



Modelling a linear flow tubular reactor for high sulphate water treatment

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DECLARATION

I, ...Jan Bonakele Witbooi.... declare that my dissertation titled: *Modelling the linear flow tubular reactor for high sulphate water treatment*, is my work in implementation and design. Complete references are given to indicate and acknowledge the sources that are used in the dissertation. Furthermore, submission of the dissertation to other institutions has not transpired. The dissertation is submitted to the North-West University (NWU).

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ABSTRACT

The increased abandonment of mines in South Africa, along with current acid mine drainage (AMD) production from operating mines, continues to pollute clean water resources. Various methods exist to treat AMD, including passive and active options. Biological sulphate reduction (BSR) is a process that employs sulphate-reducing bacteria to treat wastewater by removing sulphate and heavy metals and producing sulphides. This process requires a carbon source, which acts as an electron donor for the bacteria. A 12 L linear flow channel reactor (LFCR) was designed and used for this study. Ethanol was selected as the carbon source. AMD and the inoculum (containing SRB) were mixed with ethanol and fed into the reactor at a flow rate of 84 mg/L. The reactor operated for 100 days, maintaining constant parameters with respect to temperature, feed flow rate, and ethanol volume. Variables such as the AMD/inoculum ratio, hydraulic residence time (HRT), and sulphate/COD ratio were adjusted. The operation was divided into three periods: Period I (40 days), Period II (30 days), and Period III (40 days). Period I showed better performance compared to periods II and III. This performance was due to SRB adapting to the reactor conditions and the inoculum being highly active. During this period, the pH remained neutral between 7 and 7.5. Heavy metal removal exceeded 95%, sulphate removal reached 99%, COD reduction was 95%, and sulphide production was 195 mg/L. Period II exhibited the poorest reactor performance due to the higher AMD/inoculum ratio, resulting in improved acidic conditions in the reactor. Although Period III demonstrated good performance, the biofilm's degradation prevented it from reaching the same level as that of Period I. Variations in HRT and the sulphate/COD ratio also influenced the reactor's performance. The combined effect of lowering the HRT and increasing the sulphate/COD ratio resulted in reduced sulphate removal efficiency. Overall, the reactor performed well in treating AMD. The reactor's performance in sulphate removal was modelled using linear regression and an artificial neural network (ANN). The linear regression model's performance was as follows: test R^2 was 0.42, root mean square error (RMSE) was 735 and mean absolute error (MAE) was 541. The ANN's performance was as follows: test R^2 was 0.75, RMSE was 483, and MAE was 305. The ANN significantly outperformed the linear regression. Adding more data points could enhance the accuracy of both models.

Key terms: acid mine drainage, biological sulphate reduction, sulphate-reducing bacteria, linear flow channel reactor, hydraulic residence time, linear regression, and artificial neural network.

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List of Abbreviations

ABC	Alkaline Barium Calcium
AMD	Acid Mine Drainage
AnMBR	Anaerobic Membrane Bioreactor
ANN	Artificial Neural Network
AOP	Advanced Oxidative Process
BSR	Biological Sulphate Reduction
Cd	Cadmium
COD	Chemical Oxygen Demand
CSTR	Continuous Stirred Tank Reactor
Cu	Copper
DPBR	Degrading Packed Bed Reactor
DSR	Dissimilatory Sulphate Reduction
FBR	Fluidised Bed Reactor
Fe	Iron
FSB	Floating Sulphur Biofilm
GLD	Green Liquor Dregs
GLSS	Gas-Liquid-Solid Separator
GSR	Gas Scouring Rate
HCl	Hydrochloric acid
HDS	High-Density Sludge
HRT	Hydraulic Residence Time
ICP-OES	Inductively Coupled Plasma Optical Emission Spectroscopy
IMPI	Integrated Managed Passive System
LFMR	Linear Flow Channel Reactor
LFMTC	Liquid Film Mass Transfer Coefficient
MAE	Mean Absolute Error
MBR	Membrane Bioreactor
MF	Microfiltration
MFV	Minimum Fluidisation Velocity
MPA	Methane-Producing Archaea
MSE	Mean Square Error
NaOH	Sodium hydroxide
NF	Nanofiltration

Ni	Nickel
NWU	North-West University
OLR	Organic Loading Rate
PBR	Packed Bed Reactor
PPE	Protective Personal Equipment
PRB	Permeable Reactive Barrier
PSS	Primary Sewage Sludge
PTFE	Polytetrafluoroethylene
PVC	Polyvinyl chloride
RBSR	Recycling Sludge Bed Reactor
RMSE	Root Mean Square Error
RO	Reverse Osmosis
SA	South Africa
SFV	Superficial Fluid Velocity
SGV	Superficial Gas Velocity
SOB	Sulphate Oxidising Bacteria
SRB	Sulphate Reducing Bacteria
SRT	Solid Retention Time
SRU	Secondary Sulphate Reduction Reactor
TCE	Trichloroethylene
TMP	Transmembrane Pressure
VFA	Volatile Fatty Acid
VCC	Vryheid Coronation Colliery
VSRR	Volumetric Sulphate Reduction Rate
UCT	University of Cape Town
UF	Ultrafiltration
USA	United States of America
UV	Ultraviolet
Zn	Zinc

CHAPTER 1 INTRODUCTION

1.1 Overview

The chapter begins with background challenges in treating acid mine drainage (AMD) and the use of biological reactors for its treatment. It then identifies the problem to be addressed by the study: the use of a linear flow channel reactor (LFCR) for AMD treatment, substantiated by modelling methods such as linear regression and artificial neural networks (ANN). The chapter also outlines the study aims and objectives, emphasises its significance for high-sulphate wastewater treatment, and defines the guiding hypothesis. The chapter finally provides a comprehensive overview of the dissertation's layout.

1.2 Background information

A threat to freshwater resources is the production of acid mine drainage (AMD). In South Africa (SA), AMD is produced through the minerals-producing industry, mainly mines (Knobel & Lewis, 2002). AMD is produced by the oxidation of sulphide-rich metals linked with metal ores and coal deposits (Marais *et al.*, 2020b). Specific environmental problems, such as high sulphate, dissolved heavy metal concentrations, and low pH, are associated with AMD (Maree *et al.*, 1987). Furthermore, the environment is also affected by waste rock dumps and discard heaps (diffuse sources) linked with coal mining, which discharges continuous, low-volume AMD (Marais *et al.*, 2020b; McCarthy, 2011a, 2011b).

The management of AMD is a specific challenge that requires economically sustainable treatment options. Underground mine basins discharge high-volume AMD-contaminated water, which can be treated using established active technologies (Marais *et al.*, 2020b). These technologies do not sufficiently reduce the sulphate load but address metal removal and neutralisation (Marais *et al.*, 2020b). Other strategies used to treat AMD include membrane filtration, neutralisation, and biological methods (Bai *et al.*, 2013; Dhir, 2018). These treatment methods are effective. However, they are expensive due to the high operational costs (Bai *et al.*, 2013; Dhir, 2018).

A treatment technology such as the anaerobic biological sulphate reduction has received increasing attention for treating high sulphate waters such as AMD (Knobel & Lewis, 2002). Throughout the biological sulphate reduction (BSR) process, an electron acceptor (sulphate) utilised by the sulphate-reducing bacteria (SRB) is reduced to sulphide (Marais *et al.*, 2020a, 2020b; Zhang & Wang, 2014b). Alkalinity is generated concurrently with sulphate reduction, leading to elevated pH and increased heavy metals precipitation as sulphides and carbonates (Knobel & Lewis, 2002). Microbes can use short-chain fatty acids or ethanol as an electron donor

and a carbon source for cell growth, and some microbes can use carbon dioxide as the carbon source and hydrogen as an electron donor (Knobel & Lewis, 2002).

According to Marais *et al.* (2020b) and Kiran *et al.* (2017), metal precipitation, wastewater neutralisation, and sulphate reduction can be addressed simultaneously based on the activity of SRB by using biological sulphate-reducing bioreactors. The primary focus of the studies related to the AMD treatment through the application of SRB has been on active systems (bioreactors) (Kiran *et al.*, 2017). Sulphidogenic bioreactors that have been used include fluidised bed reactors (Kaksonen *et al.*, 2003a; Kaksonen *et al.*, 2003b; Kaksonen & Puhakka, 2007; Sahinkaya *et al.*, 2007a, 2007b), various membrane bioreactors (Kaksonen & Puhakka, 2007), anaerobic sequential batch reactors (Costa *et al.*, 2009), up-flow anaerobic sludge beds (Cunha *et al.*, 2019; Rodriguez *et al.*, 2012), and the anaerobic fixed-bed reactors (Maree & Strydom, 1985; van Rooyen *et al.*, 2021). The application of these active systems has limitations, mainly due to costly electron donors such as volatile fatty acids and ethanol, the comparatively slow SRB kinetics, and the control of the more toxic sulphide product (Marais *et al.*, 2020b; van Hille *et al.*, 2015). The packed bed reactor has limitations, such as bed degradation causing hydraulic conductivity issues, and its performance is impacted by the chemical oxygen demand (COD)-limited environment (van Rooyen *et al.*, 2021). A robust process can be developed by addressing each of these challenges.

The suitable treatment of sulphate-loaded wastewater can be achieved by having a sulphide removal step (Harrison *et al.*, 2014a; Jin *et al.*, 2007; Marais *et al.*, 2020b). Hazards associated with sulphide include its toxicity, malodour, corrosivity, and potential for re-oxidation (Harrison *et al.*, 2014a). Under oxygen-limiting conditions and the presence of sulphur-oxidising bacteria (SOB), hydrogen sulphide is oxidised to sulphates, leading to the production of elemental sulphur as an intermediate (Cai *et al.*, 2017; Kleinjan *et al.*, 2005; Marais *et al.*, 2020b). A composite linear flow channel reactor (LCFR) with the potential to integrate both the BSR process and partial sulphide oxidation can be used to treat AMD (Marais *et al.*, 2020b). The reactor delivered a satisfactory performance for the removal of sulphates in AMD, and it can handle the more toxic sulphide by using the development of the floating sulphur biofilm (FSB) (Marais *et al.*, 2020b; Mooruth, 2013). In addition, effective sulphide removal can be achieved, and harvesting the biofilm can ensure effective partial sulphur recovery (Mooruth, 2013).

In recent years, mathematical models have been developed for sulphate reduction reactors. According to Knobel and Lewis (2002), a model developed for the BSR process must account for sulphide, pH, fatty acid, and hydrogen inhibition; be applicable for popular reactor types; and be valid for several carbon sources. However, some models, similar to the model developed by

Costello *et al.* (1991), did not consider all possible electron donor substrates due to oversimplification (Knobel & Lewis, 2002). Additionally, the model by Knobel and Lewis (2002) did not consider sulphur recovery from the partial sulphide oxidation process. Therefore, mathematical models do not take into consideration all the complex dynamics that affect the biological sulphate reduction process. Several studies concluded that along the reactor height, the mathematical models do not precisely describe the liquid flow and the distribution and transport of components (Kalyuzhnyi & Fedorovich, 1998). Artificial neural networks (ANN) are used to model and predict AMD treatment performance by varying process parameters such as the influent concentration of sulphate, COD, and sulphide (Hutmacher & Singh, 2008). Finally, the present project aims to assess the effect of changes in the operating conditions of the LFCR and the changes in the SRB sizes on the hydrodynamic performance and to investigate the treatment performance of the LFCR using ANN modelling.

1.3 Problem statement

Different bioreactors, such as anaerobic membrane reactors (AnMBR), fluidised bed reactors (FBR), and up-flow anaerobic sludge beds (UASB), have utilised the BSR process for AMD treatment. However, drawbacks associated with these bioreactors include heavy sludge formation, membrane fouling, bed degradation, and accumulation of toxic hydrogen sulphide, which affect the BSR process and result in low sulphate and sulphide removal efficiencies, thus causing health and environmental risks (Austigard *et al.*, 2018).

A linear flow channel reactor (LFCR) is a cost-effective and environmentally friendly treatment system. The LFCR is considered an alternative bioreactor for use in a full-scale BSR treatment plant due to its reduced reliance on carbon sources, as the reactor has the potential to combine sulphate reduction and sulphide oxidation. Some studies have used both a small and a larger LFCR for AMD treatment, and higher sulphate removal efficiencies (>90%) have been reported (Marais *et al.*, 2022). However, the medium-sized LFCR has not been tested yet, and the study will present it as a potential treatment for AMD by removing sulphates and reducing the sulphide concentration.

1.4 Aim and Objectives

1.4.1 Aim of the study

The project aims are to assess the effect of changes in reactor operating conditions and the amount(s) of sulphate-reducing bacteria (SRB) on reactor performance, and to model the reactor performance.

1.4.2 Specific objectives

The following objectives should be adhered to the aim of the study and thus achieved:

- To assess the influence of varying operating conditions such as AMD volume, hydraulic retention time (HRT), and sulphate/COD ratio on the performance of the reactor.
- To determine the influence of the changes in the SRB community sizes on the reactor performance.
- To determine the influence of the structure of the floating sulphur film on the reactor performance.
- To model the treatment performance of the reactor using a linear regression and an artificial neural network.
- To compare the obtained artificial neural network model to the linear regression model.

1.5 Significance of the study

AMD in South Africa requires biological treatment systems that are both economically viable and effective. The study focused on applying the sulphate reduction process utilising a sulphidogenic bioreactor (LFCR) to increase the removal efficiency of metal ions and sulphates from AMD, improve effluent pH, and reduce sludge formation. The study used a medium-sized LFCR (10.4 L) with ethanol as the carbon source. There is limited literature on modelling the hydrodynamic performance of a biological reactor treating AMD, and the treatment performance of the LFCR treating AMD has not been modelled using data analytics such as ANN or linear regression. ANN and linear regression models were used to model the reactor performance.

1.6 Hypothesis

Increasing the hydraulic retention time (HRT) can result in a high sulphate removal rate. Using the large size of the SRB community can result in a high sulphate removal percentage due to increased microbial activity. A fully developed FSB can provide a suitable microenvironment, leading to increased process efficiency. The sulphide concentration in the effluent can be reduced by sulphide oxidation. Finally, the ANN models can provide treatment performance predictions that are similar to the experimental data.

1.7 Dissertation layout

This dissertation consists of five chapters, which are as follows:

Chapter 1: Introduction

In the first chapter, the background of the study is discussed, and the research path is also outlined. The study background discusses the generation and impact of AMD, as well as the potential treatment methods that can be used to treat AMD. Other issues that are outlined include the advantages and disadvantages of applying neutralisation and membrane filtration methods. The background also outlines the following applications: different bioreactors used for the biological sulphate reduction process and mathematical modelling utilised for the sulphate reduction process. The chapter also presents the problem statement, the research objectives and aim, the importance of the research, and the hypothesis.

Chapter 2: Literature Review

In the second chapter, the existing information is discussed in detail to highlight AMD production and its effect on the surroundings. In addition, treatment methods such as neutralisation and membrane filtration are analysed and reviewed, and the benefits and drawbacks of using these processes are outlined. The use of biological processes such as sulphate reduction is analysed and reviewed, and the application of this process using different bioreactors is outlined in detail. The utilisation, adaptation, and activity of the SRB are also outlined thoroughly. The existing information on the modelling of bioreactor performance is also discussed.

Chapter 3: Materials and methods used to obtain experimental data and artificial neural network models

Chapter 3 describes the materials and methods used to obtain experimental data. The materials used during experimentation are specified in terms of their size and the reagents' purity. The experimental set-up (bioreactor) used for experimental runs is presented and labelled accordingly. The bioreactor parameters that varied during the experimental runs, regarding values and periods of change, are provided in detail. The experimental run procedure is explained in detail, and the operating conditions of the reactor, along with analysis methods, are discussed, including the names of the equipment used for analysis. ANN and linear regression models are derived to model the performance of the bioreactor are presented in this section. The steps followed as well as the assumptions made to derive the models, are explained in detail.

Chapter 4: Experimental and artificial neural network modelling results and discussion

The fourth chapter focuses on the results obtained from the experiments that were conducted. The linear flow channel reactor performance was evaluated in detail using the sulphate, sulphide, and heavy metal concentrations. The results were then compared to the literature. The impact of changes in operating conditions and microbial sizes on the reactor's performance was highlighted

and thoroughly discussed. The FSB formation is described using figures, and the elements present in the FSB are provided. The ANN and linear regression models are presented and compared to literature results and experimental data. The ANN model which performed effectively for the AMD treatment, was selected to model the reactor performance.

Chapter 5: Conclusions and Recommendations

The final chapter addresses the primary outcomes recorded in Chapter 4 based on the objectives of the study. Lastly, the recommendations are presented, focusing on future research objectives that can be achieved by implementing new ideas. These recommendations are made from the knowledge obtained throughout the research.

CHAPTER 2 LITERATURE REVIEW

2.1 Overview

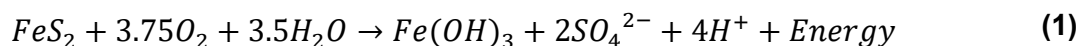
This chapter provides a comprehensive examination of AMD and its treatment, beginning with a review of AMD formation and traditional treatment methods. It then analyses the biological sulphate reduction (BSR) process, focusing on the functions of the sulphate-reducing (SRB) and sulphate-oxidising (SOB) bacteria, and the importance of carbon sources in maintaining the BSR process. This chapter also addresses the design and uses of biological reactors for AMD treatment, followed by the evaluation of the BSR process in active treatment systems. Finally, the chapter presents artificial neural networks (ANNs) as an analytical tool for modelling AMD treatment, defining their key features, applications in chemical processes and wastewater treatment, limitations and benefits, and distinguishing them from linear regression methods.

2.2 Acid mine drainage Review

In the following sections, a discussion of AMD production and mitigation strategies will be presented.

2.2.1 Acid mine drainage (AMD) production

AMD or acid rock drainage (ARD) is considered one of the primary pollutants of water in most countries with past or ongoing mining activities (Kefeni *et al.*, 2017). Pit lakes or mine laneways are expected to continue generating AMD indefinitely, as long as the mines are abandoned or closed (Tomiyaama *et al.*, 2019). AMD generation, release, mobility, and attenuation involve complex processes governed by a combination of physical, chemical, and biological factors (Chen *et al.*, 2022; Chen *et al.*, 2015). Oxidation of sulphide mineral ores is the primary source of AMD, caused by the exposure of sulphide mineral ores to the environment through intensive mining activities (Chen *et al.*, 2015; Kefeni *et al.*, 2017). The principal mineral responsible for producing AMD is pyrite (FeS_2), which oxidises when subject to microorganisms, water, and oxygen (Chen *et al.*, 2022). Pyrite oxidation involves the oxygen-dependent and oxygen-independent reaction as a multistep process (Lukovic & Stankovic, 2012). The production of AMD is shown by the overall stoichiometric **Equation (1)**.



The reactions involved in the production of AMD are amplified by mining activities where the sulphidic rocks with large surfaces are exposed to water and oxygen (Feris & Kotze, 2014; Vyawahre & Rai, 2016). The acidity produced by these reactions leads to metal ions (iron, lead,

zinc, etc) being mobilised due to the solubilising action of the acid (Choudhary & Sheoran, 2012b; Feris & Kotze, 2014). Finally, the effluent formed by oxidation has a very low pH and a high dissolved heavy metal content (Bratkova *et al.*, 2018; Neculita *et al.*, 2010; Westensee *et al.*, 2018). **Table 2-1** provides the pH and quantity of the metal ions and sulphate found in AMD as used by Zhang and Wang (2014b).

