et al*., 2011).

Sulfidogenic bioreactors are the most common active, biological remediation method. The bioreactors can be implemented in several configurations, but all use anaerobic cultures of SRB. The bioreactor configurations include, continuous stirred tank reactor (CSTR) (Panda *et al*., 2016), fixed bed reactor (FBR) (Brown, 2007; Nagpal *et al*., 2000; Kolmert and Johnson, 2001), fluidised bed reactor (FBR) (Brown, 2007; Gomez *et al*., 2013;), up-flow anaerobic sludge bed (UASB) (Bal and Dhagat, 2001; Bajpai, 2011), anaerobic filter reactor (AFR) (Morel and Diener, 2006), anaerobic hybrid reactor (AHR) (Pandian *et al*., 2011), anaerobic baffled reactor (ABR) *(*Foxon *et al.*, special edition).

Two main active technologies have been described for the active treatment of AMD using SRB: The Thiopaq and Biosulfide processes (Martinez and Cornella, 2014).

2.6.4.1. The Thiopaq Process.

The Thiopaq system consists of two distinct microbial populations and processes:

1) DSR and metal precipitation occurs in a single anaerobic bioreactor,

2) The excess H_2S produced by the DSR process is oxidised to sulfur, by sulfur oxidising bacteria in a separate aerobic reactor (Figure 2-19) (Martinez and Cornella, 2014; Johnson and Hallberg, 2005).

In the anaerobic bioreactor, SRB consumes the organic material while reducing sulfate to H_2S. The H_2S that is formed reacts with dissolved metals to form insoluble metal sulfide precipitates. In the subsequent aerobic reactor, excess H_2S is oxidised by microorganisms into elementary sulfur (Figure 2-19). This technology was successfully implemented at the Budelco Zinc Refinery in Netherlands, which achieved an average 99.7% zinc and cadmium removal with sulfate concentration levels remaining below 200 mgL^{-1} from a sulfate influent of 15 000mg/L during four years of operation (Erasmus., 2000; Boonstra *et al*., 1999; Scheeren *et al*., 1993; DeVegt and Buisman 1996) and at Kennecott UTA Copper (USA) (Boonstra *et al*., 1999). The

Thiopaq process has also been applied at an industrial scale to recover copper at Pueblo Viego gold mine in the Dominican Republic. Copper yields of up to 12 000 tons per year have been recovered through precipitation as sulfides. This process not only adds value to the treatment process, but also reduces the concentration of dissolved metals in tailings, thus, reducing the potential of tailings to generate AMD (Nancucheo *et al*., 2017)

The Thiopaq process has many variations, and the process can be tailored to a host of applications in the mining and metallurgical industry (Jamil and Clarke, 2013). The main advantages of the Thiopaq process are:

a) It produces low concentrations H_2S,
b) Most of the H_2S is in the aqueous phase rather than in the gas phase,
c) The process can be conducted at ambient temperature,
d) Flow rates can be controlled (Reinsel, 2015).

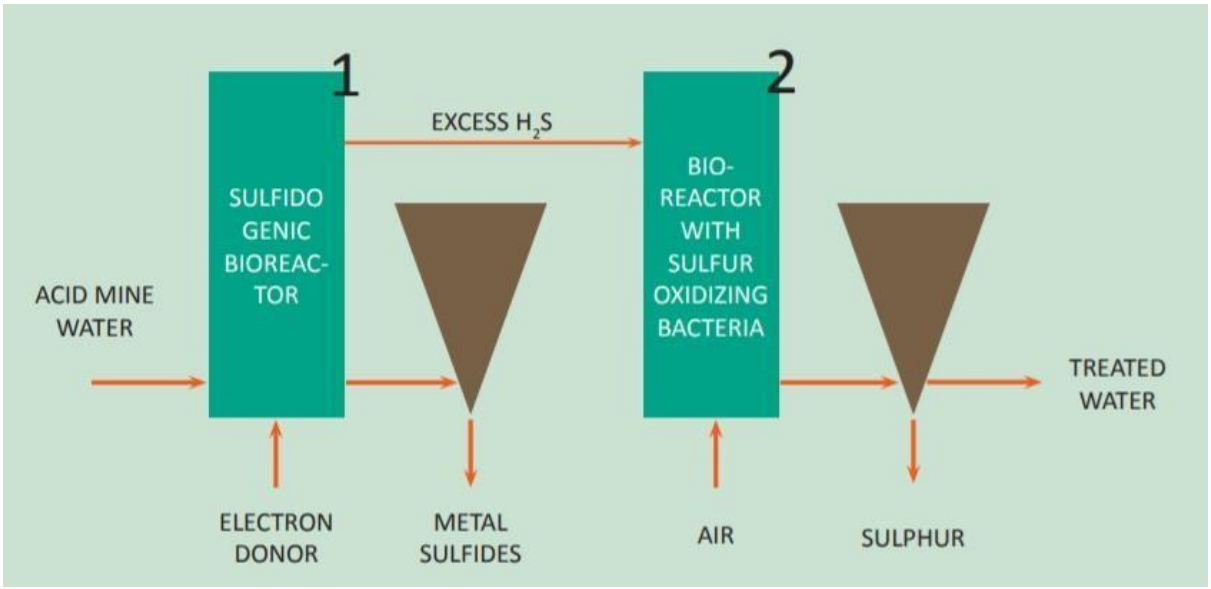


Figure 2-19: Schematic representation of the Thiopac process where sulfate reduction and metal precipitation occur in a single reactor (1) and aerobic oxidation of hydrogen sulfide to sulfur occur in a subsequent sulfur oxidizing reactor (2).

Source: Martinez and Cornella (2014).

2.6.4.2 The Biosulfide process.

The Biosulfide technology is used in the treatment of AMD with low or high metal streams (Martinez and Cornella, 2014). The Biosulfide is composed of a biological unit and a chemical unit, which operate independently (Figure 2-20) (Rowlry *et al*., 1997). AMD is introduced to the Chemical circuit where it reacts with H_2S generated in the biological circuit (Johnson and Hallberg, 2005). Some fraction of volume of treated AMD enters the biological circuit were DSR occurs, producing sulfide from the reduction of sulfate (Figure 2-20) (Martinez and Cornella, 2014). In this manner, the sulfide sludges are isolated in the chemical circuit, eliminating the problems experienced due to the build-up in the bioreactor effectively separating the biomass (Rowley *et al*., 1997). Reactor conditions (pH and sulfide concentration) may be changed to allow for the selective separation of a particular metal

sulfide, which is removed from the partially processed water ahead of further treatment (Johnson and Hallberg, 2005).

The Thiopaq and the biosulfide processes have 3 potential advantages over passive biological remediation.

1) Their performance is more predictable and readily controlled.
2) Metals of economic value may be recovered.
3) Concentration of sulfate in treated water may be lowered significantly.

The major disadvantage of these systems is high costs associated with construction and operation of the treatment plants (Taylor *et al*., 2005).

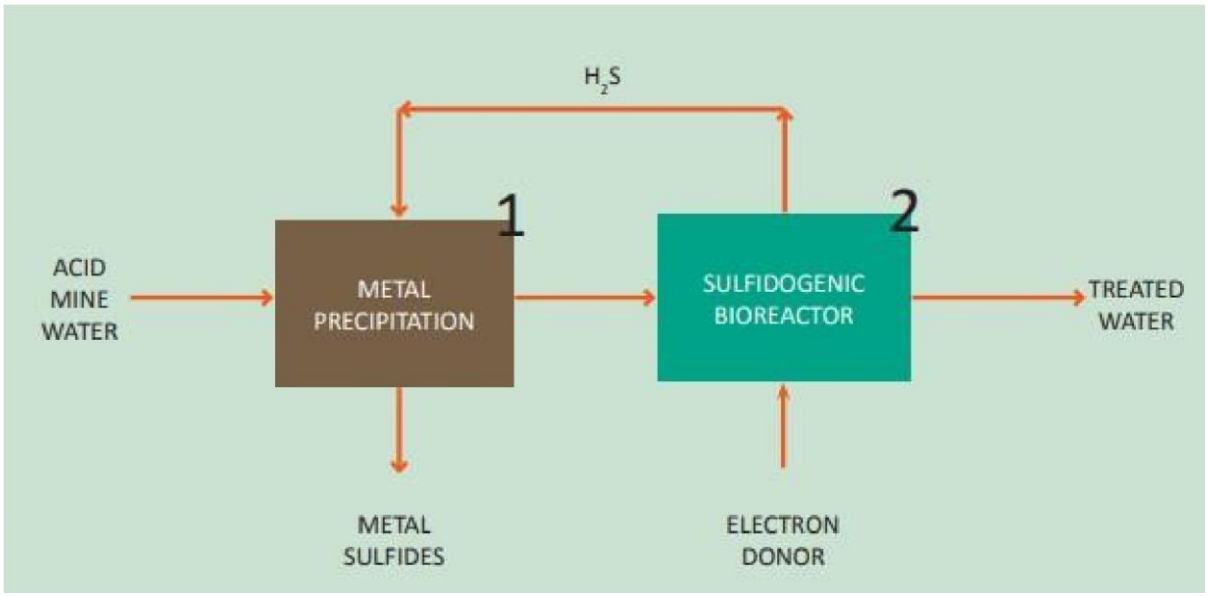


Figure 2-20: Schematic representation of the biosulfide process: (1) metal precipitation occurs and (2) H_2S is generated.

Source: Martinez and Cornella, 2014.

The biosulfide process has been successfully operated at Kennecott Utah copper in the USA, where results in Table 2-4 were obtained.

Table 2-4: Treatment of ground water with high levels of metals and sulfates at the Kennecott Utah copper in the USA using the biosulfide process.

Component	Influent (mg/L)	Effluent (mg/L)
Sulfate	30 000	<0.1
Copper	60	<0.1
Iron	675	<0.3
Zinc	65	<0.1
Manganese	350	0.3
Aluminium	2 200	<2

Source: Martinez and Cornella (2014).

2.7. Environmental factors affecting SRB activity.

2.7.1. pH.

SRB appears to survive a wide range of pH conditions but become less active below a certain pH. Below pH 6 and above pH 7, SRB is inhibited (Doshi, 2006; Poinapen, 2008). Ratanasit *et al.* (2016) showed a decrease in sulfate reduction in a decreased pH environment. SRB can survive pH values as low as 3, hence, their use in the treatment of AMD (Doshi, 2006). SRB produce bicarbonate alkalinity, which neutralises AMD to a more favourable pH for growth. The alkalinity also helps in the precipitation of metals in the AMD. Al-zuhair *et al*. (2008) reported high growth rate in a basic media than in an acidic environment. They attributed this inhibitory effect of low pH to the existence of the undissociated H_2S in acidic conditions. In acidic conditions, sulfide exist in the undissociated form as the pH increases the sulfide dissociates into HS^- and S^{2-}. Perry and Jong (2006) cited in Doshi (2006) observed that SRB sustained sulfate reduction rates of up to 1052 mMol/m^3/day when the pH was lowered from 6.0 to 4.0. They reported that SRB was able to survive at pH 2.5 in an ethanol fed reactor. Willow and Cohen (2004) cited in Cohen (2006) showed that pH of the water is more critical to reactor efficiency than the oxygen content of the influent water. Near neutral pH was

reported to enhance the activity of SRB. In low pH wastewater, additional chemical alkalinity may be required to improve the pH in addition to the alkalinity generated by SRB.

2.7.2. AMD metal chemistry.

Metals play an important role in life processes, as they are involved in important functions of the microbial cell. Some metals are involved in catalytic activities within the cell; some are structural components of DNA and protein, while some serve in maintaining osmotic balance (Bruins *et al*., 2000). Essential and non-essential metals are toxic when they exceed specific concentrations. Metals are toxic to microorganisms because they react with functional groups in proteins leading to the deactivation or denaturing of enzymes. Metals also compete with essential cations, replacing them resulting in enzymes losing their function (Kitot *et al*., 2010; Bruins *et al*., 2000).

2.7.3. Temperature.

Temperature is one of the major influences of any microbial mediated reaction. Temperature can affect both kinetics and stoichiometry via phenomena ranging from thermodynamic activity through physical enzyme conformation changes (Okabe and Satoshi, 1993). In general, the mesophilic range (25^{o}C-45^{o}C) is used in anaerobic reactor systems with optimum temperature of growth at 35^{o}C (Stronach *et al*., 1986). SRB have been reported to grow at substantially lower temperatures such as 20^{o}C. At low temperatures, there is a protracted and difficulties in the start-up of the reactors. Bacterial growth is limited at low temperature by restrictions on cellular level processes such as permeability of membranes or temperature limitation of enzyme activity (Stronach *et al*., 1986, Arnosti *et al*., 1998). As the temperature rises, chemical and enzymatic reactions in the cell proceed more rapidly and bacterial growth becomes faster. Other SRB can grow within the thermophilic range (55^{o}C-80^{o}C) (Liamleam and Annachhatre, 2007). Thermophilic sulfate reduction is applicable for treating sulfate-containing wastewater that is relatively warm. For example, sulfate wastewater discharged

from pulp and paper industry, rayon manufacturing process, and flue gas desulfurization. Furthermore, the conversion rate of thermophilic treatment is much higher than mesophilic treatment (Liamleam and Annachhatre, 2007). Thermophilic sulfate reduction is preferred over conventional mesophilic treatment because it eliminates the need for cooling.

Greben *et al.* (2002) reported a decrease in sulfate removal rates with decreasing temperatures. They showed that when the reactor temperature decreased from 20^{o}C to 5^{o}C, the specific sulfate removal rate decreased from 3.39 to 0.40 g SO_4/ (gVSS). d. Al-Zuhair *et al.* (2008) observed that the highest rate of sulfate reduction occurs at 35^{o}C. The rate of sulfate reduction at 20^{o}C was reported to be higher than the rate at 50^{o}C. Sulfate reducing bacteria have also been reported to operate at temperatures as low as 0^{o}C and as high as 110^{o}C in deep see hydrothermal vent sediments.

2.7.4. Sulfide toxicity.

Sulfide exist in different species as aqueous or gaseous H_2S, dissociated hydrogen sulfide (HS^-) and sulfide (S^{2-}). At a pH between 6-8, most sulfide exist as H_2S and HS^-, whereas above 8, the HS^- and S^{2-}species dominate (Poinapen, 2008). The undissociated molecule is thought to bring about toxicity because H_2S can enter the cytoplasm where it interferes with cell function. This may be because sulfide cause native protein denaturation through formation of sulfide and disulphide cross-links between peptide chains. H_2S may also interfere with co-enzymes A and M through formation of disulphide linkages. H_2S may also affect the internal pH of the cell (Fernandez-Polanco and Encina, 2006; Hulshoff-Pol *et al*., 1998).

Formation of insoluble heavy metal sulfides can also inhibit SRB activity. It is thought that metal sulfides deposit on the surface of the SRB or are concentrated near the bacterial cells and hinder further metabolism by preventing the contact between reactants and cells (Fernandez-Polanco and Encina, 2006). Metal sulfides may also cause denaturation of enzymes by irreversibly binding to enzymatic protein. In general, SRB have a greater tolerance for sulfide

inhibition than most bacteria, however some genera and species of SRB are more vulnerable to sulfide inhibition than others (McMahon, 2007). Ratanasit *et al.* (2016) investigated the effect of sulfide concentration on sulfate reduction and concluded that sulfate reduction rate decreased with the increase of sulfide concentration. Okabe and Satoshi (1993) reported a decrease in lactate utilisation at sulfide concentration of 150 mg/L, with sulfide levels above 250 mg/L dramatically reducing cellular production.

2.7.5. Competition between SRB and Methanogens.

In anaerobic environments, SRB and methanogens compete for the same substrates, acetate, and hydrogen/carbon dioxide (Figure 2-21) (Moestede *et al*., 2013; Van Hanadel *et al*., 2006). SRB also compete for substrates like propionate and butyrate with acetogenic bacteria. The outcome of this competition is influenced by pH, temperature, substrate affinity, specific growth rate, sulfate concentration, immobilisation properties of bacteria and the type of reactor used (Van Hanadel *et a*l., 2006). In the presents of non-limiting levels of sulfate, SRB is favoured because anaerobic respiration with sulfate yields more energy for growth compared with carbon dioxide.

Choi and Rim (1991) observed that there was active competition between SRB and methanogens at COD/SO_4^{2-} ratios from 1.7 to 2.7. At lower ratios, SRB dominate while at ratios higher than these methanogens dominate. They attributed this to two factors, which are the stoichiometric sulfate requirements for SRB metabolism and the kinetics of substrate utilisation. The first factor takes precedent when the sulfate concentration is limiting SRB growth while the second when sulfate is in excess. SRB also have a higher affinity for both hydrogen and acetate enabling them to consume substrate below levels possible for methanogens (Moestedt *et al.*, 2013, Plugge *et al.*, 2011). SRB have a higher specific growth rate than methanogens (Moestedt *et al*., 2013). Methanogens are generally poor competitors with SRB in natural environments such as marine sediments and large intestines in humans

(Raskin *et al*., 1996). Methanogens tend to be dominant scavengers of hydrogen and acetate in low sulfate environments (Figure 4-2) (Raskin *et al*., 1996; Oremland and Polcin, 1992). Some researchers have reported the co-existence of methanogens and SRB in the presents of non-limiting sulfate concentrations. Large populations of SRB have also been found in sulfate-depleted environments. Raskin *et al.* (1996), reported the presents of SRB in the absence of sulfate. This is due to the ability of SRB to function as proton reducing acetogens/or fermenters. This maybe because SRB can grow syntrophically with hydrogen and formate consuming methanogens on lactate, ethanol, propionate, fumarate, and pyruvate in defined cocultures, eliminating the need for sulfate (Raskin *et al*., 1996). According to Oremland and Polcin

(1992), sulfate reducers outcompete methanogens for hydrogen and acetate but do not compete with methanogens for methanol.

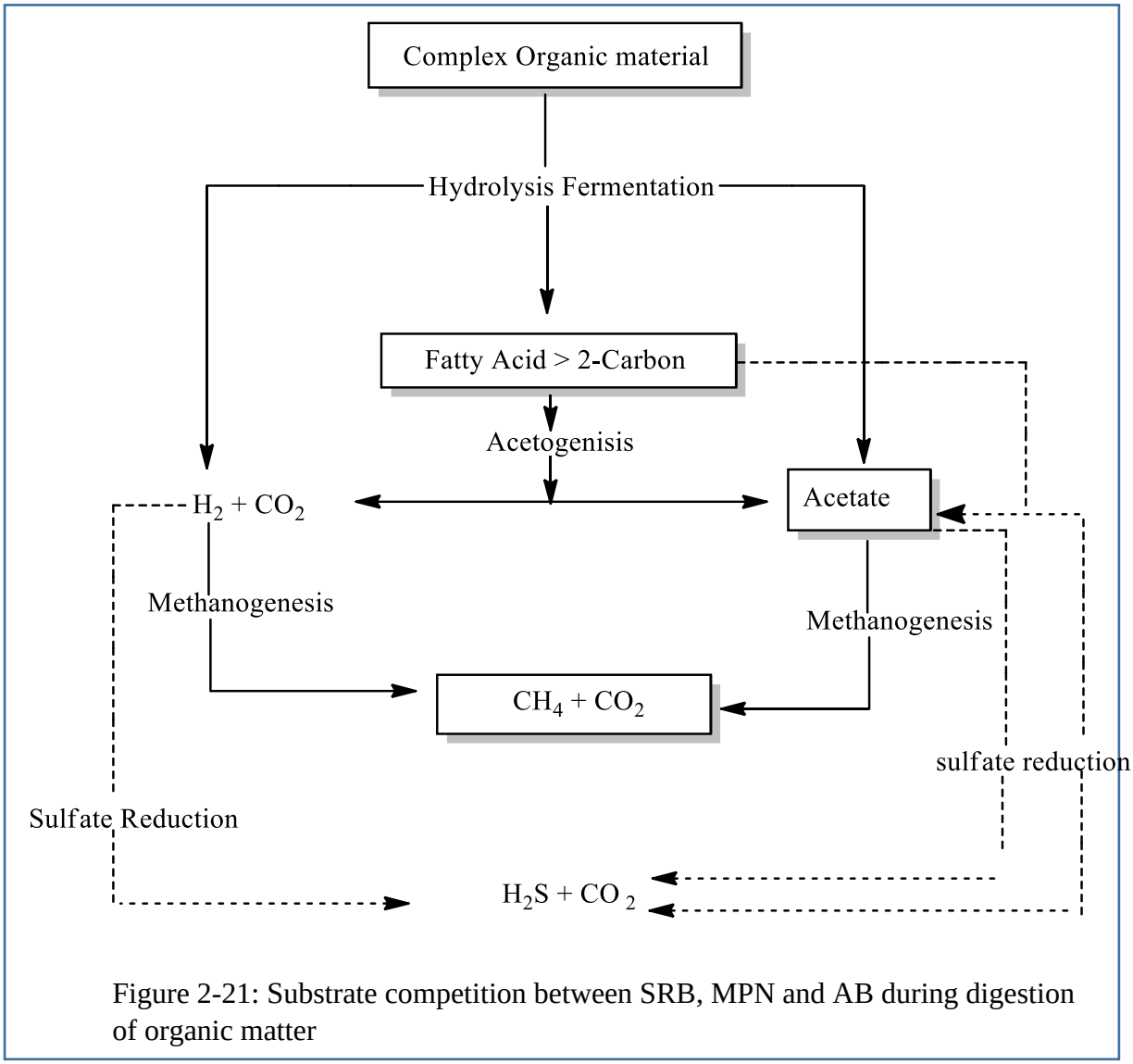


Figure 2-21: Substrate competition between SRB, MPN and AB during digestion of organic matter

2.7.6 Nutrient Availability.

Nutrient availability can affect both growth of the organism through energy limitation or through limitation of precursors as well as the amounts and types of products. Reducing the concentration of essential nutrients such as nitrogen (N) and Phosphorus (P) to below limiting concentrations is a possible means of controlling SRB (Okabe and Satoshi, 1993)

2.8. Mechanism of sulfate reduction.

All steps of sulfate reduction occur in the cytoplasm, meaning sulfate must be transported across the cytoplasmic membrane into the cell (Caspi *et al*., 2014; Gibson, 1990). Once inside the cell, sulfate dissimilation then proceeds by the action of the enzyme sulfate adenyl transferase to produce a highly activated molecule called adenosine phosphosulfate (APS), as well as pyrophosphate, which may be subsequently cleaved to yield phosphate. APS is rapidly converted to sulfite (SO_3^-) by the enzyme reductase. Sulfite may then be reduced via intermediates to form sulfide ions (Gibson, 1990; Nevalto, 2010). The intermediates are thought to include metabisulfate ($S_2O_5^{2-}$), dithionate ($S_2O_4^{2-}$), Trithionate ($S_2O_6^{2-}$) and thiosulfate ($S_2O_3^{2-}$).

The general mechanism of sulfate reduction is explained by Equation 2.7:

$$SO_4^{2-} + 8H^+ + 8e^- \rightarrow 4H_2O + S^{2-} \qquad \text{(Equation 2.7)}$$

From Equation 2.7, 8 moles of electrons are required to reduce one mole of sulfate. The electrons are obtained from an organic electron donor such as ethanol. Considering biomass yield, theoretical molar ratios of the organic substrate to be blended with sulfate can be calculated so that sulfate conversion is maximised, and residual organic substrate is minimised. For more complex substrates such as primary sewage sludge (PSS), the molecular formula for the particulates is not known and rather the total COD is measured.

From equation 2.7, one mole of sulfate requires 8 electrons. This is equivalent to (Equation 2.8):

$$2O_2 + 8H^+ + 8e^- \rightarrow 4H_2O \qquad \text{(Equation 2.8)}$$

Therefore 1 mole of sulfate (96 g/mol) requires 2 moles of oxygen (32 g/mol), or 96g sulfate require 64g COD. Therefore, a minimum chemical (excluding biomass yield) COD: SO_4 ratio of 0.67 $gCOD/gSO_4$ is required (Ristow *et al*, 2005).

2.9. Substrates used by SRB.

SRB can use a large variety of organic and inorganic compounds for their metabolism (Table 2-5). Most of these are small molecular weight organic compounds that in natural habitats are excreted by fermentative bacteria (Nevalto 2010). In bioreactors, various types of organic substances have been employed as electron donors and carbon sources including sewage sludge, leaf mulch, wood chips, animal manure, vegetal compost, sawdust, mushroom compost, whey, and other agriculture waste (Liamleam and Annachhatre, 2007; Sheridan *et al.*, 2018; Waybrant *et al*., 1998; Gilbert *et al*., 2002; Chistensen *et al*., 1996).

In active bioreactor systems where, continuous operation is required; liquid or gaseous carbon sources are usually preferred as solid organic substrates should be regularly replenished. Moreover, the use of complex organic compounds such as plant material would require the enhancing of the microbial community with anaerobic fermentative bacteria for the degradation of the organic material into very volatile fatty acids and alcohols capable of supporting SRB growth (Kousi *et al*., 2015; Doshi 2006). Several liquid and gaseous electron donors have been studied and a few are discussed below:

2.9.1.1. Hydrogen.

Growth of SRB on hydrogen requires either an external carbon source, which can either be autotrophic and requires CO_2 or heterotrophic, which requires both acetate and CO_2. Hydrogen is the most favourable electron donor for DSR due to high free energy change of sulfidogenic oxidation ($2H^+/H_2$, E_O'=-0.414) (Nevalto, 2010; Liamleam and Annachhatre, 2007). Molecular hydrogen is a key intermediate in the natural mineralisation of organic substrates in sediments, sludge digesters and other anoxic environments. Many species of SRB can use hydrogen as the sole electron donor. The oxidation of hydrogen proceeds according to Equation 3.2 (Nevalto, 2010):

$4H_2 + SO_4^{2-} \rightarrow H_2S + 2H_2O + 2OH^-$ (AG^o =38.1kj/mol) (Equation 3.2)

In wastewater treatment systems, H_2 can be directly supplied to the reactor or generated on site from other electron donors like propionate, methanol, and glucose. Consumption of hydrogen generates hydroxide as shown in equation 3.3 (Liamleam and Annachhatre, 2007):

$4H_2 + SO_4^{2-} \rightarrow H_2S + 2H_2O + 2OH^-$ (Equation 3.3)

2.9.1.2. Monocarboxylic acids.

These include formate, acetate, propionate, butyrate, and higher fatty acids up to 20 carbon atoms (C20). These are major fermentation products in many ecosystems and are key intermediates in anaerobic digesters. Formate is one of the best energy sources for the growth of SRB. The oxidation of formate proceeds as in equation 3.4 (Nevalto, 2010).

$4HCO_2^- + SO_4^{2-} \rightarrow 4HCO^{3-} + HS^-$ (Equation 3.4)

Acetate is a key intermediate in the breakdown of organic substances in anaerobic processes. SRB are generally poor competitors of methanogenic archaea (MA) for acetate. Thauer and Postgate (1982) found high activities of the Krebs cycle enzymes in *Desulfobacter Postgatei* cell extracts suggesting that acetate is oxidised through the citric acid cycle with the synthesis of pyruvate from acetyl CoA and carbon dioxide. According to Thauer and Postgate (1982), acetate is the quantitatively most important physiological electron donor for dissimilatory sulfate reduction in sulfate sufficient habitats accounting for 70% of the H_2S formed.

2.9.1.3. Dicarboxylic acids.

These include succinate, fumarate, oxalate, and malate. Malate and fumarate are used mainly by several *desulfovibrio* species and by certain *desulfobacterium* strains (Hansen, 1993).

2.9.1.4. Alcohols.

Ethanol is used as an energy source by many desulfovibrio species and several representatives of other general such as *Desulfococcus, Desulfosarcina,* and *Desulfobulbus* (Hansen, 1993). Ethanol is oxidised by most SRB either completely to CO_2 or incompletely to acetate (Equation

3.5). The sulfidogenic oxidation of ethanol proceeds via acetaldehyde to acetate (Nevalto *et al*., 2010).

$2CH_3CH_2OH + SO_4^{2-} \rightarrow 2CH_3COO + HS^- + H^+ + 2H_2O$ (Equation 3.5)

The drawback of using ethanol as an electron donor is the low growth rate of SRB on ethanol. Ethanol also produces acetate, which results in an increase in the COD of the effluent. This may be resolved by incorporating acetate utilisation by methanogens and SRB (Liamleam and Annachhatre, 2007).

Methanol is readily available and can be directly used by SRB and/or indirectly used via the involvement of other anaerobic microorganisms (Liamleam and Annachhatre, 2007). Longer chain primary alcohols are usually incompletely oxidised to yield the corresponding acid (Hansen, 1993).

2.9.1.5. Sugars.

The ability to use sugars is rarely observed in SRB. *Desulfotomaculum nigrificans* has been observed to grow on fructose and glucose as electron donors. Equation 3.6 shows the sulfidogenic oxidation of glucose (Nevalto, 2010)

$C_6H_{12}O_6 + SO_4^{2-} + H^+ \rightarrow 2CH_3COOH + 2CO_2 + HS^- + 2H_2O$ (Equation 3.6)

2.9.1.6. Complex substrates for sulfate reduction.

Cellulosic substrates can be used as substrate for biological sulfate reduction. Cellulolytic and acid forming bacteria however are required to breakdown the cellulose material into short chain acids, which can readily be used by SRB. The cellulolytic bacteria can breakdown the cellulose into lower molecular weight soluble organics (Magowo *et al*., 2014). Fermentative bacteria then use these products converting them to short chain organic acids and alcohols. The organic acids and alcohols are then used by SRB for sulfate reduction. Agricultural plant waste and

organic wastes have been used in bioreactors containing mixed cultures of SRB, cellulolytic and fermentative bacteria (McMahon, 2007).

Table 2-5: Sulfate and sulfite reduction rates during biological removal of sulfate with different donors under mesophilic conditions Source: Liamleam and Annachhatre (2007)

Electron donor	**Temperature (ºC)**	**Bioreactor type**	**SO_4^{2-} removal (g/L/d)**	**References**
Molasses	30	UASB[a]	4.3	Annachhatre and Suktrakoolvait 2001a and 2001b
Molasses	27	CSTR	0.84	Maree and Hill (1987)
Molasses	35	Anaerobic RBC	0.35	Lo *et al*. (1990)
Molasses	31	Packed bed	6.5	Maree and Strydom (1995)
Molasses + mine water	N/A	Packed bed	1.36	Maree *et al* (1991)
Synthesis gas	30	Gas-lift	12-14	Van Houten *et al*. (1994)
H_2/CO_2	30	Gas-lift	30	Van Houten *et al*. (1994)
H_2/CO	35	Pack bed	1.2	Du Preez and Maree (1995)
CO	35	Pack bed	2.4	Du Preez and Maree (1995)
Mixture of VFA	30	Baffled reactor	0.41	Hammack *et al*. (1994)
Acetate	33	Pack bed	15-20	Sucki *et al*. (1993)
Acetate	33-35	EGSB	28.5	De Samul and Verstraete (1999)
Lactate	RT	Plug Flow	0.41	Hammack *et al*. (1994)
Glucose/acetate	35	Anaerobic digester	1.92	Polpresert and Haas (1995)
Sucrose/peptone	35	Baffled reactor	23.5	Barber and Stuckey (2000)
Ethanol	33	EGSB	21	De Samul and Verstaete (1999)
High strength leachate	19-25	Anaerobic filter	0.02	Henry and Prasad (2000)

2.9.2. Fischer Tropsch Wastewater (FTWW) as a potential substrate for AMD treatment using biological sulfate reduction.

One of the major challenges which determines the success of the biological treatment of AMD is the availability and cost of the organic feedstock needed for sulfate reduction (Neculita *et al*, 2007; Van den Berg *et al*., 2016). A range of organic materials may provide the organic

substrates. The availability and degradability of these organics determines the success of the treatment process.

The costs of some feedstocks such as hydrogen, lactate, and ethanol make the application of biological treatment expensive (Neculita *et al*, 2007; Van den Berg *et al*., 2016). In this study, the proposal is to use FTWW as the organic feed for DSR.

FTWW has a high chemical oxygen demand contributed by mainly short chain fatty acids and alcohols (Majone *et al*., 2010; Greiger *et al*., 2014). Sulfate reducing bacteria readily use short chain fatty acids and alcohols for dissimilatory sulfate reduction (Cao *et al*., 2012; Greben *et al*., 2000; Eger *et al*, 2004). The coal to liquid (CTL) technology that converts coal to liquid fuels, produces FTWW. CTL plants are likely to be located on or near coal mining plants which have the potential to produce large volumes of acid mine drainage. CTL plants produces large volumes of organic rich FTWW. The FTWW and AMD waste streams can be combined and co-treated using sulfate reduction.

Using these locally available organic rich waste stream will reduce the cost of both supply, as FTWW is a waste material, which require treatment and little to no transport costs as FTWW is found on or near coal mining plants. This makes the application of FTWW in the treatment of AMD using DSR a more practical and economical treatment option.

2.9.2.1. The Production of FTWW.

The decrease in the availability of oil resources and the increase in energy demand especially in liquid fuels has generated interest in technologies to produce synthetic liquid fuels through the X to Liquid (XTL) process (de Klerk, 2011; van Steen *et al*., 2018). The XTL process is used to produce synthetic fuels from carbon containing feedstocks via syngas (van Steen *et al*., 2018; Ma *et al*., 2018; Zhang *et al*., 2018). Synthetic fuels can be generated through several pathways, which can be direct, such as, with gas to liquid (GTL) technology, coal to liquid

(CTL) and biomass to liquids (BTL). This classification is based on the feed employed for the synthesis (de Kleck, 2008). The GTL uses natural gas, The CTL uses coal and BTL uses biomass as the feedstock (Matthey, 2011; Saeidi *et al*., 2014). All these processes produce precursors for synthetic crude oil and natural gas which is then used to produce useful hydrocarbons and oxygenated products such as liquid transportation fuels including diesel fuel, gasoline, and chemicals such as alkenes using a process called the Fischer Tropsch (FT) process (Pacheco and Guirardello, 2017; Saeidi *et al*., 2014; Ma *et al*., 2018).