Table 2-1: The compounds and amounts of metal ions and sulphate that are present in synthetic AMD at a pH of between 3 and 3.5 (Zhang & Wang, 2014b)

Component	Source	Concentration range (mg/L)
Cd^{2+}	$3CdSO_4 \cdot 8H_2O$	11.2-12.2
Cu^{2+}	$CuSO_4 \cdot 8H_2O$	28.6-30.0
Fe^{2+}	$FeSO_4 \cdot 8H_2O$	586.4-598.8
Mn^{2+}	$MnSO_4 \cdot 8H_2O$	29.3-29.6
Ni^{2+}	$NiSO_4 \cdot 6H_2O$	15.2-16.0
Zn^{2+}	$ZnSO_4 \cdot 7H_2O$	49.3-50.4

2.2.2 Impact of AMD on water sources

Acids, metal ions, and sulphates are the main environmental hazards associated with AMD. A large percentage of the content in AMD is comprised of the most common metal ion, which is Fe^{2+} . The precipitation of Fe^{2+} into iron hydroxide occurs after its oxidation with dissolved oxygen in water, causing the water to turn yellowish-brown and smother life all along the way by embedding in streams or ocean beds (Han *et al.*, 2017). As a result, this will severely impact small aquatic life feeding on the bottom of ocean beds or streams (Tabelin *et al.*, 2020). Furthermore, most microorganisms existing in the ocean will be damaged by the production of the ferric hydroxide precipitate, which intensifies the situation by reducing the pH (Agrawal & Sahu, 2009). The solubility of the toxic metal is also easily decreased by the AMD's corrosive nature as it combines with rocks comprised of a variety of mineral ores (Kefeni *et al.*, 2017). The metal ion concentration in the water environment is increased by the AMD metals dissolved in the sediments and soil (Han *et al.*, 2017; Kefeni *et al.*, 2017). Therefore, the negative impact of AMD can be reduced by having low-cost and efficient treatment methods to establish long-term environmental sustainability.

2.2.3 Treatment of AMD

Active and passive methods are the two main categories for the clean-up of AMD. Adsorption, membrane technology, and the utilisation of alkaline chemicals to precipitate metals are well known active treatment methods used to treat AMD. The major difference between the active and passive methods is continuous chemical addition for neutralisation during active treatment, whilst passive treatment does not require chemical addition because AMD is cleaned using biological processes and naturally occurring chemicals (Kefeni *et al.*, 2017). Several economic and

environmental factors, such as AMD composition and pH, dictate the selection of AMD treatment options. The composition of AMD differs due to its dependence on its sources. Hence, there is no universal technology for AMD treatment (Neculita *et al.*, 2007). Consequently, the selection of the treatment methods and the waste generated during the treatment also differ.

2.2.3.1 Neutralisation

Over the past 50 years, the consequences of AMD environmentally have been minimised by using alkaline industrial chemicals for neutralisation to remove metals and sulphates (Kefeni *et al.*, 2017). Alkaline industrial chemicals such as ammonium hydroxide (NH_4OH), calcium oxide (CaO), caustic soda (NaOH), limestone (CaCO_3), magnesium hydroxide ($\text{Mg}(\text{OH})_2$), slaked lime ($\text{Ca}(\text{OH})_2$) and soda ash (Na_2CO_3) are the most commonly utilised for neutralisation (Watten *et al.*, 2005). Most of these mentioned chemicals are relatively expensive since they are produced commercially. Alkaline chemicals, such as sodium hydroxide, are hazardous and should be carefully handled when used. Using ammonium hydroxide may also lead to problems such as eutrophication and nitrification (Chowdhury *et al.*, 2015). Additionally, when limestone or slaked lime is utilised for treating AMD, there is production of a considerable quantity of sludge with excessive water content (Tolonen *et al.*, 2014), and the difficulty in recycling the metals from the waste results in the sludge having no economic value (Chen *et al.*, 2015).

A typical method that has been used for AMD neutralisation to remove metals as metal hydroxide precipitates and sulphates as gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) sludge is the use of limestone (CaCO_3) or calcium hydroxide ($\text{Ca}(\text{OH})_2$) (Tolonen *et al.*, 2014). Both methods are unsustainable due to the high cost of disposal, which can outweigh the operational costs of an AMD treatment plant (Herrera S *et al.*, 2007). According to Kefeni *et al.* (2017), the sulphate removal capacity of these chemicals is also ineffective. Furthermore, the use of $\text{Mg}(\text{OH})_2$, NH_4OH , and NaOH for the treatment of AMD is primarily chosen for the precipitation of metals, and at the same time sulphate is left in a solution (Akinwekomi *et al.*, 2016; Kefeni *et al.*, 2015). Furthermore, NaOH is the only alkaline industrial chemical with a high capacity for metal removal (Kefeni *et al.*, 2017); the main reason for this is due to the complete dissociation that results in an elevated pH value; however, the method is very costly in comparison to other methods (Akinwekomi *et al.*, 2016).

According to Clyde *et al.* (2016) and Potgieter-Vermaak *et al.* (2006), the aeration process using compressed air or oxygen with the availability of alkaline chemicals such as $\text{MgCO}_3(\text{s})$, $\text{CaCO}_3(\text{s})$ or NH_4OH leads to the oxidation of $\text{Fe}(\text{II})$ to $\text{Fe}(\text{III})$, which is removed as the ferric hydroxide precipitate. The type of alkaline chemical used to alter the pH of the solution, the aeration rate, and the chemical composition of the AMD influences the rate of removing metals from AMD (Clyde *et al.*, 2016). The alkaline barium calcium (ABC) desalination process is another efficient

active treatment that can reduce both sulphate and metals to below the toxic level (Kefeni *et al.*, 2017). The technology is interesting because it can dispose of low sludge levels after the recovery of useful metals or chemicals, and this technology is presented in detail by Kefeni *et al.* (2015) and Mulopo *et al.* (2012).

With the latest advancements in research, alkaline industrial by-products have replaced traditional alkaline chemicals for AMD treatment. The amount of waste and the cost of treatment decrease when using by-products, leading to economic and ecological benefits as well as environmental protection. Blast furnace slag, cement kiln dust, lime kiln dust, and fly ash are typical alkaline industrial by-products (Chen *et al.*, 2022). Fly ash can produce good water quality convenient for irrigation by removing toxic radioactive metals and metal pollutants in the AMD (Madzivire *et al.*, 2014). According to Jones and Cetin (2017), the treatment of AMD using recycled concrete aggregates can significantly reduce the concentrations of Fe, Zn, Cu, Cr, Mn, and sulphate in AMD. According to Sebogodi *et al.* (2020), it was found that the alkaline treatment using green liquor dregs (GLD) from two Kraft pulp manufacturers in South Africa has a competitive edge over commercial CaCO_3 because it reduces the acidity to a low dosage, and the pH of AMD can be increased to an optimum value. According to the findings of the study, it is less expensive to use alkaline industrial by-products to treat AMD.

2.2.3.2 Membrane filtration

The membrane process is the more promising technology for using wastewater reclamation to minimise water requirement and reduce effluent discharge (Liu *et al.*, 2011). The disadvantages of using membrane technology for AMD treatment are high membrane fouling and the high cost of the membrane (Al-Zoubi *et al.*, 2010). High membrane fouling is caused by the vulnerability of membrane systems to the low pH of AMD (Al-Zoubi *et al.*, 2010). According to Liu *et al.* (2011), the high capacity of salt and metal retention has led to more researchers being attracted to the application of reverse osmosis (RO) and nanofiltration (NF) processes to treat AMD. The study by Nasir *et al.* (2016) suggests that ultrafiltration (UF) and RO can be utilised after using sand filtration outfitted with coal fly-ash and rice husk-ash adsorbent columns for pre-treatment of AMD to reduce the risk of performance failure caused by high membrane fouling. The results obtained are as follows: the final pH of the RO permeation was increased to 6 by pre-treatment, and also 94.1, 95.8, and 98% of Mn, Fe, and sulphate were removed (Nasir *et al.*, 2016). Additionally, the small-scale AMD plant can meet the environmental standards and produce a high-quality permeate.

The study by Aguiar *et al.* (2016) evaluated the applicability of RO and NF for gold AMD treatment. It was observed that NF had an adequate solute retention efficiency and a higher permeate flux

than RO. Additionally, at optimised conditions, a single-step treatment using the NF270 membrane from gold AMD resulted in a maximum water recovery rate of 60%, and a sharper decrease in the permeate flux, showing higher membrane fouling (Aguiar *et al.*, 2016). According to other researchers, higher fluxes were obtained for NF when lower pressure was applied, indicating that it is one of the suitable membrane separation processes for treating AMD (Al-Zoubi *et al.*, 2010; Mullett *et al.*, 2014). In another study, conducted by Sierra *et al.* (2013), the FILMTEC™ NF-2540 membrane was used for AMD treatment to remove some of its pollutants. The results of the study are as follows: metals in AMD were removed at low pH and moderate pressure indicating the effectiveness of the membrane used (Sierra *et al.*, 2013).

The study conducted by Buzzi *et al.* (2013) also evaluated the application of cation (using HX 100 cation exchange membrane) and anion exchange (using HX 200 anion exchange membrane) membranes for AMD treatment. The study indicated that a contaminant removal capacity exceeding 97% was achieved, suggesting that electrodialysis is convenient for recovering water from AMD (Buzzi *et al.*, 2013). However, at the surface of the cation exchange membrane, precipitation of iron resulted in the blockage of the membrane, reducing process efficiency caused by membrane fouling (Buzzi *et al.*, 2013).

The study by Marti-Calatayud *et al.* (2014) reported that pure sulphuric acid was obtained when using the membrane for AMD treatment. In general, the use of membrane technology to treat AMD was minimised due to membrane fouling and operating costs, despite the high efficiency in separating pure water from contaminants (Marti-Calatayud *et al.*, 2014). Severe damage to the membranes can be reduced by pre-treating AMD utilising UF or microfiltration (MF) before RO and NF treatment to remove the fouling components (Meschke *et al.*, 2015). In general, AMD treatment using a membrane appears to be a promising technology. Appropriate fouling-resistant membranes should, however, be investigated for the full utilisation of membrane technology. In addition, it is necessary to develop a technology to treat the final waste produced as brine.

2.2.3.3 Biological methods

The biological treatment of AMD is a passive treatment that has been frequently used since the early 1990s (Yadav & Jamal, 2015). Recently, there has been a surge in the use of biological active systems for AMD treatment. Treatment methods that have been used include sulphate-reducing bioreactors, limestone drains, and constructed wetlands (Olds *et al.*, 2013; Tolonen *et al.*, 2014; Zipper & Skousen, 2010). Different types of biological methods used to treat AMD are shown in **Figure 2.1**. Biological treatment methods depend on bacterial activity by utilising organic matter to remove heavy metals and reduce sulphates (Skousen *et al.*, 2017). According to Seervi *et al.* (2017), key parameters that affect the biological treatment systems include dissolved oxygen

and metal concentration, alkalinity, acidity, and flow. Active (bioreactors) and passive (geochemical processes) treatment systems are two types of biotreatment methods that are used to treat AMD. Dissimilarities between active and passive systems are given in **Table 2-2**.

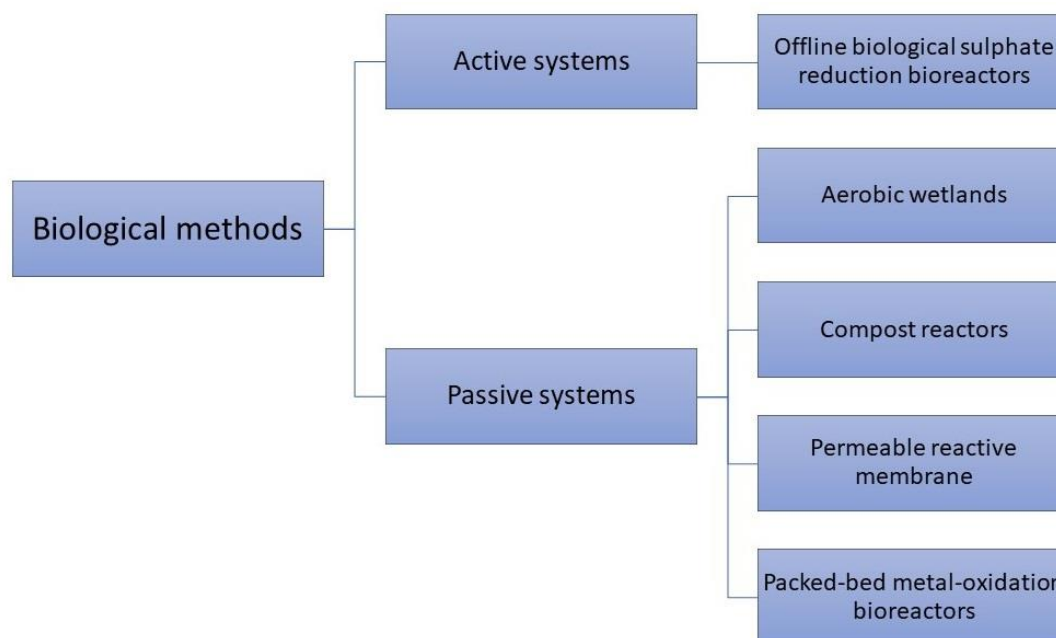


Figure 2.1: Biological treatment methods for AMD treatment (Rambabu *et al.*, 2020).

Table 2-2: Differences between passive biological treatment systems and active biological treatment systems

Passive biological systems	Active biological systems	References
More appropriate for application at abandoned mines.	Used to treat continual flow of AMD water (active mine sites).	(Song & Choi, 2015)
Widely used due to the benefits of low operational and investment costs and maintenance.	Not widely utilised due to high operational and investment costs and maintenance.	(Seervi <i>et al.</i> , 2017)
Suitable for operation for longer period, resulting in slow biological process.	Require less treatment time, and the biological process is faster.	(Rambabu <i>et al.</i> , 2020)
Requires vast land.	Does not require vast land.	(Sato <i>et al.</i> , 2018)
Suitable for treatment of AMD with low acidity.	Can treat AMD with any acidity.	(Skousen <i>et al.</i> , 2017)
Not suitable for adapting to changes in flow rate and chemistry and have a high footprint.	Can adapt to changes in flow rate and chemistry and have a low footprint.	(Trumm, 2008)

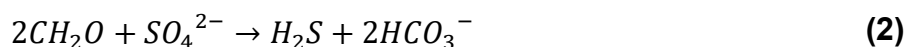
2.3 Biological Sulphate Reduction (BSR) Process

In the following sections, a discussion of different biological sulphate processes is presented.

2.3.1 BSR process description

Both the removal capacity of dissolved metals and the capacity of a specific process to generate the needed alkalinity are the basis used to evaluate the feasibility of passive treatment (Kalin *et al.*, 2006). The remediation of AMD requires the use of microorganisms that can survive and grow in severe AMD environments. The use of sulphate-reducing bacteria (SRB) for biological passive treatment has become known as the most auspicious alternative technology for AMD treatment. Some researchers (Bai *et al.*, 2013; Castillo *et al.*, 2012; Clyde *et al.*, 2016; Deng *et al.*, 2016; Hao *et al.*, 2014; Johnson, 2014; Kieu *et al.*, 2011; Zhang & Wang, 2014b) have conducted several experiments and reported that SRB is an improved, effective treatment option for AMD. The SRB requires an electron donor, such as an organic carbon source (short-chain alcohol or volatile fatty acid), since it is heterotrophic (Harrison *et al.*, 2014a). The biological process occurs under anaerobic conditions where a labile organic carbon is supplied to facilitate the reduction of sulphate to sulphide (Zhang & Wang, 2014b). The sulphate reduction process can either be dissimilatory or assimilatory. The process is dissimilatory when sulphide is discharged to the external medium, and it is assimilatory when sulphur-containing amino acids are integrated with sulphide (Harrison *et al.*, 2014a).

The reaction that takes place during the dissimilatory process is given by the **Equation (2)**, where bicarbonate ions (HCO_3^-) are formed by the oxidation of organic matter, and the energy that is generated by the reaction is utilised for the development and growth of the SRB (Bratkova *et al.*, 2018; Magowo *et al.*, 2020b; Reeder *et al.*, 2010).



From **Equation (2)**, CH_2O is a short-chain organic molecule and H_2S , which is generated, forms insoluble precipitates by reacting with metal ions (Rambabu *et al.*, 2020). The acidity in the AMD is reduced by the carbonate, and the carbonate also facilitates the precipitation of metals as carbonates. This is shown in **Equation (3)**.



There is precipitation of metal sulphides in low redox conditions. SRB growth and sulphate reduction are notably affected by the pH of the medium (Zhang & Wang, 2014b). SRB cannot survive at a pH below 4.5 since the optimum pH range is 7-7.5. Adjusting the pH leads to

sequential precipitation of individual metal sulphides, initiating a reduction of heavy metal concentration to significantly low levels. However, the downstream separation can be complicated by the precipitation of relatively small particles ($<0.2\ \mu\text{m}$) triggered by the promotion of primary nucleation caused by the low solubility due to the exceedingly high supersaturation (Harrison *et al.*, 2014a).

The review by Hao *et al.* (2014) focused on the use of SRB for the treatment of sulphur-contaminated groundwater and industrial sulphate water and the conditions suitable for the bacteria attributed to different operational parameters of the bioreactors. The review outlined that the use of SRB in sulphur bioconversion systems is advantageous over the conventional wastewater treatment method for treating industrial wastewater, by concurrently removing toxic trace metals and carbon. In addition, the use of SRB minimises greenhouse gas emissions and biological sludge production in comparison to standard carbon cycle-based treatment systems (Deng *et al.*, 2016). However, the tolerance or adaptability of SRB to a particular AMD should be investigated before using SRB for AMD treatment (Zhang & Wang, 2014b). Efficient BSR can be achieved by maintaining a high biomass concentration, which can be a challenge due to the relatively slow growth rate of anaerobic SRB in flow-through systems (Marais *et al.*, 2020b). Biomass retention is used to overcome these limitations (Marais *et al.*, 2020b). Biomass retention in several reactor configurations is promoted by the use of support matrices for biomass attachment, which facilitates the decoupling of the hydraulic residence time (HRT) and biomass retention time (Mooruth, 2013).

Bacterial metabolism occurs by using locally available organic substrates (sugarcane waste, yeast extract, sawdust, manures), mixtures of salts (CaCO_3 , CaCl_2 , K_2HPO_4 , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, NH_4Cl , $(\text{NH}_4)_2\text{SO}_4$), and other carbon sources when treating AMD using SRB (Castillo *et al.*, 2012). The reactive mixtures should contain more than one organic component source to achieve a higher sulphate reduction (Gibert *et al.*, 2011; Muhammad *et al.*, 2015; Neculita *et al.*, 2007; Waybrant *et al.*, 1998; Zagury *et al.*, 2007). Substrate mixtures are utilised as nutrients for microorganisms (Kefeni *et al.*, 2017). Furthermore, the efficiency of SRB is significantly impacted by the increase or decrease of the substrate mixtures (Bai *et al.*, 2013). Therefore, according to Muhammad *et al.* (2015) and Neculita *et al.* (2010), optimising the desirable proportion mixture of the substrate is needed to achieve optimum metal removal and sulphate reduction. The importance of substrate mixtures is based on buffering the solutions' acidity and removing metals using adsorption to reduce the detrimental consequences of toxic metals on SRB (Zhang & Wang, 2014b). Biological treatment of AMD requires SRB activities, which are promoted using the organic substrate, which is made from woody and herbaceous materials (Hao *et al.*, 2014; Kefeni

et al., 2017), while the permeability of the media in the bioreactor is increased by the wood chips (Kefeni *et al.*, 2017).

Microbially mediated reactions are used by SRB to enhance the removal of trace metals (i.e., Zn, Cd, Cu, Ni), sulphate, and Fe (Zhang & Wang, 2014b). During these reactions, hydrogen sulphide (H_2S (g)) is formed by the conversion of the first sulphate, while hydrogen carbonate is formed by the conversion of organic matter (" CH_2O "). Then the insoluble metal sulphide precipitates are created by the reaction of H_2S (g) with metal cations such as Zn, Cd, Cu, Fe, and Ni (Hauri & Schaider, 2009; Lewis, 2010). These metal sulphide precipitates are likely to form colloidal particles, which are simpler to dewater (Bratkova *et al.*, 2018; Magowo *et al.*, 2020b). Finally, sulphide precipitation is used to remove metals because metal sulphide precipitates are more stable than metal hydroxides (Hedrich & Johnson, 2014). Metal sulphide precipitation addresses the generation of hydrogen sulphide by using partial oxidation to remove excess sulphide as sulphur (Magowo *et al.*, 2020a). However, there are some cases where there is a process failure due to the treatment process being severely constrained by sulphide accumulation (Greiben *et al.*, 2005). Therefore, before discharging the treated effluent, the sulphide must be removed since it is malodorous, corrosive, and toxic. The residual sulphide will always be present if the sulphate reduction process has 50% or more efficiency, since only a portion of the sulphide is removed by metal sulphide precipitation. The partial oxidation of sulphide to elemental sulphur is an option to regulate the residual sulphide by recovering sulphide as a value-adding product (Harrison *et al.*, 2014a). The previously mentioned procedure has been successfully achieved in a passive system (Mooruth, 2013; Rose, 2013), and an active system (Zhang *et al.*, 2017).