In the CTL process, oxygen is blended with coal and steam at high temperatures and pressure to produce carbon monoxide and hydrogen gas (Figure 2-22). Sulfurous compounds are also produced and need to be removed to avoid catalyst poisoning and to produce zero sulfur fuels (Styles, 2008). The hydrogen and carbon monoxide gases are converted to liquid hydro-carbon fuel using a metal-based catalyst in the FT process (Figure 2-22) (Failey, 2012; Saeidi *et al*., 2014). The Equation 3.7 can summarize the FT process:

$$(2n + 1)H_2 + nCO \rightarrow C_nH_{2n+2} + nH_2O \qquad \text{(Equation 3.7)}$$

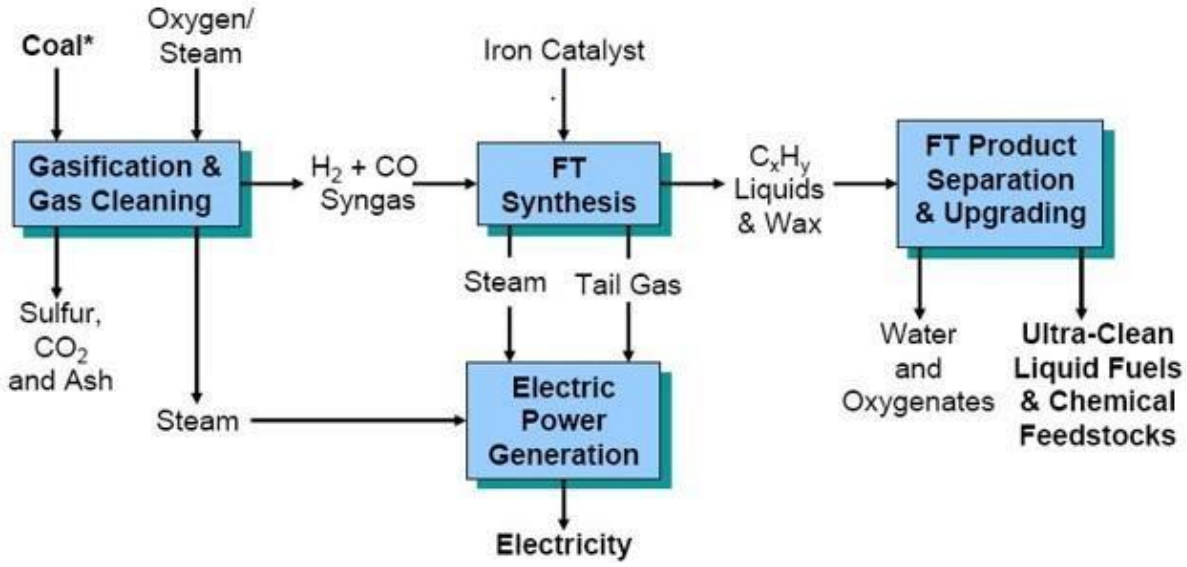


Figure 2-22: Schematic representation of the FT process

Source: IEA ETSAP- Technology brief 2010)

Franz Fischer and Hans Tropsch developed the FT process in 1920 (IEA ETSAP- Technology brief, May 2010; Makhura *et al*., 2019). During the Second World War (1936), German commissioned an FT plant to augment its fuel supplies. The production of synthetic fuel in Germany was recorded at more than 124 000 barrels per day in 1944 (Makhura *et al*., 2019). Subsequent FT plant operations were initiated in Texas ((1940) (was eventually shut down due to operational costs)), South Africa (Sasol 1 in Sasolburg (1950's) and Sasol 2 (1980) & 3 (1982), GTL plant in Mossel bay (1993) still operating). Currently, there are many plants in various stages of development in the world including Qatar, China, and the United States (Table 2-7).

The production of liquid fuels using the FT process is classified into high temperature FT (HTFT) and low temperature FT (LTFT) process (Figure 2-23) (Leckel, 2009; Makhura *et al*., 2019; Minchener, 2011). The LTFT operates at temperature ranges between 220-250^{o}C and uses a cobalt catalyst and maximises the production of paraffins, olefins and alcohols. The HTFT process operates at 300-350^{o}C, is based on an iron catalyst, and produces mainly

fractions with higher molecular weight, thus maximising the gasoline fractions (IEA ETSAP-Technology brief, May 2010; Steynberg and Nel, 2004; de Klerk, 2008; Minchener, 2011).

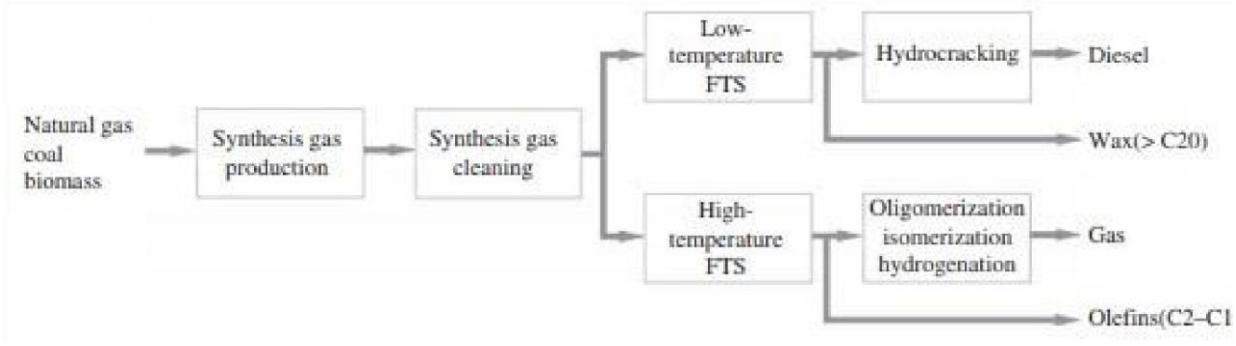


Figure 2-23: A schematic presentation of the production of fuels by HTFT and LTFT processes

Source: Saeidi *et al*. (2014)

During cooling in the FT process, water is condensed with hydrocarbons and oxygenates to produce an oil product and an aqueous product. The short chain polar oxygenates dissolve in the aqueous product and are refined separately from the oil product (de Klerk, 2008). The vast amounts of water produced makes water one of the major products from the Fischer Tropsch process (Equation 3.8). The water contains 7-10% of syncrude in the HTFT and around 3% in the LTFT process. The aqueous product in the HTFT process has small amounts of methanol, while methanol is a major compound in the LTFT aqueous product (de Klerk, 2008).

$$nCO + 2nH_2 \rightarrow -(CH2)_n- + nH_2O \qquad \text{(Equation 3.8)}$$

Some of the dissolved compounds are separated from the aqueous phase. The remaining water still contain significant amounts of short chain fatty acids (SCFA) and alcohols (C1-C6) and other organic contaminants (Table 2-6) (Greger *et al*., 2014). These cannot be separated economically from the water (van Zyl, 2008). The organic rich waste stream is called Fischer Tropsch wastewater (FTWW). FTWW has a very high chemical oxygen demand (COD) in the

range of 18000-30000 mg/L (Grieger *et al*., 2014; Majone *et al*., 2010; Moreroa *et al*., 2018). The FTWW is a low pH (pH 3.65) waste stream.

According to Moreroa *et al* (2018), for every tonne of crude oil produced by the FT process, there is 1.1 to 1.3 tonnes of wastewater produced. Because of its acidic nature, FTWW is very corrosive and is a concern to the environment as well as infrastructure (Ma *et al*., 2019). FTWW cannot be discharged into the environment in its raw form. The COD and the acidity need to be removed before discharge or reuse of the waste stream.

Table 2-6: Composition of a typical FTWW

Composition	mg/L
COD	30100
TOC	6600
Diethyl ketone	181
Alcohols C1-C10	
Butanol	446
Decanol	5.4
Ethanol	2450
Heptanol	28
Hexanol	94.2
Methanol	77.80
Nonanol	5.2
Octanol	10.2
Pentanol	251
Propanol	690
Acetic acid	261
Propionic acid	108
n-Butyric acid	22.8
n-Valeric acid	23.6

Source: Grieger *et al.* (2014*)*

2.9.2.2. Location of GTL and oil plants in the world.

The FT process may best benefit those countries with large reserves of coal but still rely on imports for liquid fuels. These countries can use their coal reserves to produce the much-needed liquid fuel, thereby alleviating the cost of fuel importation.

CTL plants are usually built at the mine mouth with adjacent reservoirs of at least 500 Mt of coal depending on the plant capacity (IEA ETSAP-Technology brief, May 2010). According to world coal institute, coal is mined in over 50 countries in the world and is present in over 70. The existing and potential CTL plants bring the major concern of environmental pollution, were large volumes of low pH and organic rich FTWW may be generated by the FT processes. In places where treatment is not done or there is inadequate treatment of the FTWW, the environment will be impacted negatively.

The mining activities also potentially generate large amounts of low pH metal and sulfate rich effluent (AMD). If these wastes are disposed without treating them to meet the local standards

for potable water, the balance in the ecosystem can be adversely affected. Combining and co-treating these two waste streams is a possibility, simultaneously removing sulfates and dissolved metals from AMD, as well as the COD from the FTWW.

2.9.2.3. Location of FT plants in South Africa -Sasol.

South Africa has been producing liquid fuels and chemicals from coal using the Fischer-Tropsch (FT) based Gas-to-Liquid (GTL) technology since 1955 (Minchener, 2011). This technology was expanded in the 1970s to counteract trade sanctions on oil supplies to the Apartheid Regime (Styles, 2008). At present, approximately 30% of liquid fuels such as gasoline and diesel come from the GTL technology, with daily production capacities exceeding 160 000 barrels. This leads to the production of large volumes of wastewater (29 ML/d FTWW produced at Secunda) (Nasr, 2012; Sasol Technology R&D, 2013). Sasol has produced more than 1.5 billion barrels of synthetic fuel and chemical products since it started operating in 1955 and has a market in over 90 countries around the world and remains at present the largest CTL plant in the world (IEA ETSAP-Technology brief, May 2010).

Sasol has plants in Secunda and Sasolburg. These are both regions where large volumes of AMD are produced due to legacy and ongoing coal mining activities (McCarthy and Humphries, 2012).

2.9.2.4. Location of FT plants in China

The Shenhua group in China have been operating a direct coal liquification plant in Erdos, Inner Mongolia China since 2008 (Rong and Victor, 2011). The plant has an operational capacity of 50 000 barrels per day. Other operating CTL projects in China includes:

The Yitai CTL plant in Ordos, Zhungeer that started operating in 2009 using FT technology. The Yitai Plants has a capacity of 160, 000mt/FT liquids. The Jincheng MTG plant which

produces 300 000 t/a methanol from the MTG process and has been operating since 2009. The Shanxi Lu'an CTL plant started operating in 2014 producing 160 000 mt/a FT liquids.

Many other projects are still in the planning stages in China while some are still to be commissioned. All these projects have the potential to produce large volumes of waste effluent from both the mining and the CTL activities.

2.9.2.5. Location of FT plants in the USA.

The USA is one of the largest consumers of oil in the world and imports most of its needs. It is thus necessary for the USA to apply CTL technology to alleviate its energy concern. USA has vast coal reserves. Though the USA has not been applying this technology, feasibility studies have been on going to harness this technology in several sites (See Table 2-7). The CTL technology is also being considered in India, Germany, Botswana, Mongolia, New Zealand, Philippines, and Indonesia, (Styles, 2008). The operating and proposed plants are shown in Table 2-7:

Table 2-7: Operating and Proposed GTL sites around the world

Project	Location	Type	Products	Status
Sasol Synfuels II and III	Sekunda, SA	CTL	160 000 BPD; primary gasoline and light olefins.	Operating
Sasol Synfuels	Sekunda, SA	CTL	3,960,000 Nm/d syngas capacity, FT liquids	Operating
Shenhua Direct coal liquefaction plant	Erdos, Inner Mongolia, China	CTL (direct liquefaction.	20,000 BPD primary products diesel fuel, liquefied petroleum gas, naptha	Operating
Yitai CTL plant	Ordos, Zhungeer, China	CTL	160,000 mt/a FT liquids	Operating
Jincheng MTG plant	Jincheng, China	CTL	300,000 t/a methanol	Operating
Shanxi Lu'an	Lu'an, China	CTL	160,000mt/a FT liquids	Operating
ICM coal to liquids	Tugrug Nuur, Mongolia	CTL	Gasoline, syngas	Proposed
Yitai Yili,	Yili, China	CTL	FT liquids	Proposed
Yitai Ordos phase II	China	CTL	FT liquids	Proposed
Yitai Urumqi	China	CTL	FT liquids	Proposed
Celanese coal/ethanol project	Indonesia, Kalimantan, or Sumatra	CTL	Ethanol	Proposed
Clean Carbon Industries	Mozambique	Coal waste to Liquids		Proposed
Arkaringa Project	Australia	CTL		Proposed
Future Fuels, Kentuky	Kentucky, USA	CTL	Coal to methanol and other products	Proposed
US Fuel	Perry count/Muhleberg Kentucky	CTL	Coal to liquid fuels.	Proposed
Secure Energy	Big Horn County, Montana	CTL	Gasoline	Proposed
Adams Fork energy	Mingo county, West Virginia	CTL	Coal to gasoline	Proposed
FEDC Healy CTL	Fairbanks, Alaska	CTL	Liquid fuels	Proposed
Tyonek	Cook inlet, Alaska	CBTL	Jet fuel/Gasoline/diesel	Proposed
Many Stars CTL	Big Horn County, Montana	CTL		Proposed

Source: Tennant (2014)

All these plants are/will be located on or close to coal mining operations, as they require large amounts of coal for CTL processes. Because of these large coal-mining operations, there is the potential to produce large volumes of AMD from mines having sulfur rich coal deposits. The CTL process produces large volumes of FTWW, which is rich in organics. Both these waste

streams are produced in proximity to each other making it possible to co-treat these streams without the need for transportation.

2.9.2.6. Current technologies of treating FTWW.

2.9.2.6.1 Aerobic Treatment of FTWW.

Most GTL plants in operation have been using the activated sludge method for the treatment of the organic rich FTWW. Sasol has been using aerobic treatment to treat GTL and CTL effluents in ORYX GTL, Qatar, and Synfuels, Secunda facilities (Sasol media centre, 2013). In most cases, the FTWW is treated in combination with two other waste streams form the FT process. These are oily sewers, which originates from plant drainage and stripped gas liquor, which comes from the gasification condensate (van Zyl, 2008). The treatment process is aerobic and is energy intensive increasing the cost of treatment. The aerobic treatment treats the effluent by using aerobic microorganisms to convert the organic material into carbon dioxide and water, leading to no carbon recovery (Brand South Africa, 2010). This process also produces large amounts of sludge, which requires further treatment before disposal (Rycroft, 2013).

2.9.2.6.2. Anaerobic treatment of FTWW.

Anaerobic technologies use microorganisms that can live without oxygen. These organisms live in environments such as sediment layers on floors of lakes, dams, and oceans (Brand South Africa, 2013). The treatment occurs in closed vessels in the absence of oxygen and produces methane gas as well as biomass (Rycroft, 2013). The biogas can be used as an energy source for electricity production. Another advantage of the anaerobic treatment technology is that it provides about 80% less waste sludge than the aerobic process (van Zyl, 2008).

Sasol has recently developed an anaerobic membrane bioreactor technology (AnMBr) for the treatment of FTWW. This technology removes the organics from the wastewater while providing biogas as a by-product for power generation (Sasol media centre, 2013).

2.9.2.8 FTWW as a substrate for sulfate reduction/DSR as a potential treatment alternative for FTWW.

FTWW is a potential substrate for DSR due to its high COD content. This project sought to co-treat both AMD and FTWW using DSR. The SRB bacteria use the organics from FTWW to reduce the sulfate in the AMD. This reduction of sulfate produces hydrogen sulfide, which reacts with dissolved metal ions in the AMD to form insoluble metal sulfide precipitates. The high acidity of both waste streams will be neutralised by biogenic alkalinity. In this way, organics are removed from FTWW, and metals, sulfates and acidity are removed from AMD.

The advantage of using FTWW for DSR is that it is readily available and is in the proximity of mining regions where there is potential to produce large volumes of AMD. FTWW is a waste material and hence will not significantly add cost to the reclamation process. FTWW is rich in organic carbon, which enables it to support microbial activities during the treatment of contaminated water.

At the end of this project, we expect the following outcomes:

1) To provide affordable methods to decontaminate wastewater streams and mines.
2) Providing cheap substrates for biological sulfate reduction.

2.9.3. Research aims and objectives:

1) To investigate the feasibility of using FTWW as an electron donor and carbon source for biological sulfate reduction in the treatment of AMD.
2) To develop and operate a bench scale sulfidogenic bioreactor.
3) To produce hydrogen sulfide and/or elementary sulfur using AMD as a sulfur source.
4) To improve the quality of water using cheap raw materials and microorganisms.
5) To investigate the effect of seasonal temperature variations on bioreactors treating AMD.

2.9.4. Motivation.

Not much literature is available on the use of FTWW as a substrate for the treatment of AMD using biological sulfate reduction, suggesting that this has not been extensively explored. FTWW is a liquid effluent, which is rich in organic acids and alcohols (Grieger *et al.,* 2014; Majone *et al*., 2010). The alcohols and acids in FTWW can be used as the substrates for DSR in the treatment of AMD. Using FTWW will significantly lower the cost of AMD treatment through DSR as FTWW is a waste material produced in large volumes in coal mining areas where there is potential to produce large quantities of AMD. The co-treatment of FTWW and AMD using DSR offers a solution to the long-term sustainability of treating AMD using biological sulfate reduction especially in the developing world where lack of skills and capital have seen inadequate treatment facilities.

Chapter 3.
Co-treatment of AMD and FTWW using biological sulfate reduction.

Modified manuscript submitted to *Minerals Engineering*

Abstract.

Biological sulfate reduction (BSR) is one of the treatment options for acid mine drainage (AMD). In BSR, the oxidation of organic substrates, coupled with the reduction of sulfate produces alkalinity and hydrogen sulfide. The alkalinity helps in improving the pH of the AMD, while the sulfide immobilizes metals. In this study, the possibility of using high COD Fischer Tropsch wastewater (FTWW) as an organic substrate and energy source for biological sulfate reduction in the treatment of AMD was explored. The AMD and FTWW were combined in the presence of sulfate reducing bacteria (SRB). There was considerable success in iron precipitation especially in the batch reactors where 99 % dissolved iron was removed from the effluent. Sulfate removal efficiencies were 95 % and more than 99 % COD was removed from the FTWW. The pH in the reactors was increased from 2.0 to 7.6 without the addition of alkalinity. The continuous reactors were not as successful as the batch reactors due to accumulation of sulfate, iron, and COD in the effluent.

Objectives.

1) To investigate the performance of batch and continuous reactors in the co-treatment of acid mine drainage (AMD) and Fischer Tropsch wastewater (FTWW) using sulfate reducing bacteria.
2) To find new and cheaper substrates for biological sulfate reduction.

3.0. Introduction.

Acid mine drainage (AMD) and Fischer Tropsch wastewater (FTWW) are two major pollutants produced because of mining activities and synthetic fuel production processes respectively. AMD is mainly produced through mining activities that expose sulfide-bearing ores to the environment. This result in the oxidation of sulfide material in the presence of oxygen and water to produce a low pH, sulfate and metal rich effluent (Sheng *et al*., 2017; RoyChowdhury *et al*., 2015; van den Berg *et al*., 2016). The disposal of this raw effluent into the environment is a major environmental concern as it has detrimental effects on water resources as well as on human, animal, and plant health (Pozo Antonio *et al*., 2014; RoyChowdhury *et al*., 2015; Yadav and Jamal, 2016; Hussain and Qazi, 2016; Vyawahre and Rais, 2016; Ochieng *et al*., 2010; Hesketh *et al*., 2010; Reis *et al*., 2013; Jencarova and Luptakova, 2012; Fig 2011).

The Fischer Tropsch (FT) process produces FTWW. The FT process converts coal to synthetic liquid fuels and generates large volumes of organic rich FTWW (Majone *et al*., 2010). The disposal of FTWW is an environmental concern as it may result in environmental pollution. It is necessary that all effluent water be treated before disposal into the environment.

Biological sulfate reduction offers prospects to co-treat a variety of organic and inorganic waste effluents because of the ability of sulfate reducing bacteria (SRB) to reduce sulfate to sulfide and generating alkalinity while using organic substrates for their metabolism (Bowell, 2004;

van den Berg *et al*., 2016; Gusek, 2004). SRB are found in sulfate rich waters such as acid mine drainage (AMD). AMD does not contain enough organic substrates to sustain SRB metabolism and growth. Commercial grade organic electron donors such as ethanol, lactate and methanol have been used successfully in the treatment of AMD but are costly to sustain long-term treatment of AMD. This calls for the exploration of cheap and locally available organic substrates as alternatives. This study therefore explores the utilization of FTWW in providing organic substrates for sulfate reduction in the treatment of AMD. FTWW has a high chemical oxygen demand contributed by mainly short chain fatty acids and alcohols (Majone *et al*., 2010). Short chain fatty acids and alcohols are readily used by sulfate reducing bacteria for dissimilatory sulfate reduction (Greben *et al*., 2000; Bomberg *et al*., 2017, Weijima *et al*., 2000, Sarti and Zaiat 2011; Cao *et al*., 2012).

Coal-to liquid (CTL) plants using coal as their feed are usually located on or near coal mining plants that have the potential to produce large volumes of AMD. CTL plants produce large volumes of organic rich FTWW. FTWW and AMD waste streams can be combined and co-treated using sulfate reduction.

This study leverages the ability of SRB to use organic substrates while reducing sulfate to hydrogen sulfide. Organic substrates are removed from the organic rich effluent through sulfate reduction, while the biologically generated hydrogen sulfide is used to precipitate dissolved metals, biogenic alkalinity attenuates pH, and hence AMD is made free of sulfates, metals, and acidity.

Iron being one of the most prevalent metals on earth is a major constituent of AMD giving AMD its characteristic orange colour. Other dissolved ions found in AMD include copper, nickel, lead, mercury, radium, and aluminium. These metals have different levels of toxicity. It is therefore necessary to remove the dissolved metals from AMD before the water can be reused or discharged into ground or surface water.

Active methods of treating AMD are often used to precipitate metals and remove them from AMD. This is done through the addition of chemicals to raise the pH of the AMD and to precipitate the dissolved metals mainly as metal hydroxide precipitate (Johnson and Hallberg, 2005; Poinapen, 2008, RoyChowdhury *et al*., 2015). The costs of chemicals and labour are some of the main reasons why this method of treatment maybe unsustainable in the long run. The chemical precipitation method also produces a high volume of unstable sludge. The sludge needs to be safely disposed as it is often hazardous. The need to dispose the hazardous sludge confers additional capital and operational costs to the treatment system (Florence *et al*., 2015).

The application of biological sulfate reduction in the treatment of AMD can provide a cheaper and environmentally friendly method of removing dissolved metals from AMD. Metal sulfides are formed due to the activities of SRB, which reduces sulfate to sulfide in the presents of an oxidizable carbon source. The sulfide reacts with dissolved iron to form insoluble iron sulfide precipitates that can easily be removed from the wastewater. Biological sulfate reduction also produces alkalinity. The alkalinity neutralizes the acidity of AMD thereby raising the pH of the water.

This chapter explores the use of biological sulfate reduction in the removal of iron from AMD through iron sulfide precipitation. AMD was used as the sulfate source. Synthetic FTWW (sFTWW) was the electron source. Through biological sulfate reduction, SRB can generate hydrogen sulfide. The sulfide reacts with dissolved iron to form insoluble iron sulfide. The chapter also looks at the iron removal efficiency of a fixed bed batch reactor in comparison to a continuous flow reactor.

3.1. Materials and Methods.

3.1.1. Sulfate reduction and iron precipitation in batch a reactor.

In this experiment, biological sulfate reduction and metal precipitation were conducted in the same reactor. The reactors were made from glass (Modified 2 litre measuring cylinders), with a height of 50cm and diameter of 10cm. Three sampling ports were located equidistant along the length of the reactor (Figure 3-1). The reactors were operated as fixed bed (30 cm bed height) with charcoal (1.2cm-2cm diameter) used as the carrier material. The charcoal produced from hardwood was sourced from Ignite Products, South Africa.

Each reactor had a working volume of 1.2 litres. Preliminary investigations whose findings are shown in Appendix A were used to optimize reactor operations. The reactors were fed with acid mine drainage obtained from Emalahleni in South Africa. The AMD was diluted by a factor of 4 to reduce the sulfate and iron concentration to 3800 mg/L and 950 mg/L respectively. The stock solution of the synthetic FTWW (sFTWW) had a concentration of 20 945 mg COD/L made from distilled water, alcohols, and organic acids (Table 3-1). The sFTWW was mixed with the AMD to obtain a COD concentration of 3400 mg/L, and a COD/sulfate ratio of 0.90 (which is more than the stoichiometric ratio of 0.67 for complete COD removal (Velasco *et al*., 2008)).

The reactors were inoculated with SRB obtained from passive bioreactors on the roof of the Richard Ward building at the University of the Witwatersrand in Johannesburg, South Africa. Vegetal compost was used as the organic source in the passive bioreactors with a sulfate feed of 2000 mg/L. The system was operated at pH 6.5-7. The SRB was transferred to an enrichment media containing trace elements which consisted of 0.5 g/L KH_2PO_4, 1 g/L NH_4CL, 1 g/L $CaSO_4$, 3.5 g/L sodium lactate, 1 g/L yeast extract and 0.1 g/L ascorbic acid (all chemicals were of analytical grade and supplied by Merck). This enrichment media was

subsequently replaced with the AMD/FTWW mixture when evidence of microbial growth on the charcoal was satisfactory (biofilm growth).

The reactors were fed with diluted concentrations of synthetic FTWW and AMD and sealed to become anaerobic. The experiments were operated in duplicate at ambient temperature and were maintained in batch mode for 1056 hours (44 days) before being converted to continuous flow. A control experiment was set up parallel to the main experiment, with the same COD/sulfate ratio and charcoal but without the SRB inoculum.

The conversion of the batch reactor to continuous flow reactor was initiated when the pH levels were above neutral (7.6), dissolved iron concentration was at 0.1 mg/L and sulfate concentration of less than 182.5 mg/L. The concentration of COD at this stage was below the sensitivity of the equipment used (<15 mg/L). Microbial growth was evidenced by the appearance of a biofilm around the charcoal. There was also evidence of iron sulfide production as a black precipitate appeared in the bioreactors (Figure 3-1). The FTWW/AMD feed had similar characteristics to those employed in the batch reactors. The FTWW/AMD feed was pumped from a reservoir into the reactor using a multichannel peristaltic pump at a flow rate of 0.74 ml/minute. The feed was introduced through an inlet pump situated at the bottom of the

reactor and the effluent was collected by gravity into an effluent tank. Inlet samples were frequently analysed for iron, sulfate and COD concentration.



Figure 3-1: Anaerobic single stage continuous reactor for the precipitation of iron in AMD using FTWW as an organic source. The biomass was immobilised on charcoal.

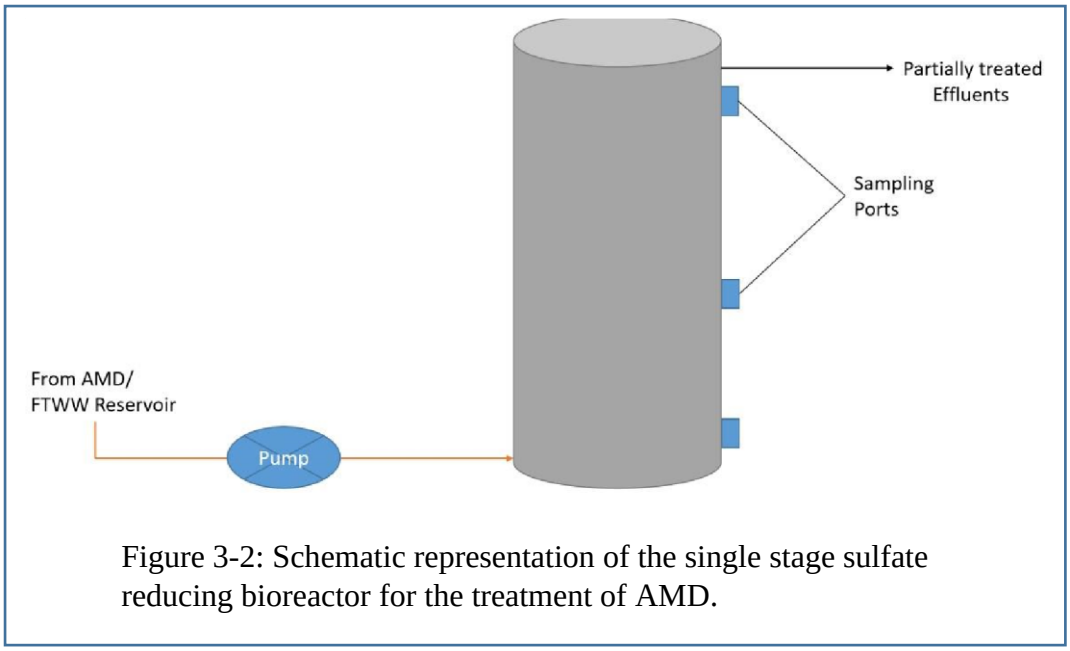


Figure 3-2: Schematic representation of the single stage sulfate reducing bioreactor for the treatment of AMD.

Table 3-1: Composition of the synthetic FTWW stock solution (Adopted from Majone *et al*., 2010).

Compound	**Carbon atoms**	**mg/L**	**mg/L COD**	**% COD**
Methanol	1	2379	3568	17,04
Ethanol	2	2523	5265	25,14
Propanol	3	2303	5527	26,39
Butanol	4	1720	4463	21,31
Propanoic acid	3	691	1046	4,99
Acetic acid	3	1076	1076	5,13
Total		**10692**	**20945**	**100**

3.2. Chemical Analysis.

All Samples were in duplicates.

3.2.1 Alkalinity.

Total alkalinity tests were done on unfiltered samples immediately after sampling, by titrating the bioreactor sample with a 0.020N solution of sulfuric acid (Buret titration, method 8221, reference number: DOC316.53.01151) using an easy sense pH probe and easy sense pH software to an end point of pH 4.3. At this pH, all the alkaline components are used up. The multiplier in Table 3-2 was used to convert the titrant volume to total alkalinity using the formula:

Total alkalinity = Titrant volume (millilitres) X multiplier mg/L $CaCO_3$ alkalinity.

Table 3-2: Sample volume and multipliers for alkalinity determination

Alkalinity Range mg/L	**Sample volume(ml)**	**Titrant-sulfuric acid**	**Multiplier**
1 – 500	50	0.020 N	20
400 – 1 000	25	0.020 N	40
1 000 – 2 000	10	0.020 N	100
2 000 – 5 000	5	0.020 N	200

3.2.2 Sulfate.

The sulfate evaluation was conducted using a PF-12 photometer (Macherey-Nagel, Germany). All the reagents' kits for determinations were supplied by Macherey-Nagel. The procedures followed were as per the supplier's manual. The kit used for sulfate determinations was Viscolor Eco test 592. The PF-12 was factory calibrated.

3.2.3. COD analysis.

COD was determined photometrically using a PF-12 photometer following the digestion of the samples in a Nanocolor Vario mini compact heating block. The test is based on the photometric determination of the decrease in chromate concentration after the oxidation of the wastewater

sample with a strong oxidising agent such as potassium dichromate. The COD analysis was conducted on unfiltered samples. The procedures followed were as per supplier's manual. The kits used were Viscolor eco test 0-26 for COD range 15 mg/L-160 mg/L and Viscolor eco test 0-28 for COD ranges from 1000 mg/L-15000 mg/L. Appropriate dilutions were made on samples whose COD concentration range was between 160 mg/L and 1000 mg/L.

3.2.4. Dissolved iron assays.

Dissolved iron was assayed photometrically using a PF-12 photometer. The iron testing kit (viscolor eco test 5-25) was supplied by Macherey-Nagel. The test kit performs colorimetric determination of Fe(II) and Fe(III) ions with a triazine derivative. The procedures followed were as per supplier's manual.

3.2.5. pH determination.

pH was determined using the Data Harvest easy sense pH electrode and easy sense software.

3.4. Results and discussion.

The sulfate concentration, dissolved iron concentration and pH analysis were done at 24-hour intervals. COD analysis was done at the commissioning of the batch bioreactor. The initial COD concentration was 3400 mg/L. This was done to determine the initial COD/SO_4^{2-} ratio (0. 9), once during the operational time to make sure there was still enough organic material to sustain DSR (the COD sulfate ratio at this point (400hours) was 0.8), and at the end of the bioreactor operation to see if all the COD was removed (Figure 3-3). The concentration of COD remaining in the effluent at the end of the batch operation was below the detection limit of the equipment used (< 15 mg/L), indicating more than 99 % COD removal efficiency. The COD/sulfate ratio used in the bioreactors was above the stoichiometric ratio for sulfate reduction, which is 0.67. This implies the COD was in excess. The near complete utilisation of the COD suggests the existence of other microorganisms such as methanogens, consuming the excess COD apart from those involved in sulfate reduction. This is possible as there was no

selection of a pure culture during the inoculation. In addition, no sterilisation was done on both the equipment and media used. Alternatively, high COD/SO_4^{2-} ratios were needed to improve the efficiency of metal removal. Velasco *et al*. (2008) suggested a feed COD/SO_4^{2-} ratio of 1.5 or above was needed for high metal removal efficiencies above 90%. The control experiment confirmed that 360mg/L (10%) COD was removed possibly through adsorption on charcoal (Figure A9 in appendix A). Charcoal has a high porosity that may provide large surface area for removal of pollutants through adsorption.

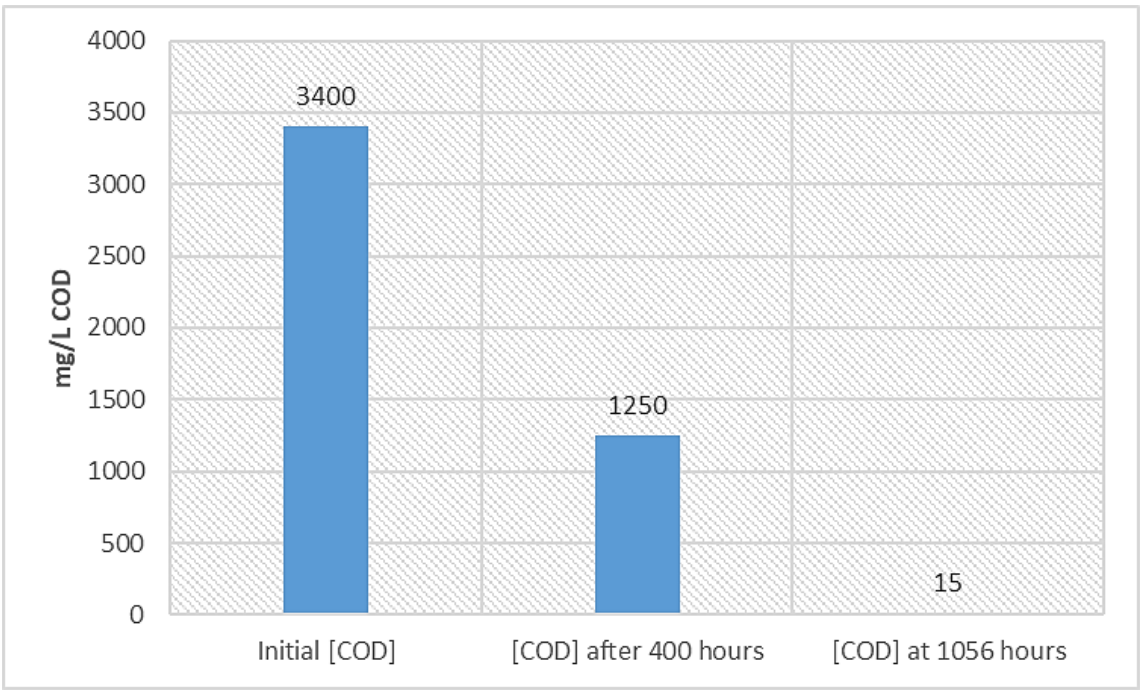


Figure: 3-3: COD concentration at various stages of batch reactor operation

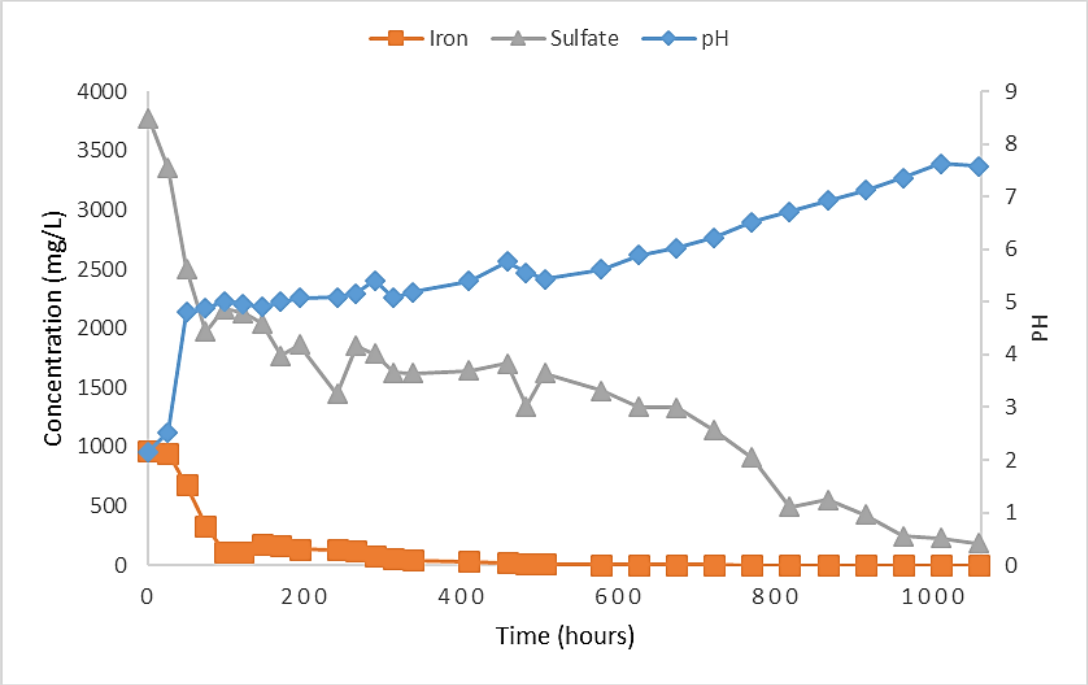


Figure 3-4: Reduction of sulfate and Iron concentration in an anaerobic DSR reactor fed with FTWW.

Figure 3-4 shows the removal of dissolved iron and sulfate as well as the pH increase in the bioreactor. Iron removal in the reactor started gradually at a rate of 4 mg/L/hr, with the iron concentration dropping from 965 mg/L to 680 mg/L within the first 72 hours, a 30% drop in the dissolved iron concentration. A further 345 mg/L of dissolve iron was removed within the next 24 hours, equating to an iron removal rate of 14 mg/L/hr, a cumulative 65 % removal efficiency within the first 96 hours, thereafter there was a gradual removal of dissolved iron to a residual iron concentration of 0.1 mg/L at the time of decommissioning of the bioreactor. An iron removal efficiency of 99 % with the bulk of the iron removed between pH 2.5 and pH 5.0 (Figure 3-4). A strong odour of hydrogen sulfide and a black iron sulfide precipitation was observed in the bioreactors, confirming that sulfate reduction was occuring (Figure 3-5). The precipitates were found settled at the bottom of the reactor, on the biofilm surfaces as well as fine solids in solution. The iron removal efficiencies obtained in this study confirm those of Viera *et al*. (2014) who found iron removal efficiencies around 88%, with 98% of zinc and 99% of copper removed from the AMD.



Figure 3-5: Shows the AMD/FTWW mix (A) before inoculation with SRB, (B) shows the contents of the reactors showing evidence of iron precipitation at the end of the batch operations.

In terms of sulfate removal efficiencies, sulfate removal rates of 19 mg/L/hr were observed in the first 96 hours with the bulk of the sulfate (51 %) removed within the first 200 hours. The rate of sulfate removal thereafter slowed down to 2 mg/L/hr, with a further 36 % sulfate being removed in the following 660 hours. The slow rates of sulfate removal after 200 hours might be due to the inhibitory effects of sulfide to the system. At the time of decommissioning, the residual sulfate concentration was 182.5 mg/L, a 99 % removal efficiency. There was evidence of hydrogen sulfide as a characteristic rotten egg smell was emitted from the reactors. Tests confirmed 120mg/L of hydrogen sulfide at the time of decommissioning of the reactors. These results agree with the findings of Sarti and Zaiat (2011), who obtained up to 99 % sulfate removal efficiency using butanol as the sole carbon source for sulfate reduction.

In the early stages of reactor operation, a rapid decline in sulfate and iron concentration was observed which could not be explained by sulfate reduction alone considering the low pH of

the reactor. Though the emission of a rotten egg smell confirmed the production of hydrogen sulfide at this stage, additional removal mechanism may have been responsible for aiding the remediation process. The control experiment showed limited amounts of iron and sulfate being absorbed on the charcoal (Figure A7 and A8 in appendix A), therefore charcoal was ruled out as playing a significant role in the remediation process, confirming Moodley *et al*., (2015) observations that charcoal did not contain any bioavailable carbon source for DSR and therefore does not play a significant role in the bioremediation process. Other possible mechanisms of iron and sulfate removal such as through adsorption on biofilm were not investigated; however, there is a strong possibility that adsorption on biofilm, could aid the remediation process. The oxidation of Fe(II) to Fe(III), followed by the hydrolysis of Fe(III) and precipitation of oxyhydroxides under anaerobic conditions has been credited as an important iron and sulfate removal mechanism (Genty *et al*., 2017).

There was a sharp increase in pH from 2.0 to 4.8 within the initial 48 hours. The generation of alkalinity through biological sulfate reduction and the removal of sulfate, organic acids, and hydrogen ions through adsorption on biofilm may explain the rapid increase in pH in the first 48 hours. Figure A6 (control) shows that charcoal did not significantly aid in the remediation process as pH was raised from pH 2.01 to pH 2.49 in the reactor with no biological activity. Acidophilic SRB are responsible for sulfate reduction at low pH (Tran *et al*., 2021). This allows the pH to increase to the point where most SRB would not be inhibited allowing more time for the SRB to acclimatise. The pH then gradually increased to a high of 7.6 after 1008 hours as SRB activity increased (Figure 3-4). Similar results were obtained by Neto *et al*. (2018) who reported pH increases to above 6.5 from an influent pH of 4.0. The increase in pH was attributed to alkalinity produced through dissimilatory sulfate metabolism by SRB. The sulfide and bicarbonate ions, formed during sulfate reduction and carbon source oxidation equilibrate into

a mixture of H_2S, HS^-, S^{2-}, CO_2, HCO_3^-, and CO_3^{2-}, which buffers the pH around neutral to slightly alkalinity (Kousi *et al*., 2015).

The rapidness of the processes occurring in the bioreactor is evidence that the simple organics, found in FTWW, can be easily metabolized by SRB, and can be used up quickly in the bioreactor. This means that a continuous supply of FTWW is required to sustain long-term treatment of AMD. The rapid processes occurring in the bioreactor also indicate a quicker treatment rate. Enough sulfide was generated to aid the removal of 99% of iron. These results show that the SRB were able to tolerate the initial low pH in the reactors and were able to attenuate pH to levels close to or above neutral (Neto *et al*., 2018). pH values above neutral detoxifies the reactor by reducing the amount of non-ionized sulfide, which is the toxic form of sulfide (Papiro *et al*., 2013).

3.4 Results from the Continuous Reactor.

The conversion of the batch reactor was initiated when the pH was above neutral (7.6), dissolved iron concentration was at 0.1 mg/L and sulfate concentration of less/equal to 182.5 mg/L. The concentration of COD at this stage was below the sensitivity of the equipment used (<15 mg/L) (Figure 3.3). Microbial growth was evidenced by the appearance of a biofilm around the charcoal and the appearance of a black iron sulfide precipitate. The FTWW/AMD feed had the same profile as the feed used in batch reactors. Inlet samples were frequently analysed for iron, sulfate and COD concentration as well as pH to determine if there was any microbial activity occurring in the reservoir. These parameters remained constant in the feed as well as in the control experiment throughout the treatment process. The effluent was also analysed for the same parameters with results showing a decrease in iron, sulfate and COD concentration as well as an increase in pH. The black precipitate of iron sulfide was an indicator

that biological sulfate reduction played a significant role in the changes that occurred in the bioreactor.

3.4.1. Removal of dissolved iron and sulfate.

The continuous reactor was able to lower the concentration of dissolved iron in the effluent to levels below 0.6 mg/L compared to an influent concentration of 965 mg/L (Figure 3-7) - a removal efficiency above 99 %. The sulfate concentration in the effluent was maintained below 500 mg/L (Figure 3-6), at a sulfate removal rate of 138 mg/L/hr. Though this study focused on the removal of only dissolved iron from AMD, Sato *et al* (2018) obtained similar trends in terms of Zn (83.6 %- 100 % removal efficiency), copper (99.1 % - 100 % removal efficiency) and cadmium (93.3 %- 100 % removal efficiency) using biological sulfate reduction. Glombitza *et al*., (2001) observed sulfate reduction rates of up to 132 mg SO_4^{2-}/h, with close to 100% metal removal in a fixed bed up flow reactor fed with methanol. The effluent pH of the bioreactor was recorded at an average 6.9 against an influent of 3.0.

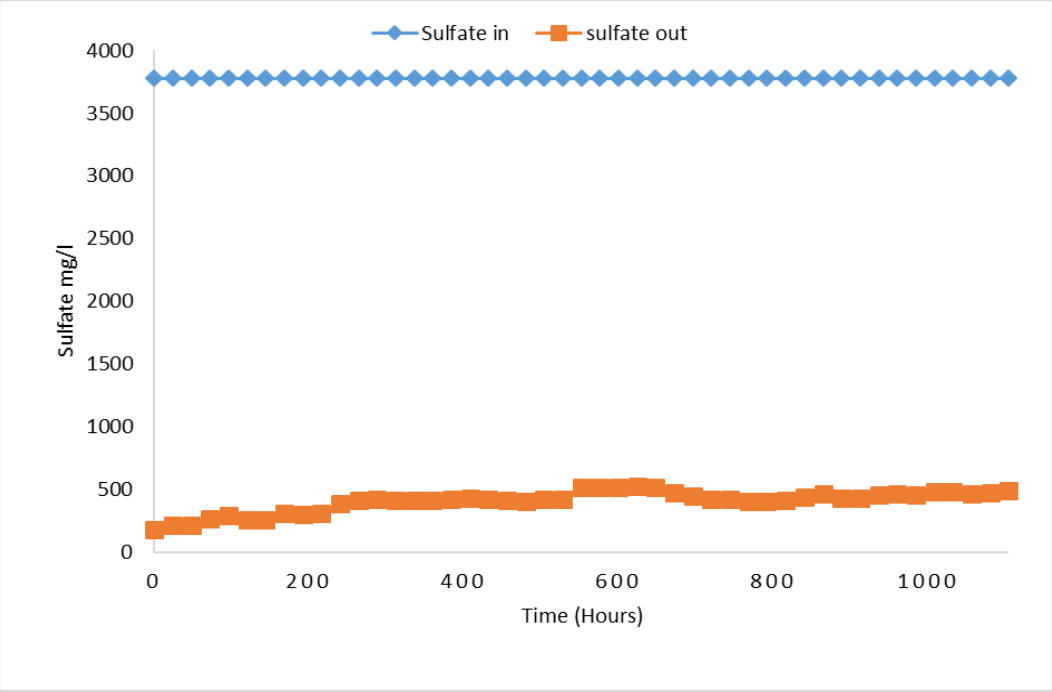


Figure 3-6: The removal of sulfate from AMD in a continuous sulfate reducing fixed bed reactor.

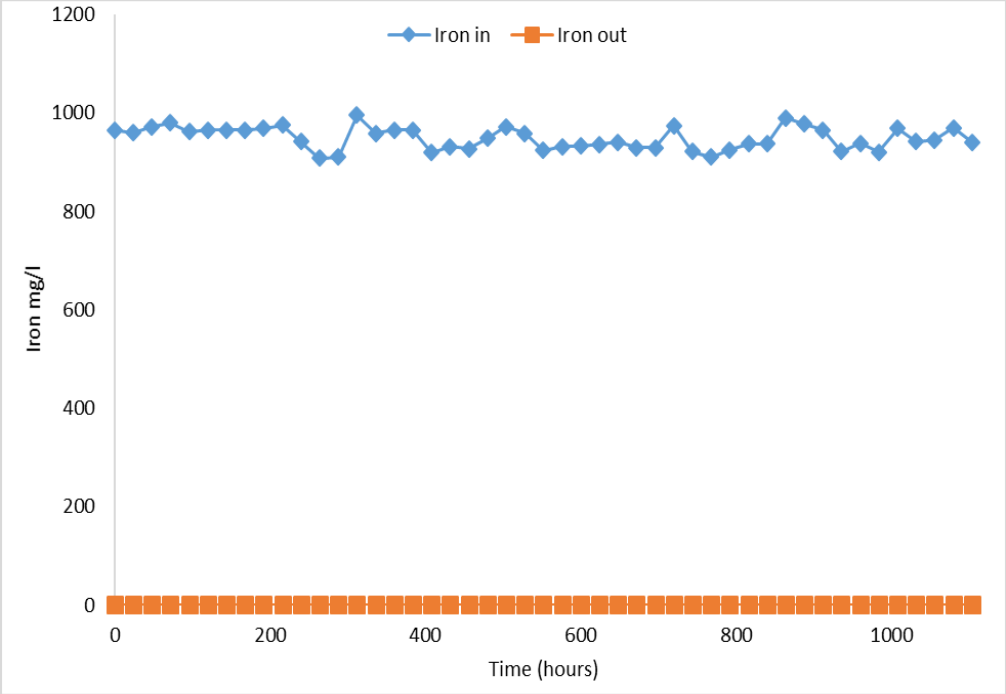


Figure 3-7: The removal of iron as iron sulfide in a continuous bioreactor treating AMD.

Although Sato *et al.*, (2018) attributed metal sulfide precipitation as the main mechanisms for metal removal in a sulfate reducing bioreactor, other metal removal mechanisms such as sorption onto biofilms, complexation and precipitation with other compounds have been acknowledged to contribute to the remediation process (Gunatilake, 2015; Dula and Duke, 2019). As metal sulfide precipitation is the main mechanism of metal removal, it is important that the system generate enough sulfide to precipitate all the dissolved metal ions. The findings by Sato *et al.*, (2018) show that this is possible; however, this study did not confirm if other dissolved metals like Zn, Cu, PB can be effectively removed from the bioreactor using biological sulfate reduction.

3.4.2 pH changes

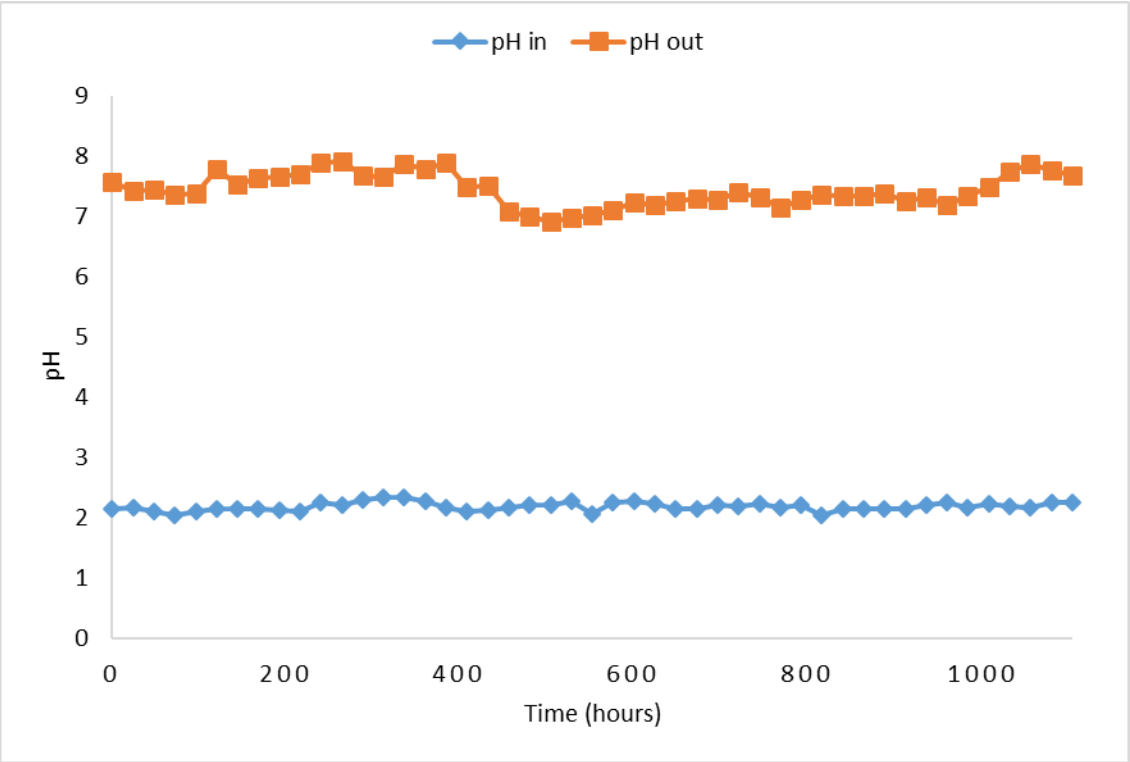


Figure 3-8: pH attenuation in a sulfate reducing batch bioreactor treating AMD.

The effluent pH remained above neutral throughout the operation period with the highest pH recorded at 8.0 from an influent pH of 2.0 (Figure 3-8). The pH increase can be attributed to alkalinity generation and proton consumption due to the metabolic activities of sulfate reducing bacteria, indicating that despite the low influent pH, SRB were able to work well under these conditions, removing acidity from the system. According to Brahmacharimayum *et al* (2019), most sulfidogenic bioreactors have been operated at pH ranges between pH 5 and pH 8 to allow SRB to survive. Many bioreactors fail below pH 4. This study demonstrated that a mixed culture of SRB could effectively treat AMD at pH values as low as pH 2.0. These results are supported by Elliot *et al* (1998) and Kolmert and Johnson (2001) who reported SRB activity at

pH below 3 using a mixed culture of SRB, showing that mixed cultures can withstand harsh environmental conditions compared to pure cultures (Brahmacharimayum 2019).

3.4.3 COD Removal.

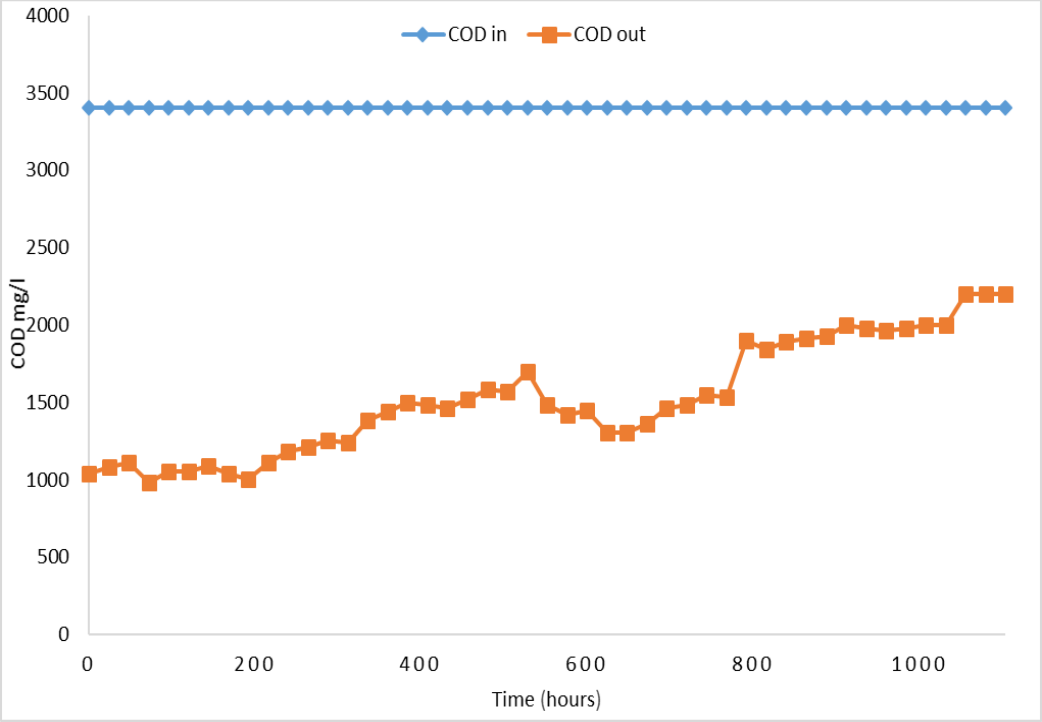


Figure 3-9: The removal of COD from a continuous bioreactor treating AMD.

Despite sulfate removal by the SRB, Sulfate and COD concentrations accumulated as their concentrations increased with time during the treatment process (Figures 3-6 and 3-9). The sulfate concentration steadily increased to levels slightly above 500 mg/L during the operational period decreasing the maximum sulfate removal efficiency to 86 % (Figure 3-6). The removal of the COD from the reactor slowed down with time in the continuous reactor leading to the accumulation of COD. The bioreactor initially maintained steady state for the first 200 hours, thereafter COD started to continuously accumulate (Figure 3-9), indicating reactor failure. At decommissioning of the reactors, COD levels in the effluent were at 2200 mg/L, showing a removal efficiency of 35 %.

These results suggest that either the residence time was not sufficient to remove most of the COD, or there was an inhibition of SRB and other microorganisms by the accumulation of hydrogen sulfide and metal sulfide precipitates. Though most of the hydrogen sulfide produced in the reactor was removed as metal sulfide precipitates, tests confirmed residual hydrogen sulfide concentration below 40mg/L which may have inhibitory effects on the activity of SRB and other resident bacteria (Paulo *et al*., 2015; Utgikar *et al*., 2002). Some bacteria species such as acetogens are acutely inhibited by low hydrogen sulfide (Barber and Stuckey, 2000). This may have negative impacts on the metabolism of acetate produced by incomplete sulfate reducers leading to the accumulation of acetate as well as the slight decrease in pH observed, as acetate is a weak acid. Chao-Hai *et al*. (2007) put the threshold concentration of free H_2S that causes irreversible process failure in bioreactors fed with acetate and propionate to be around 60 mg/L. The toxicity of sulfide in bioreactors is attributed to the un-dissociated H_2S since it can diffuse through the cell membrane. Once in the cell the H_2S reacts with the native proteins by forming sulfide or disulphide cross-links between polypeptide chains leading to denaturation of the proteins (Chen *et al*., 2008).

The concentration of sulfur species in the bioreactor depends on the prevailing environmental conditions in the reactor. The pH of the bioreactor is crucial to the speciation of sulfide (Hulshoff Pol *et al*., 1998). According to Neto *et al*., (2018), at a pH value of 6.5, 50% of sulfide species is present in the form of bisulfide (HS^-). This pH value is necessary for the precipitation of metals as sulfides. At lower pH however, the sulfide exists in the un-dissociated form and as the pH increases the H_2S dissociates into HS^- and S^{2-}. At pH of 8.5, the HS^- dissociates into S^{2-} form and becomes the predominant sole species above pH 10 (Figure 3-10) (Brahmacharimayum *et al*., 2019).

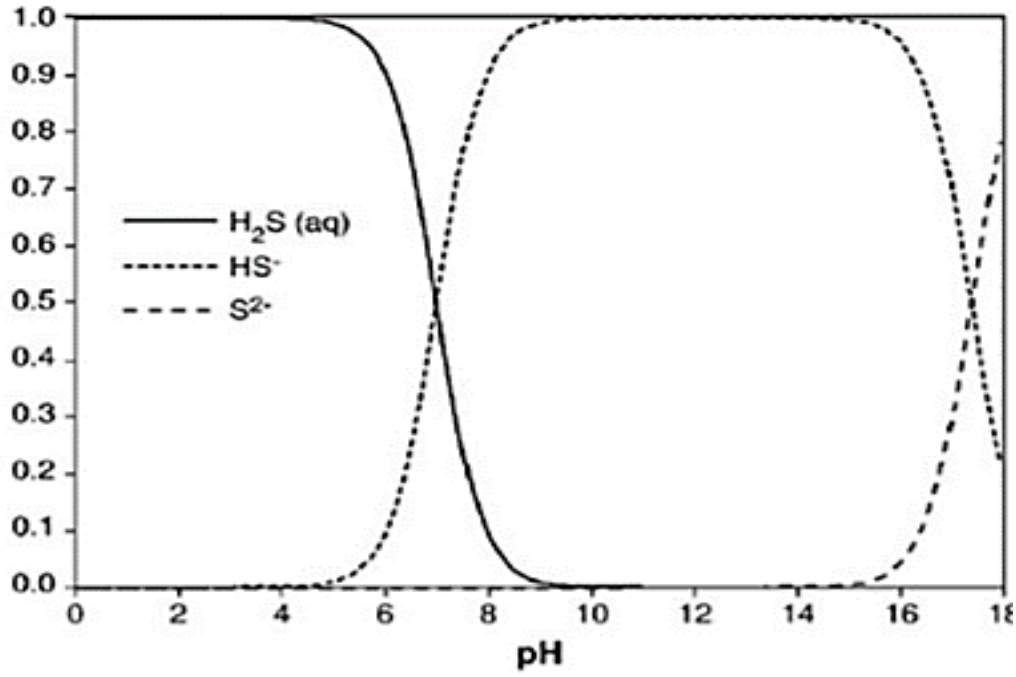


Figure 3-10: pH dependant sulfide speciation (Neto *et al*., 2018)

Heavy metal ions present in AMD such as iron, lead, copper, zinc, and mercury also contribute to bioreactor failure, as they may be toxic to the resident microbial community. Heavy metals inhibit enzymes by binding with organic substrates or by replacing naturally occurring metals in the prosthetic groups of enzymes (Singh, 2015).

Another factor that might contribute to the reduction in the efficiency of the bioreactor is the possible deposition of metal sulfide precipitates on the surface of the biofilm. This may have the effect of reducing the mass transfer of substances into the biofilm, decreasing the rate of consumption of both sulfate and COD. Utgikar *et al*. (2002) used scanning electron microscopy to prove the mechanism of inhibition by metal is external, acting as barriers to prevent access of reactants (sulfate and organic matter) to the necessary enzymes. It is thus necessary to immediately remove any metal precipitates formed in the bioreactor to maintain high performance levels (Utgikar *et al*., 2002).

3.5. Conclusion.

The objective of this study was to evaluate the feasibility of using FTWW as a carbon source in the treatment of AMD using biological sulfate reduction. The results show that FTWW contains enough COD to be used as the carbon source in the treatment of AMD using SRB. Considering the inability of SRB to utilize complex organic substrates, the utilization of FTWW and other industrial effluents containing simple organics should be prioritized in the search for organic substrates in the treatment of AMD.

The results also show the robustness of a mixed culture of SRB to withstand low pH and high dissolved metal concentrations. SRB activity continued as the pH increased implying that SRB can work over a wide range of pH. Though the AMD had a very low pH, there was no need to add chemical additives to increase the pH as the SRB were able to generate enough alkalinity to improve the pH. This removes or reduces the cost of chemicals thereby significantly reducing the cost of treatment. The batch reactor was more effective than the continuous reactor in improving the quality of the combined FTWW/AMD effluents, as it was able to remove almost all the dissolved iron, COD, and sulfate from the influent. This is due to the unlimited residence time as the batch reactors were operated until the minimal parameters were obtained. The residence time in the continuous reactor was enough to maintain effluent pH above 7 and sulfate concentration below the standard recommended for disposal, however, the COD levels remained very high to warrant a subsequent treatment step to remove the organic matter.

The precipitation of metals as sulfides offers some advantages over metal hydroxide precipitates as metal sulfides have lower solubility, are more stable, have better settling properties and can therefore be easily removed from the bioreactors. Metal sulfide precipitation also offers the opportunity for the selective removal/recovery of important minerals.

Chapter 4: The biological removal of hydrogen sulfide from the effluent of sulphate-reducing bioreactors.

Modified from an article published in the *Journal of Water Process Engineering*

Abstract.

The treatment of acid mine drainage (AMD) using dissimilatory sulfate reduction (DSR) leverages the ability of sulfate reducing bacteria (SRB) to reduce sulfate to sulfide using organic compounds as electron donors. Given that AMD contains relatively low levels of organic matter, the electron donors need to be supplied externally to facilitate the bioremediation process. In this, research Fischer Tropsch wastewater (FTWW) was recognized as a potential organic source because of its richness in organic acids and alcohols. The FTWW was combined with synthetic AMD at an initial COD/SO_4^{2-} ratio of 1.8 and was maintained in batch mode for 800 hours before being turned to a fed batch mode, with an aerobic reactor receiving the sulfide rich effluent from the sulfate-reducing reactor. The purpose of the aerobic reactor was to remove hydrogen sulfide through biological oxidation. The influent oxygen was regulated to avoid complete conversion of hydrogen sulfide to sulfate. The sulfate removal efficiency was 92 % and 99 % COD removal in the anaerobic sulfate-reducing reactor. The pH in the anaerobic reactor was raised from 2.0 to levels above neutral. The subsequent aerobic sulfur oxidising reactor was able to convert up to 96 % of the sulfide produced in the anaerobic sulfate reducing reactor to sulfur. An average 102 mg/L/d sulfur was produced.

Objectives:

1) Produce hydrogen sulfide from acid mine drainage (AMD) using biological sulfate reduction in a bench scale sulfate reducing bioreactor (SRBR).
2) To produce elemental sulfur through the incomplete biological oxidation of hydrogen sulfide produced in the SRBR.

4.1. Introduction.

Large amounts of hydrogen sulfide (H_2S) are generated during the treatment of acid mine drainage and other sulfate rich wastewaters using biological sulfate reduction (Yu *et al*., 2014). In the petroleum industry, the activity of SRB produces H_2S. The H_2S contaminate gas and stored oil. Hydrogen sulfide also precipitates iron as ferrous sulfide. The ferrous sulfide precipitate is responsible for the plugging of injection wells in the oil industry. Sulfide is also responsible for the corrosion of iron, metal, and concrete infrastructure (Cord-Ruwisch *et al*., 1987).

In addition to its unpleasant odour, H_2S can also cause adverse effects to health (Lee *et al*., 2000). Exposure to low concentrations of H_2S (15-20 ppm) may cause irritation of the mucous membranes, headaches, dizziness, and nausea. H_2S concentration between 200 and 300 ppm may lead to respiratory arrest causing unconsciousness and coma. Concentrations of H_2S more than 700 ppm may be fatal (Syed *et al*., 2006). In biogas, H_2S when burned forms sulfur dioxide - a very poisonous gas and an environmental hazard (Magomnang and Villanueva, 2014). All these factors necessitate the removal of hydrogen sulfide from anaerobic treatment plants (Midha *et al*., 2012; Lee *et al*., 2000).

In the treatment of AMD, hydrogen sulfide is usually removed through metal sulfide precipitation. In cases where the AMD does not have sufficient dissolved metal concentration to remove all the hydrogen sulfide as metal sulfide precipitates, alternative sulfide removal techniques should be applied to remove the hydrogen sulfide. The conventional physico-chemical processes for H_2S control have many drawbacks such as large energy requirements, high capital and operational costs, production of secondary wastes, need to replace poisoned catalysts, contaminated reagents, and corroded reaction vessels (Mahmood *et al*., 2007; Syed *et al*., 2006).