The study by Lefticariu *et al.* (2015) applied the controlled bio-mineralisation in sulphidogenic bioreactors to remove the two primary metals available in AMD, namely iron (400 mg/L) and Zn (460 mg/L). During this process, Zn was converted to ZnS(s) , and iron was converted to schwertmannite ($\text{Fe}_8\text{O}_8(\text{OH})_6(\text{SO}_4) \cdot n\text{H}_2\text{O}$). Finally, iron was removed as a ferric hydroxide precipitate using microbial iron oxidation (Lefticariu *et al.*, 2015). According to Lefticariu *et al.* (2015), it is more efficient to use organic substrate-based reactors than to use only limestone reactors. The study also revealed that the AMD produced by coal mining can be treated using anaerobic organic substrate bioreactors. Furthermore, the authors outlined that the contaminant removal efficiency can be enhanced by improving the pre-treatment of AMD and organic substrate input to sustain the reduction of the elevated concentrations of Al and Fe content.

The use of SRB, rather than metal hydroxide precipitate, is due to the lower solubility of the metal sulphides over a wide pH range (Choudhary & Sheoran, 2012b). In general, treating AMD using SRB is recommended for abandoned mines since it is a more cost-effective treatment option for

AMD (Muhammad *et al.*, 2015). In addition, Zn is entirely removed using SRB, and the mobility of all metals present in AMD are reduced by it (Castillo *et al.*, 2012). Bioreactors can conveniently recover and reuse beneficial chemicals. However, more time is needed to remove metal precipitates (Muhammad *et al.*, 2015). There is also a suggestion by Castillo *et al.* (2012) to treat synthetic AMD with metal ions concentration using immobilised SRB granules that are toxic resistant.

One of the disadvantages of using SRB is that the bioreactor's performance is impacted by the growth of microorganisms, which are significantly affected by the low pH of AMD (Hao *et al.*, 2014; Sanchez-Andrea *et al.*, 2014). The addition of an organic substrate and CaCO_3 can reduce this problem (Kieu *et al.*, 2011). The pH of the AMD is increased by adding CaCO_3 . Both chemicals can be utilised as nutrients for microorganisms and to remove specific toxic trace metals (Kieu *et al.*, 2011). Another disadvantage of using SRB is the cause of environmental pollution over time due to the inability of the process to recover and reuse metal sulphide precipitates (Hao *et al.*, 2014). Yet, some literature reports have identified the precipitation of metal sulphides from aqueous solutions in bioreactors as a solution to the possibility of recovering and reusing metal sulphides (da Costa *et al.*, 2012; Liu *et al.*, 2013). Controlling the reaction conditions brings about this recovery to obtain a nanoparticle-sized sulphide precipitate. In bioreactors, further precipitation of other metal sulphides can be done using the recovered sulphide (da Costa *et al.*, 2012). The economic advantage of this process is reducing the use of toxic or expensive chemicals by the metal sulphides that are recovered and reused (da Costa *et al.*, 2012). Therefore, advanced research is essential to overcome the drawbacks of the application of SRB for AMD treatment. The process parameters that can impact the BSR process are given in **Table 2-3**.

Table 2-3: Summary of process parameters that affect the BSR process (van Rooyen *et al.*, 2021)

Parameter	Range/Condition
Operating cost	4.5-11 (ZAR.m ⁻³)
Relative capital cost	Low
Possible cost drivers	Carbon source or electron donor
Characteristics of the water effluent	Sulphates less than 200 mg/L
Waste considerations	Presence of H ₂ S and metal sulphates
Application: Wastewater	High sulphate wastewater
Application: Capacity	Low/Medium

2.3.2 SRB and SOB characteristics

The key bacteria in the BSR process are SRB, which reduce sulphate to sulphide and help degrade organic matter. The other bacterium, SOB, oxidises sulphide to sulphur or sulphate, which helps prevent sulphide build-up (Shuqing *et al.*, 2011). Sulphide can be oxidised by abiotic or biotic oxidation. In the case of abiotic oxidation, this process occurs under microaerophilic conditions, where sulphide is oxidised via chemical reaction with dissolved oxygen (Pirieh & Naeimpoor, 2019; Willard & Kelly, 2021). Notably, this process is characterised by low oxygen levels, which allow the gradual conversion of sulphide into less hazardous compounds without the direct involvement of living organisms (Chatterjee *et al.*, 2017). Conversely, biotic oxidation involves SOB, which acts as a catalyst to partially oxidise sulphide to elemental sulphur (Chatterjee *et al.*, 2017; Singh & Chakraborty, 2022). Here, the SOB uses sulphide as an energy source.

SRB is from Clostridia and Deltaproteobacteria, and it has dominant genera including Desulphotomaculum, Desulphovibrio, Desulphomicrobium, Desulphobulbus, and Desulphotignum (Castillo *et al.*, 2012; Tian *et al.*, 2017). These genera are temperature-dependent; some, such as Sulphorimonas and Desulphovibrio, are active at both low and high temperatures, while others, such as Desulphotignum, are active only at high temperatures (Tian *et al.*, 2017). The SOB belongs to the Alphaproteobacteria and Betaproteobacteria, with dominant genera including Thiohalomonas, Thiococcus, Thiobacillus, and Sulphurimonas (Andriyanto *et al.*, 2020; Tian *et al.*, 2017; Xia *et al.*, 2014; Zhang *et al.*, 2017). SRB is found to be more sensitive to environmental conditions, such as air, than the SOB. The summary of the comparisons of key characteristics of SRB and SOB is given in **Table 2.4**.

Table 2-4: Summary of the comparison between the SRB and SOB

Characteristic	SRB	SOB	References
Metabolic pathway	Uses sulphate as an electron acceptor.	Oxidise sulphide into sulphur using aerobic conditions.	(Tian <i>et al.</i> , 2017) (Molwantwa & Rose, 2013)
Carbon Sources	Uses different organic electron donors such as ethanol and acetate.	Sulphide acts as an electron donor.	(Corbett, 2001) (Rose, 2013)
Byproduct	It produces hydrogen sulphide.	Generate sulphur, sulphuric acid, and sulphate.	(van den Brand <i>et al.</i> , 2018) (Kushkevych <i>et al.</i> , 2021)

Favourable surroundings	Anaerobic conditions	Active in microaerobic and aerobic conditions.	(Aguilar <i>et al.</i> , 2008) (Singh <i>et al.</i> , 2011)
Environmental impact	Has an essential contribution to the sulphur cycle. Contribute to the deterioration of concrete and metal pipelines.	Has an essential contribution to the sulphur cycle. Causes the concrete corrosion in sewers	(Adekanmbi <i>et al.</i> , 2020) (Tripathi <i>et al.</i> , 2021)

2.3.3 Carbon source and electron donor

During the process of using SRB to remove metals from AMD, the metal removal efficiency is affected by the nature of the electron donor and carbon source (Alam & McPhedran, 2019). The demand for continuous operation for active bioreactors requires solid substrates rather than gaseous or liquid substrates (Greben *et al.*, 2005). The use of simple organic substrates such as methanol, lactate, and ethanol is efficient in treating AMD, as they accelerate the rate of treating AMD and encourage the growth of SRB (Magowo *et al.*, 2020a). The complexity of the microbial community is reduced using simple organic substrates, which leads to a favourable COD/sulphate ratio for sulphate reduction by improving the frequency of treatment (Magowo *et al.*, 2020a). According to Kaksonen *et al.* (2004), 60% of the sulphate can be removed by having an adequate stoichiometric COD/sulphate ratio of 0.67. Many studies (Alam & McPhedran, 2019; Kiran *et al.*, 2018) have recommended using lactate because it maintains a higher metal removal efficiency than other carbon sources, and SRB growth is supported by it. Other studies have utilised various popular carbon sources, such as methanol and ethanol (Castro Neto *et al.*, 2018; Janyasuthiwong *et al.*, 2015; Natarajan, 2017; Sahinkaya *et al.*, 2013; Sierra-Alvarez *et al.*, 2006; Ucar *et al.*, 2011). The study by Costa *et al.* (2017) used the reactor experiment to confirm that ethanol is effective in an anaerobic sequential batch reactor (ASBR); the effectiveness of ethanol was also verified by (Zhao *et al.*, 2018), who used an anaerobic reactor. These studies have stated that the capacity of SRB to remove metals can be enhanced by using ethanol. Under room temperature conditions, ethanol is more competitive, in contrast to lactate, in terms of kinetics (Alam & McPhedran, 2019), and thus more scalable. The disadvantage of using simple substrates is that they are predicted to be depleted quickly, leading to increased maintenance and operational costs (Hiibel *et al.*, 2011; Magowo *et al.*, 2020b).

Some studies have used carbon sources that are made up of organic waste (e.g., cow manure, leaves, wine and cheese industry waste, and maize straw) and are called complex organic matter (Choudhary & Sheoran, 2012a; Giachini *et al.*, 2018; Kim *et al.*, 2014; Muhammad *et al.*, 2018;

Sahinkaya *et al.*, 2013; Viggi *et al.*, 2010; Zhang *et al.*, 2016). Most of these organic sources can remove metals since they are porous and cheap. The study by Nielsen *et al.* (2019) has provided evidence that using complex organic carbon sources can enhance SRB. Complex organic carbon sources undergo a more gradual degradation; thus, they can last longer in contrast to simple carbon sources (ethanol and lactate) (Nielsen *et al.*, 2019). However, the sulphate yields of complex carbon sources are below those of simple carbon sources. Therefore, this is the disadvantage of using complex carbon sources (Nielsen *et al.*, 2019). The other study by Zhang and Wang (2014b) conducted experiments in a sulphidogenic batch reactor using three carbon sources (sawdust, dairy manure, and chicken manure). It was found that sawdust attained a sulphate reduction of 50.3%, dairy manure reached 64.8%, and chicken manure reached 79.1%. These results indicated that SRB activity could be stimulated using chicken manure as a carbon source. The study also showed that using organic waste can promote acidity buffer and metal sorption. Thus, the adverse effects of high acidity and heavy metals can be reduced on SRB activity (Zhang & Wang, 2014b). However, using organic waste as a carbon source may lead to incomplete oxidation and the release of abundant organic carbon that can cause pollution problems (Zagury *et al.*, 2007). Therefore, high organic loads can be reduced by designing a second stage of treatment.

The use of cellulose as a substrate requires the supply of acetate, ethanol, glucose, and lactate by utilising degraders and fermenters (Aoyagi *et al.*, 2017; Jamil & Clarke, 2013; Magowo *et al.*, 2020b; Rezadehbashi & Baldwin, 2018). Cellulose degradation is the rate-limiting step when treating AMD due to the growth of competitors that are enhanced by the slow degradation of cellulose (Magowo *et al.*, 2020b). According to Hiibel *et al.* (2011), lignocellulose bioreactors result in a 2-3% enrichment of SRB compared to 70% of ethanol. Thus, SRB cannot directly use cellulose. Therefore, low-flow passive treatment systems should use lignocellulosic material.

2.4 Biological Reactors for AMD Treatment

The biological reactors used to treat AMD using the sulphate-reduction process are fluidised bed, anaerobic membrane bioreactor, batch reactor, and upflow anaerobic sludge blanket reactor. The fluidised bed and membrane bioreactors are reactors that will be discussed in this review. These bioreactors are less commonly used for AMD treatment, and the discussion will provide more details on why these reactors are not frequently used. The sensitivity of SRB to moderate acidity ($\text{pH} < 5$) is one of the critical factors that limit the design and use of sulphidogenic treatment systems (Sahinkaya *et al.*, 2007b).

2.4.1 Fluidised bed reactor

The fluidised bed reactor (FBR) is a system with a basic design, and the processes can be controlled adequately. This reactor has been used to treat wastewater from industries, mines, and municipalities. The main advantages of using the FBR include catalyst (microorganisms) renewability, less sludge generation, and less HRT for conversion of pollutants (Sharma *et al.*, 2021). The FBR is chosen for the AMD treatment due to sufficient contact being achieved in the middle of the wall and phases (Subramanian, 2002). The bioreactor (FBR) used by researchers to treat AMD is shown in **Figure 2.2**. AMD is fed at a specific superficial fluid velocity (SFV) through the reactor bed. The SFV should be sufficient to support media suspension (Bello *et al.*, 2017). The reactor can consist of a sparger used to distribute the effluent uniformly along the bed of the reactor (Karadag *et al.*, 2015). Furthermore, microorganisms are used as a fluidised bed medium during bioprocessing, such as the BSR. The benefits and drawbacks of using the FBR are given in **Table 2-10**.

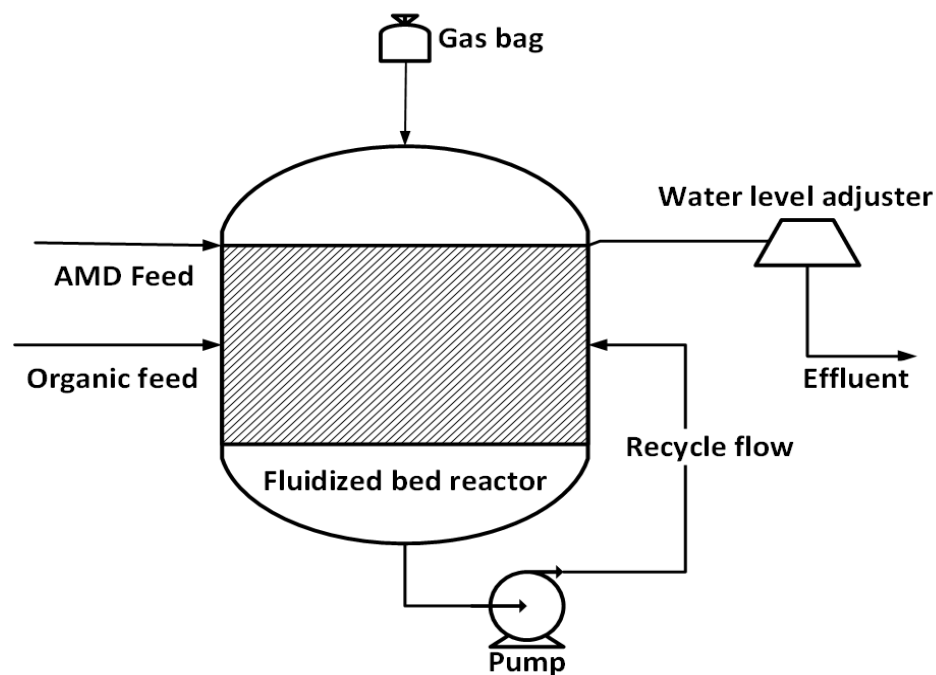


Figure 2.2: Schematic of the fluidised bed reactor (Sahinkaya *et al.*, 2007b)

Four types of FBR have been used for wastewater treatment. These FBRs include a conventional FBR, inverse-FBR, two-phase, and three-phase FBR. The selection criteria for the FBRs application are shown in **Figure 2.3**. The FBRs are chosen based on the used particle density in comparison to the fluid density, such that the conventional FBR is more suitable for denser solid particles, and the inverse-FBR is used for less dense particles (Janyasuthiwong *et al.*, 2015;

Sahinkaya *et al.*, 2007a). Furthermore, in the conventional FBR, the fluid flows upward, whilst in the inverse FBR, the fluid flows downward (Subramanian & Saravanan, 2019). The BSR treatment process used a two-phase FBR (liquid-solid), where the liquid is the AMD, and the catalysts or support material, is a solid phase (Bello *et al.*, 2017; Ucar *et al.*, 2011). In this reactor, the flow of AMD across the catalyst bed provides fluidisation. The addition of aeration to a two-phase FBR induces the development of the three-phase (solid-liquid-gas) FBR, where the gas and liquid counter-current flow causes fluidisation (Bello *et al.*, 2019). The reactor has vast applications but has drawbacks, such as reactor failure caused by elevated agitation and gas flow rate (Sharma *et al.*, 2021).

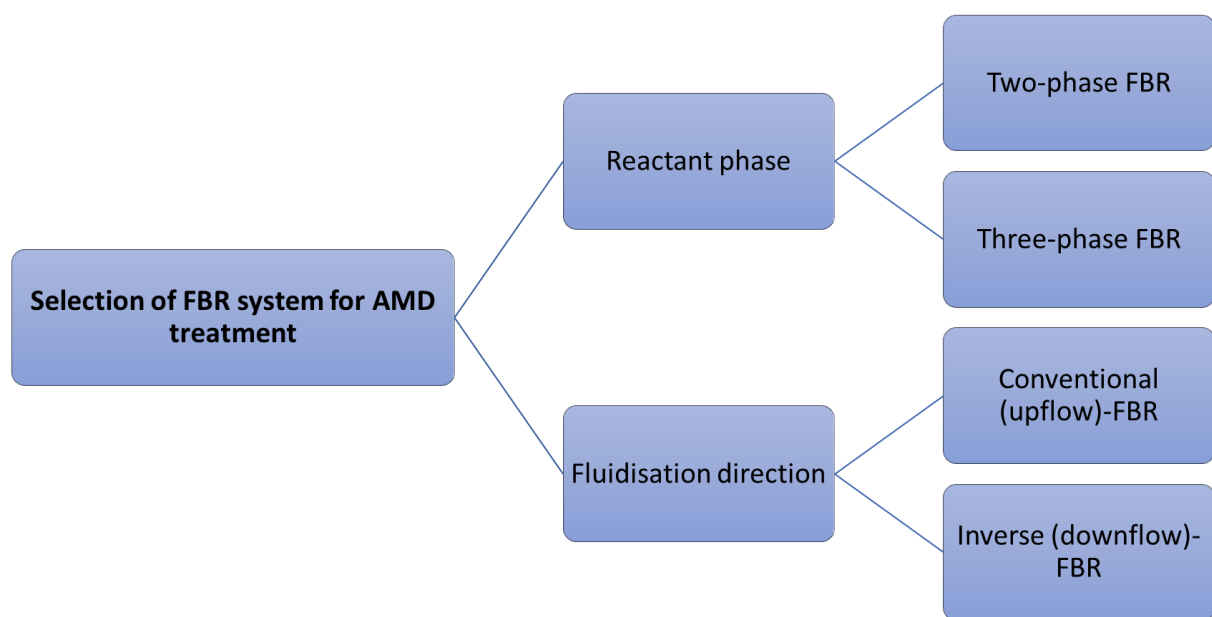


Figure 2.3: Selection of different types of FBR systems used for AMD treatment (Bello *et al.*, 2017)

The performance of the FBR treating the AMD, using the BSR process, is mainly affected by the pressure drop and minimum fluidisation velocity (MFV) (Anantharaman *et al.*, 2018; Sahinkaya *et al.*, 2011). According to Sharma *et al.* (2021), a pressure drop in the FBR is created by an increase in the flow rate. The pressure drop is higher with an increased flow rate. The MFV affects the bed density and bed voidage, where an increase in the MFV results in an increased bed voidage and decreased bed density (Anantharaman *et al.*, 2018). The MFV is dependent on the fluid and solids properties and the SFV (Mishra, 2013). The increase in the SFV results in a moderate decline in the MFV; smaller particles have a lower MFV compared to larger particles.