In this investigation Sulfur Oxidizing Bacteria (SOB) were used to oxidise the sulfide rich effluent from the sulfate reducing bioreactor. SOB of the genera *Thiobacillus, Sulfolobus, Beggiatoa,* and *Acidothiobacillus* can use reduced inorganic sulfur compounds as their electron donors and carbon dioxide as carbon source (Mahmood *et al*., 2007; Syed *et al*., 2006). The two most important bioconversions in an aerobic sulfide oxidising bioreactor are shown in Equations 4.1 and Equation 4.2 (Mahmood *et al.,* 2007):

$2HS^- + O_2 \rightarrow 2S^0 + 2OH^-$ $G^{0'}$=-129.50kJ/ ($molHS^-$) Equation 4.1

$2HS^- + 4O_2 \rightarrow 2SO_4^{2-} + 2H^+$ $G^{0'}$=-772.43 kJ/ ($molHS^-$) Equation 4.2

The presented $G^{0'}$ values (STP) are given for physiological conditions at pH 7. Under oxygen limiting conditions, i.e., at oxygen concentrations below 0.1 mg/L, sulfur is the major product of the sulfide oxidation (Equation 4.1), whereas sulfate is formed under sulfide limiting conditions (Equation 4.2) (Mahmood *et al*., 2007; Dumont, 2015; Bierre and Fullerton, 2015; Janssen *et al*., 1995).

Some SOB have the ability to grow under low pH. According to Syed *et al*., (2006), Many *Thiobacillus* species can develop in conditions of low pH (1-6). However, many SOB have an optimum pH between 6 and 8. pH values in the alkaline range allow more hydrogen sulfide to dissolve and consequently be more available to SOB (Syed *et al*., 2006; Rattanapan and Ounsaneha, 2012). In terms of temperature, the mesophilic range with optimum between 35^{o}C and 37^{o}C is often reported. In addition, nutrients such as nitrogen, phosphorus and trace elements are required for growth.

In this study, the conversion of sulfide to sulfur (Equation 4.1) was of utmost importance because sulfur is of economic importance as it can be used in the manufacture of fertilizers and other industrial products. The removal of sulfide as sulfur is also important in that unlike the

complete oxidation of sulfide to sulfate, the production of sulfur does not lead to the acidification of the bioreactors.

4.2. Materials and Methods.

4.2.1. Sulfate Reducing Bioreactors.

The reactor setup and inoculation technique described in chapter 3 was utilized. There was no selection of a pure culture; therefore, a mixed culture of bacteria was used in the reactors. The use of a mixed culture for the treatment of AMD may have advantages over using a pure culture, as mixed cultures may be able to adjust to different operational conditions. The high diversity of bacteria in a mixed culture might also mean that the bioreactor may be able to remove a large variety of contaminants.

The initial concentration of sulfate in the bioreactors was 3000 mg/L, which was initially made up of 307 mg/L introduced as H_2SO_4 and the remainder 2693 mg/L was introduced as Na_2SO_4. The stock solution of the sFTWW had a concentration of 20 945 mg COD/L made from alcohols and organic acids, mainly methanol, ethanol, propanol, butanol, propionic and acetic acid. The composition of the sFTWW was adopted from Majone *et al*. (2010) as reported in the previous chapter (table 3-1). The sFTWW was diluted to provide a COD/SO_4^{2-} ratio of 1.8.

The SRB was initially grown in an enrichment media containing growth factors which consisted of 0.5 g/L KH_2PO_4, 1 g/L NH_4CL, 1 g/L $CaSO_4$, 3.5 g/L sodium lactate, 1 g/L yeast extract and 0.1 g/L ascorbic acid. This enrichment media was subsequently replaced with the AMD/FTWW mixture when evidence of microbial growth on the charcoal was satisfactory (biofilm growth). The SRB was allowed to acclimatise during preliminary investigations that were done to optimize the bioreactor conditions. The bioreactors were operated at ambient temperature. A control experiment was set up parallel to the main experiment, with the same COD/sulfate ratio and charcoal but without the SRB inoculum.

The batch reactor was run for 840 hours before it was changed to a fed batch reactor. The initiation of the fed batch reactor was done when the sulfate levels were at 28 mg/L, COD was at 96 mg/L and the pH was above neutral at 7.5. 50 mL of the combined influent of AMD/FTWW at pH 2, 5700 mg/L COD and 4150 mg/L sulfate concentration (a COD/sulfate ratio of 1.4) was pulsed into the anaerobic sulfate reducing bioreactor each day. The sulfide rich effluent from the anaerobic reactor was then collected into the aerobic sulfur oxidising reactor using gravity (Figure 4-1). The aerobic reactor was initially allowed to run without additional oxygen as the volume rose. This was to allow the oxygen contained in the reactor to be depleted due to consumption by SOB. The pulsing of oxygen was only initiated when the reactor was 75% full. Samples were collected and analysed for COD, sulfate, sulfide and pH.

4.2.2. Sulfur Oxidising Bioreactors.

The aerobic sulfur oxidising bioreactors were connected to the sulfate reducing reactors such that the influent of the sulfur oxidising bioreactor was from the sulfate reducing reactor. The reactors were of similar make and structure to the anaerobic reactors (Figure 4-1). Charcoal was used as the immobilizing matrix in the anaerobic sulfate reducing bioreactor while polyurethane (obtained from bedding material) was used as the immobilising matrix in the sulfur oxidising bioreactor to allow the mass movement of O_2 and H_2S. An air inlet and influent inlet were placed near the bottom of the sulfur oxidising bioreactor. The effluent exited the reactors at the top. The SOB was obtained from constructed wetlands on the roof of the Richard Ward building at the University of the Witwatersrand.

The sulfur oxidising bacteria were initially grown in liquid medium containing: 5.1 g/L $Na_2S_2O_3$, 2.0 g/L KH_2PO_4, 0.4 g/L NH_4CL, 0.2 g/L $MgCl_2.7H_2O$, and 0.01 g/L $FeSO4.7H_2O$. The pH of the solution was adjusted to 7 using sodium hydroxide. The media was regularly removed when the pH fell to levels below 4, which was presumed to be an indication of

thiosulfate oxidation to sulfate. Fresh media to replenish the necessary trace elements replaced the media. Upon the formation of biofilm on the polyurethane, the media was replaced by a semi-continuous flow of effluent from the sulfate reducing bioreactor. Micro-aerobic conditions were obtained by pulsing air regularly using a labotec air pump.

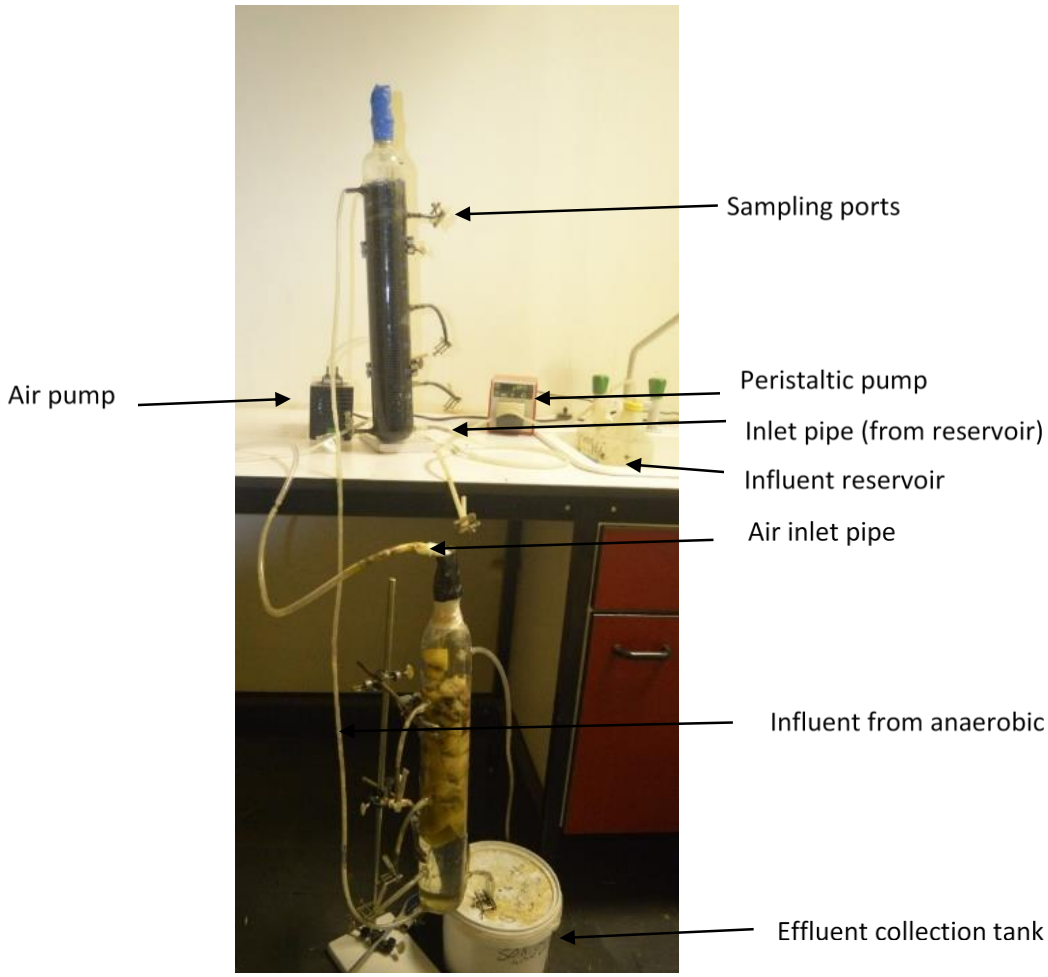


Figure 4-1: Showing the reactor set up. On top is the anaerobic reactor producing hydrogen sulfide for the aerobic bioreactor (bottom).

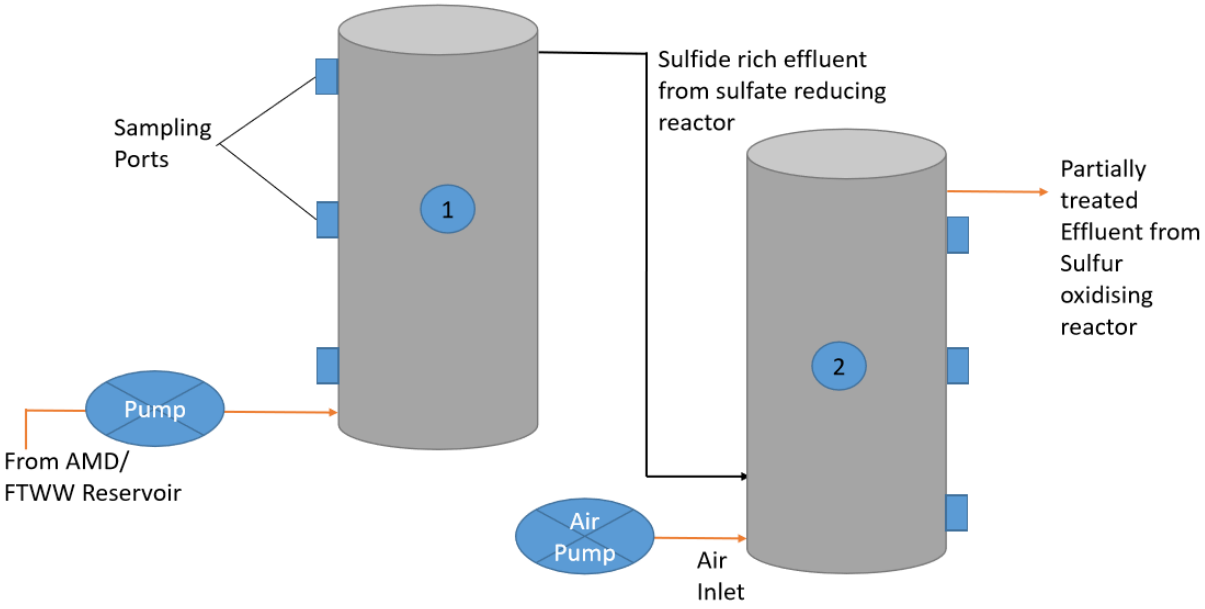


Figure 4-2: Flow diagram of the sulfur (2) oxidising bioreactor connected in series to a sulfate reducing bioreactor (1).

4.2.3. Chemical Analysis.

All chemicals were of analytical grade and supplied by Merck.

4.2.3.1 Alkalinity.

Total alkalinity tests were done on unfiltered samples immediately after sampling, by titrating the bioreactor sample with a 0.020N solution of sulfuric acid (Buret titration, method 8221) using an easy sense pH probe and easy sense pH software to an end point of pH 4.3. At this pH all the alkaline components are used up. The multiplier previously reported in section 3.2.1 was utilized.

4.2.3.2 Sulfate and sulfide.

The sulfate and sulfide evaluations were conducted using a PF-12 photometer (Macherey-Nagel, Germany). All the reagents' kits for determinations were supplied by Macherey-Nagel. The procedures followed were as per the supplier's manual. The kits used for sulfate and sulfide

determinations were Viscolor Eco test 592 and Viscolor Eco test 594 respectively. The PF-12 was factory calibrated.

4.2.3.3. COD analysis.

COD was determined photometrically using the PF-12 photometer following the digestion of the samples in a Nanocolor Vario mini compact heating block. The test is based on the photometric determination of the decrease in chromate concentration after the oxidation of the wastewater sample with a strong oxidising agent such as potassium dichromate. The COD analysis was conducted on unfiltered samples. The procedures followed were as per supplier's manual. The kits used were Viscolor eco test 0-26 for COD range 15 mg/l-160 mg/L and Viscolor eco test 0-28 for COD ranges from 1000 mg/L to 15000 mg/L. Appropriate dilutions were done to samples whose COD concentration range was between 160 mg/L and 1000 mg/L.

4.2.3.4. pH determination.

pH was determined using the Data Harvest easy sense pH electrode and easy sense software.

4.3 Results and discussion.

4.3.1. Batch Results.

The sulfide odour was detected 24 hours after commissioning the reactor and was present throughout the operation of the reactor. There was a sharp decrease in sulfate concentration from 3000 mg/L to 612 mg/L within the first 48 hours, a sulfate removal rate of 50 mg/L/hr. Thereafter, sulfate concentration gradually decreased to 25 mg/L after 840 hours, a total 99 % sulfate removal efficiency.

The rapid decline in sulfate concentration in the first 24 hours was accompanied by a rapid increase in sulfide concentration (Figure 4.3), indicating that sulfate reduction was occurring

within the first 48 hours of reactor operation. These observations compare well with Kolmert and Johnson (2001), who observed instant sulfate removal in an ethanol fed bioreactor with pH 2.5 following acclimation at low pH. Kolmert and Johnson (2001), observed that a pH below 3.0 caused loss of sulfate reduction. They suggested an acidophilic culture was responsible for SRB activity.

The sulfate removal was accompanied by a rapid decrease in COD concentration from 5450 mg/L to 1974 mg/L within the first 168 hours of bioreactor operation. Thereafter the COD concentration gradually decreased to 97 mg/L after 840 hours, a 99 % COD removal efficiency (Figure 4-3). As explained in the previous chapter, SRB were able to consume both COD and sulfate through dissimilatory sulfate reduction. However, due to the high COD/SO_4^{2-} ratio, some of the COD was potentially removed through methanogenesis and other routes since the available sulfate would not support complete removal of COD. The control experiment showed 10% of COD being removed through adsorption to charcoal (Figure A9 in appendix A), however charcoal did not significantly remove sulfate and iron from the AMD (Figures A6-A8 in appendix A). The process was instant due to the availability of readily usable organic sources and that the SRB was given enough time to acclimatise. The sulfate reduction process produced carbonate alkalinity, which helped in neutralising the acidity in AMD improving the pH of the effluent (Figure 4-4). The removal efficiencies (RE) were calculated using the formula:

$$RE = (1 - C_{(out)}/C_{(in)}) \text{ X } 100$$

Where $C_{(in)}$ is influent concentration and $C_{(out)}$ is effluent concentration.

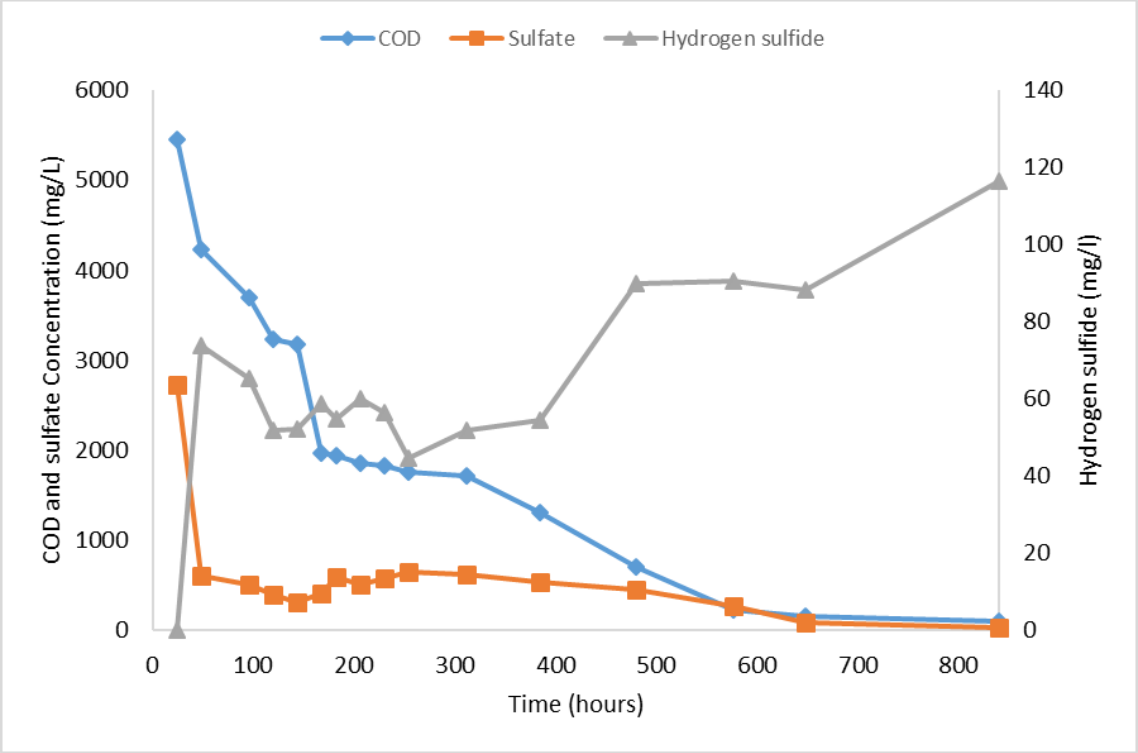


Figure 4-3: Hydrogen sulfide production in a sulfate reducing batch reactor fed with simulated AMD and FTWW.

Although there was no evidence of alkalinity generation within the first 100 hrs of operation and below pH 4, the alkalinity gradually increased to concentrations above 3920 mg/L CaCO3 alkalinity, before remaining at a constant level (Figure 4-4). From the results shown in Figure 4-4, enough alkalinity was generated to increase the pH from 2.0 to 7.5. The pH increased rapidly from 2.0 to 6.7 within the first 182 hours, and then gradually increased, reaching a peak at pH 7.5. The rapid increase in pH corresponds with high COD and sulfate consumption in the bioreactor.

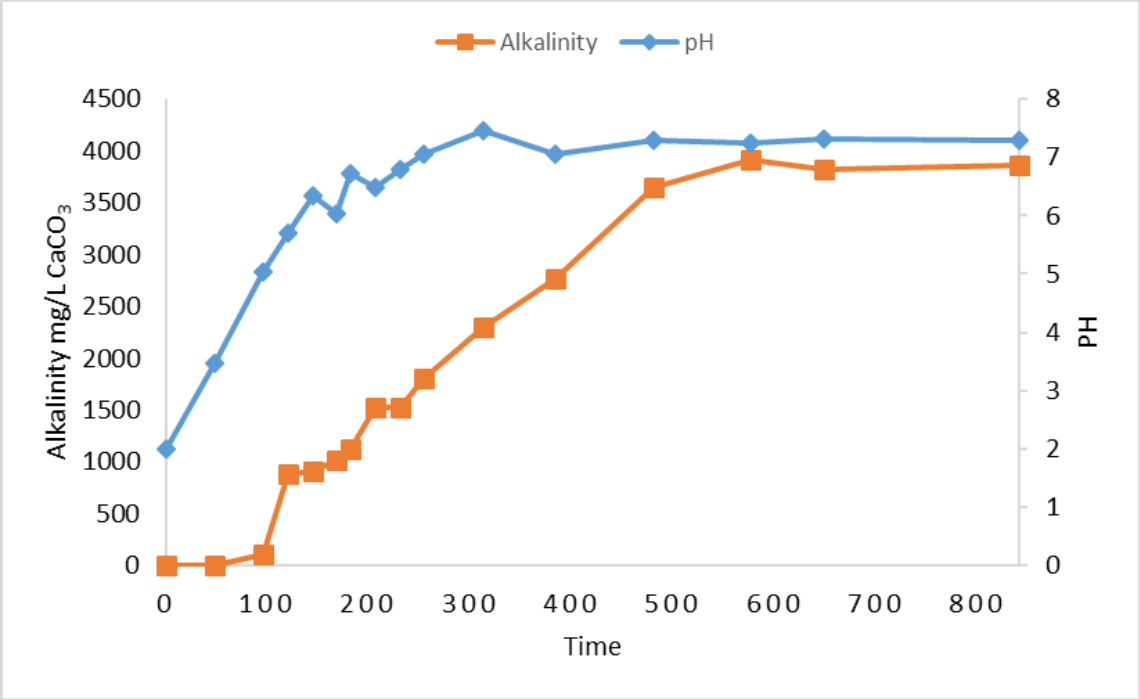


Figure 4-4: Alkalinity and pH changes in a sulfidogenic batch bioreactor for the treatment of AMD

Figure 4-5 shows the relationship between pH and alkalinity generation in sulfate reducing batch reactor, where increase in alkalinity is accompanied by a gradual increase in the pH of the wastewater up to pH 7.5 and further increase in alkalinity does not lead to further pH increase. This suggests that the wastewater had acquired enough buffering capacity to resist any pH changes as the alkalinity increased.

The increase in pH was attributed to alkalinity generation through microbial activity and possibly through the removal of sulfate and hydrogen ions by adsorption to the bed matrix and biofilm (Figure A6 and A7 appendix A), as there were no neutralising agents added to the reactors. This is an indication that the SRB used was able to work at extremely low pH values in the bioreactor. This contrasts studies done by Kaksonen *et al*., (2007) who reported inhibition of SRB at low pH and high metal concentrations. The tolerance of low pH by SRB in this study can be attributed either to the resilience of immobilised cells or to the wide variety of SRB strains and the availability of several substrates for sulfate reduction.

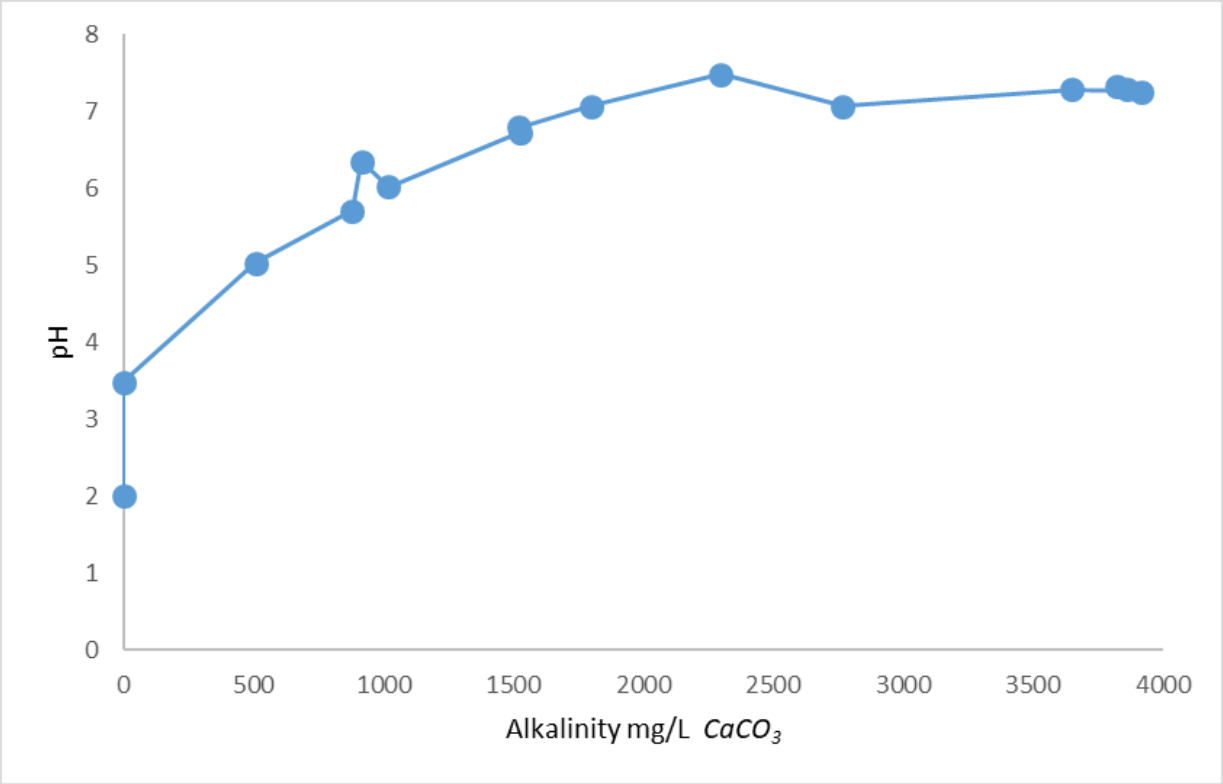


Figure 4-5: Relationship between pH and alkalinity generation in a sulfidogenic batch reactor treating AMD.

The H_2S produced was difficult to quantify. Hydrogen sulfide exists in different forms which may be aqueous or gaseous depending on the pH of the bulk liquid (Loma *et al*., 2016; Neto *et al*., 2018), with 50% of the sulfide species existing as HS^- at pH 6.5 (Neto *et al*., 2018). According to Neto *et al.* (2018), this pH is conducive for metal precipitation as sulfides or hydroxide. Marchan and Plumb (2005), reported the production of large quantities of sulfide using biological sulfate reduction, but a small amount of the sulfide evolved as hydrogen gas due to elevated pH. At low pH, the solubility of H_2S decreases; thus, H_2S is driven out of the liquid and remains in the gaseous form (Rachbauer *et al*., 2015). Hence, the rotten odour observed during the operational period was due to a fraction of H_2S being released from the reactors in gaseous form. This made quantifying of the total sulfide species produced in the reactor difficult especially at low pH.

Therefore, the method used to quantify H_2S measured only dissolved hydrogen sulfide and yielded a maximum concentration of 116 mg/L (Figure 4-3). These results were support Nagpal *et al*. (2000) who obtained a maximum sulfide production of 100 mg/L with a sulfate influent concentration above 2500 mg/L, showing no correlation with the amount of sulfate removed from the reactor. In a similar investigation, Subtil *et al*. (2012) got similar variations in the amounts of sulfide produced in comparison to the total amount of sulfate reduced and attributed this to the fact that sulfide may exist in different forms such as liquid and gas. Therefore, the measured sulfide cannot account for the total sulfide produced. At a COD/SO_4^{2-} ratio of 0.67, Velasco *et al*. (2008) obtained a maximum dissolved sulfide concentration of 140 mg/L using ethanol as the sole carbon source. The maximum dissolved H_2S increased as the COD/SO_4^{2-} ratio was increased to 2.5.

This study utilized a two-step reactor configuration where sulfate reduction and sulfur oxidation occurred in different bioreactors. Sulfur formation was seen to occur in the anaerobic bioreactor as some yellow solid substance started to appear in the bioreactor after 30 days of operation (Figure 4-6). The formation of sulfur in the anaerobic reactor suggests the occurrence of some micro-aerophilic pockets in the reactor allowing for the oxidation of the produced hydrogen sulfide to elemental sulfur. According to Brahmcharimayum *et al*., (2019), some sulfur oxidising bacteria such as *Thiomicrospina sp.* and *Thiobacillus sp*. can perform sulfide oxidation even in an anaerobic sludge digester depending on the oxygen availability. Micro-aerobic conditions can also be created in the headspace of anaerobic bioreactors to allow for the oxidation of sulfide to sulfur. The quantity of oxygen should not be inhibitory to the anaerobic processes in the bioreactor (Brahmcharimayum *et al*, 2019). The formation of sulfur in the anaerobic reactor and the loss of hydrogen sulfide to the environment could have contributed to the low amounts of sulfide in the effluent of the sulfate reducing bioreactor.

Table 4-1 shows the effluent characteristics of the batch reactor before it was turned into a fed batch reactor. The results show that the batch reactor was able to remove both sulfate and COD from the wastewater through sulfate reduction, in the process generating enough alkalinity to improve the pH of the effluent.

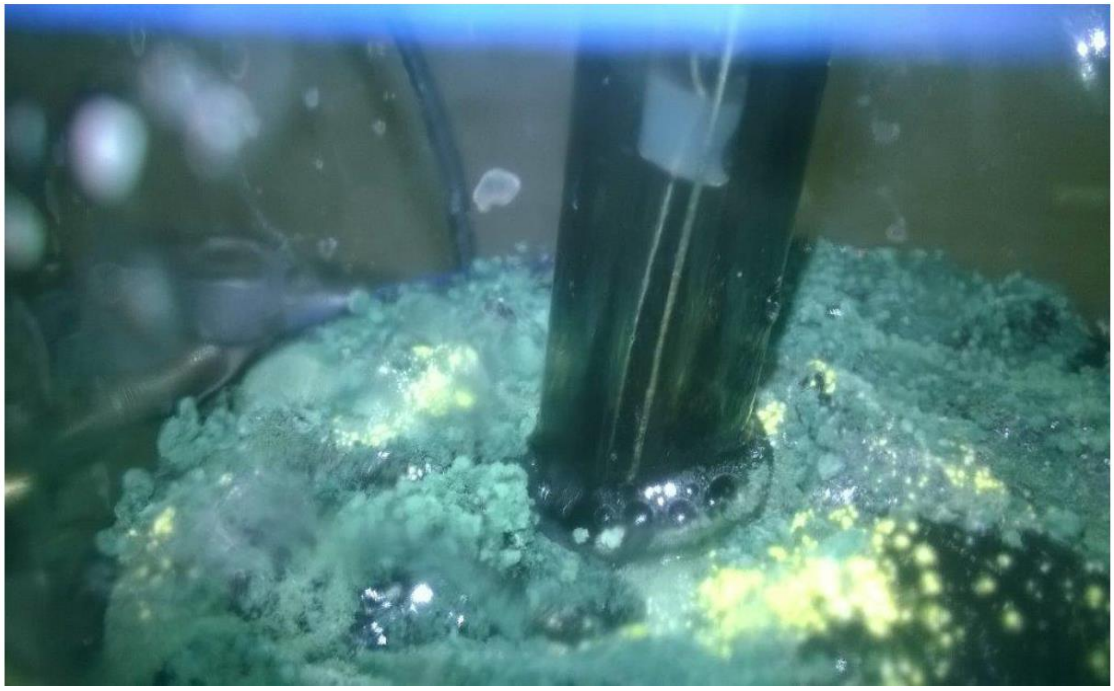


Figure 4-6*:* Image of the surface of the anaerobic bioreactor after 30 days of operation showing microbial growth on the surface. The yellow colouring might be evidence of sulfur production.

Table 4-1: Effluent characteristics of the batch reactor after 840 hours of operation.

Determinant	**Units**	**Discharge**
Sulfate	mg/L	28
COD	mg/L	96
Hydrogen sulfide	mg/L	116
Alkalinity	mg/L $CaCO_3$	3920
pH		7.45

4.3.2. Fed batch results

Upon reaching the discharge concentrations in Table 4-1, the batch reactor was turned into a two-stage fed batch reactor with an aerobic sulfur oxidising reactor connected to the sulfate reducing bioreactor.

In the fed batch reactor, effluent sulfate concentration was maintained at levels within the recommended range (250-500 mg/L) against a sulfate influent concentration of 4150 mg/L (Figure 4-7). The sulfate removal efficiency was determined to be 92.6 % in the fed batch reactor and was lower than the 99 % obtained in the batch reactor due to less residence time. Similar sulfate removal results were obtained using ethanol (Nagpal *et al*., (2000), above 95 % sulfate removal; Buccambuso *et al*., (2007), greater than 95%; Velasco *et al*., (2008), 94% sulfate removal); Butanol (Sarti and Zaiat, (2011), 99% sulfate removal); Propionate (Greben *et al*., (2004), 78 % sulfate removal) and acetate (Greben *et al*., 2004, 55.5% sulfate removal). Guo *et al*., (2019), reported sodium acetate to be the optimal substrate to achieve the highest sulfide production rate, followed by ethanol, methanol, glycerol, pyruvic acid, acetic acid, N-propanol, N-butanol, lactic acid, sodium lactate, propionic acid, and sodium propionate in that order. Other low molecular weight organic acids and alcohols have also been used as sole electron providers in the removal of sulfate with satisfactory removal efficiencies being obtained.

The near complete removal of COD (Figure 4-7) in the sulfate reducing bioreactor was not expected considering that the COD/SO_4^{2-} ratio of 1.4 which was used in the fed batch reactor was above the stoichiometric requirement for the total removal of COD from a bioreactor treating sulfate rich wastewater using biological sulfate reduction. This contrasts Velasco *et al*. (2008) who reported acetate accumulation at high COD/SO_4^{2-} ratios because of sulfate limiting

conditions. Most studies suggest a COD/SO_4^{2-} ratio of 0.67 is sufficient for the purpose of complete organic matter removal (Kaksonen *et al*., 2004; Vela *et al*., 2002; Lens *et al*., 1998).

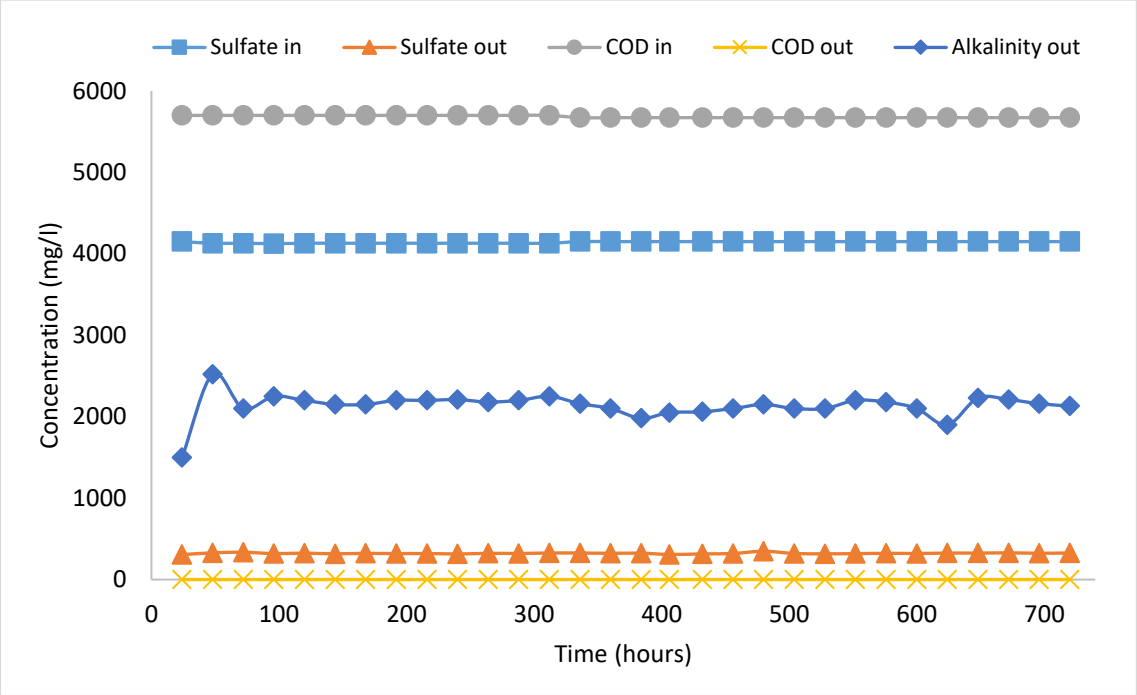


Figure 4-7: Changes in COD, Sulfate and alkalinity in a fed batch bioreactor fed with simulated AMD and FTWW

The complete removal of COD suggests the removal of COD by other bacteria such as methanogens, acetogens and Fe(III) reducing bacteria which compete, with SRB for substrates in the FTWW. As the COD/SO_4^{2-} ratio shifts due to consumption, methanogens may dominate SRB leading to the exhaustion of COD before complete sulfate removal. Lens *et al*. (1998) reported similar findings and suggested that complete COD removal from wastewater with COD/SO_4^{2-} ratio of above 0.67 also requires methanogenic COD digestion. The low H_2S concentration may not have been inhibitory to methanogens thus allowing them to proliferate in the reactors. Choi and Rim (1991) observed that at higher COD/SO_4^{2-} ratios, methanogenic archaea tend to compete with SRB for hydrogen and acetate that is produced in the anaerobic reactor because of the incomplete oxidation of ethanol to CO_2. The pH in the sulfidogenic bioreactor was maintained above neutral against an influent pH of 2.0 (Figure 4-8). pH

improvement was because of alkalinity production by SRB. The high alkalinity in the effluent of the bioreactor can be recycled back to neutralize and improve the pH of the influent. The bioreactor was able to maintain the pH within the neutral range (pH 7-8) because the sulfide and bicarbonate system generated through sulfate reduction buffers the pH of the solution.

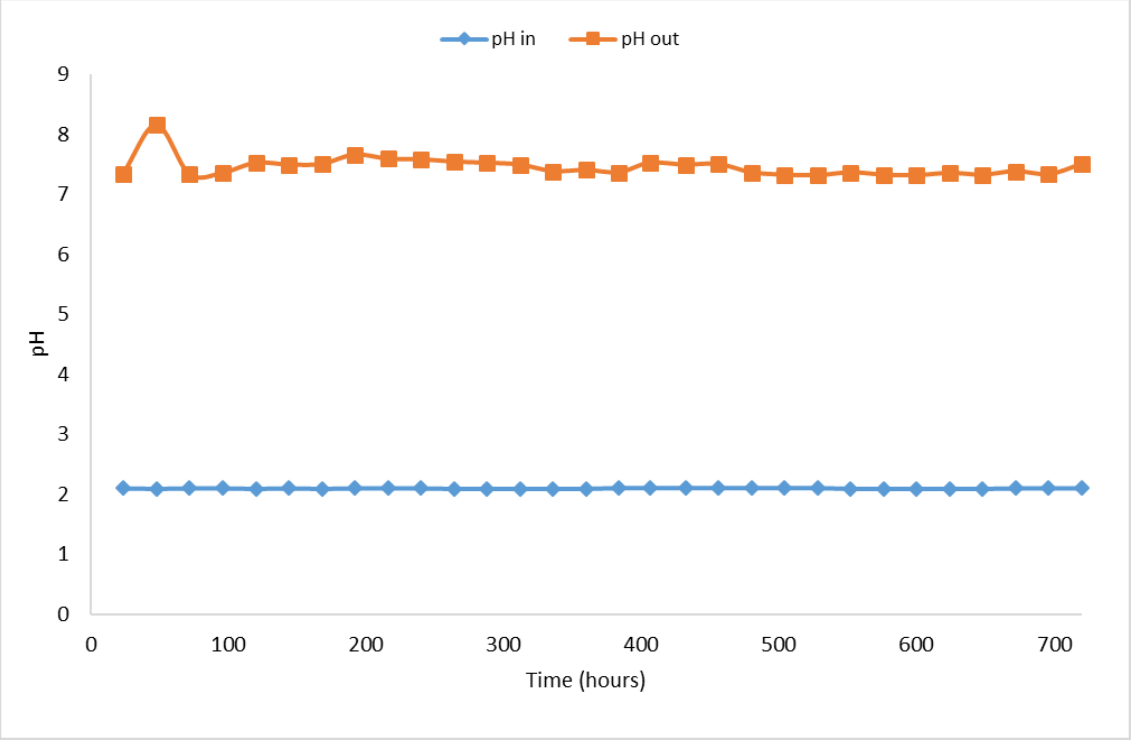


Figure 4-8: pH changes in a sulfate reducing bioreactor fed with simulated AMD and FTWW.

4.3.3 Removal of H_2S in the sulfur oxidising bioreactor

In the sulfur oxidising bioreactor, the levels of hydrogen sulfide were significantly lowered with up to 96.8% sulfide being converted to sulfur (Figure 4-10) and a very small amount (<5%) of sulfide being converted to sulfate (Figure 4-9). The sulfate levels in the bioreactor effluent remained below the limit (600mg/L) for disposal. A small amount of dissolved H_2S was detected in the effluent of the sulfur oxidizing bioreactor, meaning some of the influent sulfide was not oxidized to either sulfur or sulfate.

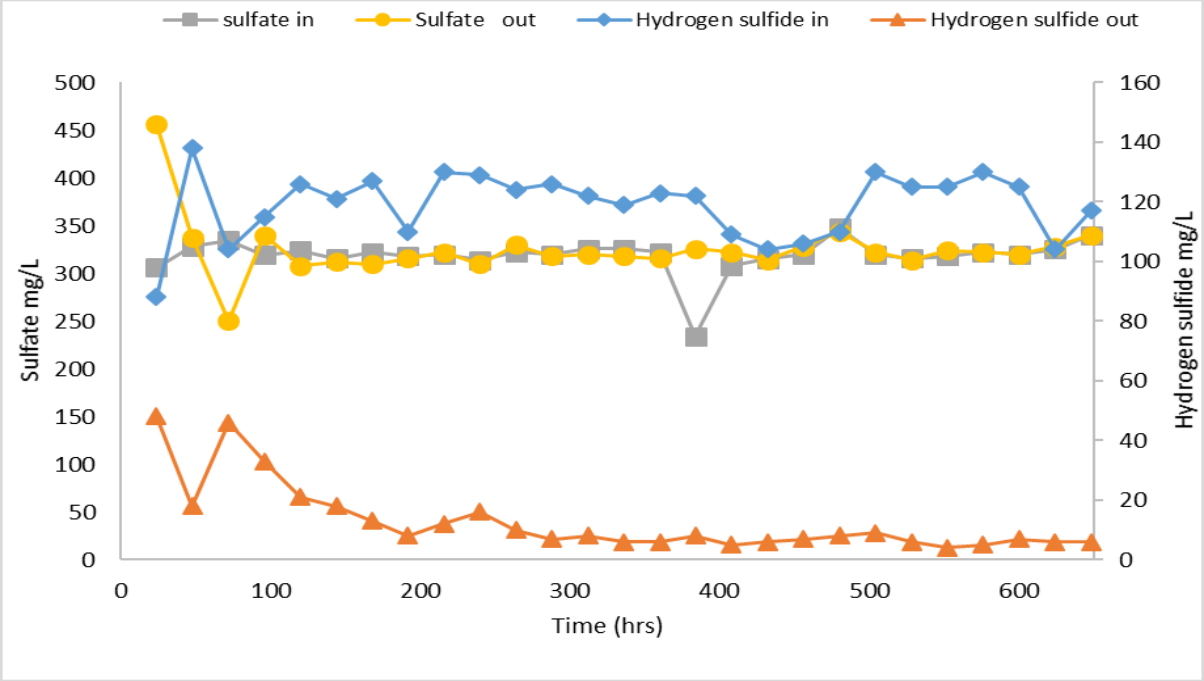


Figure 4-9: Removal of hydrogen sulfide from a sulfur oxidising bioreactor fed with effluent from the sulfate reducing bioreactor.

The residual H_2S in the effluent of the sulfur oxidising reactor suggests that the concentration of oxygen was too limiting to convert all the hydrogen sulfide to sulfur or sulfate. The residual sulfide concentration however was too low to warrant a further sulfide removal step. The quantity of the other species of sulfur such as polysulfides and thiosulfides were not determined, though de Rink *et al*., (2019) opined that their formation could not be prevented. This might have led to the theoretical quantity of the sulfur yield being inflated. In an investigation similar to this, Molwantwa and Rose (2013) reported 88% sulfide removal through biological oxidation of sulfide to sulfur and polysulfides, with 66% sulfur recovery. De Rink *et al*., (2019) used chemolithoautotrophic haloaliphilic SOB to oxidise HS^- to S_8 particles in oxygen limiting conditions. They obtained 80-90 mol % conversion to S_8, with some HS^- being converted to $S_2O_3^{2-}$ and SO_4^{2-}, corroborating the results of this investigation which showed the possibility of sulfur generation from the biological oxidation of sulfide. The removal of sulfide as sulfur is preferred as it removes odours, the corrosive potential of the

effluent and above all prevents the complete oxidation of sulfide to sulfate. Sulfur is a non-corrosive solid, which can form precipitates, which can easily be removed from the bioreactors; however, the packing matrix (polyurethane) used in this study may trap some of the sulfur particles in the pores preventing maximum sulfur recovery. This drawback maybe overcome by using suspended growth bioreactors that do not have packing material (Zytoon *et al*., 2014).

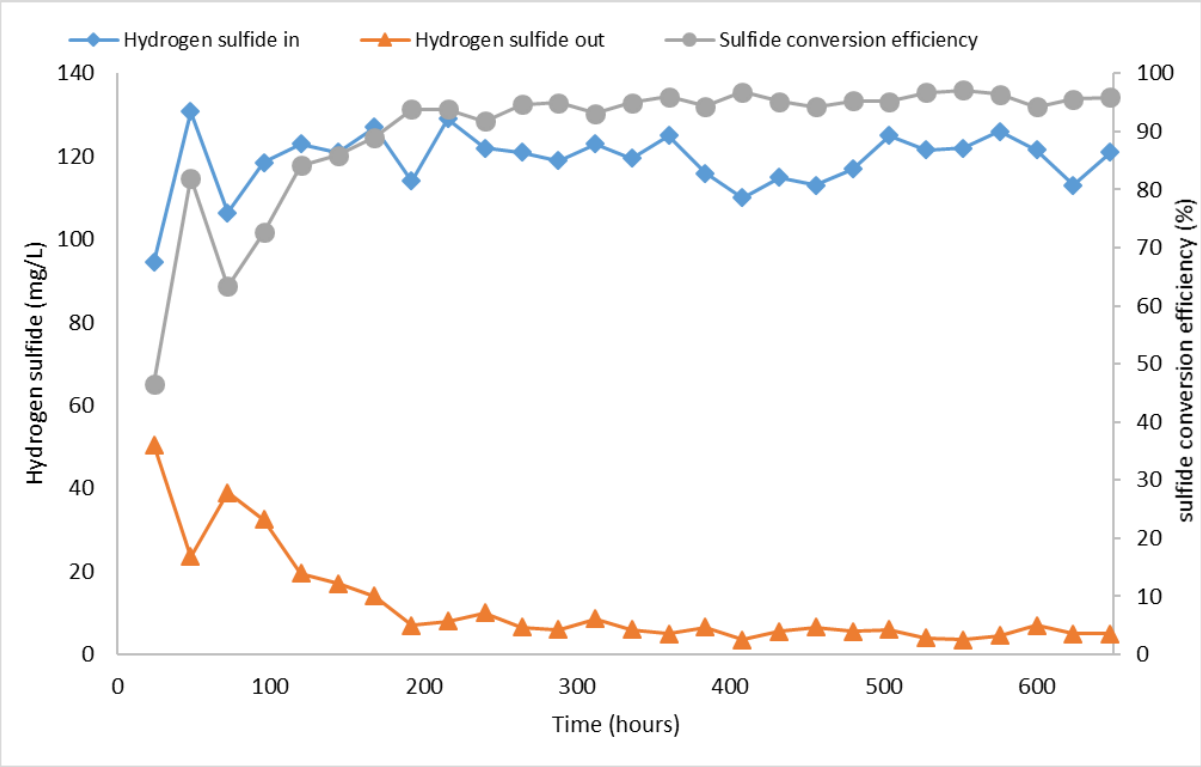


Figure 4-10: Sulfide conversion efficiency in the sulfur oxidising bioreactor

4.3.3.1. Sulfur production and mass balance.

Initially the production of sulfur in the sulfide oxidising reactor was slow as most of the sulfide was converted to sulfate due to excess oxygen in the bioreactor. Once the residual oxygen was depleted, controlled amounts of oxygen was pulsed into the reactors, leading to oxygen limiting conditions. Under oxygen limiting conditions sulfur was the primary product. Janssen (1996) determined that at molar $(O_2/S_2)_{consumption}$ ratio between 0.5 and 1.0, thiosulfate and sulfur are formed, while at molar ratios above 1.0, sulfate is produced. Sulfur production is at its maximum at molar $(O_2/S_2)_{consumption}$ ratio between 0.6 and 1.0. In this study, at the end of

reactor operation (Figures 4-11 and Figure 4-12), an average 99.4 mg/day of sulfur was produced in the bioreactor.

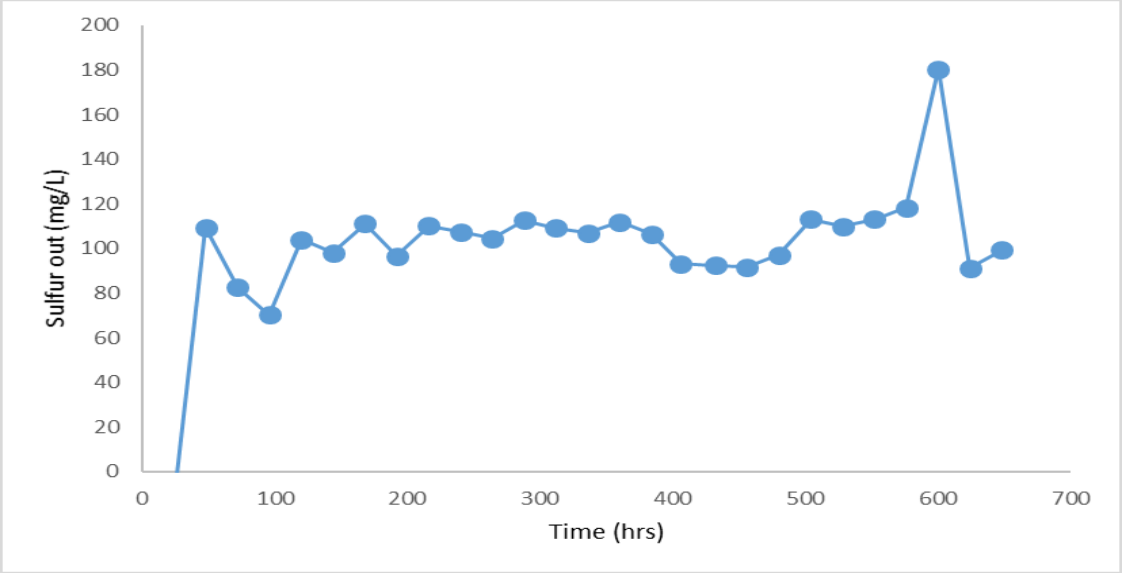


Figure 4-11: Sulfur production in the sulfur oxidising bioreactor

Sulfur mass balance in the sulfate oxidising reactor was estimated using the mole fraction of sulfur, sulfate and sulfide in the effluent against the influent sulfate and sulfide concentration. The sulfate mass balances did not consider the elemental sulfur produced in the sulfate reducing bioreactor. The total amount of sulfur produced in the whole system is thus an underestimate.

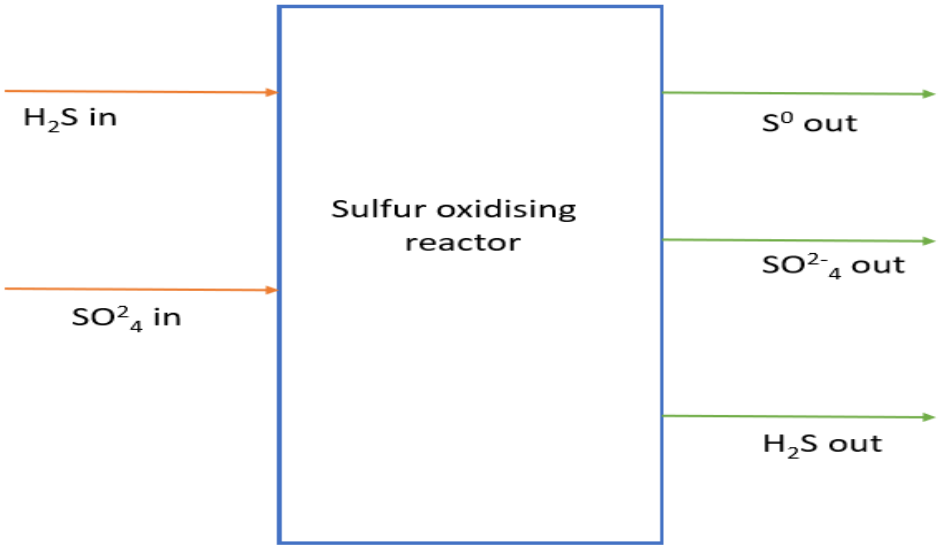


Figure 4-12: Schematic representation of sulfur balances in the sulfur oxidising reactor

From Figure 4-12, Mass of sulfur in = mass of sulfur out.

From Figure, 4-11 at time 648 hrs the above equation translates to:

H_2S (in=117mg/L) +SO_4^{2-}(in=325mg/L) = S^o(out) + SO_4^{2-}(out = 340 mg/L) + H_2S (out=6 mg/L)

32/34 X 117mg/L + 32/96X 325 mg/L = S^o(out) + 32/96 X 340 + 32/34 X 6

$$S^o = 99.39 \text{ mg/L}$$

Therefore, at 648 hours, the total amount of sulfur in the reactor was equivalent to 99.4 mg/L. This calculation was used to determine sulfur concentration at different times in the reactor. The average theoretical sulfur output was 102 mg/L/d. The performance of the sulfur oxidizing bioreactor may be overstated because intermediate sulfur species such as polysulfides were not determined. According to Sun *et al*., (2018, 2019), polysulfides exist in sulfur oxidising reactors and they would lower the efficiency and recovery of sulfur; but the amount of sulfur produced is significant to consider biogenic sulfur an income generating resource.

4.5 Conclusion.

The results indicate that it is possible to produce elemental sulfur from the hydrogen sulfide produced in the sulfate reducing reactor using SOB under oxygen limiting conditions. Sulfur recovery during AMD/FTWW treatment removes hydrogen sulfide, preventing the potential damage to equipment through corrosion. Elemental sulfur is easy to remove from the effluent as it is hydrophilic and can be recovered through sedimentation. Hydrogen sulfide is also a potential health risk to wastewater treatment plant operators as it poses a health hazard at high concentrations. The application of micro-aerophilic conditions to recover sulfur from sulfide-rich effluent is not only limited to the treatment of AMD, but also can be extended to other fields such as biogas purification, oil refineries and domestic wastewater treatment plants. The recovery of sulfur also adds value to the treatment process as sulfur is marketable and can be used to produce some important industrial products.

Chapter 5: Effects of seasonal temperature variation on the efficiency of a continuous sulfate reducing bioreactor treating AMD.

Abstract.

Bioreactors treating AMD use sulfate reducing bacteria to generate hydrogen sulfide and alkalinity using biological sulfate reduction. The sulfide is used to precipitate metals as metal sulfides while the alkalinity attenuates pH. The biological treatment of AMD using SRB is subject to temperature changes. The effect of seasonal temperature variations on the performance of the bioreactors was measured by the efficiencies of the reactors in the removal of sulfate, COD, and iron as well as pH attenuation. Sulfate removal rates at an average of 140 mg/L/hr were higher in summer, compared to an average of 123 mg/L/hr in winter. Average iron removal rates were 36.6 mg/L/hr in summer and 31.4 mg/L/hr winter while COD removal rates were higher in summer at 74.3 mg/L/hr and 36mg/L/hr in winter. pH was maintained above neutral in the bioreactor operated in summer while the pH decreased with time to a low of 5.7 during the winter operational period.

5.1 Introduction.

The treatment of AMD using biological sulfate reduction is influenced by diurnal or seasonal temperature variations at the treatment site. This is because microorganisms are sensitive to changes in temperature. South Africa like most of the countries located in the southern hemisphere has cold winters occurring in the months from May to August, while the summers are warmer and extends from September to April. In Johannesburg where this research was conducted, winter temperatures ranged between 2^{o}C and 18^{o}C while summer temperatures ranged between 21^{o}C and 30^{o}C during the operational period.

Temperature is one of the major influencers of any microbial-mediated reaction as it can affect both kinetics and stoichiometry via phenomena ranging from thermodynamic activity to physical enzyme conformation changes (Okabe and Satoshi, 1993). In general, the mesophilic range (25 ^{o}C - 45 ^{o}C) is used in anaerobic reactor systems with the optimum temperature for growth at 35^{o}C (Stronach *et al*., 1986). Some SRB have been reported to grow at substantially lower temperatures of around 20^{o}C. At low temperatures, there are difficulties in the start-up of the reactors. Ingnoversen *et al* (2003), found that the exponential phase of bacterial growth is shorter (6 hours) at higher temperature compared to 20 hours at 5^{o}C. Bacterial growth is

limited at low temperature by restrictions on cellular level processes such as permeability of membranes or temperature limitation of enzyme activity as well as bioavailability due to decrease in diffusion of reactants and products (Stronach *et al*., 1986, Arnosti *et al*., 1998). As the temperature increases within an appropriate range, chemical and enzymatic reactions in the cell proceed more rapidly and bacterial growth becomes faster (Tekere, 2019). According to Maree and Strydom (1987), a temperature quotient (Q_{10}) of 2 is obtained when temperature is increased from 20^{o}C to 30^{o}C with optimum for mesophilic SRB growth rate occurring at 30.5^{o}C. This temperature dependence is essential for modelling bacterial growth in bioreactors. The effect of temperature on the activities of SRB is described by the equation:

$$K = Ae^{-E/RT}$$

Were K = rate constant, Ae = frequency factor, E = activation energy R = Universal gas constant, and T = absolute T^{o}C (in Kelvin). Source Middleton and Lawrence, 1977.

Other SRB can grow within the thermophilic range (55 ^{o}C – 80 ^{o}C) (Liamleam and Annachhatre, 2007). Thermophilic sulfate reduction is applicable for treating sulfate-containing wastewater that is relatively warm such as sulfate wastewater discharged from pulp and paper industry, rayon manufacturing and flue gas desulfurization. Furthermore, the conversion rate of thermophilic treatment is much higher than mesophilic treatment (Liamleam and Annachhatre, 2007). In this study, mesophilic and psychrophilic temperature ranges were applied. Thermophilic ranges may be used to FTWW treatment, as the FTWW is a high temperature effluent because of the high temperatures used in the FT process. The FTWW may be diluted to bioreactor temperatures using a cooler AMD waste stream.

Many studies on sulfidogenic bioreactors and other biotechnological processes have shown that the efficiency of these processes strongly depend on operational temperatures (Li and Lu, 2017). Greben *et al.* (2002) reported a decrease in sulfate removal rates with decreasing

temperatures. They showed that when the reactor temperature decreased from 20°C to 5°C, the specific sulfate removal rate decreased from 3.39 to 0.40 g SO_4^{2-}/(gVSS).d. Al-Zuhair *et al.* (2008) observed that the highest rate of sulfate reduction occurs at 35°C. The rate of sulfate reduction at 20°C was reported to be higher than the rate at 50°C. Sulfate reducing bacteria have also been reported to operate at temperatures as low as 0°C and as high as 110°C in deep see hydrothermal vent sediments.

This chapter investigates how seasonal temperature variations may affect the efficiency of treatment of AMD using SRB in a bioreactor fed with FTWW. The assessment was based on the removal of COD, sulfate and iron as well as the improvement of pH in the bioreactor.

5.2. Materials and Methods.

5.2.1. Bioreactor Operation.

Two bioreactors were operated at different seasons of the year. One was operated in warmer summer temperatures (average temperature of 26°C) while the other was operated in cooler winter temperatures (average of 10°C). Sulfate reduction and metal precipitation were conducted in single stage continuous reactors (Figure 3-1 in section 3.1) with charcoal as the carrier material. The reactors were inoculated with SRB from a previous reactor treating AMD with FTWW as the organic feed. The bioreactors were sealed and allowed to obtain anaerobic conditions. The reactors were initially operated in batch mode for 500 hrs in a medium containing AMD and FTWW at COD/SO_4^{2-} ratio of 0.9 with nutrient supplementation to allow for the growth and acclimatisation of SRB. After evidence of biofilm growth, a continuous influent of FTWW/AMD, COD/SO_4^{2-} ratio of 0.9, at a flow rate of 0.74 ml/hr was introduced to the reactors using a peristaltic pump. The reactors were allowed to reach steady state. The bioreactors were operated for a period of 1104 hours in summer and 1104 hours in winter.

5.2.2. Chemical analysis.

Influent and effluent water analysis was monitored at 24 hr intervals. Samples were taken and analysed for pH, sulfate, COD, and iron. Refer to section 3.2 for methods.

5.3 Results and Discussion

5.3.1. Influence of seasonal temperature variation on pH changes in fixed bed sulfate reducing bioreactor treating AMD

The results presented in Figure 5-1 shows how the pH of the reactor effluent was affected by changes in temperature. During the summer, when temperatures ranged between 20^{o}C - 30^{o}C, the effluent pH was maintained above neutral pH from an influent pH of 2.15. However, as temperatures dropped to below 20^{o}C in winter, the effluent pH gradually dropped to pH levels as low as pH 5.2. These results agree with Ali *et al*., (2018) who observed slight decreases in pH as the temperature decreased from 22^{o}C to 5^{o}C.

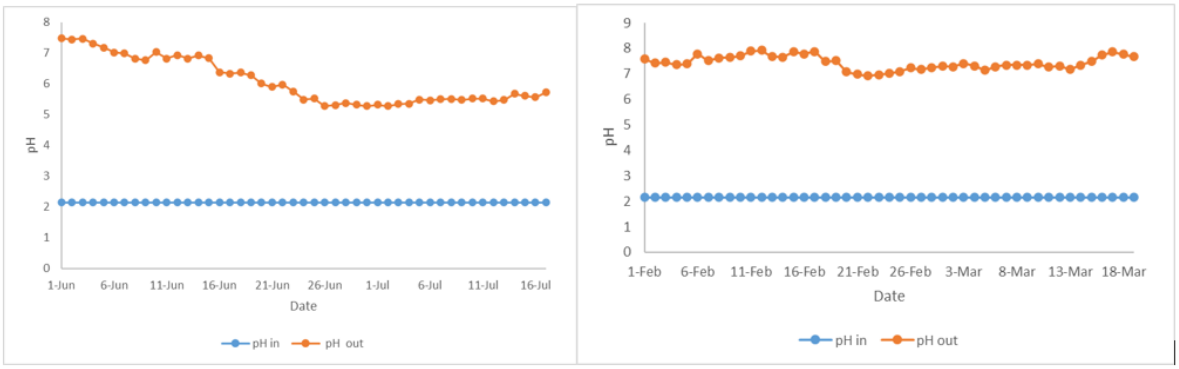


Figure 5-1: Effect of seasonal variation temperature on the effluent pH of bioreactors operated in summer and winter.

5.3.2. Differences in sulfate removal efficiencies in bioreactors treating AMD in summer and winter.

Figure 5-2 shows the residual sulfate in the bioreactors during the two temperature seasons (summer (average 26^{o}C) and winter (average 10^{o}C)) investigated. Both reactors maintained high rates of sulfate removal (Table 5-1) during the operational period. The reason for the high

performance may be that immobilized cells offer high cell density and higher endurance in adverse reactor conditions. According to Xu and Chen (2020), SRB immobilization can enhance the rate of sulfate reduction by 33-40%. The mechanism of enhancement involves synergistic effects such as the provision of large surface area for growth, and adsorption of sulfate and heavy metals onto available surfaces. Additionally abiotic and biologically mediated processes that result in precipitation of hydrated oxide and sulfate phases contribute to the overall contamination attenuation process (Lefticariu *et al*., 2015). Baskaran and Nemati (2006) obtained sulfate removal rates of 1.7 g/ (L.h), at a residence time of 0.5 hours using sand as the immobilization matrix with lactate as the organic feed. They attributed the high sulfate removal rates to the higher surface area offered by the sand particles.

The summer effluent maintained residual sulfate concentration below 600 mg/L from a continuous influent sulfate concentration of 3800 mg/L. The change to winter resulted in an increase of up to 1110 mg/L residual sulfate in the effluent. These results agree with Al-Zuhair *et al*. (2008) who reported an increase in sulfate reducing activities as the temperature was increased from 20^{o}C to 35^{o}C. Praharaj and Fortin (2004) obtained sulfate reduction rates of 100 to 1000nmol cm^3 day^{-1} in samples from mine tailings in Northern Ontario, Canada during summer temperatures of around 30^{o}C. The rate of sulfate reduction decreased to less than 10nmol cm^3 day^{-1} as the temperature dropped to 12^{o}C during springtime. The drop in temperature was given as the reason for lower biological activity. Low temperatures slow down microbial enzymatic activity. This can be seen in the decrease in efficiency as the temperatures dropped in winter (Figure 5-2).

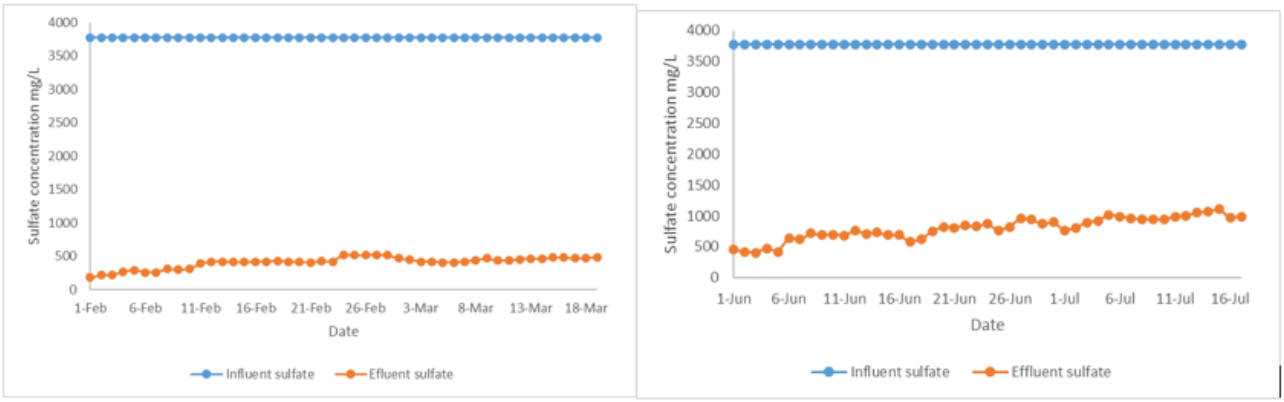


Figure 5-2: Comparison of effluent sulfate in bioreactors operated in summer and winter.

Table 5-1 shows the comparison of the sulfate removal rates in the summer and winter operational periods. As expected, the sulfate removal rates were higher in summer than in winter. Within the first 200 hours, the average sulfate removal rates were 146 mg/L/hr in the bioreactor operated in summer compared to 134 mg/L/hr in the winter operational period. As time increased, the sulfate removal rates decreased from 146.6 mg/L/hr and 134.9 mg/L/hr in the first 200 hrs to 137.4 mg/L/hr and 114.0 mg/L/hr after 1000 hrs in summer and winter respectively. The lower sulfate removal rates resulted in lower sulfate removal efficiencies in winter than in summer (Figure 5-3).