Other parameters that affect the FBR performance include Fe^{2+} concentration, organic loading rate (OLR), HRT, and the pH (Bello *et al.*, 2017; Makhathini *et al.*, 2021; Sahinkaya *et al.*, 2011; Yan *et al.*, 2020). In summary, efficient BSR is provided by low Fe^{2+} concentration, low OLR, long

HRT, and higher pH. The recycle ratio affects FBR performance as well as the acidity and metal concentrations in the reactor's influent, which is decreased by a high recycle ratio (Cassidy *et al.*, 2017). The FBR performances of the studies that used the BSR process for AMD treatment are given in **Table 2-5**.

Table 2-5: Summary of the results obtained by researchers who used FBR for the AMD treatment

Type of FBR	Days of operation	Carbon source	COD removal%	Sulphate removal%	Sulphide removal%	pH	References
Inverse (Lab-scale)	220	Ethanol	64	67	42	7.5	Sahinkaya <i>et al.</i> (2007a)
Inverse (Lab-scale)	266	Acetate	80	90	35	7.9	Sahinkaya <i>et al.</i> (2011)
Two-phase (Lab-scale)	110	Ethanol	98	91	50	7.8	Ucar <i>et al.</i> (2011)
Inverse (Lab-scale)	110	Activated sludge	68	88	17	7.1	Villa-Gomez <i>et al.</i> (2011)
Inverse (Lab-scale)	250	Glucose	60	50	60	7	Cirik <i>et al.</i> (2013)
Inverse (Lab-scale)	150	Landfill leachate	95	96	84	7.7	Sahinkaya <i>et al.</i> (2013)
Inverse (Lab-scale)	90	Ethanol	73	74	38	5-7	Janyasuthiwong <i>et al.</i> (2015)
Inverse (Lab-scale)	60	Lactate	85	84	34	6-7	Cassidy <i>et al.</i> (2017)
Inverse (Lab-scale)	140	Sodium lactate	78	75	55	7	Reyes-Alvarado <i>et al.</i> (2017)
Inverse (Lab-scale)	160	Lactate	83	79	49	6.9	Reyes-Alvarado <i>et al.</i> (2018)
Inverse (Lab-scale)	20	Sodium lactate	19	79	41	6.8	Torbaghan and Torghabeh (2019)
Inverse (Lab-scale)	115	Wastewater sludge	96	97	53	7.7	Makhathini <i>et al.</i> (2021)
Inverse (Lab-scale)	125	Phosphogypsum	53	75	-	7.9	Melgaço <i>et al.</i> (2020)
Inverse (Lab-scale)	360	Ethanol	80	73	57	7.5	Yan <i>et al.</i> (2020)
Inverse (Lab-scale)	150	Sodium lactate	80	65	48	7.1	Kumar and Pakshirajan (2021)
Inverse (Semi-pilot)	1	Wastewater sludge	92	99	-	7.2	Afsar <i>et al.</i> (2022)

2.4.2 Anaerobic membrane bioreactors

Recently, anaerobic membrane bioreactors (AnMBR) have been used for both industrial (Yurtsever *et al.*, 2016; Zhou *et al.*, 2021) and municipal wastewater treatment (Yue *et al.*, 2018), due to the low capital costs. The AnMBR integrates the membrane filtration treatment with the biological method to critically separate liquids. According to Naghizadeh *et al.* (2011), most AnMBRs use an aeration tank with the membranes submerged in the tank. Equal aeration distribution is provided by the air sparger, and gas is pumped into the reactor to ensure the cake layer is scrambled and mixed. The bioreactor's pressure gauge is for pressure flow measurements and maintaining pressure along the membrane. Few studies have investigated the performance of AnMBR for sulphate reduction; however, the available studies have demonstrated high process performance (Sahinkaya *et al.*, 2018; Vallero *et al.*, 2005; Yurtsever *et al.*, 2016). During the AMD treatment, a sulphate reduction process occurs in the biofoulants of the AnMBRs (Zhou *et al.*, 2021). The bioreactor used by researchers (Sahinkaya *et al.*, 2018; Sahinkaya *et al.*, 2019) is displayed in **Figure 2.4**. The AnMBRs can consist of two types of membrane systems (Naghizadeh *et al.*, 2011; Stephenson *et al.*, 2000). The first system is the in-pipe cartridge process (side-stream AnMBR) that is pressure-driven and is placed outside the bioreactor (Judd, 2016).

Inside the side-stream AnMBR, water from the membrane is pushed to the permeate by increasing the transmembrane pressure (TMP). The second system is the immersed process (submerged AnMBR), which is vacuum-driven and is installed inside the reactor due to its design (Judd, 2016). In the submerged-AnMBR, pressure is applied to extract treated water from the membrane (Kharraz *et al.*, 2022). The permeate side of the bioreactor uses suction pressure, and the retentate side uses a static head (Judd, 2016). The membrane used inside the reactor uses biodegradation to thoroughly retain the biomass by eliminating contaminants (Naghizadeh *et al.*, 2011). Different membranes used in previous studies incorporate nanofiltration (NF), microfiltration (MF), and ultrafiltration (UF) (Lin *et al.*, 2011; Naghizadeh *et al.*, 2011; Quang *et al.*, 2016; Sahinkaya *et al.*, 2018; Wang *et al.*, 2018). The membranes are required for biomass retention, leading to a higher microbial concentration in the reactor. Cylindrical shape and flat sheet are two membrane shapes that can be utilised in the bioreactor. The cylindrical shape is used for scale-up applications due to its advantages of having a packing density and a lower footprint compared to a flat sheet (Kharraz *et al.*, 2022). However, a flat sheet is used by researchers for the development of lab-scale submerged AnMBR systems.

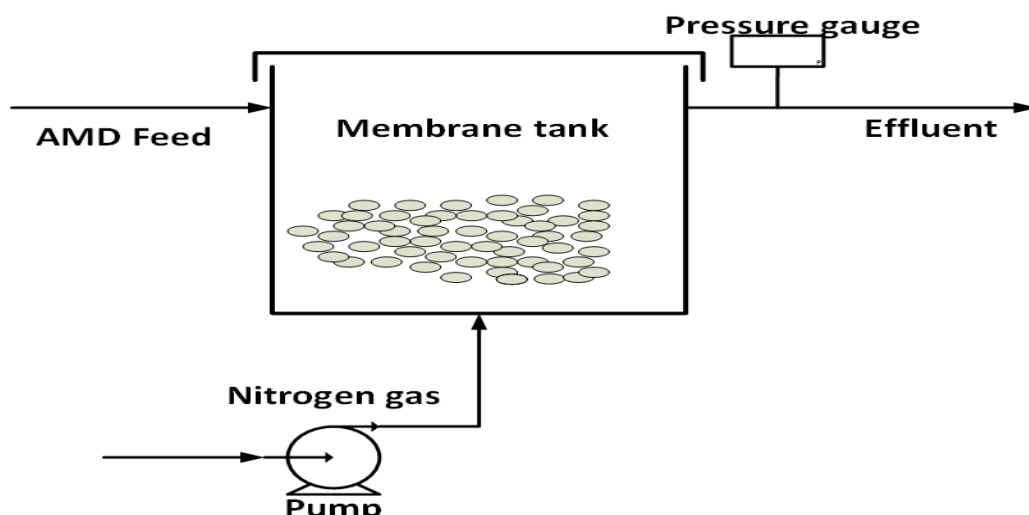


Figure 2.4: Anaerobic membrane reactor used to treat AMD (Sahinkaya *et al.*, 2019)

In general, the use of attached growth reactors for AMD treatment is due to the slow-growing SRB requiring a solid retention time (SRT) (Sahinkaya *et al.*, 2019). The biomass accumulates inside the AnMBR, providing a suitable environment for SRB to grow (Yurtsever *et al.*, 2016). According to Zhou *et al.* (2021), operating the bioreactor for ten months results in a gradual decrease in the sulphate concentrations in the effluent. However, sulphide concentrations may increase in the effluent due to the dissimilatory sulphate reduction (DSR). Indicating that a specific bacterial group in the biofoulants possesses a pathway required for complete DSR, producing sulphide (Zhou *et al.*, 2021). The results of the different AnMBRs used in recent studies for the high levels of sulphate in wastewater purification are given in **Table 2-6**.

The AnMBR systems have an HRT as the critical operating parameter for AMD treatment (Naghizadeh *et al.*, 2011). The HRT has a notable relationship with the organic loading rate (OLR), and it is strongly correlated with the treatment efficiency and biomass characteristics. According to Wang *et al.* (2018), a short HRT is required when there is an increase in membrane flux. However, severe membrane fouling will occur as the membrane flux increases. Other parameters affecting the process performance in the bioreactor include the membrane flux, transmembrane pressure (TMP), and gas scouring rate (GSR). The decrease in membrane flux leads to an efficient filtration performance, and the increase in the gas scouring rate results in a stable bioreactor performance (Sahinkaya *et al.*, 2018). The cake layer density also influences process performance and membrane fouling, as a dense cake layer causes membrane fouling (Sahinkaya *et al.*, 2018). The cake formation can be regulated by performing intermittent filtration (suction and relaxation).

Table 2-6: Summary of the results of the studies that used AnMBR for AMD treatment

Type of AnMBR	Carbon source	Days of operation	HRT (days)	COD removal%	Sulphate removal%	Sulphide removal%	pH	References
Submerged (Lab-scale)	Acetate	92	1.5	80	85	41	7.2	Vallero <i>et al.</i> (2005)
Submerged (Lab-scale)	Formate	20	5	62	49	58	6	Bijmans <i>et al.</i> (2008)
Submerged (Lab-scale)	Sodium acetate trihydrate	60	5	69	69	40	7.5	Nevatalo <i>et al.</i> (2010)
Submerged (Lab-scale)	Glucose	160	2	94	94	75	7.5-8	Yurtsever <i>et al.</i> (2015)
Submerged (Lab-scale)	Glucose	410	1.75-2	85	76-95	55	7.2	Yurtsever <i>et al.</i> (2016)
Submerged (Lab-scale)	Glucose	200	3	72	80-90	67	7.3	Yurtsever <i>et al.</i> (2017)
Submerged (Lab-scale)	Ethanol	165	2	90	90	40	7.5	Sahinkaya <i>et al.</i> (2018)
Submerged (Lab-scale)	Ethanol	290	1-2	94	95	67	7.3	Sahinkaya <i>et al.</i> (2019)
Submerged (Lab-scale)	Ethanol	127	1-2	87	98	75	7.8	Oztemur <i>et al.</i> (2020)
Submerged (Lab-scale)	Ethanol	131	1	96	95	67	7.1	Yigit <i>et al.</i> (2020)
Submerged (Full-scale)	Wastewater sludge	290	5	-	83-92	25-40	6.8	Zhou <i>et al.</i> (2021)
Submerged (Lab-scale)	Glucose	243	2	86	94	35	6.5	Siddiqui <i>et al.</i> (2022)
Submerged (Full-scale)	Wastewater sludge	180	5	-	77.4	60.3	7.3	Zhou <i>et al.</i> (2022)

Membrane fouling is a notable drawback of using this bioreactor. The factors that affect membrane fouling are shown in **Figure 2.5**. According to Sahinkaya *et al.* (2018), heavy metal deposits form on the surface by the accumulation of metal-sulphide precipitates during high-sulphate wastewater treatment. High TMP indicates membrane fouling (Sahinkaya *et al.*, 2018). In the past two decades, progress has been made in reducing membrane fouling (Meng *et al.*, 2007). Membrane fouling can be reduced by extracellular polymeric material (Shi *et al.*, 2017) and can be controlled by using quorum quenching (Huang *et al.*, 2016). Other methods used to reduce membrane fouling include using recent hybrid bioreactors such as the microbial electrolysis cell-AnMBR, forward osmosis-AnMBR, and granular-AnMBR (Anjum *et al.*, 2021). These methods have been tested in treating domestic wastewater, and high efficiencies have been achieved.

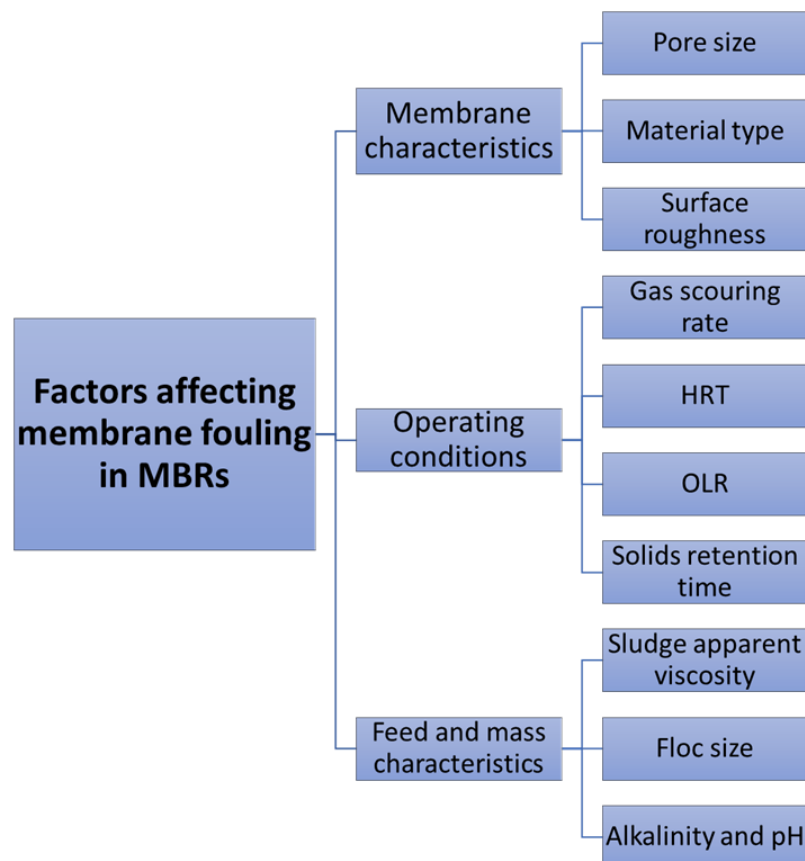


Figure 2.5: Factors affecting membrane fouling during the operation of AnMBR (Iorhemen *et al.*, 2016)

2.4.3 Upflow anaerobic sludge blanket bioreactor

The upflow anaerobic sludge blanket (UASB) reactor is a high-rate system used to treat industrial, mine, and domestic wastewater. Several researchers have utilised a UASB bioreactor (Chaparro

& Bayona, 2021; Lew *et al.*, 2011; Najib *et al.*, 2017; Zhou *et al.*, 2009) for the treatment of AMD, as shown in **Figure 2.6**. Inside the UASB is a biologically activated sludge blanket, through which the influent flows upward (Daud *et al.*, 2018). This sludge is comprised of granular aggregates that are highly stable and contribute to the treatment efficiency (Banihani & Field, 2013). The production of the methane-biogas enhances internal mixing in the reactor, which maintains biologically formed granules (Crone *et al.*, 2016). Chong *et al.* (2012) recommend a gas-liquid-solid separator (GLSS) to effectively remove granules, liquid, and gas located at the top of the UASB reactor. Bio-granules analyses indicate that specific substrate kinetics and degradation thermodynamics are required for microbial dispersions in granules (Wu *et al.*, 2022). Two distributions are observed from these analyses: a layered distribution is found on organically degrading granules, and an even bacterial distribution is discovered when substrates are present (Ngwenya *et al.*, 2022). When a high organic content is available for digestion by bacteria, AMD treatment using a UASB reactor is effective (Singh *et al.*, 2013). According to Van Lier *et al.* (2010), AMD toxic components such as heavy metals, sulphates and hydrogen sulphide are overcome by the resistance of the organic matter-degrading granule, compared to suspended sludge (Wu *et al.*, 2022).

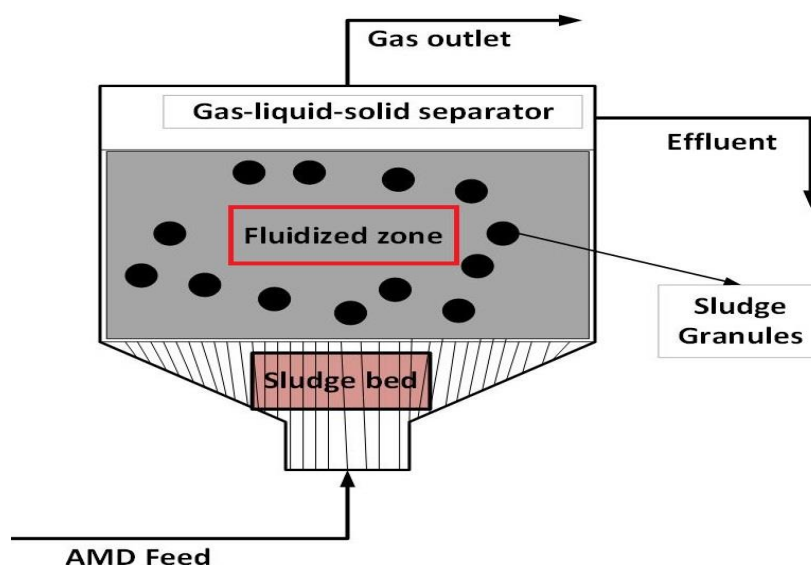


Figure 2.6: An Upflow anaerobic sludge blanket (UASB) reactor used for the treatment of AMD
(Guerrero-Barajas *et al.*, 2014)

The UASB reactor has been preferred for the treatment of AMD due to the following advantages: short period (24 hours) required for effective treatment, efficient biogas generation, less sludge generation, solids can be stabilised in the reactor, pollutant load reduction, can reduce high COD loads, and rapid biomass recovery (Daud *et al.*, 2018; James & Kamaraj, 2002). Economic advantages associated with the reactor include, amongst others a lower construction and operational cost, excess sludge can be removed after 3 to 4 years.

Parameters that affect the performance of the UASB reactor are given in **Figure 2.7**. Effective mixing increases biogas generation, contact time of wastewater microbes, and reduces by-product growth (Rizvi *et al.*, 2015). A low HRT leads to reduced treatment performance due to the washout of organic matter, and less contact time of wastewater and sludge (Abdelgadir *et al.*, 2014; Farghaly & Tawfik, 2017). Granules are essential in reducing start-up time, and a layered granule structure enhances bacterial growth, improving the process performance (Farghaly & Tawfik, 2017). High organic loading rate (OLR) is found to decrease the treatment efficiency due to sludge flotation, elevated influent COD, and sludge washout (Wu *et al.*, 2022). Low temperatures in the reactor have been found to reduce the maximum bacterial growth and rates of substrate usage (Gao *et al.*, 2024; Lew *et al.*, 2011). According to Rizvi *et al.* (2015), it is recommended that the UASB reactor be operated at high temperatures between 20 °C and 30 °C when using biological processes to enable optimal bacterial growth.

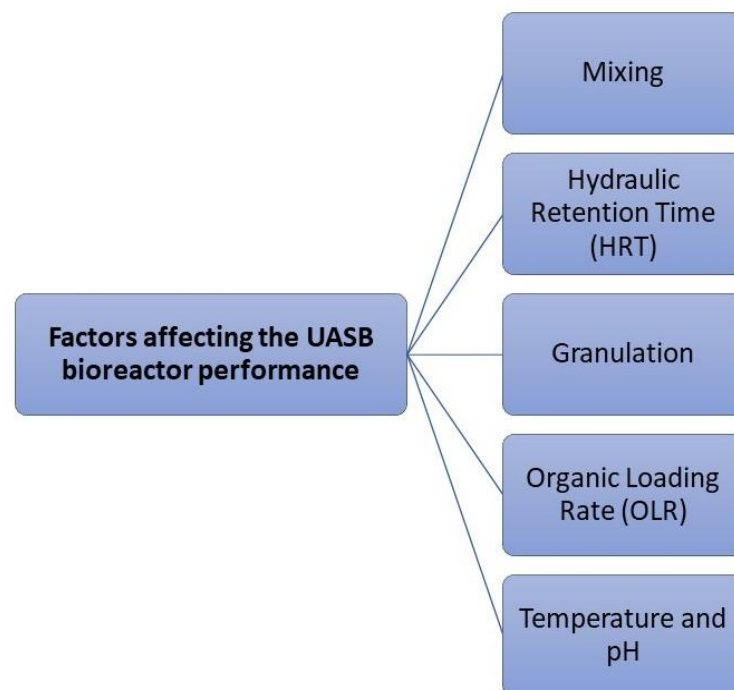


Figure 2.7: Factors affecting the performance of the UASB bioreactor during the operation

In a UASB reactor, settling characteristics of the anaerobic sludge should be efficient to maintain the aggregates and reduce immobilised active microbial aggregation (Lew *et al.*, 2011). The effluent quality can be improved by having a post-treatment system to remove existing pathogens, nutrients, and organic matter (Daud *et al.*, 2018). Post-treatment processes that have been used include advanced oxidative processes (AOPs), downflow hanging sponge, rotating biological contactors, and constructed wetlands (Nair & Ahammed, 2015; Possmoser-Nascimento *et al.*, 2014). The success of a UASB reactor depends on the contact between wastewater and sludge,

as well as the quality of biofilms that facilitate pollutant removal through microflora (Vassalle *et al.*, 2020). The results of the performance of a UASB used in different studies are given in Table 2-7. UASB drawbacks, such as prolonged granulation, can be overcome by adding calcium sulphate to enhance the granulation rate of methanogenic activity and expedite granulation (Liang *et al.*, 2020; Mainardis *et al.*, 2020).

Table 2-7: Summary of the Results of the Studies that used UASB for AMD Treatment

Carbon source	Days of operation	HRT (hours)	COD removal%	Sulphate removal%	Sulphide removal%	pH	References
Ethanol	340	24	68	86	50	6.4	(Rodriguez <i>et al.</i> , 2012)
Lactate	580	24	41	89	44	7.3	(Subtil <i>et al.</i> , 2012)
Acetate and ethanol	180	6	90	36	25	7.8	(Qiu & Ting, 2013)
Trichloroethylene (TCE)	340	24	93	98	68	7.7	(Guerrero-Barajas <i>et al.</i> , 2014)
Ethanol	20	-	87	98	75	7.2	(Najib <i>et al.</i> , 2017)
Acetate and ethanol	330	6	93	98	61.2	7.9	(Wu <i>et al.</i> , 2018)
Acetate and ethanol	329	6	84	88	-	7.0	(Chen <i>et al.</i> , 2019)
Methanol	285	6	93	38	80	7.5	(Mahmod <i>et al.</i> , 2019)
Acetate	148	24	91	99	75	7.3	(Yildiz <i>et al.</i> , 2019)
Acetate	180	12	91	43	58	7.1	(Wang <i>et al.</i> , 2021)
Hydrogen	105	10	76	82	49	7.0	(Zhang <i>et al.</i> , 2021)
Biochar	100	14	59	97	28	6.7	(Wu <i>et al.</i> , 2022)
Acetate and ethanol	360	6	71	99	55	7.5	(Gao <i>et al.</i> , 2023)

2.4.4 Fixed-bed bioreactor

The fixed-bed bioreactor comprises a packed bed of inert media through which sulphate-rich wastewater flows in an upward direction. The feed is introduced at the bottom of the reactor, ensuring maximum contact between the microbial biofilm attached to the media and the wastewater (Viggi *et al.*, 2010). The bioreactor is also fitted with gas vents designed to release hydrogen sulphide gas and other biogases (Dev *et al.*, 2017). The accompanying **Figure 2.8** illustrates the design of the fixed-bed bioreactor.

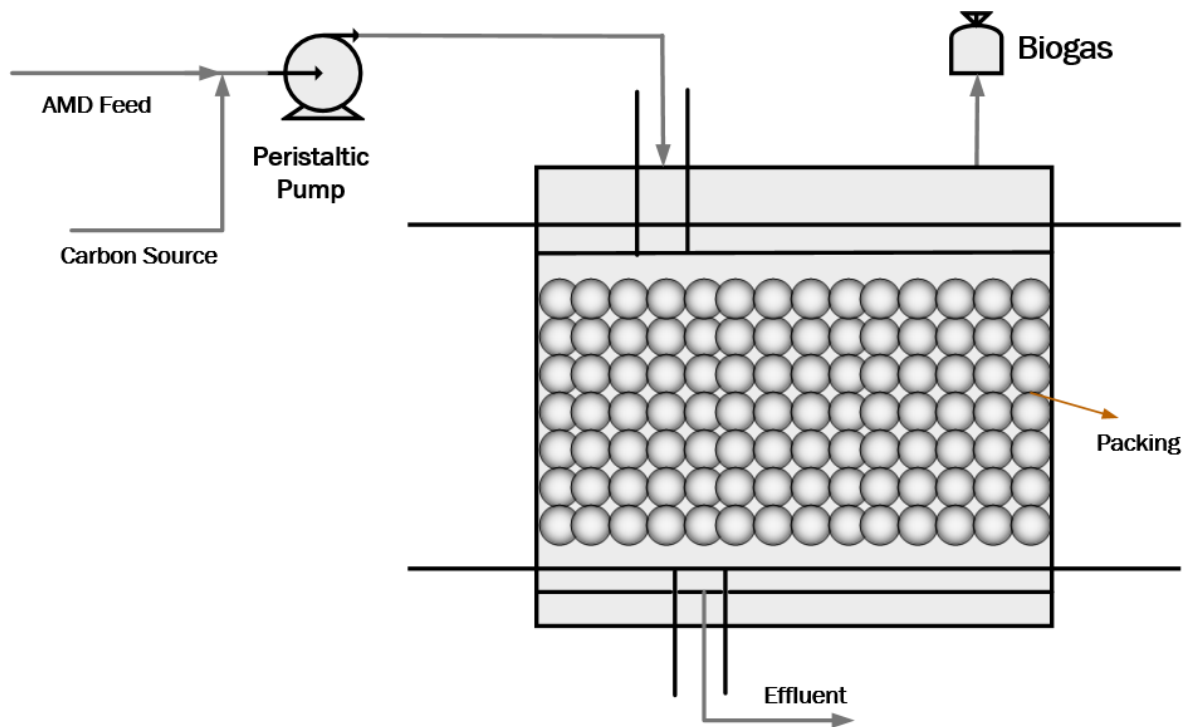


Figure 2.8: A fixed-bed reactor used for the treatment of AMD (Viggi *et al.*, 2010)

The packing media plays a crucial role in SRB biofilm, as it provides a high surface area support structure. Three key factors: cost-effectiveness, durability, and porosity - are considered when selecting materials (Kousi *et al.*, 2007). These materials are categorised into three types: inorganic, organic, and synthetic. **Figure 2.9** displays examples of each category. Inorganic materials, such as limestone and pervious concrete, help buffer pH levels, making them suitable for acidic wastewaters (Thisani *et al.*, 2021). Organic materials are more prone to breakdown, which can lead to clogging (Camiloti *et al.*, 2014).

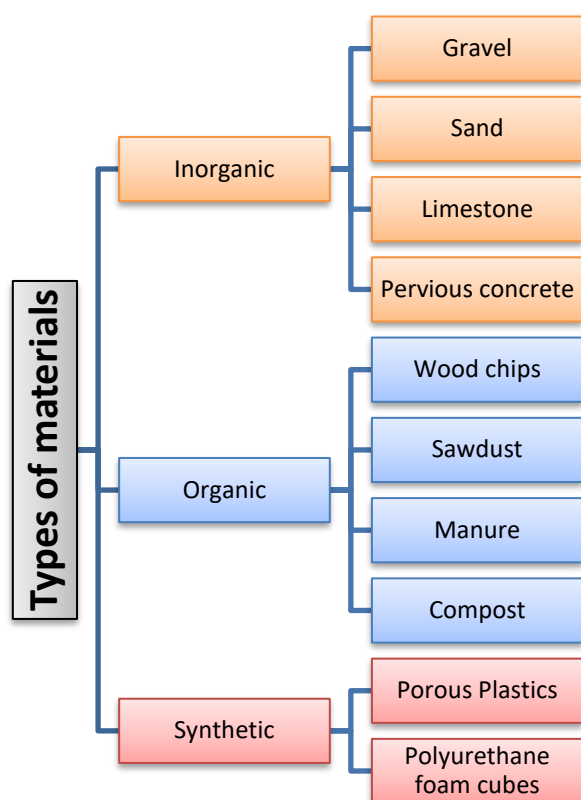


Figure 2.9: Different types of materials used for the packing media (Viggi *et al.*, 2010)

The wastewater is pretreated before entering the reactor to prevent inhibition of SRB caused by heavy metal toxicity. Some researchers have used permeable reactive barriers (PRB) for this stage, which include an alkaline medium to neutralise the acidic wastewater and partially precipitate certain metals (Thisani *et al.*, 2021). There are two strategies for removing heavy metals: either through single-stage or two-stage treatment (Khelifi *et al.*, 2009). In a single-stage process, one reactor is used for both metal precipitation and sulphate reduction simultaneously. Although this approach is simpler, it may lead to the accumulation of sulphide sludge at the bottom of the bioreactor, potentially causing clogging (Khelifi *et al.*, 2009). The two-stage process involves two reactors; one is used to generate sulphide, and the effluent is then transferred to another reactor for metal precipitation (Dev *et al.*, 2017). The method is beneficial as it reduces clogging within the bioreactor and decreases toxicity, thereby supporting the growth of SRB (Hernández *et al.*, 2022).

The parameters influencing the performance of the fixed-bed bioreactor are shown in **Figure 2.10**. Two types of electron donors are used: alcohol, such as ethanol and methanol, and solid organic waste, including manure. The latter can produce toxic compounds and requires a longer HRT (Ganesh *et al.*, 2010). The COD/sulphate ratio typically ranges from 0.67 to 2. A higher ratio leads to the dominance of methanogens, which decreases sulphate reduction by diverting carbon

(Camiloti *et al.*, 2014). The optimal pH for the process is maintained between 6.8 and 7.2. pH levels can be adjusted using chemical dosing or limestone (Hernández *et al.*, 2022). Temperature conditions are classified as mesophilic or thermophilic. For mesophilic conditions, SRB is active between 20 and 45 °C, while for thermophilic conditions, it is active between 55 and 70 °C (Kousi *et al.*, 2007). Toxic sulphide is managed through a multistage process. The HRT is longer for highly concentrated acid wastewater to ensure adequate contact between SRB biofilm and the wastewater (Kousi *et al.*, 2007). The results from studies using the bed bioreactor are provided in **Table 2-8**.

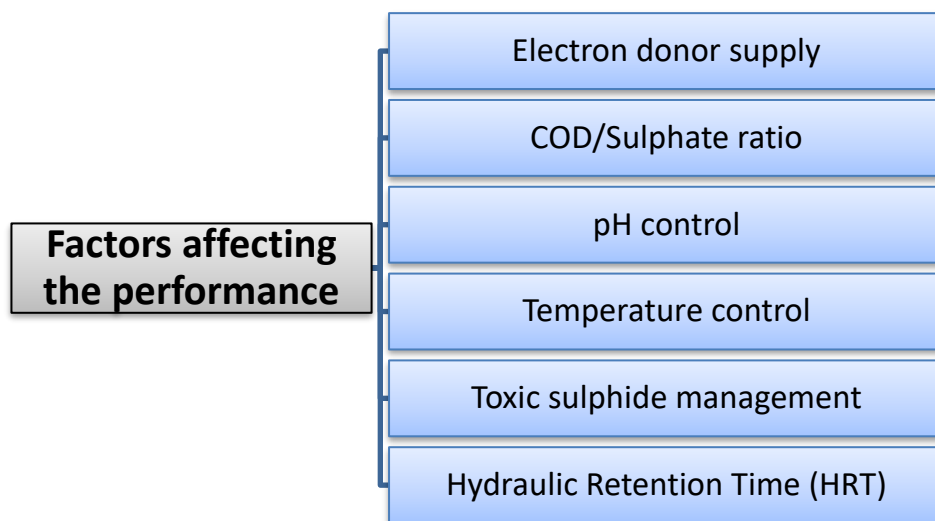


Figure 2.10: Factors affecting the performance of the fixed-bed bioreactor (Kousi *et al.*, 2007)

Table 2-8: Summary of the results of the studies that used a fixed-bed bioreactor for sulphate-rich wastewater treatment

Carbon source	Days of operation	HRT (hours)	COD removal%	Sulphate removal%	Sulphide removal%	pH	References
Ethanol	275	48	-	92	88	7	(Sarti <i>et al.</i> , 2010)
Cheese whey	39	192	55.4	68.1	47	7	(Jiménez-Rodríguez <i>et al.</i> , 2010)
Butanol	130	48	97	95	-	6.2	(Sarti <i>et al.</i> , 2011)
Zeolite	92	17	19	93	36	6.2	(Bratkova <i>et al.</i> , 2013)
Polyurethane	51	16	82	89	-	7.1	(Camiloti <i>et al.</i> , 2014)
Sodium lactate	110	24	32.3	89.2	77	6.8	(Teiri <i>et al.</i> , 2016)
Hospital wastewater	115	8	96	97	-	6.5	(Makhathini <i>et al.</i> , 2020)
Zeolite	7	168	-	77	-	6.8	(Andriyanto <i>et al.</i> , 2020)
Glucose	21	48	45	96	-	7.5	(Thisani <i>et al.</i> , 2021)
Microbial fuel cells	63	24	94.8	93	47.8	6.8	(Kieu <i>et al.</i> , 2023)

2.4.5 Batch bioreactor

Batch bioreactors are closed, anaerobic systems designed to accommodate a fixed volume of sulphate-rich wastewater for a predetermined period. The bioreactor type is appropriate for laboratory studies, small-scale operations, or scenarios with highly variable wastewater flow or composition (Zhang & Wang, 2013). It features a sealed single-tank chamber to facilitate all biological reactions, thereby maintaining optimal anaerobic conditions necessary for the survival of sulphate-reducing bacteria (SRB) (Gusek, 2002). For laboratory applications, a glass column is typically employed, whereas larger-scale implementations utilise enclosed plastic or steel tanks. An example of a batch reactor used by previous studies is shown in **Figure 2.11**. An essential requirement of the batch reactor is a well-mixed environment, which is achieved through mechanical agitation methods such as recirculation pumps or magnetic stirrers (Salo & Bomberg, 2022). Additionally, the bioreactor is equipped with a thermostat to maintain a mesophilic temperature range of 20 to 45 °C. There is also the option to incorporate inert support materials—such as glass beads, mineral coal, and polyurethane—to improve solid retention, increase the surface area for SRB attachment, thereby enhancing biological activity, and reduce cycle time (Greben *et al.*, 2005). Further design features include vents and gas meters to monitor and regulate the release of gases such as H₂S and CO₂. The bioreactor also contains sampling ports for collecting liquid samples to track parameters such as pH and sulphate levels.

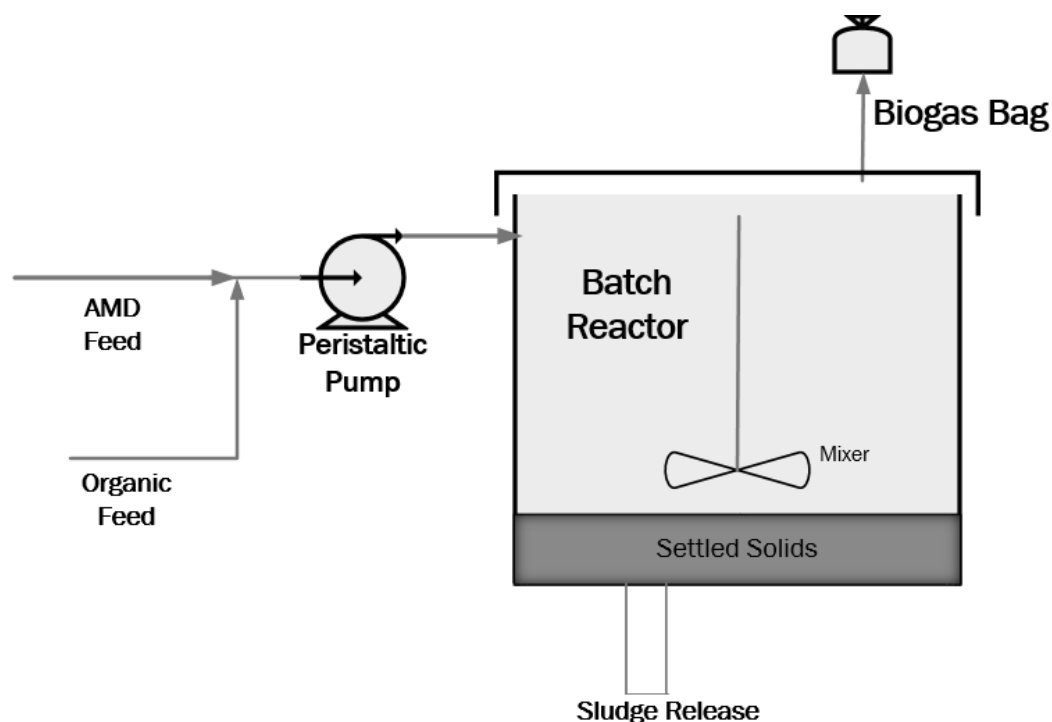


Figure 2.11: A batch reactor used for the treatment of sulphate-rich wastewater (Zhang & Wang, 2013)

The batch treatment process begins with feeding sulphate-rich wastewater alongside the external electron donor. Inside the reactor, the activity of SRB and its dominance over methane-producing microbes are encouraged by maintaining the correct carbon-to-sulphate ratio (Oyekola *et al.*, 2012). Additionally, limestone, as an alkaline substrate, can be added to help neutralise highly acidic wastewater. The reaction time is set (for example, 24-28 hours) to preserve anaerobic conditions. Once the reaction ends, mixing is halted, and heavier materials such as solid biomass and precipitated metal sulphides are allowed to settle at the bottom of the reactor (Dutta & Sarkar, 2015). All the treated water is then removed, while the accumulated sludge containing precipitated metals remains in the reactor. The process cycle is then repeated (Zhang & Wang, 2013).

The parameters influencing the performance of the batch bioreactor are shown in **Figure 2.12**. There are two types of electron donors used: liquid substrates, such as ethanol and methanol, and solid organic waste, including manure. Liquid substrates have higher reaction rates. The COD/sulphate ratio typically ranges from 0.67 to 2. Less COD can lead to insufficient sulphate reduction (Salo & Bomberg, 2022). The optimal pH for the process is maintained between 6.8 and 7.8 for effective operation. Chemical dosing or limestone can be employed to adjust pH levels. Temperature conditions are categorised into mesophilic and thermophilic ranges. For mesophilic conditions, SRB is active between 20 °C and 45 °C. Temperature affects the bacteria's metabolic rate; lower temperatures slow the process, while higher temperatures can kill the bacteria (Lamssali *et al.*, 2024). Toxic sulphide is controlled through a multistage process. The HRT is longer for highly concentrated acid wastewater to ensure adequate contact between SRB biofilm and the wastewater (Grubba & Majtacz, 2020). The results from previous studies using the batch bioreactor are provided in **Table 2-9**.