Table 5-1: Comparison of average sulfate removal rates at different periods of reactor operation in summer and winter

Hours of operation	Average SO_4^{2-} removal rates (mg/L/hr) in summer (average 26ºC)	Average SO_4^{2-} removal rates (mg/L/hr) in winter (average 10ºC)
0-200	146.6	134.9
200-400	140.5	128.8
400-600	138.4	124.4
600-800	138.4	120.6
800-1000	138.7	116.7
1000-1104	137.4	114.0

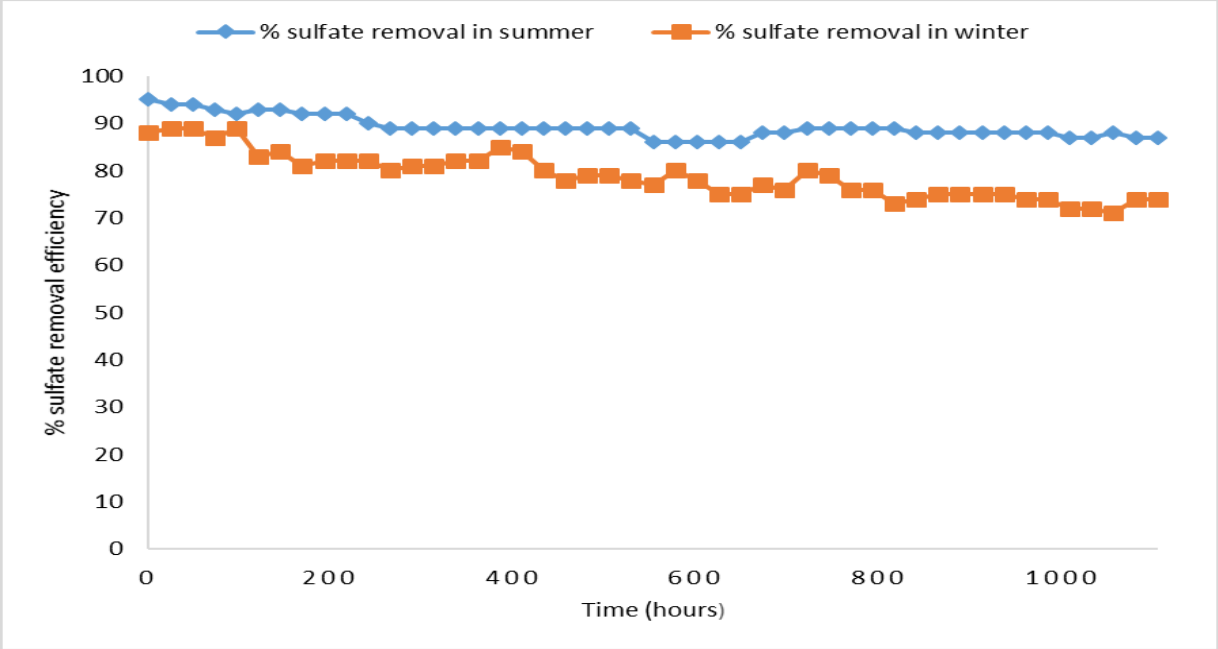


Figure 5-3: Differences in sulfate removal efficiencies between sulfate reducing bioreactors operated in summer and winter.

5.3.3. Effect of seasonal temperature variation on iron precipitation in a continuous sulfidogenic bed reactor treating AMD.

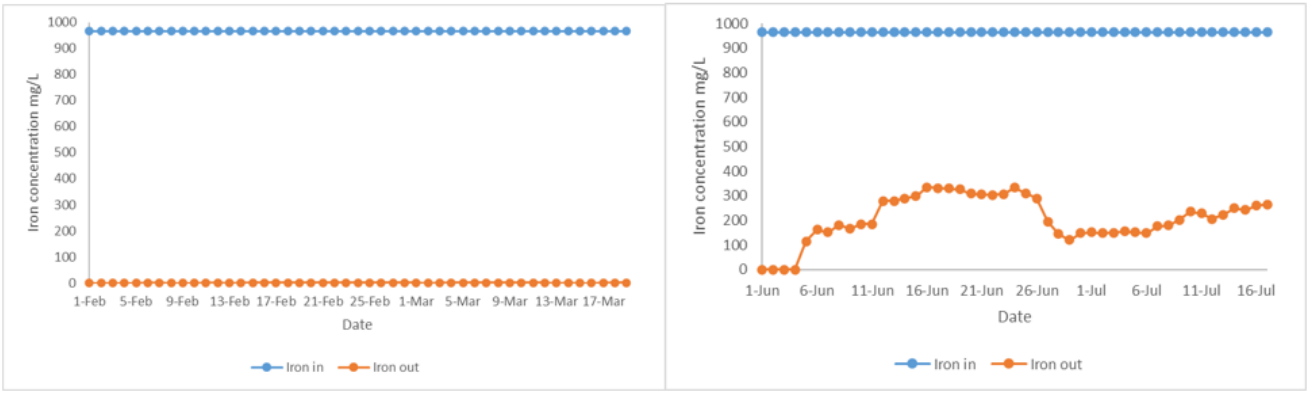


Figure 5-4: Effluent iron from sulfate reducing bioreactors in different seasons.

The highest iron removal rates were observed in the initial stage of operation (first 200 hrs) of the bioreactor - 51.9 mg/L/hr in summer and 36.6 mg/L/hr in winter (Table 5-2). The iron removal rates decreased with time in both summer and winter operations, with iron removal rates of 51. 9 mg/L/hr and 26.9 mg/L/hr after 1000 hours in summer and winter respectively. In summer the bioreactors maintained the effluent iron concentration at below detectable levels against an influent iron concentration of 965 mg/L (Figure 5-4) - an iron removal efficiency above 99 %. The efficiency of iron removal decreased to 65 % (Figure 5-5) during the winter, a 34 % decrease in efficiency with a maximum 335 mg/L residual iron in the effluent (Figure 5-4). Stoichiometrically, if the removal of iron and sulfate was solely due to SRB activity, there should be no residual iron in the winter operated bioreactor. The reason could be that some sulfide may have been converted to other intermediates such as elemental sulfur, thiosulfate, and sulfite in microaerophilic pockets within the reactor. Another possible explanation could be that some sulfate was removed through other mechanisms such as adsorption to surfaces, and the remnant sulfate was metabolized slowly to effect complete iron removal due to the decrease in temperature during winter periods resulting in the production of insufficient

quantities of sulfide required for the complete removal of dissolved iron through sulfide precipitation.

Table 5-2: Comparison of iron removal rates at different weather seasons.

Hours of operation	Average iron removal rate (mg/L/hr) during summer ($21^{o}C$-$30^{o}C$)	Average iron removal rates (mg/L/hr) in winter ($2^{o}C$-$18^{o}C$)
0-200	51.9	36.6
200-400	40.2	28.6
400-600	40.2	27.3
600-800	31.9	33.9
800-1000	26.8	32.2
1000-1104	26.6	29.9

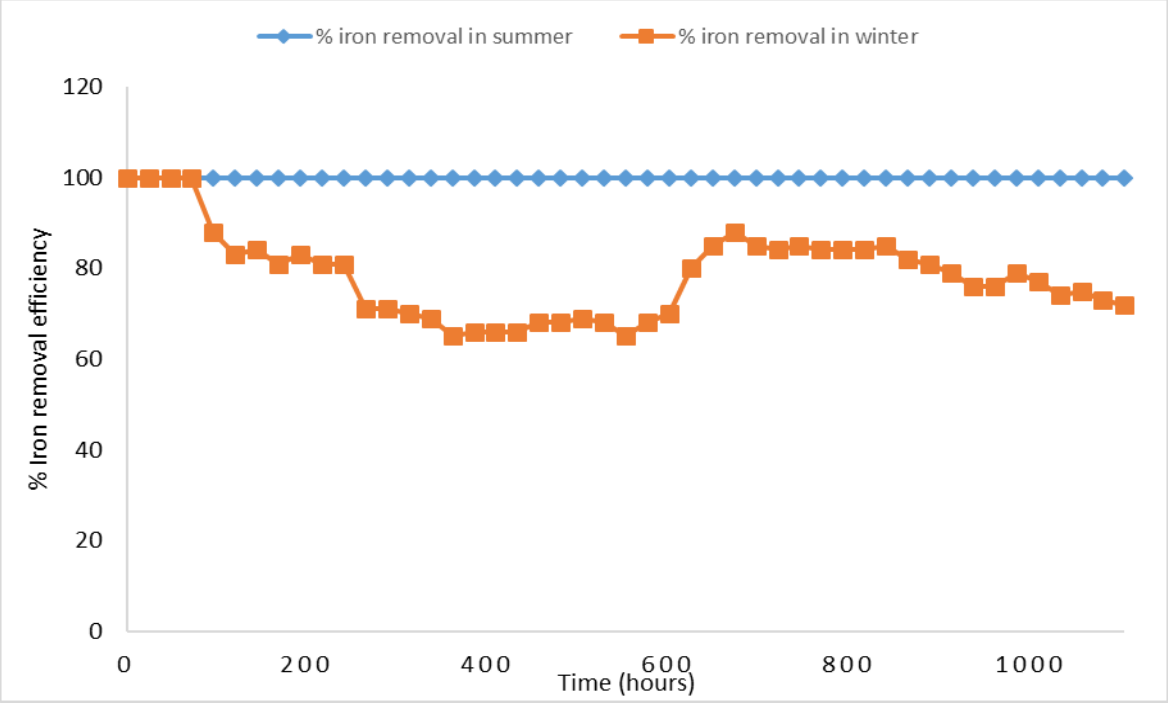


Figure 5-5: Comparison of iron removal efficiencies in bioreactors operated in summer and winter.

5.3.4. Effect of seasonal temperature variation on COD removal efficiencies in a continuous sulfidogenic bed reactor treating AMD.

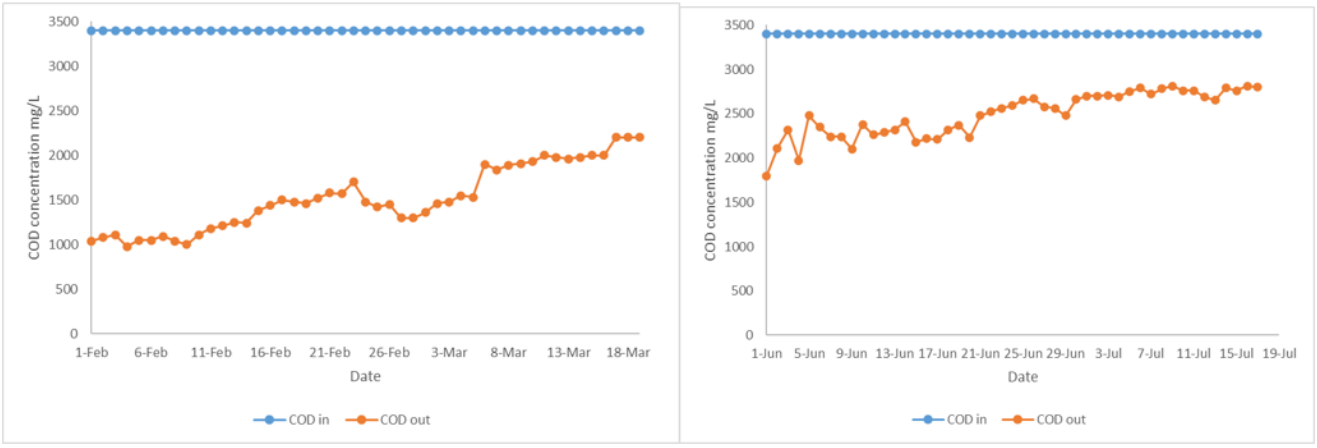


Figure 5-6: COD effluent in sulfate reducing bioreactors operated in different seasons.

The COD removal efficiency was higher in summer than in the winter (Figure 5-7), however in both cases there was accumulation of COD in the effluent as the COD concentration increased with time (Figure 5-6). The efficiency of the bioreactors in summer ranged from 69 % to 35 %, while the efficiency in winter dropped from 47 % to 18 % COD removal. As a result, about 2800 mg/L residual COD was left in the effluent of the winter operated bioreactor compared to a residual COD concentration of 2200mg/L in summer (Table 5-3). These observations are supported by Li and Lu (2017) who investigated the removal of COD under seasonal variation of temperature and found that the efficiency of COD removal changed with temperature. They reported an average COD removal efficiency of 76+/- 7.2 % and 52 +/- 5.9 % in summer and winter respectively. The highest rates of COD removal, 88.9 mg/L/hr in summer compared to 50.9 mg/L/hr in winter were recorded within the first 200 hrs (Table 5-3). Thereafter COD removal rates began to decrease, at the tail end of reactor operation (after 1000 hrs), the rates were recorded at 53.3 mg/L/hr in summer and 26.6 mg/L/hr in winter. The control experiment results showed that charcoal contributed 10% towards the removal of COD

but did not play a significant role in pH improvement and in the removal of sulfate and dissolved iron (Figures A6-A9 in appendix A).

Estimation of removal rates and efficiencies.

The removal rates and efficiencies were estimated using Equations 5.1 and 5.2.

$$\text{Removal Rate} = 1/24\text{hr}\ (C_{in}-C_{out}) \qquad \text{Equation 5.1}$$

And

$$\text{Removal efficiencies} = (C_{in}-C_{out}t)/C_{in}\ X\ 100 \qquad \text{Equation 5.2}$$

Where: C_{in} is influent concentration and C_{out} is effluent concentration.

These equations were used to estimate the sulfate, COD and iron removal rates and efficiencies (Figure 5-7)

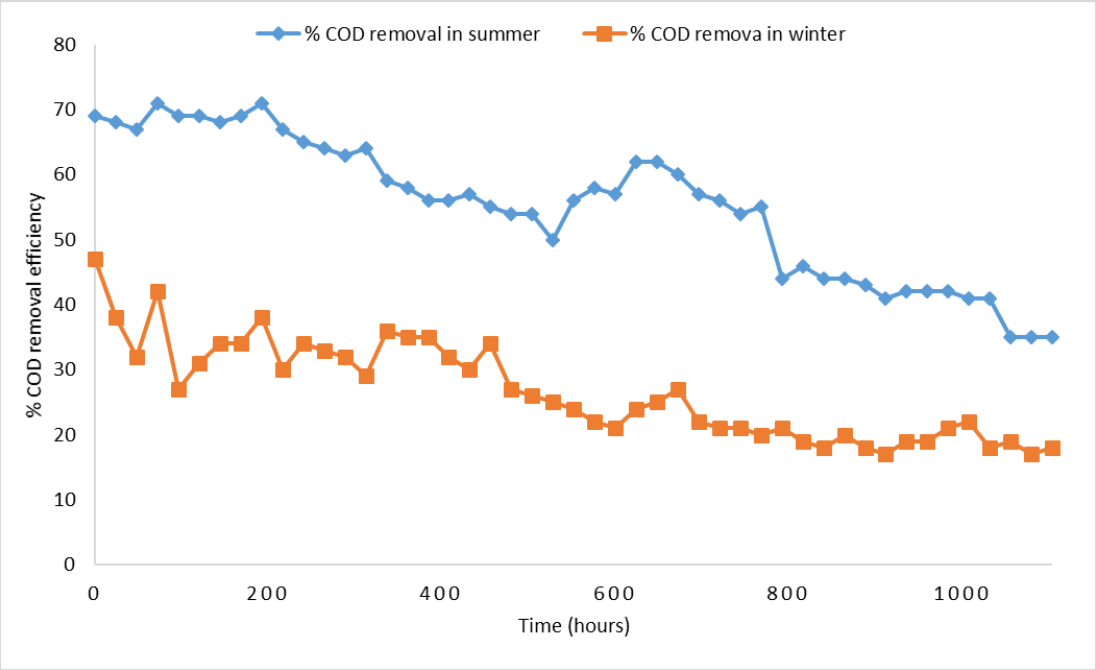


Figure 5-7: COD removal efficiencies in sulfate reducing bioreactors operated different seasonal temperatures.

Table 5-3: Comparison of COD removal rates during summer and winter operational periods.

Hours of operation	Average COD removal rates (mg/L/hr) summer (21^{o}C-30^{o}C)	Average COD removal rates (mg/L/hr) winter (2^{o}C-18^{o}C)
0-200	88.2	50.9
200-400	87.1	46.3
400-600	78	37
600-800	79	31.9
800-1000	61	26.8
1000-1104	52.3	26.6

Table 5-4: Comparison of effluent characteristics from bioreactors operated in summer and winter.

Parameter	**Summer (20^{o}C-30^{0}C)**	**Winter (2^{o}C -18^{o}C)**
Sulfate (mg/L)	490	990
COD (mg/L)	2200	2800
Iron (mg/L)	0.48	266
pH	7.86	5.72

Generally, researchers agree that low temperatures decrease the efficiency of bioreactors treating AMD; yet some researchers are of the opinion that the efficiency of bioreactors is only affected during acclimatisation of the microbial community to the new temperatures. Once acclimatization is achieved, the efficiency will improve (Tsukamoto *et al*., 2004). This statement is supported by the work of Ingvorsen *et al* (2003), who found out that the exponential phase of SRB growth is significantly shortened to 6 hours at 20^{o}C compared to 20 hours when the temperature drops to 5^{o}C. Highlighting the presence of mesophilic SRB.

High sulfate reduction rates have been reported at low temperatures (1^{o}C to 16^{o}C) meaning SRB are active and functional in cold temperature. According to Virdiranta *et al*., (2019), though SRB can tolerate temperatures from -5^{o}C to 75^{o}C, the majority of SRB have optimum

growth in the 28^{o}C to 30^{o}C range. This is in line with the findings of this investigation, with higher treatment efficiency obtained in warmer summer temperatures while a decrease in efficiency of treatment was observed in winter due to lower temperatures. Results from a study by Marais *et al* (2018), also confirms that temperature plays a critical role in the activities of SRB. Their results show a decrease in sulfate removal efficiency from 66.73 % to 53.86 % due to a decrease in temperature from 30^{o}C to 10^{o}C, with the highest sulfate conversion occurring at 30^{o}C.

Generally, the rates of sulfate, iron and COD removal were higher in summer than in winter, indicating that temperature changes affected the activities of microorganisms involved in the treatment process. At lower temperatures, bacterial growth is slow. This was evidenced by the poor biofilm formation observed during winter operations. This negatively affects the metabolic activities occurring within the reactors resulting in reduced efficiency. On the molecular level, significant changes in temperature affects enzyme activities. A decrease in temperature is usually accompanied by a decrease in the rate of enzyme-meditated reactions. Because cell membranes are made of phospholipids, fluctuations in temperature also affect the fluidity of the cell membrane limiting nutrient uptake in mesophilic microorganisms. Elevated temperatures increase the fluidity of the cell membrane allowing the easy transportation of nutrients and substrates across the membrane, thereby promoting high rates of metabolism and cell growth (Moreroa *et al*., 2018).

According to Li and Lu (2017), lower temperatures might also increase the wastewater viscosity, making it difficult for substrate and nutrients to enter the cell thus slowing down the reactions occurring in the bioreactor. The remediation of AMD through biological sulfate reduction depends on the metabolic products of sulfate reduction. Any factor that slows down the production of these products have a negative impact on the remediation process. The products of biological sulfate reduction are hydrogen sulfide and alkalinity. Hydrogen sulfide

is used to precipitate and remove metals from the AMD while the alkalinity is needed for pH attenuation. Low temperatures reduce the concentration of hydrogen sulfide and alkalinity, thus affecting the metal removal efficiency as well as the neutralization capacity of the reactors.

In terms of iron removal efficiency, there was almost total removal of iron in summer presumably as iron sulfide precipitates. This suggest that enough sulfide was generated to allow total precipitation of iron. The iron removal efficiency decreased in winter, meaning that the amount of sulfide generated in the reactors was not sufficient to allow for total iron removal. There was COD and sulfate accumulation in the reactors. Sahinkaya *et al*. (2007) observed similar trends in the performance of ethanol fed sulfate reducing fluidised bed reactor operated at 8^{o}C. They reported total iron removal through metal sulfide precipitation. However, sulfate removal efficiency was averaged at 35% +/- 4% between day 30 and day 130 of operation. This was associated with acetate accumulation in the reactor. The reason for the accumulation of acetate was attributed to the fact that most of psychotropic SRB are incomplete oxidisers and cannot use acetate as the sole carbon leading to accumulation of both COD and sulfate, thus reducing the efficiency of sulfate reducing bioreactors at low temperatures (Kaksonen *et al*., 2008). Marais *et al* (2018), observed complete utilization of lactate at 30^{o}C, However the lactate was incompletely oxidised resulting in acetate accumulation. This led to high residual COD in the effluent. A further decrease of temperature to 20^{o}C resulted in the presence of lactate in the effluent indicating incomplete source utilization and reduced microbial activities.

During the summer, the effluent pH in the bioreactor was maintained above neutral despite an influent pH of 2.0. This suggest that the system had enough buffering capacity to neutralise the incoming acidity. The buffering capacity was produced through the metabolic activities of SRB that generates alkalinity. Because microbial activity is slowed down because of lower temperature, not enough biogenic alkalinity was generated at low winter temperatures. This resulted in the pH dropping to below neutral in winter (Table 5-4). To minimize the effect of

low temperatures, the reactors may need insulation, which adds costs to the treatment process. Another option is to build the reactors underground or alternatively to increase the residence time in the bioreactor.

The wastewaters coming from the FT processes have elevated temperatures because of the high operating temperatures of the FT process (above 200 ^{o}C). Treating FTWW using biological sulfate reduction under mesophilic conditions requires precooling. This may be done by diluting the warmer FTWW wastewater with the cooler AMD to obtain mesophilic temperatures, removing the costs required for maintaining optimal mesophilic bioreactor operating temperatures during winter times when the temperatures are lower.

An undesirable characteristic of the reactors was the decrease in rates with time, for example the COD removal rates decreased from a high of 82.2 mg/L/hr within the first 200 hrs of operation to a low of 52.3 mg/L/hr in summer after 1000 hrs of operation. This trend was also observed in winter where high COD removal rates of 50.9 mg/L/hr were obtained in the first 200 hrs of operation. The rate of COD removal decreased to 26.6 mg/L/hr after 1000hrs of operation. This decrease in efficiency with time may be attributed to the inhibitory effects of hydrogen sulfide and/or metal sulfide precipitates. Hydrogen sulfide interferes with enzyme activities by disrupting sulfide bonds in the protein structure of the enzymes. The accumulation of metal sulfide in the reactor is likely to slow the mass movement of reactants into the cell by coating the cell surface. It is thus necessary to periodically flash and remove the metal precipitates to maintain reactor efficiency. Aging of the biofilm may also be major contributor to the decreasing efficiency of the bioreactor.

5.4 Conclusion

The objective of the study was to evaluate the effect of seasonal temperature variations on the efficiency of sulfate reducing bioreactors. The results show that seasonal variations in

temperature affect the efficiency of AMD treatment using SRB. Lower temperature decreases microbial activity in the reactors, because the SRB will not grow fast enough to maintain a large population in the bioreactors to remove large amounts of COD and sulfate. Temperature is thus a critical parameter in the efficiency of bioreactors. To keep the sulfate reduction rates high, it might be necessary to increase the residence time in the reactor, or to maintain the reactor temperatures through heating and insulation of the bioreactors.

Chapter 6
Comparison of the efficiency of sulfate and COD removal in a continuous fed single fixed bed bioreactor to a two-stage continuous bioreactor system.

Abstract.
The application of biotechnology has great potential in the treatment of acid mine drainage (AMD). The biological treatment of AMD uses sulfate reducing bacteria (SRB) to generate hydrogen sulfide and alkalinity through sulfate reduction. The sulfide is used to precipitate metals while acidity is neutralized through biogenic alkalinity. Single stage bioreactors usually suffer from inhibition due to the accumulation of metal sulfide precipitates and hydrogen sulfide. This study aimed to investigate if an improved reactor performance would be achieved using a two-stage reactor system in comparison to a single stage bioreactor system. The results showed sulfate and COD accumulation in the single stage reactor. The concentration of sulfate and COD in the effluent increased with time to 500 mg/L and 2000 mg/L respectively. The efficiency of the system was improved by connecting two sulfidogenic bioreactors in series. Incorporating the second bioreactor achieved sulfate and COD removal efficiency of 99 %. Both single stage and two stage systems achieved almost total iron removal. In both systems, the pH of the effluent was maintained above neutral.

6.1 Introduction.

Several bioreactor designs have been investigated in the treatment of AMD. These include aerobic sludge blanket (USAB), (Lens *et al*., 1998), anaerobic baffled reactor (ARB) (Barber and Stuckey, 1999), and up flow packed bed reactors (Greben and Maree, 2000). These reactors may be operated as batch, sequential batch, or continuous flow reactors, with each reactor configuration conferring its own advantages in terms of operation and efficiency (Brahmachariyum, 2019).

A major concern with using continuous flow single stage sulfidogenic bioreactors for the treatment of AMD is their susceptibility to inhibition due to product accumulation. The microbial consortium in the bioreactor may be inhibited by reactor conditions, such as low pH, hydrogen sulfide or metal sulfide precipitates (Dar *et al*., 2009). Most of the SRB have their optimum pH between 7.5 and 8, while below a pH value of 5.0; the activity of SRB is significantly reduced. At low pH, acidification of the cytoplasm occurs inhibiting the formation of a proton motive force. pH mainly affects the speciation of hydrogen sulfide and heavy

metals which may be toxic to SRB. In a single stage reactor, the effect of inhibitory substances is high because the reactions are occurring in the same reactor resulting in the accumulation of products. A multi-stage bioreactor system ensures that reactor conditions are improved as the effluent is passed from one reactor to the next. This means that the subsequent reactor will have improved pH, less hydrogen sulfide and less dissolved metals and metal sulfide precipitates compared to the previous reactor.

Furthermore, an important objective in water treatment systems is to ensure that the concentration of pollutants left in the effluent at the end of the treatment process are removed to levels which are recommended for the disposal or reuse of the wastewater. Even though the single stage continuous sulfate reducing bioreactor in chapter 3 was able to remove 99 % of the iron, the reactor failed in terms of COD removal. The concentration of COD in the bioreactor was increasing with time to levels beyond the recommended limits for safe disposal. It is therefore necessary to add another sulfate reducing reactor to the existing one to improve COD removal efficiency. This study therefore sought to evaluate the performance of a fixed bed single stage continuous sulfidogenic bioreactor and a two-stage fixed bed continuous bioreactor for the combined treatment of AMD and FTWW.

6.2 Materials and Methods.

Two up-flow reactors with inlet pipes located near the bottom of the reactors and effluent pipes on top were utilized. Three Sampling ports were located at regular intervals along the length of each reactor (as reported in chapters 3 and 4). The two reactors were connected in series. The first reactor (labelled 1 in Figure 6-2) was tilted at a 60^{o} angle with the influent pipe on the upper side of the reactor (Figure 6-1) to minimise blockage from metal sulfide precipitates. Both reactors were inoculated with SRB from a previous bioreactor treating AMD using FTWW as the organic feed. Charcoal (1.5cm-2cm) was used as the carrier matrix. The reactors

were initially operated in batch mode with nutrient supplements containing 0.5 mg/L KH_2PO_4, 1 g/L NH_4Cl, 1 g/L $CaSO_4$, 3.5g /L sodium lactate, 1 g/L yeast extract and 0.1 g/L ascorbic acid to promote microbial growth. Microbial growth was evidenced by the appearance of a biofilm. At this stage, the bioreactors were turned into continuous flow until steady state conditions were achieved.

A combined FTWW/AMD influent was pumped using a peristaltic pump. The influent AMD/FTWW mixture with a COD sulfate ratio of 0.90 was pumped into the reactors at a rate of 0.74 millilitres per minute. The influent entered the system through an inlet pipe situated near the bottom of reactor 1. The effluent exited at the top of the reactor 1 into an effluent pipe leading to reactor 2 (Figure 6.2). The partially treated water entered reactor 2 through an influent pipe situated near the bottom of the reactor and exited at the top of the reactor. Samples were collected at regular intervals from both reactors for analysis.

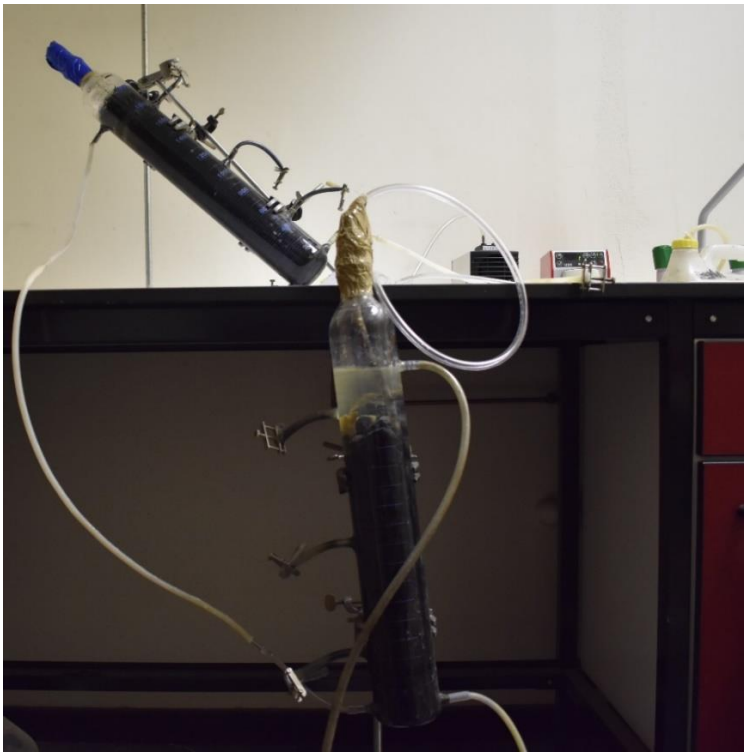


Figure 6-1: Two stage continuous bioreactor for the treatment of AMD

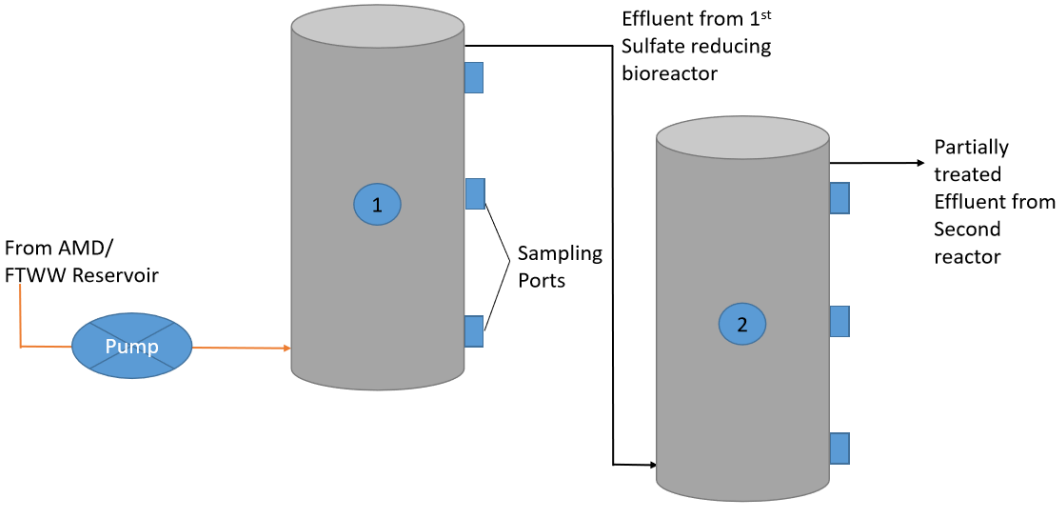


Figure 6-2: Schematic representation of a two-stage bioreactor treating AMD.

6.3 Results and discussion.

6.3.1 Iron removal.

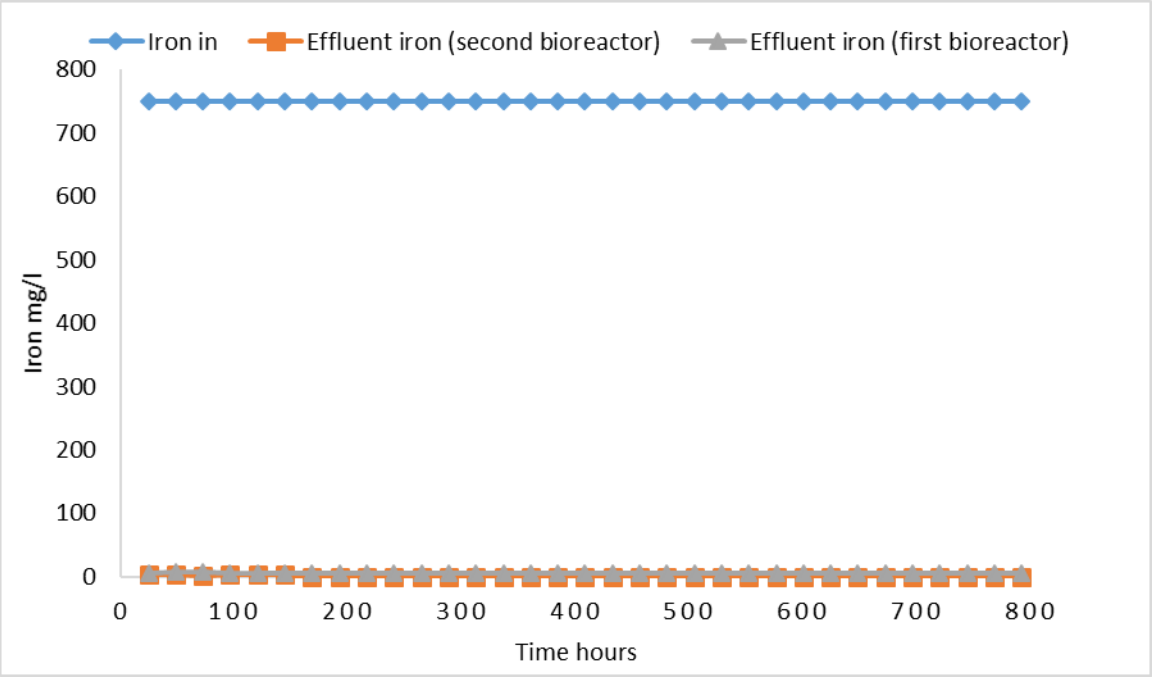


Figure 6-3: Comparison of Iron removal efficiencies in a two-stage continuous sulfate reducing bioreactor treating AMD.

The first bioreactor (reactor 1) was highly effective in removing iron with an efficiency above 99 %. The removal rates in reactor 1 remained at steady state during the operational period. An average iron removal rate of 30.9 mg/L/hr was observed throughout the operational period. An average concentration of 0.5 mg/L of dissolved iron was left in the reactor at the end of the operation time. Black precipitation was observed in reactor 1 that may be confirmation of the presence of iron sulfide, meaning the mechanism of metal removal was as metal sulfide precipitates. The results show that enough hydrogen sulfide was generated in reactor 1 to allow almost all the dissolved iron to be removed as iron sulfide precipitates. These iron removal trends were support those observed in section 3.5.1 and section 5.3.3 were iron removal efficiencies of 99% were obtained. Although biological sulfate reduction and metal sulfide

precipitation maybe the main metal removal mechanisms in sulfate reducing bioreactors, metals can also be removed through hydroxide precipitation and adsorption to available surfaces in the bioreactor. The second anaerobic reactor (reactor 2) maintained the iron concentration below 0.5 mg/L (Figure 6-3).