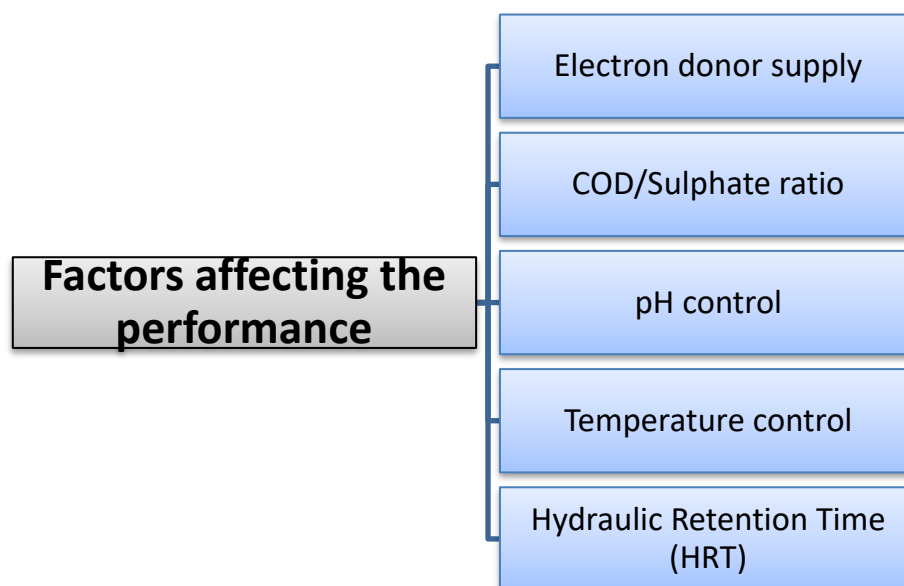


Figure 2.12: Factors affecting the performance of the batch bioreactor (Salo & Bomberg, 2022)

Table 2-9: Summary of the results of the studies that used a batch bioreactor for sulphate-rich wastewater treatment

Carbon source	Days of operation	HRT (hours)	COD removal%	Sulphate removal%	Sulphide removal%	pH	References
Ethanol	7	8	-	84.3	97.2	6.2	(Zhang & Wang, 2013)
Chicken manure	25	24	-	71.2	51.8	6.6	(Zhang & Wang, 2014a)
Methanol	200	12	90	99	99	8	(Dutta & Sarkar, 2015)
Ethanol	218	24	90	65	40	7.4	(Castro Neto <i>et al.</i> , 2018)
Ethanol	245	48	78	71	-	7.3	(Costa <i>et al.</i> , 2020)
Ethanol	90	6	-	92	-	7.8	(Grubba & Majtacz, 2020)
Ethanol	172	192	90.7	84.7	-	6.8	(Gomez <i>et al.</i> , 2023)
Sodium lactate	110	48	-	95	70	7.5	(Xue <i>et al.</i> , 2023)

2.4.6 Summary of the advantages and disadvantages of the bioreactors used to treat AMD

The table below outlines the advantages and disadvantages of bioreactors used for treating AMD or sulphate-rich wastewater.

Table 2-10: Summary of advantages and disadvantages of different anaerobic bioreactors.

Bioreactor	Advantages	Disadvantages
AnMBR	• Robust and reliable operation¹ • High effluent quality (permeate)² • Retention of microorganisms is brought about by ultra- and microfiltration membranes¹ • Possess a negligible footprint¹ • Has modularity³ • Reduction in the downstream infection needs²	• High membrane costs¹ • Membrane fouling caused by biofoulants (bacterial products)⁴ • Membrane fouling causes continual backwash/cleaning, low permeate flux, and short membrane life⁵ • Membrane fouling decreases bacterial diversity inside the AnMBR⁴ • Biofouling results in increased pressure needs⁶ • High hydraulic shear force causes high energy consumption³ • Elevated shear stress can negatively impact biological activity³ • Microbial metabolic activity can be affected by excess sulphate⁴ • Changes in operating conditions (flux, Transmembrane pressure (TMP), and gas scouring rate) affect process stability² • In-pipe cartridge AnMBR has high energy consumption due to circulation⁵ • Increasing sulphide concentrations decrease the removal efficiency¹
FBR	• Substantial solid-liquid contact zone⁷ • Negligible clogging⁸ • Low energy demand compared to CSTRs⁸ • High turbulence⁹ • Favourable mass transfer attributes¹⁰ • Uniform mixing¹¹	• Particle attrition⁹ • Particle's carryover¹² • Particle entrainment¹² • Require elevated SFV¹⁰ • Upward flow causes unconstrained biomass growth⁸ • Upward flow impacts the hydrodynamics of bioparticles¹²

UASB	• Low energy needs, short HRTs, and high OLRs can be managed¹³ • Elevated loading capacity can be facilitated¹⁴ • Can withstand an organic shock load¹⁵ • More reactor volume is required to achieve a high removal efficiency¹⁶	• Insufficient biodegradation can be caused by complex wastewater¹⁵ • Removal of pathogens requires post-treatment¹⁴ • Absence of activated sludge leads to a slow microbial growth rate¹³
Batch	• Suitable for variable compositions¹⁷ • Sequential stages with accurate control over the reaction time¹⁸ • Treat small wastewater volumes¹⁹	• Has a lower production rate compared to continuous reactors¹⁹ • Challenges with scaling up¹⁹ • Requires increased manual intervention²⁰ • High risk of inhibition due to accumulation of H₂S¹⁸

References:

¹ Domańska *et al.* (2019)

² Kharraz *et al.* (2022)

³ Lin *et al.* (2011)

⁴ Quang *et al.* (2016)

⁵ Xu *et al.* (2019)

⁶ Zhou *et al.* (2021)

⁷ Bello *et al.* (2017)

⁸ Che *et al.* (2016)

⁹ Nelson *et al.* (2017)

¹⁰ Papirio *et al.* (2013)

¹¹ Qian *et al.* (2019)

¹² Tisa *et al.* (2014)

¹³ Crone *et al.* (2016)

¹⁴ Khan *et al.* (2015)

¹⁵ Lew *et al.* (2011)

¹⁶ Oliveira and Von Sperling (2009)

¹⁷ Castro Neto *et al.* (2018)

¹⁸ Dutta and Sarkar (2015)

¹⁹ Gusek (2002)

²⁰ Moreira (2018)

2.5 Application of Sulphate Reduction on Active biological technologies in South Africa

The active biological technologies utilised to treat AMD require the use of two or more bioreactors, and some technologies use two or more microbial groups. The reactor units used need constant energy input. The addition of non-renewable resources such as lime or limestone is a dependent factor in many active treatment technologies (Harrison *et al.*, 2014a). Gypsum precipitation is caused by adding lime to sulphate-laden effluents (Harrison *et al.*, 2014a). However, precipitation management is required for the long-term sustainability of treatment technologies.

2.5.1 Rhodes BioSURE process

Using sulphate-reducing tannery resulted in increased degradation of the complex organic wastes. The Rhodes BioSURE process was implemented in the 1990s to address this problem (Harrison *et al.*, 2014a). Firstly, the algal sulphate-reducing ponding (ASPAM) process is an integrated bioprocess developed to treat wastewater with acidic metals (Rose, 2013). During this process, sulphate reduction requires an electron donor supplied by the algal primary production (Rose, 2013). The process was initially used to treat primary sewage sludge (PSS) due to the high AMD pollution on the East Rand, which required the use of more electron donors (Harrison *et al.*, 2014a). The recycling sludge bed reactor (RSBR) was then developed, as improved results were obtained from treating PSS, and the PSS degradation mechanisms were further studied (Rose, 2013).

Different RSBR configurations (3 to 23 m³, 10 L, 2 L) were used by studies that investigated the operation of the second-stage unit using the multi-compartment baffle reactor (Harrison *et al.*, 2014a). The electron donor or carbon source was supplied by: the fragmented PSS flocs from the RSBR; and the suspended and soluble COD obtained from the second reactor (Harrison *et al.*, 2014a). The sulphate reduction process was optimised separately using the obtained electron donor or carbon source. The particulate organics were entrapped, and SRB were immobilised by the sludge bed in a baffled reactor (Rose, 2013). The terminal electron acceptor (sulphate) used resulted in complete anaerobic digestion (Harrison *et al.*, 2014a) and achieved by preventing the deviation from methanogenic conditions by controlling the COD: sulphate ratio to be approximately 2:1 (Corbett, 2001). The methanogenic conditions can cause process failure because the sludge bed and SRB can be washed out by the disturbance of the upflow sludge bed caused by the evolving gas (Harrison *et al.*, 2014b).

The findings obtained prompted the design of a pilot plant for AMD treatment at 40 m³/day (Harrison *et al.*, 2014b; Rose, 2013). The plant was designed near Springs at the Grootvlei Gold Mine in South Africa. During that time, Grootvlei was the only operating mine left, and the whole

eastern basin was dewatered by it (Rose, 2013). The high-density sludge (HDS) process was then used to treat the pumped AMD (70-100 ML/day) (Rose, 2013). The pilot plant was fed with neutralised, metal-free effluent from the HDS process containing a high sulphate load (Corbett, 2001). The sulphide produced by the sulphate reduction was used to form iron sulphide by reacting the sulphide and an iron hydroxide sludge (Corbett, 2001; Harrison *et al.*, 2014b). During the pilot operation, concerns regarding the exposure of iron sulphide in the air, along with a challenge in managing the sludge, materialised (Harrison *et al.*, 2014a).

The pilot plant improved the performance process, which led to the use of an upflow RSBP by developing a demonstration-scale plant that can treat 1.6 ML/day of wastewater (Rose, 2013), followed by the development of a full-scale plant. This plant treated the iron hydroxide sludge (2 ML/day) and the HDS effluent (10 ML/day) from Grootvlei. The treatment was based on using upflow RSBPs with an external sludge recycle stream (Harrison *et al.*, 2014a). The process removed 12 t/day of sulphate, and this meant that the operation was successful. However, the plant was decommissioned after a few years because of utility charges (Rose, 2013).

2.5.2 Biosulphide and Thiopaq process

The Biosulphide and Thiopaq processes are the two primary technologies used to treat AMD. These processes are selected based on various parameters, such as flow rate, topology, and quality of AMD (Ali *et al.*, 2019; Magowo *et al.*, 2020b). The Kennecott Utah Copper Mine (USA) has a highly efficient Biosulphide process operation where hydrogen sulphide is generated through sulphate reduction, using a biological process, and there is a chemical circuit where metal sulphide precipitates are formed by the reaction between dissolved metals and hydrogen sulphide (Johnson & Hallberg, 2005; Matavacas Martínez, 2014). The process is shown in **Figure 2.13**. According to Taylor *et al.* (2005), the advantage of using these processes is that valuable materials such as sulphur and metals are recovered. However, the Biosulphide process may not be applicable for remote sites due to high operating and construction requirements. It is recommended that the capital and operating costs can be lowered by using a single bioreactor system (Magowo *et al.*, 2020b). Thus, the sulphidogens that are acid and metal-tolerant should be used to treat AMD with high metal concentrations and low pH (Holanda & Johnson, 2020).

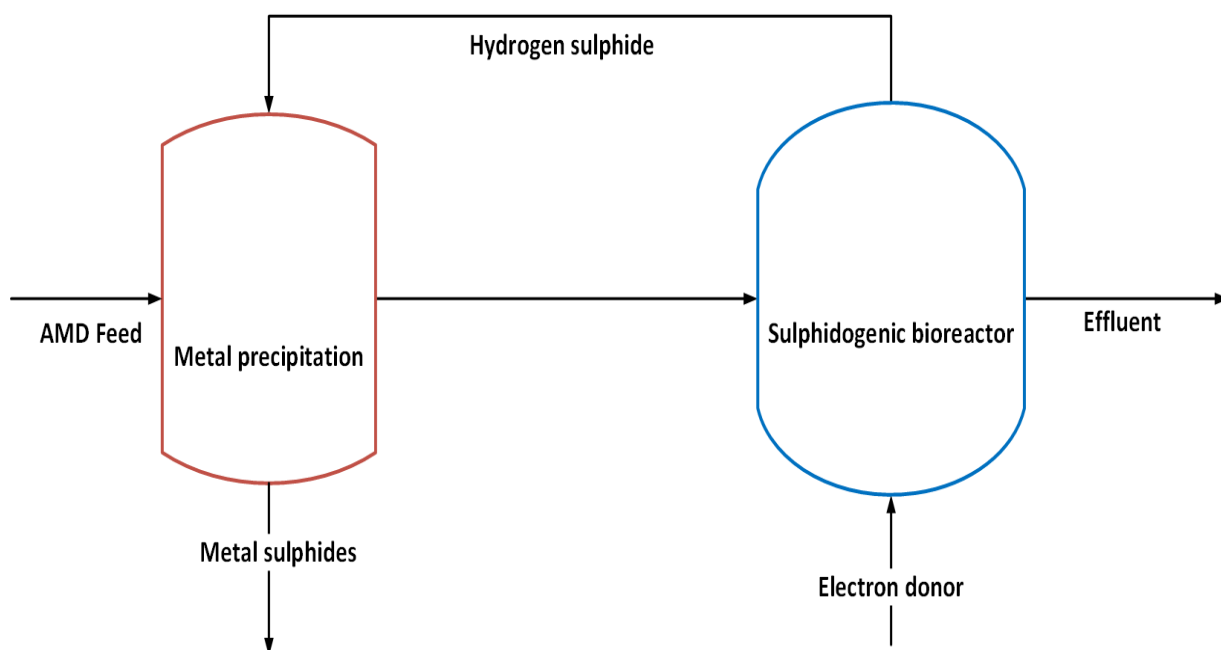


Figure 2.13: Schematic of the biosulphide process with metal precipitation and H₂S recycle stream (Matavacas Martínez, 2014)

The Thiopaq process consists of two distinct processes. Microbial populations are shown in **Figure 2.14**. The operation of the Thiopaq process has been successful at the Pueblo gold mine (Dominican Republic) and the Budezco zinc mine (Netherlands). During the anaerobic process, sulphate is reduced to hydrogen sulphide as SRB consumes the organic material (Magowo *et al.*, 2020b). The reaction between the dissolved metals and hydrogen sulphide leads to the formation of insoluble metal sulphides. In the second reactor, there is an aerobic process where SOB convert the excess hydrogen sulphide to elemental sulphur (Matavacas Martínez, 2014). When sulphide is removed as sulphur, the corrosive ability of the effluent is significantly reduced, then odours are removed, and the complete oxidation of sulphide to sulphate is prevented (Brahmacharimayum *et al.*, 2019; Magowo *et al.*, 2020b). Sulphur is a solid that is not corrosive, so the precipitates that are formed by it can be recovered conveniently from the bioreactors (Magowo *et al.*, 2020b).

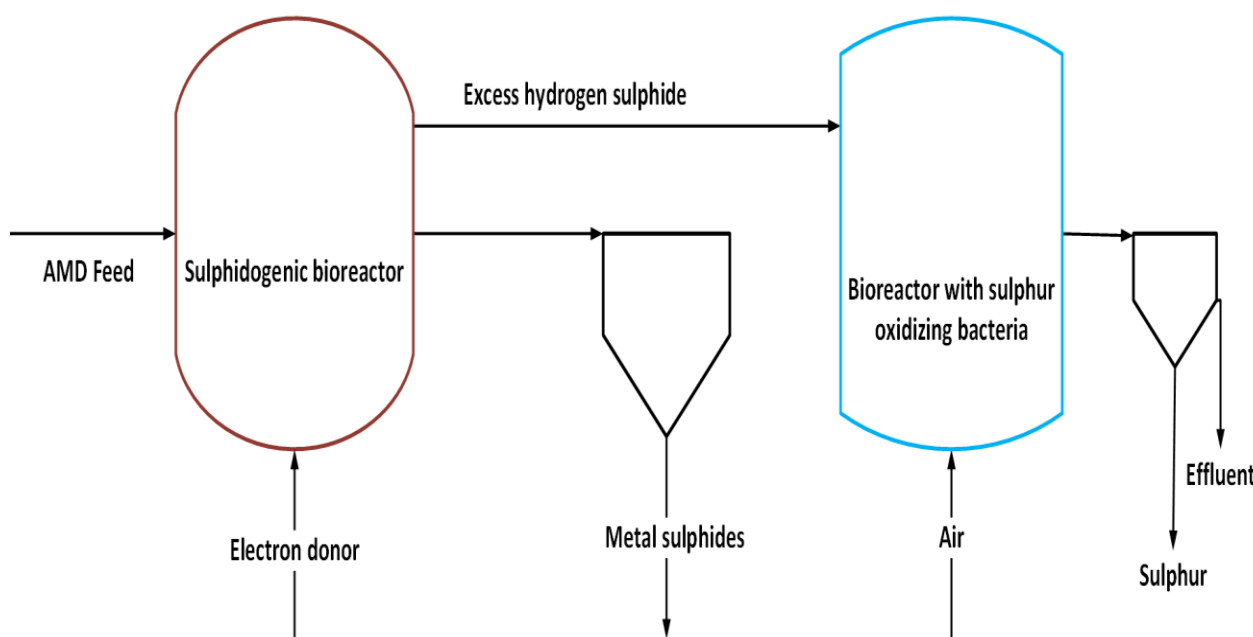


Figure 2.14: Schematic of the Thiopaq process with sulphate reduction and aerobic oxidation occurring in separate reactors (Matavacas Martínez, 2014)

2.5.3 Integrated Managed Passive (IMPI) process and passive bio-neutralisation

2.5.3.1 Integrated Managed Passive system (IMPI)

The implementation of the IMPI process was based on the disadvantages of the active treatment methods. The two main disadvantages highlighted were as follows: the pH levels under 4.5 significantly inhibited the existing systems, and the sulphate reduction efficiencies were low for the methods developed overseas. The IMPI process was established in 1995. The objective of implementing the system was to use an electron donor, such as the lignocellulosic material, to achieve high rates of sulphate reduction (Pulles & Heath, 2009). It was determined that the rate-limiting step of the process was the hydrolysis of lignocellulose (Harrison *et al.*, 2014b).

Some studies utilised the passive systems to characterise five different phases of sulphate reduction (Coetser *et al.*, 2005; Molwantwa *et al.*, 2010). The phases of sulphate reduction are presented as follows: lag phase, high-performance phase, crash phase, and sustained phase. The adaptation and establishment of the microbial community occurred during the lag phase (90-150 days), where high sulphate reduction rates were obtained during the high-performance phase, and low sulphate reduction rates were finally attained during the crash and sustained stage (Harrison *et al.*, 2014b). Ultimately, the consumption of all the hydrolysable lignocellulose led to the end of the sulphate reduction process (Molwantwa *et al.*, 2010). According to Pulles and Heath

(2009), the long-term performance of the process can be projected by reaching the crash phase, which can only be achieved by conducting studies over a prolonged period.

The initial objective of the IMPI research was to develop an economically viable system that can maintain over 600 mm/m³.day of sulphate reduction (Molwantwa *et al.*, 2010). The knowledge obtained regarding the rate-limiting step led to the investigation of the capability of the pre-treatment to decompose the lignin matrix utilising the white-rot fungi (Harrison *et al.*, 2014b). The mechanisms of lignocellulose hydrolysis were further studied in partnership with Rhodes University, where it was found that sulphidogenic conditions enhanced the breakdown of lignocellulose (Roman, 2004). It was also found that the sulphate reduction levels during the sustained phase can be increased by the organic carbon (molasses) that is provided by the degradation of lignocellulose, and the consequence of the crash phase can be substantially reduced by this molasses.

The degrading packed bed reactor (DPBR) was then developed based on the findings that were obtained. The reactor plays a significant role in the IMPI process and is shown in **Figure 2.15**. According to Coetser *et al.* (2005), the DPBR was packed with layers of distinct carbon sources, which increased the performance efficiency of the reactor. The upper section of the reactor was responsible for alkalinity generation, sulphide, and the removal of dissolved oxygen. The volatile fatty acid (VFA) was produced in the lower section of the reactor, and a rapid breakdown of lignocellulose occurred (Harrison *et al.*, 2014b). The overall process consists of the secondary sulphate reduction reactor (SRU), DPBR, and two sulphate oxidising reactors. As shown in **Figure 2.15**, organic carbon was not supplied to the sulphide oxidation units, and these units produced elemental sulphur from partial sulphide oxidation. The influent added to the reactor was generally combined with molasses (0.05-0.1%), which was used as a carbon source to establish an efficient operation. The IMPI process was applied at the pilot plant at Vryheid Coronation Colliery (VCC), and the process performance was stable for four years, obtaining higher sulphate reduction rates (>600 mM/m³/day) (Molwantwa *et al.*, 2010). The sulphate reduction rate was found to be affected by temperature during the summer season because performance improved during that period.