6.3.2. Sulfate.

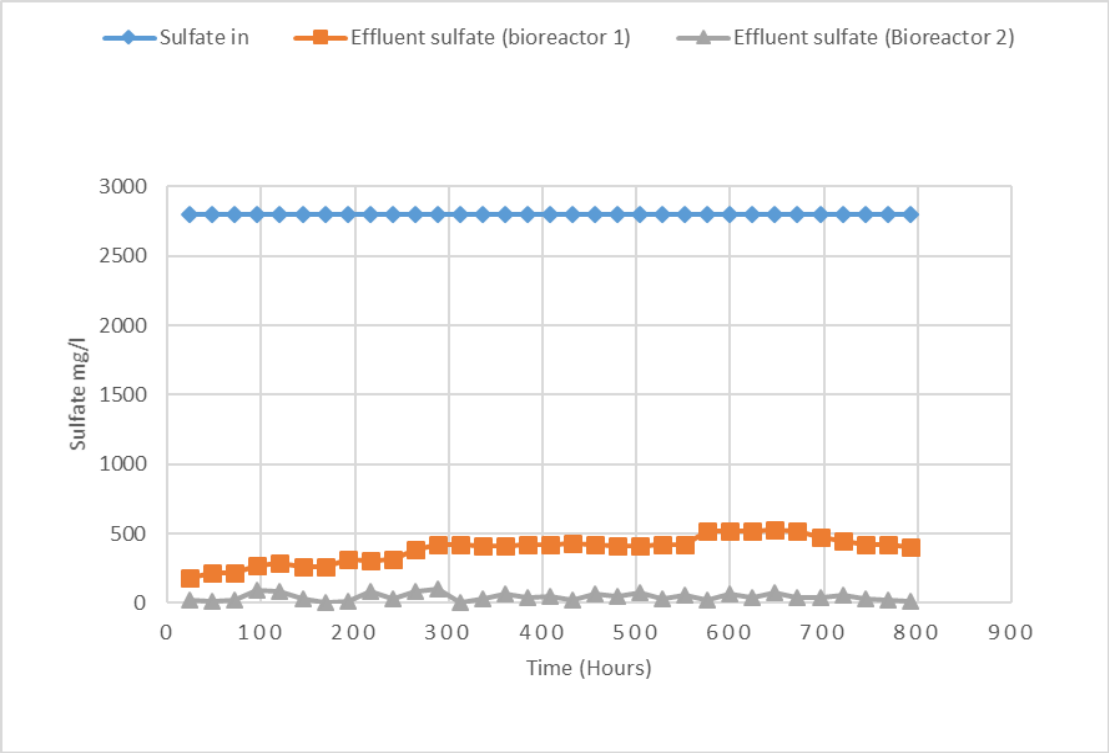


Figure 6-4: Comparison of sulfate removal efficiencies of a two-stage sulfate reducing bioreactor connected in series.

The sulfate concentration in the effluent of reactor 1 was maintained at 200 mg/L against an influent of 2800 for the first 200 hours, at an average sulfate removal efficiency of 93 %. The highest sulfate removal rate took place during this period with an average removal of 108 mg/hr, decreasing to an average of 96 mg/hr thereafter. These findings agree with Beckmezi *et al.*, (2011), who reported overall sulfate removal rates of up to 3.7 g L^{-1} d^{-1}, in a four-compartment ethanol-fed anaerobic baffled reactor treating a low pH synthetic AMD.

In this study, the high sulfate removal rate observed in reactor 1 may not be attributed to biological sulfate reduction alone because other processes such as adsorption of sulfate to the biofilm or onto ferric oxyhydroxides precipitates also contribute to the removal of pollutants from AMD. The removal of sulfate from the bioreactor was accompanied by a decrease in COD concentration (Figure 6-6). The COD/sulfate usage was maintained at a ratio of 0.9 during the first 200 hours, decreasing gradually to 0.8 after 400 hours. The COD/sulfate removal ratio was consistently maintained above 0.67, which is the ideal stoichiometric ration for sulfate removal.

Because of the decrease in removal rates, sulfate started to steadily build up with time after the initial 200 hours where conditions were maintained in steady state (Figure 6-4). This led to a partial removal of sulfate in the reactor, with 86.5 % removal efficiency with residual sulfate at below 500 mg/L. This indicates a slowing down of microbial metabolism in the reactors. These observations confirm earlier studies that showed sulfate accumulation in continuous bioreactors treating AMD using FTWW as an organic feed (Figure 3-6 and Figure 5-2).

Two phenomena might have taken place to prevent complete removal of sulfate from the bioreactor in the presence of excess COD. The inhibitory effects of the metabolic products of biological sulfate reduction (H_2S and metal sulfide precipitates) might have led to bioreactor failure or there was a shift in the microbial community due to the fluctuating COD/SO_4^{2-} ratio. The results also showed that incorporating a second anaerobic reactor (reactor 2 in Figure 6-2) ensured that 99 % of sulfate was removed from the AMD, with a further 13.7% of sulfate removed in reactor 2 (Figure 6-4).

Reactor 2 received partially treated influent from reactor 1. The sulfate concentration in the influent ranged from as low as 150 mg/L in the initial stages of bioreactor operation to as high as 500 mg/L towards the decommissioning of the bioreactors. Reactor 2 maintained the sulfate concentration in the effluent to below 100 mg/L, achieving a combined efficiency above 96 %.

Since the reactor 2 was receiving effluent with low sulfide and low metal concentration, there was less inhibition of the microbial community in the bioreactor. Therefore, the bacteria were able to grow faster leading to high metabolic rates.

6.3.3 pH.

Fig 6-5 shows the pH profiles of the influent and effluent in both reactor 1 and reactor 2. The first bioreactor (1) improved the pH of the medium to above 7 indicating that biological activity in reactor contributed enough alkalinity to neutralize the influent acidity. The effluent pH remained between 6.5 and 8.0 during the operational period. This confirms earlier studies where pH was maintained around neutral (Figure 3-8, Figure 4.8, and Figure 5-1). In a similar study, Cunha *et al*., (2019) achieved neutral effluent pH (6.3-7.3) from an effluent pH of 4 using ethanol as the only electron donor. Bekimezci *et al*., (2011) achieved near neutral pH from an influent pH of 3 due to alkalinity production during oxidation of ethanol. The neutral pH range allow for removal of dissolved iron through sulfide precipitation.

The partially treated wastewater was then fed into reactor 2 were marginal improvements in pH to levels below 8.0 were observed, though the reactor was biologically active as evidenced by the removal of both COD and sulfate. This suggests that the influent into reactor 2 had generated enough buffering capacity to resist any further pH changes because of biogenic alkalinity.

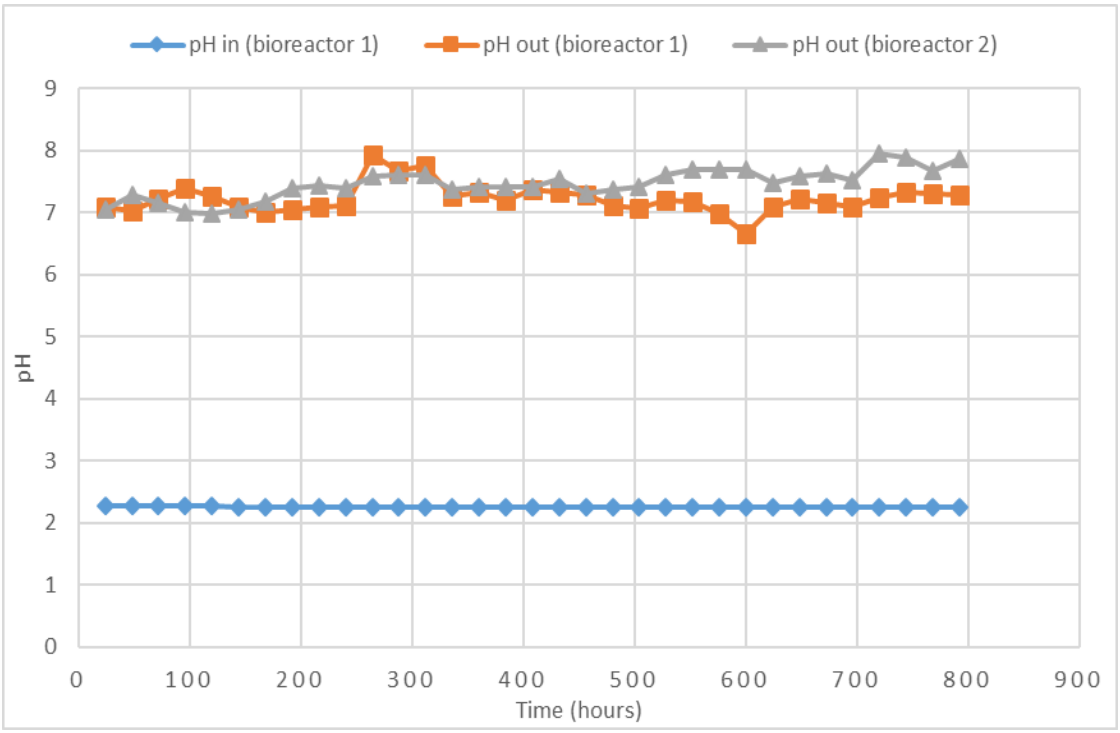


Figure 6-5: Improvement of pH in a two-stage sulfate reducing bioreactor treating AMD.

6.3.4 COD.

In the first 200 hours of continuous operation, reactor 1 maintained steady state, with an average COD concentration of 1038 mg/L in the effluent, an average COD removal rate of 98 mg/hr. The COD removal rate gradually decreased to 78 mg/hr by the decommissioning of the reactor. The gradual decrease in the COD removal rate led to an increase in COD concentration to a high of 2200 mg/L. The trend of COD accumulation in the bioreactors was observed in earlier results (Figure 3-9 and Figure 5-6).

Being the most abundant dissolved metal in AMD, it was important that reactor 1 was able to remove above 99 % of the dissolved iron through biological sulfate reduction and other physico-chemical processes occurring in the bioreactor. It can also be assumed that the other dissolved metals were similarly removed in the first reactor so that the second reactor has minimum precipitation of metals occurring. The presence of metal precipitates might have contributed to the low COD removal efficiency in reactor 1. This might be because metals

inhibit microbial reactions or interfere with the mass transport of metabolites into the bacterial cells. Figueoroa *et al*., (2007) suggested clogging by metal precipitates as one of the causes of bioreactor failure long before the substrate has been depleted. Installing a second sulfate reducing bioreactor that receives low metal concentration influent from the first reactor ensures that the inhibitory effects of metals and metal sulfide precipitates is removed. Another factor that might have contributed to the improved efficiency of the second sulfidogenic reactor might be that the improved influent pH might be suitable for optimal microbial activities.

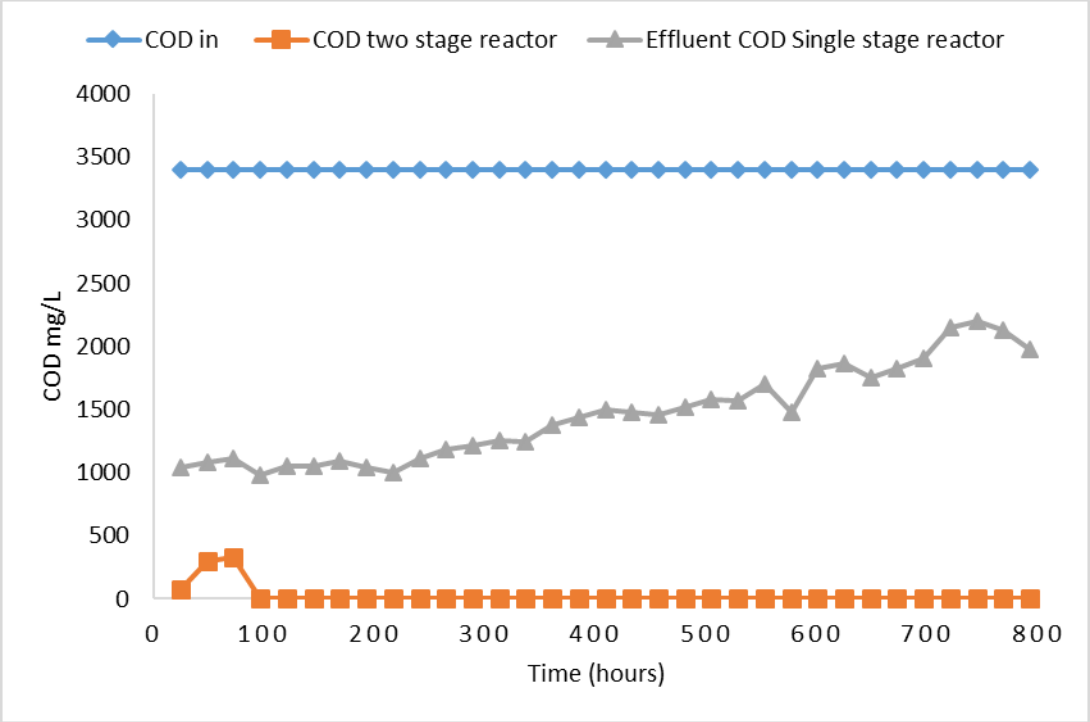


Figure 6-6: Comparison of the COD removal of a single stage reactor to a two-stage system.

The removal of COD and sulfate in both the first and second bioreactor are shown in Table 6-1, Figure 6-6, and Figure 6-4. The COD/sulfate ratio in the influent of reactor 1 was at an average of 0.9. This COD/sulfate ratio is sufficient for complete sulfate and COD removal; however, because of other factors such as inhibition, complete removal was not possible with 2200 mg/L COD and 500 mg/L sulfate remaining in the effluent of the sulfate reducing

bioreactor. This means that reactor 2 received a minimum COD/ sulfate ratio of 4.4 indicating that the amount of sulfate available was insufficient to remove all the COD through biological sulfate reduction. The second bioreactor however removed COD to levels below the detection limits (15 mg/L) of the equipment. Figure 6-4 shows that at least 99 % of sulfate was removed due to the incorporation of a second reactor, suggesting that sulfate reducing bacteria were active in the bioreactor despite the high COD/Sulfate ratio. In Figure 6-6, a minimum 99 % COD removal efficiency was observed though the amount of sulfate present in the bioreactors did not support complete COD removal, suggesting the presents of bacteria other than sulfate reducing bacteria in the second reactor. According to Figueoroa *et al*., (2007), the depletion of sulfate might result in the development of a different synergistic microbial community of fermenters, acetogens and methanogens that are able to utilize the COD in their metabolism; this explains the near complete removal of COD in the second reactor.

Table 6-1: Influent and effluent characteristics in the two components of the two-stage continuous bioreactor system connected in series.

Parameter	Influent	Effluent (single stage reactor)	Effluent (two stage reactor)
Sulfate (mg/L)	2800	<500	<100
COD (mg/L)	3400	2200	<15
Iron (mg/L)	950	<0.5	<0.5
pH	2	7.9	>8

6.4 Conclusion.

The bioremediation of AMD using a two-stage bioreactor system showed great potential in terms of the removal of COD, iron, and sulfate. The pH of the AMD was also improved to pH

above neutral due to biogenic alkalinity generated through the activities of SRB. Charcoal did not play a significant role in the removal of dissolved iron and sulfate from the AMD.

Compared to the performance of the first unit of the system, the combined two-stage process showed that bioremediation might be improved through multistage systems. Multistage systems are important in that they do not carry much of the metal precipitates to the next stage reducing the inhibitory effects of metal precipitates in the next reactor. In single stage bioreactors treating AMD with high sulfate and dissolved metal ion concentration, large amounts of hydrogen sulfide and metal sulfide precipitates are produced. Hydrogen sulfide and metal sulfide precipitates are toxic to the biomass. The accumulation of the products in the bioreactor leads to system failure.

Chapter 7
Cost evaluation for designing, constructing, and operating a pilot plant for the treatment of AMD using FTWW as an organic electron and carbon source.

7.0. Introduction.

In this study, waste effluent produced by Fischer Tropsch process was identified as the organic feed in a 2-litre bench scale bioreactor capable of treating 2 litres of AMD per day. Scaling of the bioreactor is necessary to improve on the quantity of wastewater to be treated. An ideal bioreactor should be able to treat at least 10 000L of AMD per day.

The choice of bioreactor used in the treatment of AMD should allow for ease of operation maintenance and waste removal. The bioreactors should be tailor made according to the water chemistry and the type of carbon source used for treatment. To treat large flows of AMD, the bioreactor should be able to retain biomass. Fixed bed bioreactors (FBRs) are good biomass retainers because they contain material on which bacteria can attach and grow to form a biofilm. Cheaper biofilm matrix such as charcoal can be used for microbial attachment. This removes the need for special mechanisms such as membrane filters to separate the biomass from the effluent (Tekere, 2019) In this chapter, the design and construction and operational costs of a fixed bed bioreactor capable of treating 10 000 litres of AMD are considered.

7.1. Cost considerations.

The cost of constructing and operating a bioreactor for the treatment of AMD is based on the AMD chemistry, the type of materials required to build the reactor, the level of automation of the bioreactor, the cost and availability of the organic material to be used as the organic feedstock, the cost of transporting the organic feedstock to the treatment site and the terrain of

the area where the treatment plants will be located. Once the reactor is in operation labour and maintenance costs should be factored in.

7.1.1. Construction material.

Because of the corrosive nature of AMD, the pilot plants should be made from acid and corrosion-resistant material. Stainless steel, carbon steel or plastic tanks and pipes may be used because they are resistant to corrosion (Willquist *et al*., 2015). High-density polyethylene (HDPE) or polypropylene material may be used for the construction of the bioreactors, with modifications to allow for the accommodation of the carrier matrix (charcoal). The capacity of the tanks also contributes to the cost as larger tanks cost more to construct than smaller tanks. Other secondary processes in reactor construction include temperature controls and process automation. Indeed, incorporating temperature control mechanisms will add to the cost of construction, but the overall long-term cost will be lower because temperature-controlled reactors are smaller and more efficient compared to reactors operated at ambient temperatures. This is because the temperature can be adjusted to suit the microorganisms in the bioreactor thus improving the efficiency of the bioreactor (Willquist *et al*., 2015). Costs of insulation or temperature control can be totally avoided by building underground reactors. Maintenance and energy costs are reduced by building the reactors underground (Willquist *et al*., 2015). Pumping costs may be reduced by designing gravity-controlled channel flows.

The removal of toxic hydrogen sulfide should be a consideration in the design of bioreactors treating AMD. This may be done by incorporating a second sulfur oxidising reactor after the sulfate reducing bioreactor (Figure 7-1). The sulfur oxidising reactor should be equipped with air pumping mechanism to allow for controlled aeration to create oxygen-limiting conditions. Under oxygen limiting conditions, the hydrogen sulfide produced in the sulfate reducing bioreactor is oxidised to hydrophilic (non-clogging) elemental sulfur. Elemental sulfur is

valuable, helping to potentially recover capital and operational costs through its sales. The treated water can be recycled to dilute the AMD/FTWW feed inorder to reduce the sulfate, metal as well as the organic load.

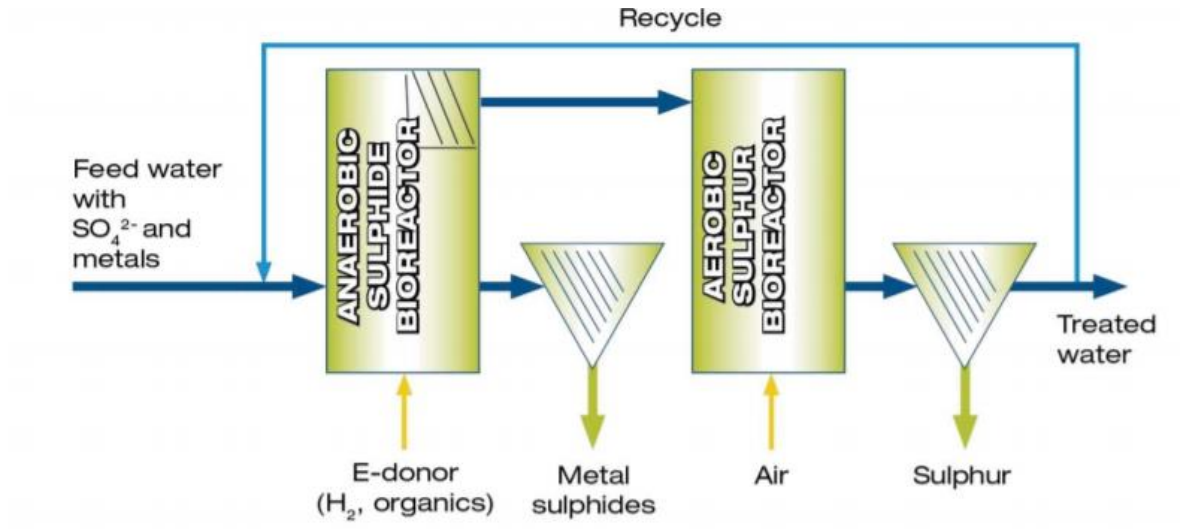


Figure 7-1: Schematic representation of a two-stage bioreactor system for AMD treatment and sulfur recovery (Source: Dama-Fikir, 2017).

7.1.2. Cost of Organic feed.

A wide variety of organic substrates has been tested for their ability to sustain biological sulfate reduction. These include simple organic material that are readily available to SRB. These substrates include short chain fatty acids and alcohols, soluble sugars, and amino acids (Skousen *et al*., 2017). These substrates are easily metabolised by SRB and can be depleted quickly. Continuous supply of the organic material is therefore required. Complex organic substrates such as lignocellulose are not available to SRB. These substrates need to be degraded and fermented to alcohols and acids before they are used by SRB. These processes are facilitated cellulose degraders and fermenters. The degradation of cellulose is a rate-limiting step and slows down the rate of treatment of AMD (Magowo *et al*., 2014). To improve on the treatment of AMD, it might be necessary to use simple organic compounds. Commercial

organic compounds such as methanol, ethanol and lactate are expensive. The large volumes of these simple products needed for biological sulfate reduction makes the treatment unsustainable due to high procurement costs. The costs involved in procuring substrates may be avoided if industrial or agricultural wastes rich in simple organic substrates are used, as these are waste products that needs to be disposed.

In this study Fischer Tropsch wastewater (FTWW) was used as the organic source for sulfate reduction. FTWW is rich in short chain fatty acids and alcohols that can easily be metabolised by SRB. The assumption is that AMD and FTWW can be potentially produced close to each other. FTWW is a waste material therefore will not have a great impact on the overall operational costs. The FT process produces large volumes of FTWW, meaning the substrate is readily available in sufficient quantities to sustain long-term treatment processes.

7.1.3. Transportation of organic feed to the treatment site.

FTWW is produced by the FT-process. The FT process produces liquid fuels from the liquefaction of coal. The coal to liquid (CTL) process requires large amounts of coal. It is for this reason that CTL plants are likely to be located near coalmines. Significant amounts of coal can only be found in areas with abundant coal reserves. In South Africa, the production of liquid fuels is done by SASOL. According to Hook *et al*., (2014), Sasol hosts the largest coal to liquids operation globally producing about 150 000 barrels of fuel per day, which requires over 150 000 tonnes of coal per day. The environmental concerns associated with both the coal-mining activities and the CTL process include the production of large amounts of wastewater. The mining of coal has the potential to lead to the production of large volumes of AMD. AMD can potentially introduce large amounts of inorganic pollutants including metals and salts to receiving waters if proper management is not practiced (Johnson *et al*., 2016). The production

of liquid fuels through the liquefaction of coal produces FTWW. These two effluents are likely to be produced in large volumes close to each other, shortening the distance required for transportation.

The organic feed (FTWW) in the AMD treatment plants needs to be transported to the treatment site. Since FTWW is a liquid, waterproofed drains or pipework may be used to transport the FTWW. The costs of constructing the drains or laying the pipe works depend on the terrain and the distance between the source of FTWW or AMD and the treatment site. The material used for the drains and/or the pipework should be acid proof to avoid corrosion. Pumping might be required in cases where the terrain does not allow for gravity flow. In such cases, additional costs for pumping equipment and power supply should be considered. Power requirements can be met by generating solar energy using photovoltaic cells; this can be included in the capital costs.

7.1.4. Microbial Kinetics.

The microbial kinetics also have an influence in the size and therefore the costs of reactor construction and operation. The microbial kinetics are affected by temperature, pH, chemistry of the AMD, flow rate and residence time. Higher metal concentration requires larger SRBR, a lower flow rate and higher residence time.

7.1.5. Downstream processing costs AMD effluent.

The sludge produced in the treatment of AMD using biological sulfate reduction contains sulfides of iron, zinc, copper, lead, magnesium, and other heavy metals. The sludge is potentially toxic due to the presents of heavy metals and radioactive material (Cirne *et al*., 2008). Though the sludge has a lower volume than the sludge obtained from chemical treatment plants, it still needs to be processed to ensure environmental safety. The volume of the sludge can be further reduced by removing water through evaporation or crystallization. The sludge

can then be disposed in landfills. Dissolved hydrogen sulfide should be removed from the effluent, as hydrogen sulfide is a health hazard and may cause damage to equipment due to its corrosive nature.

The treatment of AMD can produce some valuable resources, such as metals. Valuable metals can be selectively recovered from the reactors by manipulating the pH in the reactor (Buisman *et al*., 1999; Kousi *et al*., 2015). However, this result in cost addition in the construction and operation of the reactors, the costs can possibly be recovered from the sale of the valuable metals.

The removal of excess hydrogen sulfide is of utmost importance as it reduces the toxicity and corrosive characteristics associated with hydrogen sulfide. More metals can be used to remove the hydrogen sulfide as metal sulfide precipitate. This increases the costs of treatment, as more chemicals need to be added to the effluent. Sulfur oxidising bacteria may be used to oxidise the hydrogen sulfide to sulfur, which is easier to remove from the bioreactors and may be used as a raw material for the manufacture of fertilizers. This can be used to offset the construction and operational costs. Removal of hydrogen sulfide also ensures longevity to the treatment system as the threat of corrosion is removed, reducing costs associated with maintenance. Costs can also be recovered by using the effluent for irrigation or can be reused for cooling in the FT process.

7.1.6. Storm water and precipitation.

The rainy season brings the challenge of exposing the SRBR to high loads of influent water due to precipitation and storms. The SRBR should be constructed to resist such water influxes. Gusek (2004) suggested the application of runoff diversion channels to divert water from the bioreactor. The construction of these channels is an unavoidable cost addition.

7.1.7. Labour costs.

The labour costs are expected to be high during the construction and installation of the reactors. These include the cost involved in excavation and laying the drainage system and installation of bioreactors and other accessories such as power. Once the installation of the bioreactors is complete, the costs are expected to be significantly lowered during operation with the occasional maintenance being the most significant operational cost. Automation of the bioreactors would significantly lower the labour costs however larger capital costs will be required.

7.2. Costing Methodology.

The design, construction and operational costs were based on the application of an underground fixed bed sulfidogenic reactor, where the organic material provides the carbon and electrons necessary for sulfate reduction and an above ground sulfur producing bioreactor. In the sulfidogenic reactor, sulfide is generated accompanied by metal precipitation as metal sulfides and alkalinity generation. The sulfidogenic bioreactor is connected in series to a second sulfur oxidising reactor, where excess hydrogen sulfide is oxidised in limiting amounts of oxygen to hydrophilic non-clogging sulfur, which can be separated from the liquid (Damir-Firker *et al*., 2017). The cost estimates were obtained from a bill of quantities from DEWdrop Ecological Treatment Systems (South Africa). For affordability, the study evaluated the cost of construction using the cheapest materials available. The cost will be significantly high if expensive material such as stainless still were used instead. Gusek (2002) estimates for designing and complete construction of bioreactors treating AMD ranges from $300 000 to $1.36 million excluding operating costs, which is very high compared to the proposed system, where the total cost for installation of both the sulfidogenic bioreactor and the sulfur-oxidizing reactor is estimated at 113845.18 Rand. However, these costs excluded the costs of laying the

pipe works (drainage system). Which were quoted at 135 Rand /meter including labour. The costs of installing the pipe work are determined by the distances between the point of collection of the FTWW and/or the AMD and the point of discharge. The terrain of the specific site should also be considered. Table 7-1 and Table 7-2 provides the cost description of the installation of the sulfidogenic and the sulfur oxidizing bioreactors respectively.

The total capital costs are also determined by the chemistry of the water and the flow rate. High sulfate and dissolved metal loads may require that several sulfate reducing bioreactors be connected in series or parallel to each other to allow for increased efficiency in the removal of sulfate, metals, and COD from the wastewater. This is the case in this study where single stage continuous bioreactors were not able to completely remove both COD and sulfate. A second bioreactor connected in series was needed to improve the performance of the system.

Table 7-1: Capital cost estimates for a 2000 L sulfidogenic bioreactor treating AMD.

Item description	**Unit**	**Quantity**	**Rate (Rand)**	**Amount (Rand)**
Supply and install bioreactor, including excavating and backfilling, complete with provision for bedding		1	44775	44775
Supply and install (110mm) diameter sewer pipes (Inlet and outlet), including backfilling	m	5	135	675
Provision for the construction of small outlet headwall for 110 mm		2	2500	5000
Total				**50450**

Table 7-2: Capital costs for the supply and installation of an aerobic sulfur oxidising bioreactor

Item description	Quantity	Unit	rate (Rand)	Total (Rand)
Supply 2.5KL natural roto moulded tank	1		3190	3190
Provision of modification of tanks	1		333,5	355,5
Supply 1200mm X 1200mm thick fibreglass grating, cut to fit tank and accommodate pipe entry points	2		955,34	1910,68
Provision for supply and installation of 110mm diameter PVC inlet and outlet pipes	10		850	8500
Installation of 110mm floating siphon discharging to 50mm outlet pipe	1		750	750
Supply and install 400 mm photovoltaic system complete with solar cable and charge controller	1		32133	32133
Supply and install 200W invertor	1		2900	2900
Provision for supply and installation distribution board, complete with switches and digital time switches	1		1500	1500
Supply weatherproof electrical box	1		2156	2156
Supply and install air blowers complete with diffusers	1		10000	10000
Total				**63395,18**

7.3 Conclusions

The capital costs involved in the installation of biological treatment plants treating AMD are site specific, and based on the water chemistry, land terrain and the availability of low-cost organic carbon sources. Semi active treatment plants and passive bioreactors have the least construction costs, though treatment efficiency maybe compromised.

Chapter 8
Overall Study Conclusions

Objective 1: Combined remediation of AMD and FTWW (FTWW as an electron source for biological sulfate reduction).

Biological sulfate reduction appears to be a suitable process for the co-treatment of AMD and FTWW and or other organic waste materials. Its novelty lies on the application of FTWW to supply electron requirements for the co-treatment of the two waste streams using biological sulfate reduction. In this study, iron was effectively removed from AMD through iron sulfide precipitation and sulfate was removed to levels below disposal limits. The removal of other dissolved metal ions such as cobalt, nickel, manganese, and magnesium from the AMD was not determined. However, various researchers have indicated that these metals can be removed through metal sulfide precipitation at different pH values. Johnson *et al*., (2016) mentioned that manganese (II) forms insoluble sulfide or hydroxide precipitates at relatively higher pH values. Manganese removal cannot therefore occur at low pH values. At moderately lower pH, the manganese removal efficiency ranged from 40-80%. Though this study did not confirm the removal of other dissolved metals, many studies have confirmed that copper, cobalt, nickel, and zinc can be removed effectively from bioreactor through metal sulfide precipitation (Hussain and Qazi, 2016).

The main objective of this study was to evaluate the possibility of using FTWW as an electron and carbon source for biological sulfate reduction in the treatment of AMD. Bioreactors were operated as batch, fed batch and continuous flow reactors. Both single stage and two stage bioreactors were assessed for their ability to remove COD, sulfate and iron and to attenuate pH. The results in all the trials were promising and showed that FTWW support biological sulfate reduction in plants treating AMD. To the best of the authors Knowledge, this is the first time were the feasibility of using FTWW as a feedstock for biological sulfate reduction has

been assessed. The study shows the possibility of the combined treatment of the two waste streams (AMD and FTWW) using biological sulfate reduction. Biological sulfate reduction provides a cheaper treatment alternative of these waste streams in areas where they are prevalent. The anaerobic sulfate reduction methods remove the need for aeration, thus significantly reducing sludge production and removing the energy costs required for aeration. The results of this study can be a basis for further development of the strategy.

Objective 2: Recovery of valuable products (Production of elemental sulfur from biogenic hydrogen sulfide).

The use of biological sulfate reduction in the treatment of AMD can potentially produce substances of economic value. In this study, the hydrogen sulfide produced in the sulfate reducing bioreactor was oxidised under oxygen limiting conditions to produce elemental sulfur. Substantial amounts of sulfur were produced to consider biogenic sulfur as an income source. The benefits of removing sulfur from the bioreactor is that it is easy to remove and has more value than sulfuric acid. An additional economic importance is that it removes hydrogen sulfide, thereby preventing corrosion of equipment downstream. Another economic benefit of the treatment of AMD is the potential recovery of valuable metals through selective precipitation of metal sulfides in different pH gradients. The sale of these valuable products would offset capital and operational costs in the treatment process.

Objective 3: To find new cheaper substrates for biological sulfate reduction.