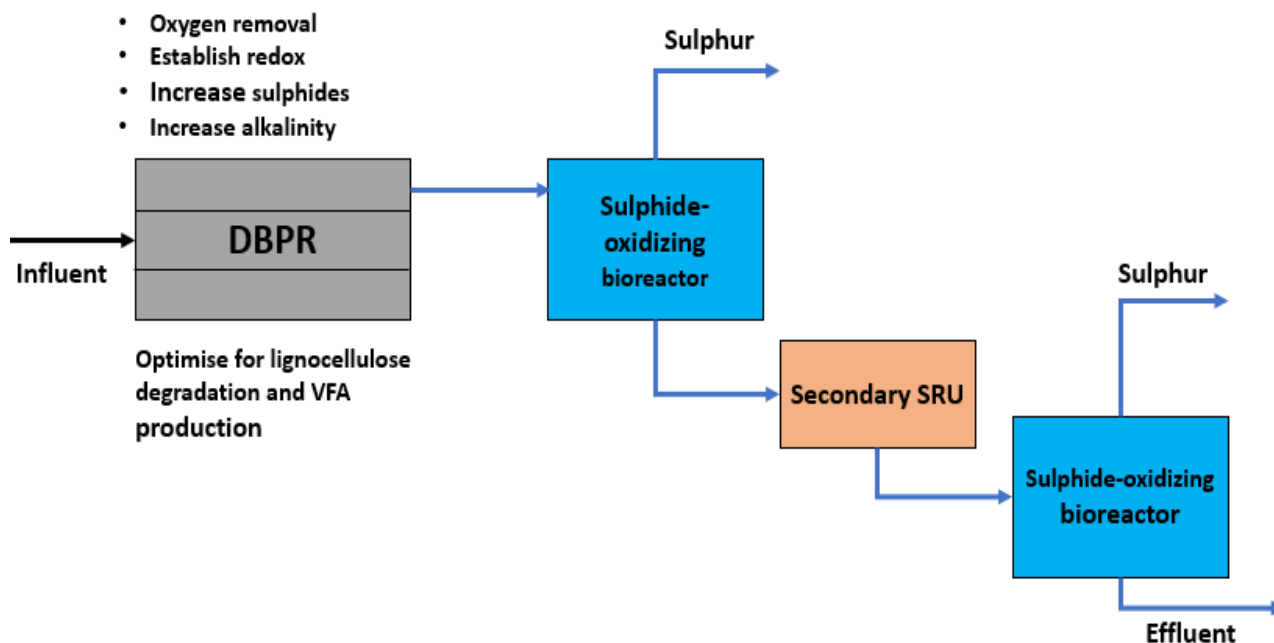


Figure 2.15: Schematic of the IMPI process used to treat acid mine drainage (Pulles & Heath, 2009)

2.5.3.2 Passive bio-neutralisation process

The objective of launching the passive bio-neutralisation system was to treat acid mine waters with a pH of less than 3 (Harrison *et al.*, 2014a). This treatment system used the degrading packed bed reactor (DPBR) as the main reactor. Firstly, a study identified three different microbial communities through a microbial ecology study (Pulles *et al.*, 2016). The study also noted that the system depended on the tight, distinctive orientation and interaction of microbial communities. To establish mandatory redox conditions (-250 to 350 mV), the microbial community initially managed to remove residual oxygen from the system (Pulles *et al.*, 2016). The *Clostridium* species were used as the second community, and these species resulted in the production of small charge-neutral carbon compounds (substrate); the breakdown of lignocellulose was enabled by these species (Harrison *et al.*, 2014b; Pulles & Heath, 2009). The third community was SRB, which led to the sulphate reduction reaction, generation of alkalinity as bicarbonate; the electron donor was oxidised (Pulles *et al.*, 2016). All the studies based on this system indicated an improved performance and produced pH values of the effluent of approximately 5.5 and 7 (Pulles & Heath, 2009; Pulles *et al.*, 2016).

2.5.4 Mintek Integrated cloSURE™ system

The cloSURE™ technology was developed by Mintek to apply a process in South Africa that can reduce the discharge of acid mine drainage (AMD) from the point sources in remote locations (du

Preez, 2021; Rambabu *et al.*, 2020). The other aim of developing the system was to produce an effluent suitable for irrigation in the agricultural industry. The system can be implemented in remote locations with insufficient infrastructure, for small point sources, such as closed mines and legacy mines (Neale *et al.*, 2018). According to van Rooyen *et al.* (2021), the advantage of using this system, in contrast to the conventional chemical precipitation process, is the production of considerably less solid waste with increased stability and reduced toxicity. The other advantages of using the cloSURE™ system are the requirement of low capital costs, and the use of semi-passive or passive treatment designs and less expensive carbon sources (waste substrate materials such as straw, wood chips, and cow manure) can sufficiently reduce the operating costs (du Preez, 2021; Neale *et al.*, 2018).

The cloSURE™ process consists of two stages: biological sulphate reduction (BSR) used to remove sulphide and the oxidation step to produce biosulphur (du Preez, 2021). This process uses the BSR column (stage 1) and the sulphide oxidation tank (stage 2). The process setup is shown in **Figure 2.16**. The first stage of the process uses sulphate-reducing bacteria, resulting in the removal of sulphate and metals, and an increase in pH and alkalinity (van Rooyen *et al.*, 2021). Sulphate is used for cellular respiration during this stage as the terminal electron acceptor by SRB, and energy is supplied by the consumption of the simple organic substrates by SRB (Knobel & Lewis, 2002). The second stage of the process was implemented to remove residual metals and sulphide and to recover biosulphur (du Preez, 2021). Sulphide is removed from the process because it can corrode the equipment and pose a threat to the plant operators. SOB is used for the sulphide oxidation to sulphur and sulphate with rising oxygen concentrations. According to Van Hille and Mooruth (2014), when there is a high oxygen concentration, partial oxidation of the sulphide to sulphur takes place, given by **Equation (4)**. And, when there is a high oxygen concentration, sulphide oxidises readily to form sulphate, indicated by **Equation (5)**. The second stage of the process utilises partial sulphide oxidation to reduce sulphate production.



The second stage of the process utilises partial sulphide oxidation to reduce the production of sulphate. In the oxidation tank, floating biofilm forms, which consists of sulphur oxidisers. This biofilm prevents the escape of hydrogen sulphide into the atmosphere. The floating biofilm also ensures minimum oxygen concentrations and maximum sulphide concentrations by minimising the diffusion of oxygen into the tank (Neale *et al.*, 2018).

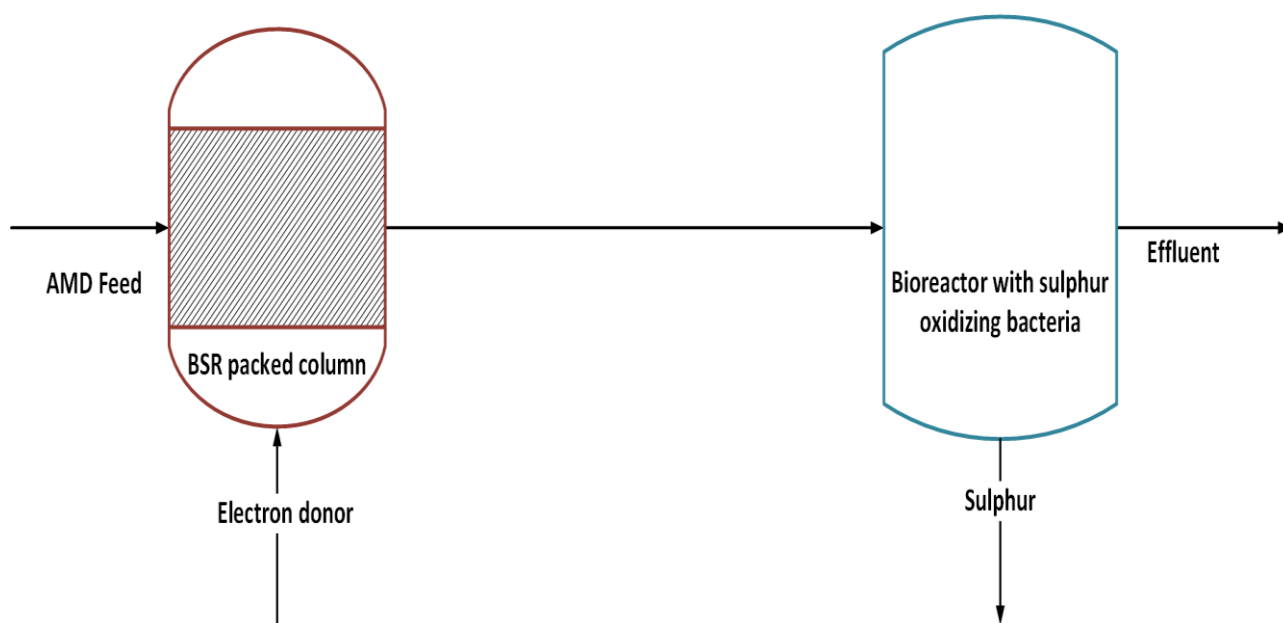


Figure 2.16: The Mintek cloSURE™ process is used to reduce the sulphate load and remove sulphides (du Preez, 2021)

The results of the cloSURE™ process are given in **Table 2-11**. From these results, it can be concluded that using two stages of the process can reduce the sulphides and produce water suitable for irrigation. Therefore, the cloSURE™ process can efficiently treat AMD in remote locations. However, it should be noted that both studies reported degradation of the packed bed column, as the research was conducted over a long duration. Mintek has investigated alternative bed designs to minimise costs and improve process performance.

Table 2-11: The summary of the results of the cloSURE process from two studies that used the pilot plant and laboratory scale tests

Study	Stage	Influent volumetric flow (L/d)	Sulphate reduction values (g/m ³ /d)	Sulphate removal%	Sulphide removal %	pH	References
Pilot	1	250	140	95	62	7.2	(Neale <i>et al.</i> , 2018)
Laboratory	1 and 2	5.2	196	87	98	7.5	(du Preez, 2021)

2.6 Use of Artificial Neural Networks (ANN) to model process performance

This section focuses on the ANN's key features, its applications in wastewater treatment modelling, the assessment of its performance, its advantages and disadvantages, and a comparison with linear regression.

2.6.1 Key Features of ANN

ANNs are computational models derived from the structure and operation of biological neural networks, although they are executed as mathematical systems rather than as exact duplicates of the human brain. ANNs consist of linked artificial neurons or nodes, which are organised into layers (Krenker *et al.*, 2011). An artificial neuron is a basic mathematical model and a fundamental component of the ANN (Krenker *et al.*, 2011). The ANN consists of an input layer that takes in initial data, numbers, or text. The subsequent layer is the hidden layer, which processes the input data through mathematical computations; here, the input to the next layer is the output of the previous one (Dongare *et al.*, 2012; Yang, 2009; Zou *et al.*, 2008). The typical structure of the artificial neural network is shown in **Figure 2.17**. ANNs have two parameters, weights and biases, which are adjusted during training to improve the network's accuracy. Weights are numerical values that represent the interactions between neurons, while biases are used by neurons to modify their outputs (Zou *et al.*, 2008).

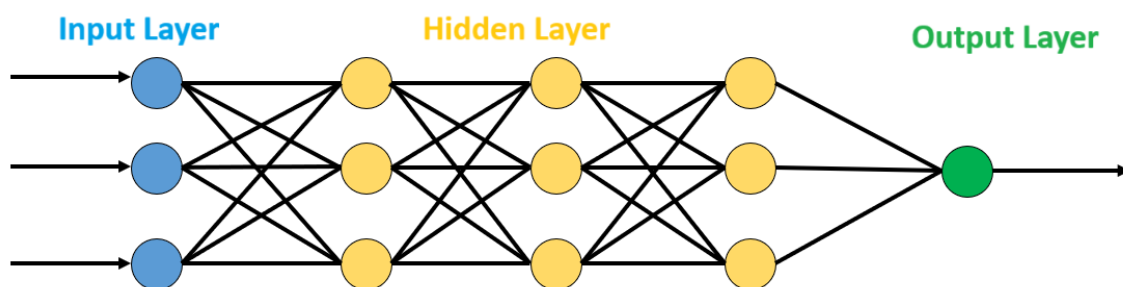


Figure 2.17: Structure of the ANN used for modelling

In the initial stage of an artificial neuron, the individual weights are multiplied by the input values. The middle of the artificial neuron is the sum of all weighted inputs and the bias. The final portion of the artificial neuron is where the activation function receives the preceding weighted inputs and bias sum. There are four activation functions, namely, Sigmoid, Rectified Linear Unit (ReLU), and hyperbolic tangent (tanh). Characteristics of each function are given in **Table 2-12**. The flow of information of an artificial neuron is shown in **Figure 2.18**. An example of a single node is given in **Appendix A (Example of a single node)**.

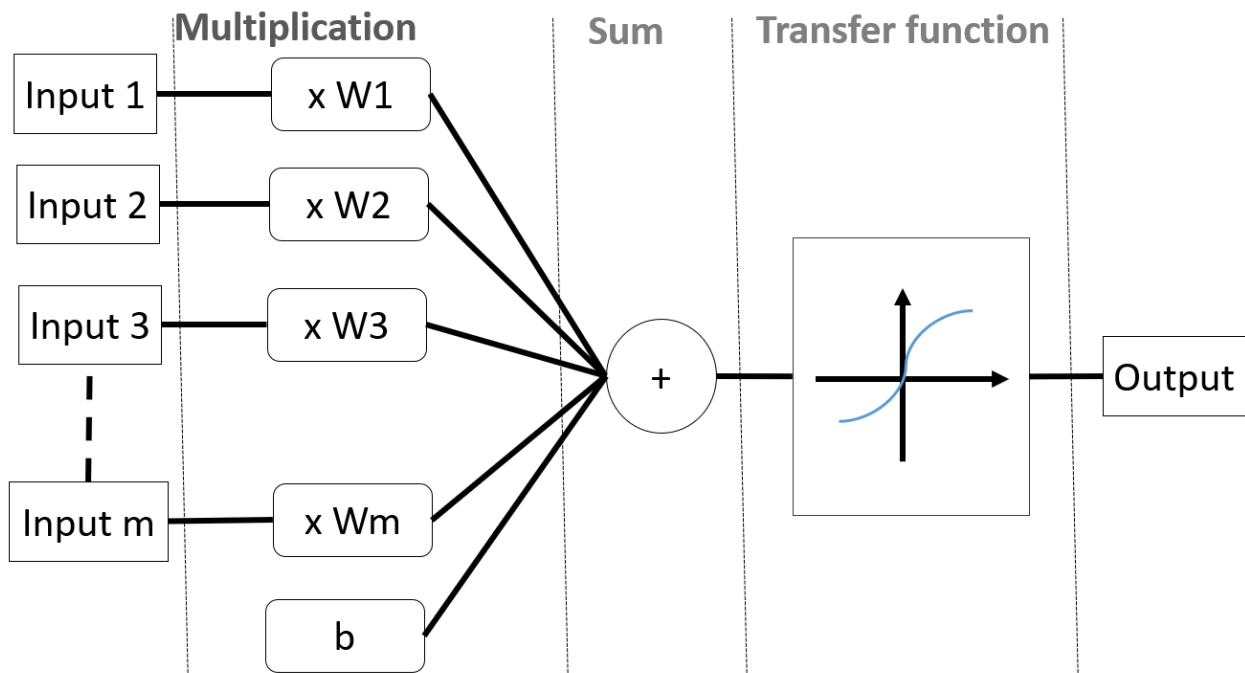


Figure 2.18: The flow of information of the artificial neuron (W is the weight and b is the bias)

Table 2-12: Mathematical form and characteristic of each activation function (Kim, 2008). Where z is the weighted sum of inputs and the bias.

Activation function	Mathematical form	Characteristics
Sigmoid	$\sigma(z) = \frac{1}{1 + e^{-z}}$	Compress values to a range of 0 to 1.
ReLU	$\tanh(z) = \frac{e^z - e^{-z}}{e^z + e^{-z}}$	Compress values to a range of -1 to 1.
tanh	$f(z) = \max(0, z)$	If positive, the input equals the output; otherwise, zero.

Networks with many hidden layers are known as deep neural networks (DNNs) (Dongare *et al.*, 2012). An activation function determines whether and to what extent a neuron transmits its output to the subsequent layer (Dongare *et al.*, 2012; Grossi & Buscema, 2007). Additionally, there are processes known as backpropagation and forward propagation. During backpropagation, the network calculates the error between the actual result and the prediction, and then adjusts the weights based on the backward propagation of this error through the network (Asadollahfardi, 2014). This process aims to prevent future network errors. Forward propagation involves generating a prediction by passing data from the input layer to the output layer via the hidden layers (Grossi & Buscema, 2007).

2.6.2 Applications of ANN in Chemical Processes and Wastewater Treatment

In industrial process environments, soft sensors are essential for measuring real-time variables such as product quality, as methods like analysers or lab analysis are complex and costly. ANNs are employed to address this by developing models that predict these variables from other measurable parameters, like pressure and temperature. In industrial settings, ANNs leverage live plant data to forecast final product quality, eliminating the need for slow, resource-intensive lab tests and enabling faster process control (De Filippis *et al.*, 2018; Otero, 2016). ANNs have also been integrated with model predictive control (MPC) to optimise key variables in distillation columns, such as reflux and reboil, thereby improving column performance (Ochoa-Estopier *et al.*, 2013; Osuolale & Zhang, 2016; Shin *et al.*, 2020). The ANN has been used in wastewater treatment to optimise operations and ensure compliance with environmental standards by predicting variables that determine water quality. Some uses of ANN in wastewater include forecasting suspended solids and biological oxygen demand (BOD₅) based on other measurable parameters, such as flow rate, temperature, and pH (Nagpal *et al.*, 2024; Qiu, 2023). There have been cases where ANNs have been used to predict key wastewater processes, such as sludge recycling, chemical dosing, and aeration. Some studies have utilised ANNs to improve flow rates in the activated sludge process, particularly for internal recycling. Other researchers have also evaluated the optimisation of coagulant dosage (Al-Ghazawi & Alawneh, 2021; Hamed *et al.*, 2004; Mihály *et al.*, 2022). ANN has been employed to reduce energy usage by aerators by predicting dissolved oxygen (DO) levels (Du *et al.*, 2018; Mihály *et al.*, 2023). Additionally, an ANN can be trained on normal operation data to help promptly detect events that cause equipment and process failures. Such models are useful for sensor fault identification, especially for DO sensors, and for predicting sludge bulking, enabling operators to intervene before process failure (Fuente *et al.*, 2012; Mihály *et al.*, 2023; Moral *et al.*, 2008). ANN models can also address complex relationships among various input variables and the removal performance of pollutants, such as heavy metals and organic matter, in wastewater processes. Some hybrid models, including integrated frameworks utilising RNNs, neuro-fuzzy models, and ANN combined with genetic algorithms (ANN-GA), have been used by other studies to predict these efficiencies (Fujail & Das, 2023; Honggui *et al.*, 2014).

2.6.3 Basics of Process Performance Modelling

The ANN workflow includes multiple steps to deploy the definitive, verified model. The objective is to apply historical plant operation data to train the ANN to forecast and improve the process performance. The steps followed in the development of ANN models are presented in **Table 2-13**.

Table 2-13: Steps followed to develop ANN models (Asadollahfardi, 2014; Hamed *et al.*, 2004; Hu, 2011; Zou *et al.*, 2008)

Design steps	
Define the problem and collect data	• Determine inputs & outputs • Collect historical plant data (large dataset)/experimental data
Data cleaning	• Remove outliers and missing values • Normalise data • Divide the dataset into train, validation, and test ratios
Network structure design	• In software such as MATLAB or Python, select the network type • Choose hidden layers and neurons. • Choose activation function (ReLU or hyperbolic tangent)
Network training, validation, and testing	• For training, calculate the mean squared error and look at the epochs • For validation and testing, check parameters such as mean squared error, root mean square error (RMSE) and coefficient of determination (R²).
Simulation and implementation	• Create a simulation using new process conditions • Implement the model into the plant control system

There are several train-test-validation split ratios, but the main ones that have been reported in the literature include 80-10-10 (used for large datasets), 70-15-15 (used for moderate-sized datasets), and 60-20-20 (used to emphasise more on validation and testing) (Gong & Zhang, 2023; Wu *et al.*, 2012). According to Walczak and Cerpa (1999) and Brandt and Lin (1998), it is recommended to start with a single hidden layer and introduce more layers if there is an improvement in validation metrics, such that two hidden layers are used when the single layer leads to underfitting of the validation data. For the number of neurons, it is recommended to begin with Baum's rule (neurons = sqrt (training data size)) for large datasets. For small datasets, it is recommended to start with neurons in the range of 10 to 20. It should be noted that an increase in neurons escalates the risk of overfitting (Goethals *et al.*, 2007; Rivals & Personnaz, 2000); fewer neurons pose a high risk of underfitting for advanced problems. It is recommended to use ReLU for medium datasets (1,000–50,000 samples) and large datasets (>50,000) as it enables faster training of data and prevents diminishing gradients (de Moraes *et al.*, 2023; Dellaferrera *et al.*, 2022).