This study is very significant in that it reinforces the use of organic rich industrial and/or agricultural wastes in the treatment of AMD and other sulfate rich effluents using biological sulfate reduction. This technology is especially important in developing countries where complex technologies for wastewater treatment are either unavailable or costly to implement. In developing countries, industrial effluent is considered of no economic importance or is costly to treat and is therefore disposed with inadequate or no further treatment resulting in dire

environmental consequences. FTWW is produced in large quantities by the FT process. FT plants are usually located in areas with potential to produce large volumes of AMD. Although at Sasol for example, FTWW is used to generate power through the production of methane, most of it is treated aerobically resulting in the loss of carbon and energy as well as the production of large amounts of sludge. The low molecular weight organic substrates provided by FTWW are readily available for sulfate reduction and can therefore be used as substrates for biological sulfate reduction. Because of the large variety of short chain fatty acids and alcohols found in FTWW, it can support a wide variety of SRB improving on the efficiency of treatment. In this study, FTWW was used successfully with up to 99 percent sulfate and iron removal efficiency depending on the reactor's operational conditions. FTWW is a liquid, thus it is suitable for use in continuous reactors.

Objective 4: To develop and operate a bench scale sulfate reducing bioreactor for the co-treatment of acid mine drainage (AMD) and Fischer Tropsch wastewater (FTWW) using sulfate reducing bacteria.

Biological sulfate reduction was successfully carried in bioreactors. In this study, several bioreactor configurations were assessed for their ability to co-treat organic rich and sulfate rich effluents. The reactor configurations included a single stage batch reactor, a single stage fed batch reactor, a single stage continuous reactor and a two-stage continuous reactor. All the reactors were attached growth. The results showed that the single stage batch reactor was more effective in terms of COD, sulfate and iron removal as well as pH attenuation. The pH was raised to values above 7.0, while COD, sulfate and iron were removed to concentrations below the limits of the testing equipment. The performance of the single stage continuous reactor deteriorated with time because of the possibility of the accumulation of toxic metal sludge and hydrogen sulfide that might be inhibitory to the resident microbial community. These phenomena were also evident in the fed batch and batch reactors; however, because of the increased residence times in these reactors the effects were not as pronounced. Considering the

results, the batch reactor might be the ideal configuration to use. The disadvantages of using the batch reactor are that low volumes of wastewater are treated per time and there is need to remove the metal sludge before the new batch is fed into the reactor. This increases the labour costs as the reactor needs to be cleaned of sludge and new inputs need to be added. The start-up of each batch operation may be slow because the SRB will need to acclimatise to the new environment. Multistage sequential continuous reactors offer a solution to this setback. In this study, the two-stage treatment system achieved significant improvements in pH as well as COD, sulfate and iron removal compared to the single stage continuous reactor. The sequential arrangements of the sulfide reducing bioreactors means that the inhibitory sludge and sulfide are removed in the preceding reactor, therefore the subsequent reactor will have less sludge and hydrogen sulfide than the preceding one. Large volumes of AMD/FTWW waste can be treated to recommended standards for reuse or disposal because of the extended residence time in the multistage bioreactors (Damar-Fikir *et al*., 2017). The treatment of AMD can also be carried out in-situ due to the natural occurrence of SRB. The FTWW can be introduced directly to flooded underground mine workings or open pits. Passive AMD treatment systems are also an option. Constructed wetlands may be used were the AMD supplemented with the FTWW flows through. As the AMD passes through the wetland, natural processes such as biological sulfate reduction helps to remove the pollutants in the waste streams. This may remove the costs associated with construction, labour, power, and maintenance of bioreactors.

Objective 5: To investigate the effects of seasonal temperature variation on the efficiency of a continuous sulfate reducing bioreactor treating AMD.

Seasonal temperature variations are critical in the performance of bioreactors as they affect microbial growth and metabolism. Warmer temperatures encourage microbial growth and metabolism resulting in improved bioreactor efficiency. The bioreactors were negatively impacted in cooler temperatures where microbial reactions are slowed down. The average rate

of sulfate reduction decreased by 17% in winter, while COD removal rates decreased by as much as 40 %. In summer, the bioreactors produced an effluent with an average pH above 7.0, while due to the drop in temperature, the average pH in winter fell below 6.0, showing that low temperatures can negatively affect the performance of sulfate reducing bioreactors. Lower temperatures decrease the kinetics of biochemical processes. Bioreactors should therefore have mechanisms for temperature controls. The proposal would be to build the bioreactors underground to avoid energy costs.

Conclusions.

Biological sulfate reduction can be used to remove pollutants from AMD and FTWW and other organic rich effluents with a possibility to recover valuable substances. Attached growth bioreactors were successfully used for the treatment of a low pH, high metal and sulfate concentration AMD using FTWW as the electron donor for biological sulfate reduction. SRB can effectively remove COD, sulfate, and metal ions while raising the pH of the wastewater. However, the longevity of the bioreactors was not assessed on a pilot plant scale.

The use for biologically produced hydrogen sulfide to precipitate metals can substantially lower the cost of treatment as compared to using commercially available chemicals. The discharge of a hydrogen sulfide rich effluent can be prevented by converting the hydrogen sulfide to sulfur. Sulfur is marketable and can potentially add value to the treatment process.

Besides providing water with improved qualities and reducing the environmental impact associated with AMD, the treatment plants can provide employment to local communities during construction and operation of the reactors.

Because of the huge potential in removing organics, metals, acidity, and sulfates, at low costs, biological sulfate reduction appears to be the most suitable process for the co-treatment of organic rich wastewater and AMD.

Reactor configuration is important in obtaining a high degree of treatment efficiency. Batch reactors produce a much more improved effluent than continuous reactors; however, the treated volumes maybe low and additional labour costs may be required for the frequent clean-up of the bioreactors. A two-stage continuous flow bioreactor treating AMD showed great capacity in terms of sulfate, COD and iron removal compared to a single stage continuous flow bioreactor. Multi-stage sulfate reducing bioreactors are therefore recommended.

Temperature is a critical parameter in bioreactors treating AMD as low temperatures decrease the treatment efficiency by affecting the biochemical activities in the reactors. Specialized bioreactors with provision for temperature control or the construction of underground bioreactors could be used to eliminate the effects of seasonal temperature variations. This study also showed the resilience of sulfate reducing bacteria in the treatment of low pH and high metal and sulfate load wastewater under a non-limiting COD feed. The COD/sulfate ratio is important in dissimilatory sulfate reduction as limiting amounts of COD (COD/sulfate ratio below 0.67) may lead to reactor failure. In this investigation, the COD/sulfate ratio was kept above 0.9. The influent pH was 2.15, compared to an effluent pH above 7. Meaning the reactors were able to generate enough alkalinity to improve the pH of the wastewater.

The study reinforces the use of organic rich industrial and/agricultural waste as electron and carbon source for biological sulfate reduction. This study offers a promising biological co-treatment solution for AMD and FTWW, indicating that there is potential and merit in co-treating the two waste streams, thereby eliminating the risk posed by either one, with the potential to create a valuable product.

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Appendix A

Bioreactor optimization

The optimization of the bioreactors was done manually. The COD concentration (3400 mg/L) was kept constant in all the preliminary investigations while the sulfate concentration was varied to obtain COD/sulfate ratios of 0.9 (R1), 1.4 (R2) and 1.8 (R3). Bacterial activity was monitored through sulfate utilization and sulfide production as well as pH improvement in the reactors. The initial sulfate concentration did not significantly affect the sulfate removal efficiencies. In all the batch reactors, the sulfate concentration decreased rapidly in the first 200 hours. This was accompanied by a rapid increase in the concentration of sulfide (Figure A1-A4). The production of sulfide seems to be independent of the initial sulfate concentration (Figure A5). The sulfide concentration in all the reactors levelled off at 110 mg/L. The concentration of sulfate was lowered to less than 100 mg/L.

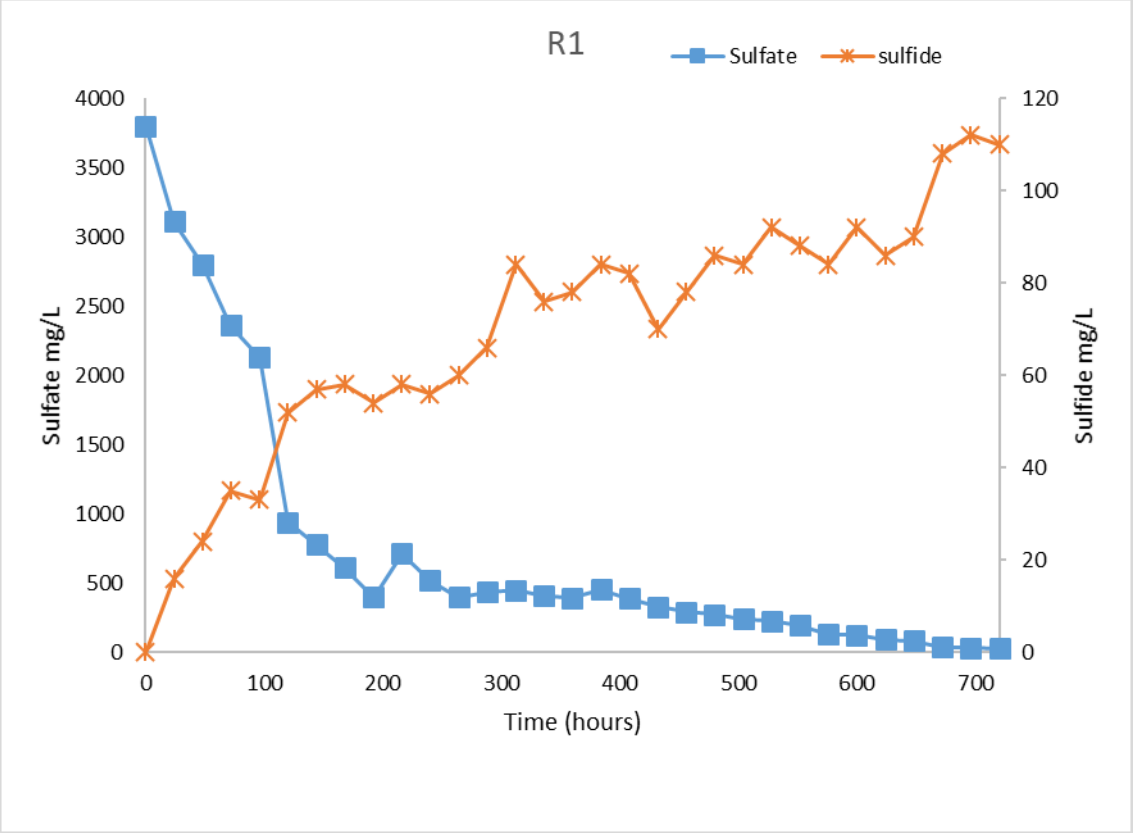


Figure A1: Sulfate removal at COD sulfate ratio 0.9.

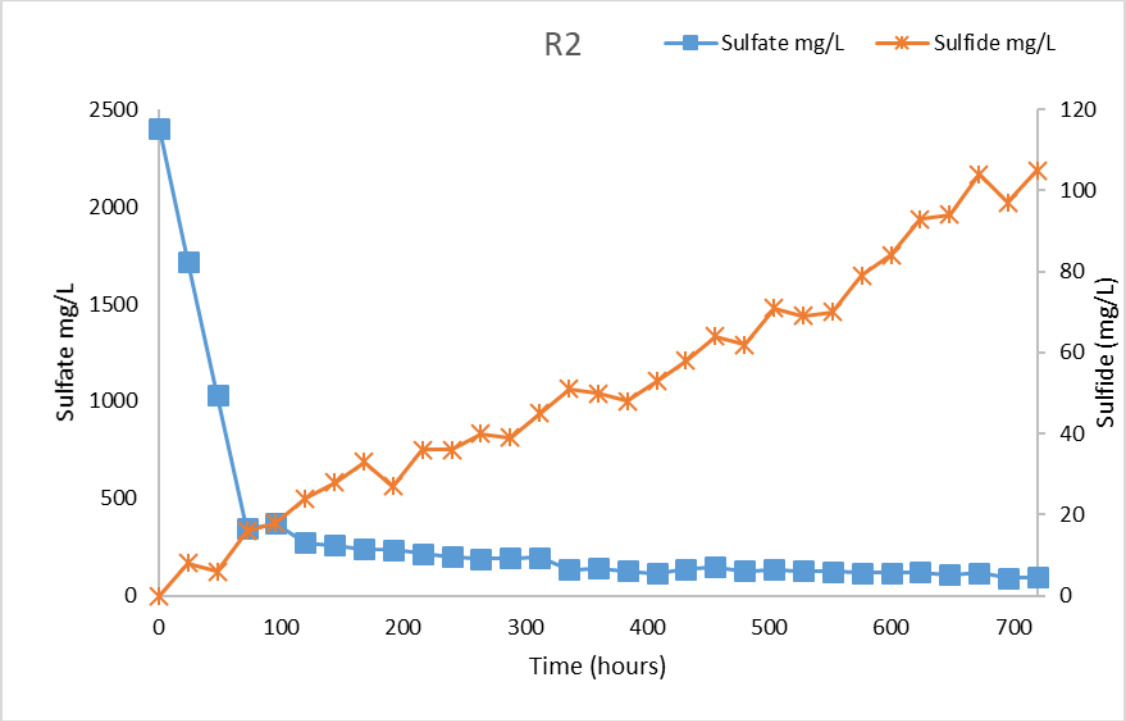


Figure A2: Sulfate removal at COD/sulfate ratio 1.4

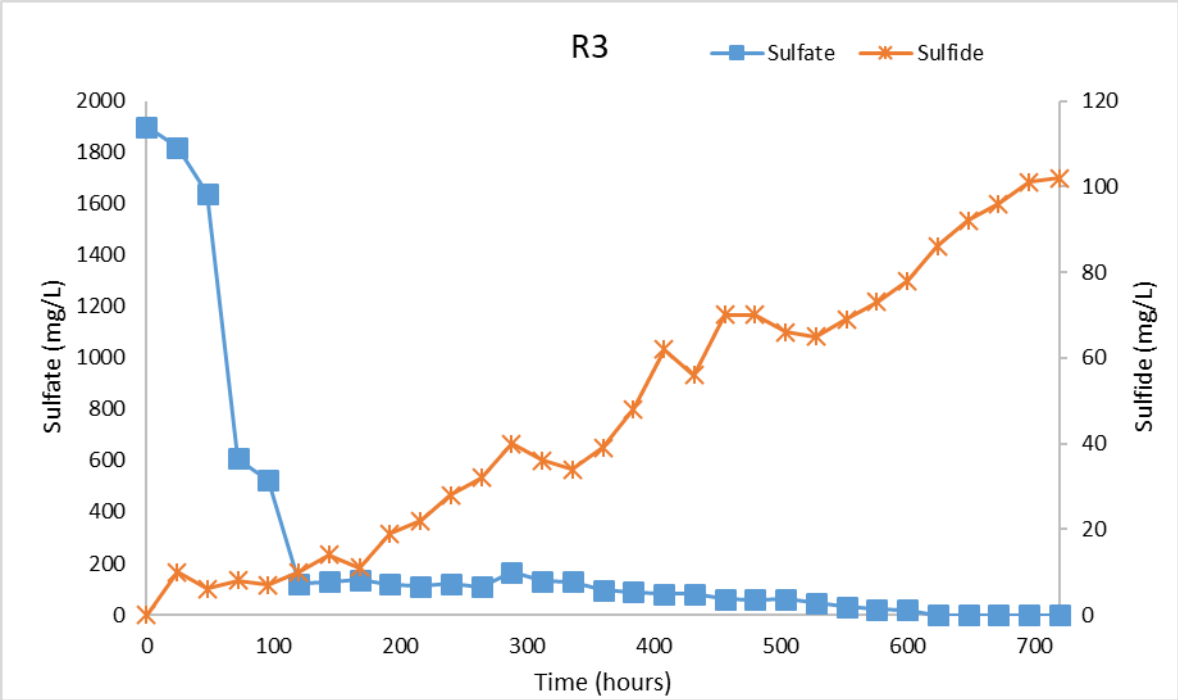


Figure A3: Sulfate removal at COD/sulfate ratio 1.8

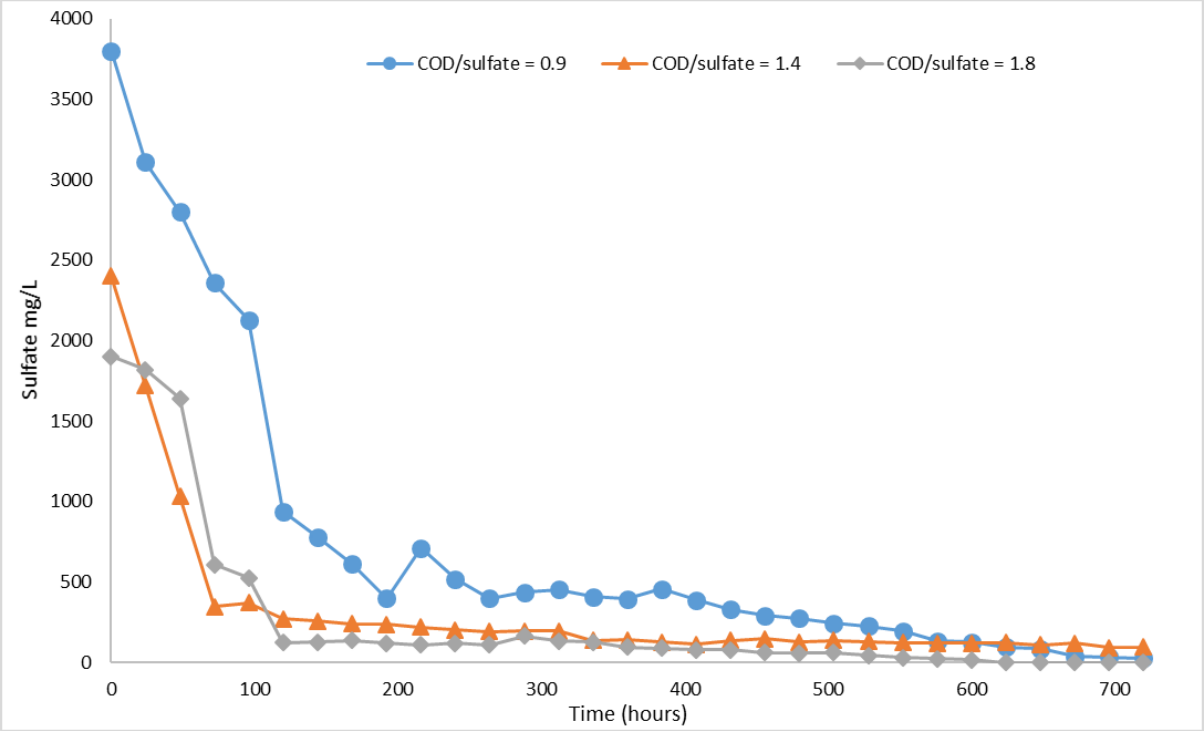


Figure A4: sulfate removal at different COD/sulfate ratios

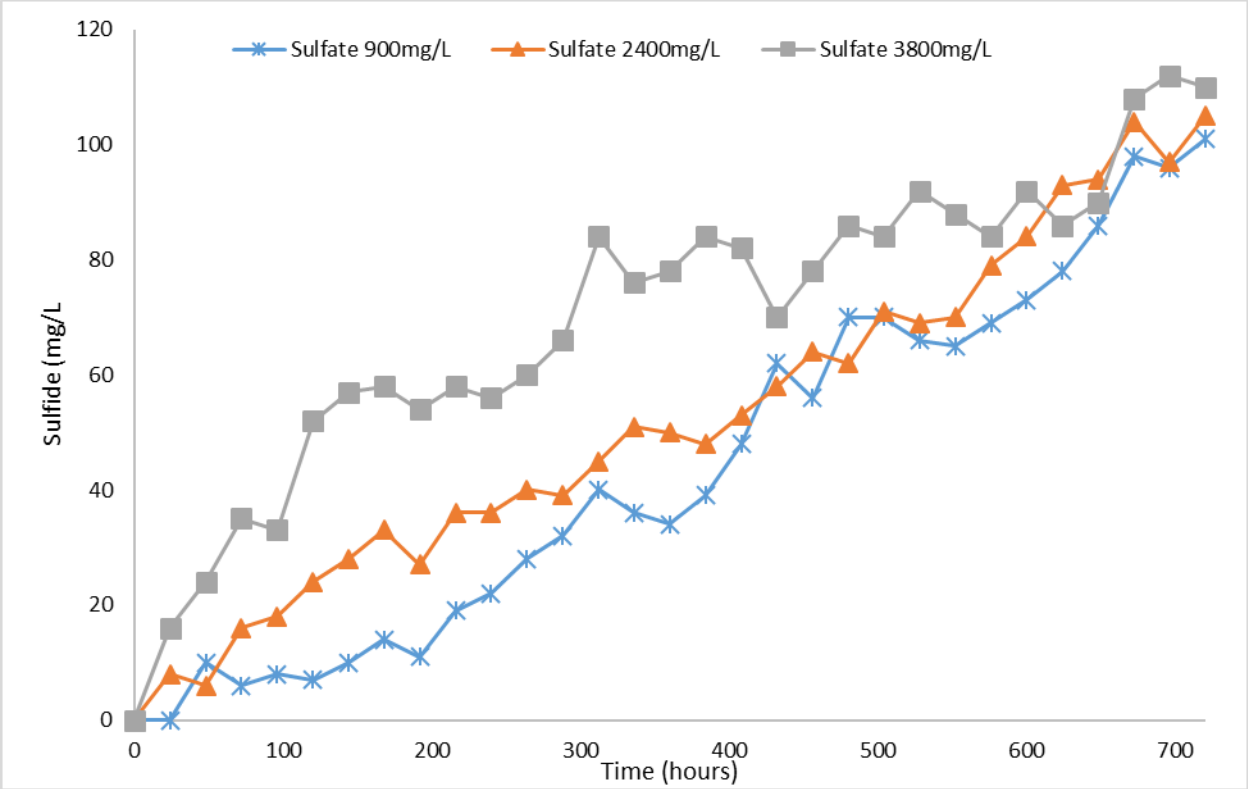


Figure A5: Sulfide production at different COD/sulfate concentration

Continuous operation

Preliminary trials were done to determine the most appropriate flow rate for maximum performance. A flow rate of 0.78 mls/minute was chosen for the experiments. The continuous reactors were initiated after steady state (t = 0). The reactors were operated under anaerobic conditions for 20 days at different COD/SO_4^{2-} ratios. Results indicate a good performance within COD/SO_4^{2-} ratios 0.9 to 1.8 (Table 1).

Table A8: Performance of reactors at different sulfate concentrations

	COD_{in} (mg/L)	$SO_4^{2-}{}_{in}$ (mg/L	COD/SO_4^{2-}	Ph_{in}	COD_{out} mg/L	$SO_4^{2-}{}_{out}$ mg/L	$S^{2-}{}_{out}$ mg/L	pH_{out}
R1	3400	3800	0.9	2.0	< 15	160	116	7.6
R2	3400	2400	1.4	2.0	< 15	120	100	7. 8
R3	3400	1900	1.8	2.0	< 15	86	110	7.5

Control: Investigating the effect of charcoal on removal of pollutants from AMD.

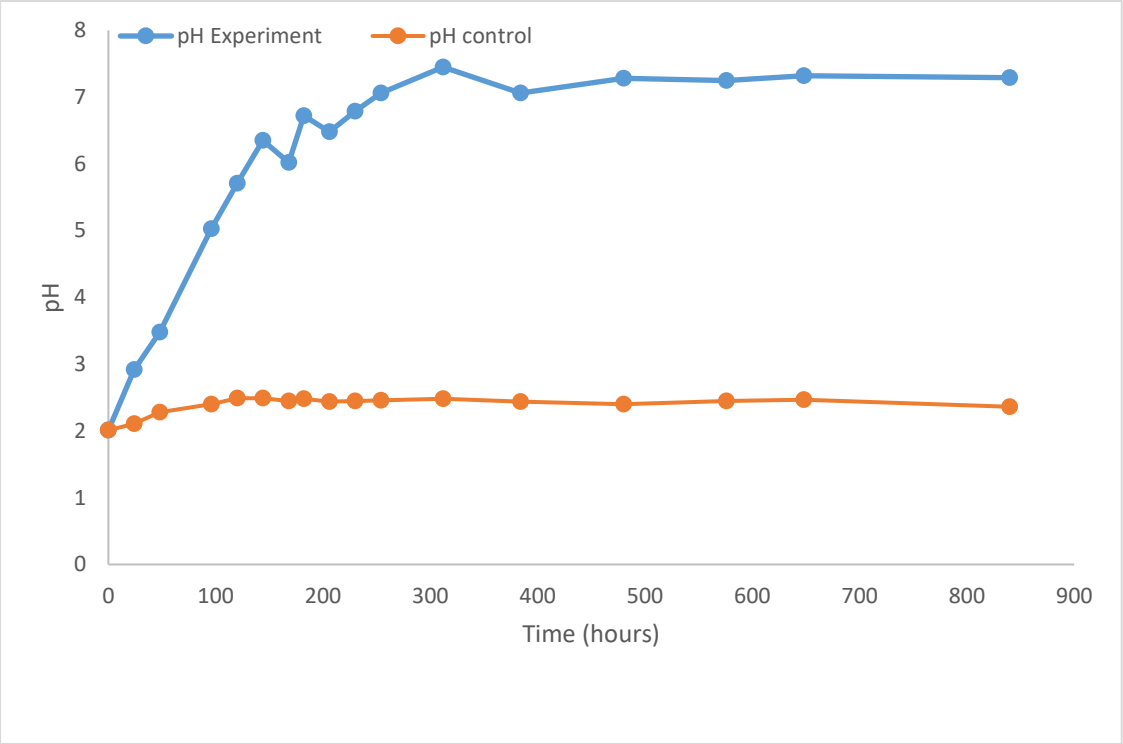


Figure A6: Changes in pH in the experiment and control of a bioreactor treating AMD using DSR.

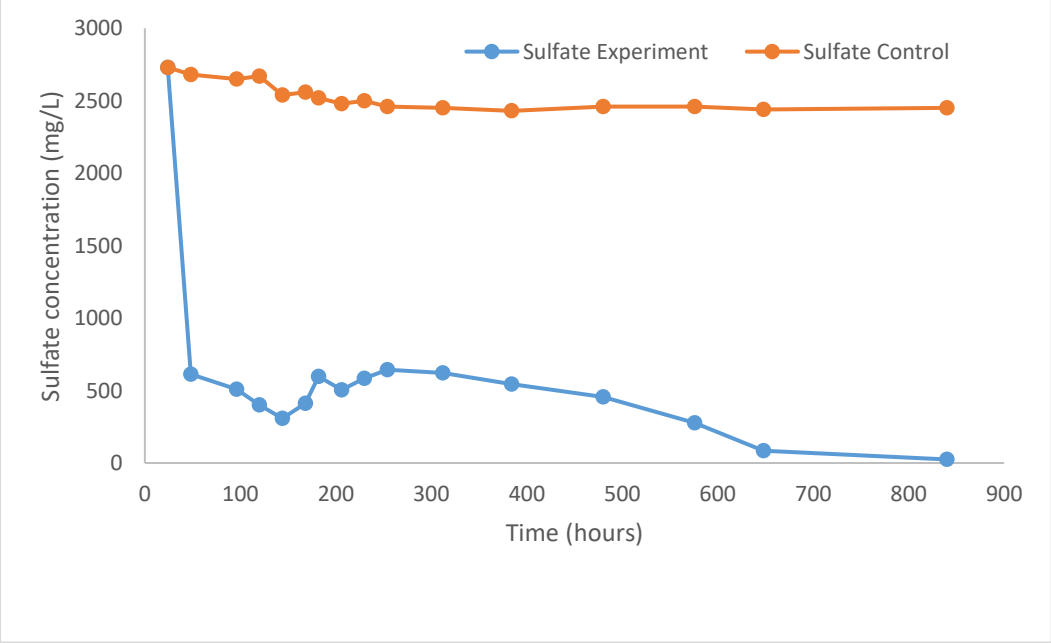


Figure A7: Changes in sulfate concentration in the experiment and control of a bioreactor treating AMD using DSR.

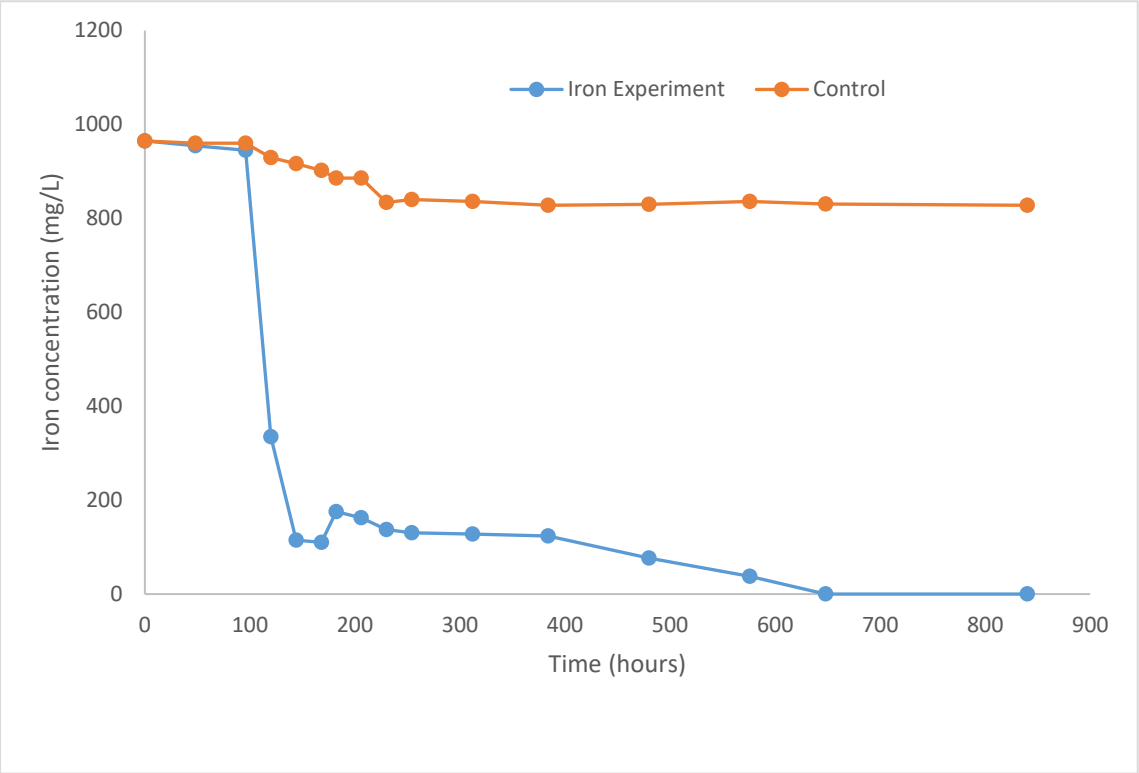


Figure A8: Changes in iron concentration in the experiment and control of a bioreactor treating AMD using DSR.

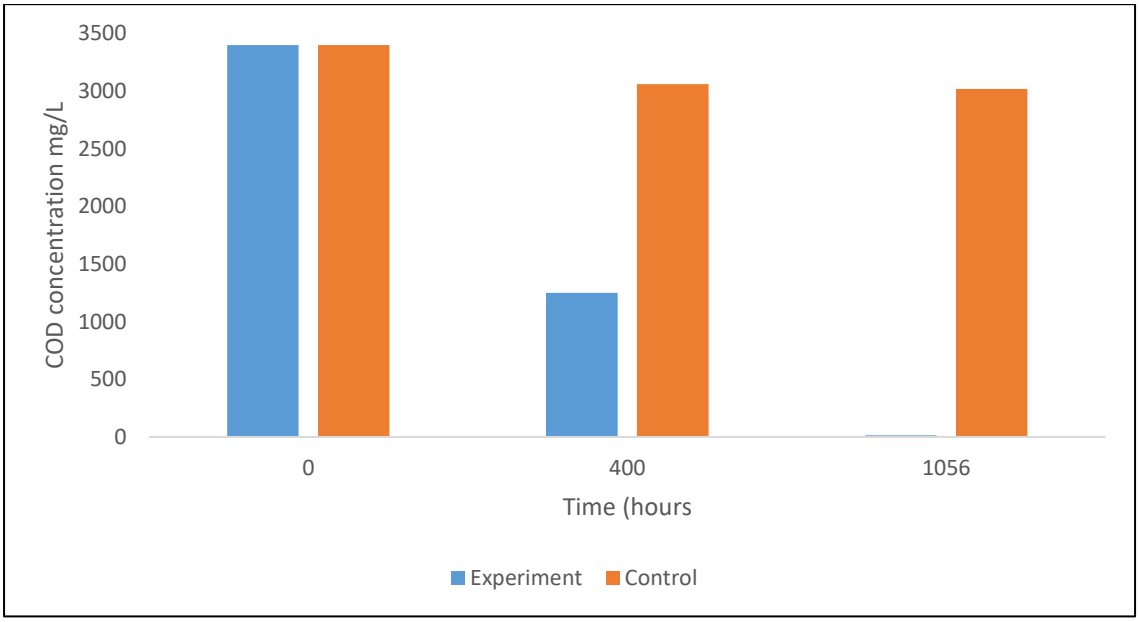


Figure A9: Changes in COD concentration in the experiment and control of a bioreactor treating AMD using DSR.

Appendix B: Organisms and reactions in biological sulfate reduction.

Table B9: Characteristics of some key Genera of sulfate reducing bacteria.

Group I Species	Electron donor	End Product	DNA (mol% GC)
Desulfovibrio	ethanol	H_2S	46-61
Desulfomonas	lactate, pyruvate, ethanol, glucose	H_2S	NA
Desulfotomaculum	lactate, pyruvate, ethanol	H_2S	37-46
Desulfobacula	Various aromatic compound, aromatic hydrocarbon toluene	H_2S, CO_2	42
Desulfobulbus	propionate	acetate, CO_2, H_2S	59-60
Desulforhopalus	Propionate, lactate, alcohols	H_2S	48
Group II Species	Electron donor	End Product	DNA (mol% GC)
Desulfobacter	acetate	oxidation to CO_2 via citric acid cycle, S^{2-}	45-46
Desulfococcus	C_1 to C_{14} fatty acids	complete oxidation to CO_2, S^{2-}	57
Desulfonema	C_2 to C_{12} fatty acids	complete oxidation to CO_2, S^{2-}	35-42
Desulfosarcina	C_2 to C_{14} fatty acids	complete oxidation to CO_2, S^{2-}	51