It is recommended to use the hyperbolic tangent for networks with 1 to 2 layers or small datasets (<1,000 samples) (Dellafrera *et al.*, 2022; Sayin *et al.*, 2025).

2.6.4 The difference between ANN and Linear Regression

In this section, the ANN is compared with linear regression on key modelling characteristics such as data dependency, interpretability, use case, etc. The comparisons are presented in **Table 2-14**.

Table 2-14: The comparison between ANN and linear regression based on the features

Feature	ANN	Linear regression	References
Data reliance	Training requires more data.	Less intensive as data is needed for fitting variables.	(Kramer & Morgado-Dias, 2018) (Khashei <i>et al.</i> , 2012)
Data volume	Suitable for large data as it is prone to overfitting for datasets that are less than 100.	Suitable for small data sets (<100 samples).	(Kim, 2008)
Model clarity	A black box, it is challenging to interpret internal decisions.	The coefficients are straightforward to understand.	(Ahmad <i>et al.</i> , 2019) (Khademi & Behfarnia, 2016)
Non-linear behaviour	ANNs offer more robust control, as they can learn and adapt to regular changes in conditions and complex nonlinear process dynamics.	It has limited capabilities since it was created for linear behaviour.	(Frontistis <i>et al.</i> , 2023) (Tosun <i>et al.</i> , 2016)
Estimation precision	In most studies, ANN has been 40% more accurate than linear regression, achieving a root mean square error (RMSE) that is 2 times lower and a mean squared error (MSE) that is 3 times lower than linear regression.		(Ahmad <i>et al.</i> , 2019; Khademi & Behfarnia, 2016; Norouzian <i>et al.</i> , 2021)
R ² improvements	R ² values of ANN are 1.5 times greater than those of linear regression in most wastewater treatment modelling, indicating high model accuracy.		(Mata, 2011; Zare Abyaneh, 2014)
Algorithmic complexity	More resources are needed to train the data, and it is generally easier to validate and test. It can handle features that are greater than 10.	It is faster to train the data. It is limited to N+1 features (N is the feature number).	(Abdipour <i>et al.</i> , 2019) (Kim, 2008)
Generalisation capacity	Outside the training range, the model's performance becomes poor.	Outside the training range, the prediction becomes less accurate.	(Ibrahim <i>et al.</i> , 2024)
Application scenario	Used for complex relationships or processes.	Mainly used for simpler models with known interactions.	(Doicin <i>et al.</i> , 2024)

CHAPTER 3 MATERIALS AND METHODS

3.1 Overview

This chapter reviews the materials, experimental setup, and analytical methods used to examine the application of a linear flow channel reactor (LFCR) for treating acid mine drainage (AMD) with sulphate-reducing bacteria (SRB). The study focused on monitoring effluent parameters, including pH, sulphate, sulphide, and chemical oxygen demand (COD) for more than 100 days.

3.2 Materials

3.2.1 Acid mine drainage (AMD) and inoculation

AMD used in this study was supplied in a 25 L container by the Council for Mineral Technology (Randburg, Gauteng province, South Africa), henceforth referred to as Mintek. The AMD, which was collected from the coal mines in Mpumalanga province (South Africa), was stored at room temperature (25 °C). The main characteristics of this AMD, are provided in **Table 3-1**. These characteristics were determined using the APHA standard methods (APHA, 2005).

Table 3-1: Characterisation of AMD from Mintek before the treatment process

Characteristic	APHA Method	Value
pH	Electrometric method (using a pH meter)	2.9
Fe (mg/L)		52.4
Mn (mg/L)		11.6
Cu (mg/L)	Inductively coupled plasma optical emission spectrometry (ICP-OES)	0.30
Zn (mg/L)		0.59
Ni (mg/L)		0.22
Sulphate (SO_4^{2-}) (mg/L)	Turbidimetry	3451
Hydrogen sulphide	Methylene blue method	0.001
COD (mg/L)	Closed reflux, colorimetric method	22

3.2.2 Inoculum source and development

Following AMD characterisation, Mintek also provided the inoculum for the reactor. This inoculum, originally developed as reported by Neale *et al.* (2018) and Preez and Marumo (2024), had previously been used in studies such as du Preez (2022) and Weideman *et al.* (2024). Consequently, it was further assumed that the well-established microbial culture would require less of the carbon source.

3.2.3 Carbon source and other materials

Ethanol was used as a sole carbon source. Due to its availability in the laboratory, different types of ethanol were used, i.e. 95% or 99%. Other materials used included a 5 L container, which was used to prepare the feed.

3.2.4 Experimental equipment

3.2.4.1 Peristaltic pump

The pump was chosen because it has no seals or valves, can also handle suspended solids and sludge, and does not clog or suffer excessive wear from complex fluids. The suitable tubes for the pump were polyvinyl chloride (PVC) latex tubes, which are soft and fit into the pump and reactor.

3.2.4.2 Fume cabinet

A fume cabinet was used to capture gases such as H_2S , and CO_2 released by the biological sulphate reduction (BSR) process and expel these gases into the atmosphere. The experiments were conducted in a fume cabinet to protect laboratory personnel from inhaling these harmful gases and to maintain a good air quality. Another advantage of the fume cabinet was that it served as a physical barrier against potential spills during experiments, especially when feeding the AMD and taking samples. The H_2S , CO_2 , and sulphate safety data sheets are provided in **Appendix B (Safety data sheets)**.

3.2.5 LFCR design

3.2.5.1 LFCR construction

The design of the LFCR was based on reactors used by Mooruth (2013) (a large reactor of 25 L) and Marais *et al.* (2022) (a smaller reactor of 2.125 L). This reactor was scaled to be in the middle range of the two previous reactors. North-West University (NWU) designed and built a new LFCR with a total capacity of 12 L, corresponding to an operational volume of 9 L. Perspex with a thickness of 11 mm was used for the construction, which made it easier to observe the mixing profile (Mooruth, 2013). The reactor designs are shown in **Figure 3.1** to **Figure 3.5**. The dimensions of the reactor are 800 mm x 100 mm x 150 mm. A silicon gasket was used to seal the reactor lid, preventing hydrogen sulphide gas leakages. For liquid sampling, the reactor was fitted with 15 ports facing the front (Mooruth, 2013). Polytetrafluoroethylene (PTFE) Teflon plugs and

rubber stoppers with holes were used for the sampling ports, allowing multiple samples to be withdrawn using a hypodermic needle and syringe.

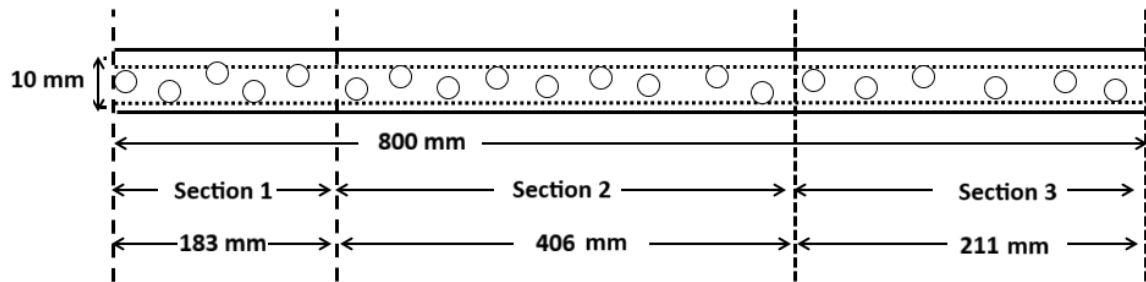


Figure 3.1: The entire length of the top view of the LFCR is shown in three sections

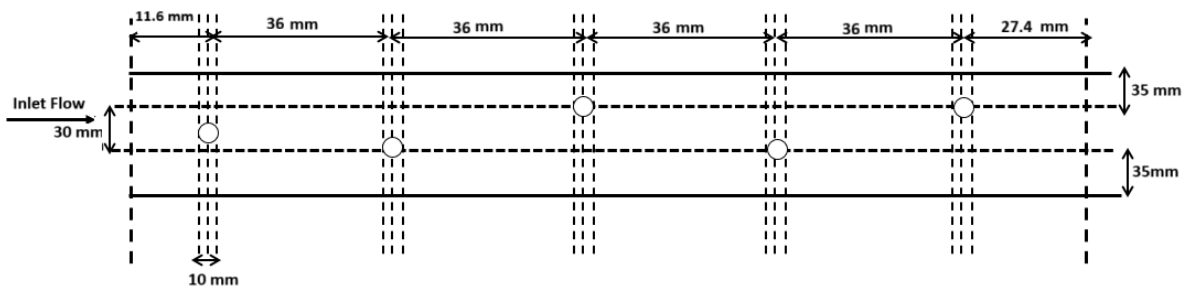


Figure 3.2: Section 1 of the top view of the reactor

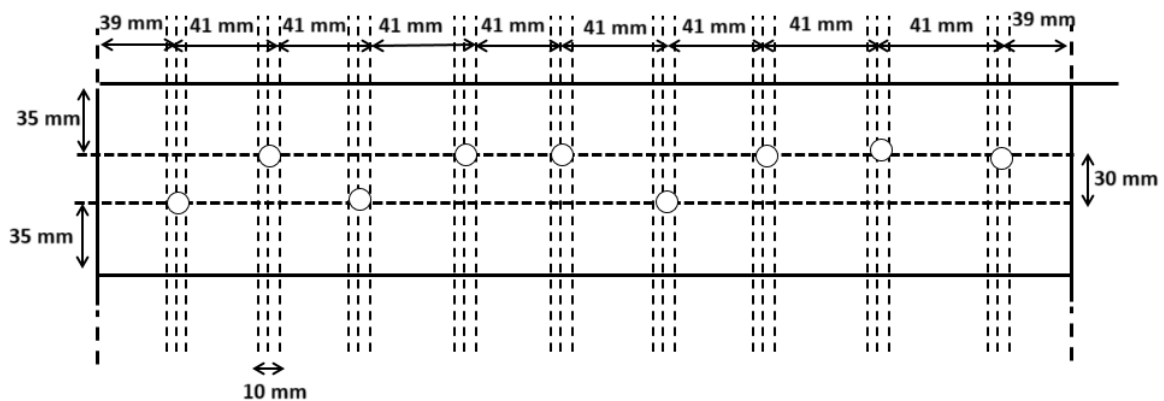


Figure 3.3: Section 2 of the top view of the reactor.

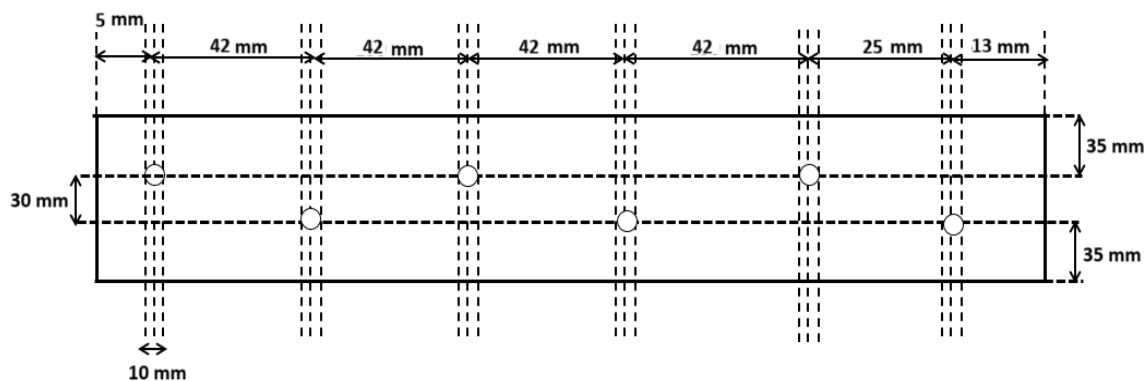


Figure 3.4: Section 3 of the top view of the reactor

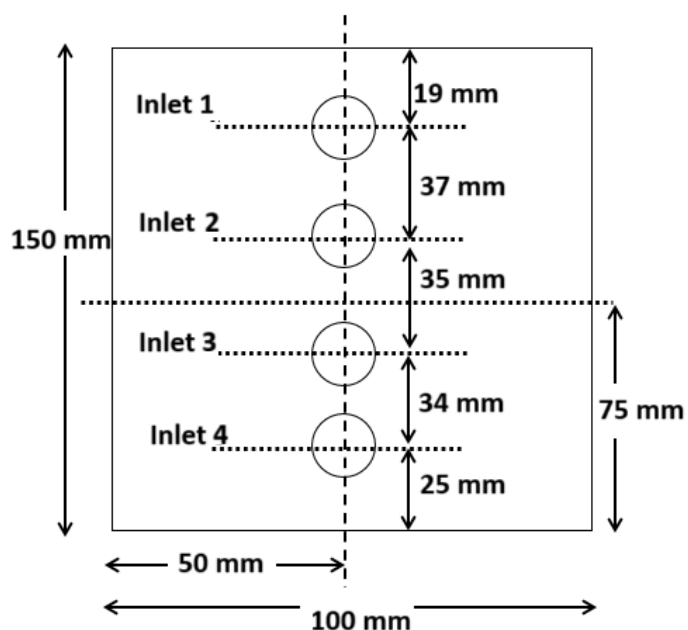


Figure 3.5: Drawing of the facade and back wall of the LFCR

3.2.5.2 Final Design of LFCR

The final design of the reactor, constructed by the NWU Technical team, was completed, primarily of plexiglass, and schematically shown in **Figure 3.6**. A photograph of the LFCR is shown in **Figure 3.7**. The reactor had tubing and connectors as shown in the figure; everything was placed inside the fume cabinet. The LFCR had four inlets or outlet ports with plastic valves to control the flow in or out of the reactor.

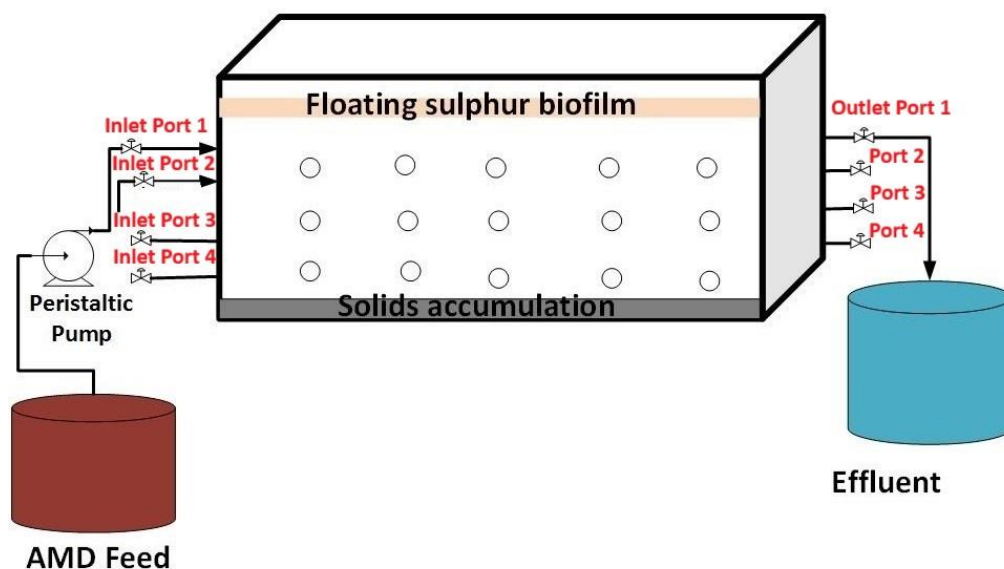


Figure 3.6: The proposed LFCR design

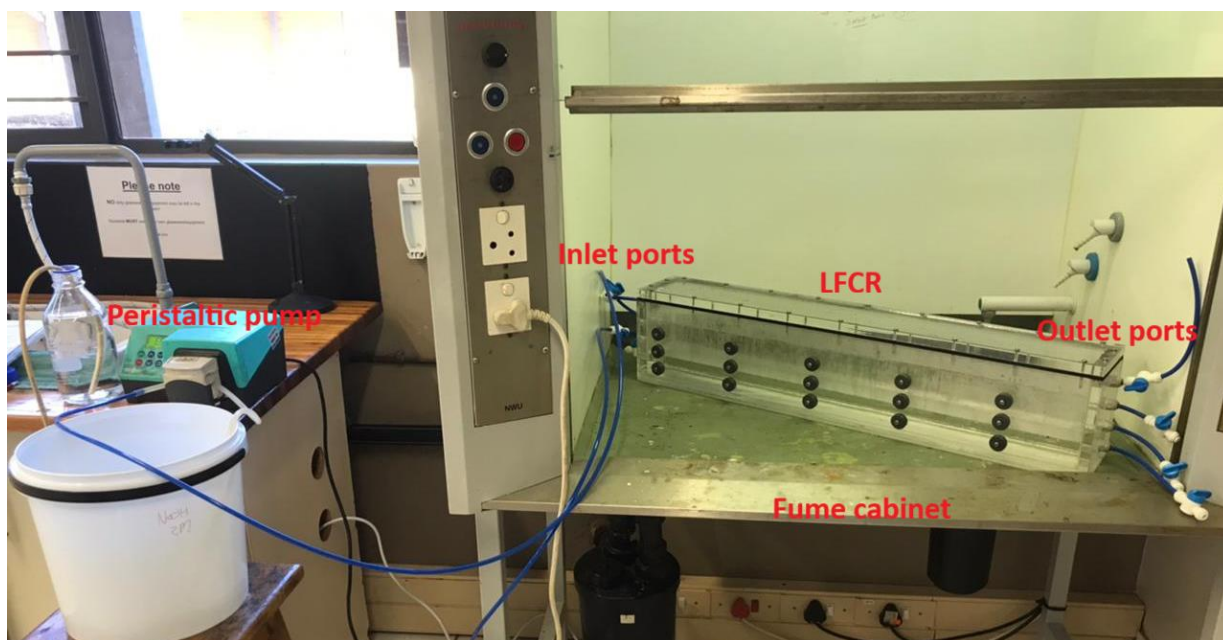


Figure 3.7: The LFCR was constructed alongside other experimental equipment, including the pump and fume cabinet

3.2.6 Personal Protective Equipment (PPE)

Suitable personal protective equipment (PPE) was worn during the experiments due to the existing hazardous chemicals; this included chemical splash goggles for eye protection, nitrile gloves as hand protection, a white lab coat for body protection, fully enclosed shoes worn in the lab, and a mask worn when working with hydrochloric acid (HCl) to protect against inhalation of fumes.

3.3 Pre-Tests

Two tests were conducted to ensure the reactor's operational integrity and verify its mixing efficiency. The tests conducted were the water leakage test and the phenolphthalein mixing test.

3.3.1 Water Leakage Test

The water-leak test was conducted to evaluate the reactor's leak-tightness before the BSR experiments could commence, to ensure the reliability of the LFCR system. The materials used for this test included clean tap water. The reactor was filled with water using the pump at a flow rate of 84 mL/hour. To reach a volume of 9 L, the height in the reactor was marked at 113 mm.

Equation (6) was used to calculate the height.

$$H_{working\ volume} = H_{Total} \times \frac{V_{working}}{V_{Total}} \quad (6)$$

After a volume of 9 L was reached, the pump was stopped, and the valves were closed. The signs of leakage were detected by inspecting all the ports, tubing, seals, and joints. The reactor was monitored the whole day. After the test, it was confirmed that there were no leaks and all reactor components functioned as intended, verifying that no operational disruptions were anticipated during the experiments.

3.3.2 Phenolphthalein Mixing Test

The test was conducted according to the reactor described by Mooruth (2013). The test used a visual pH indicator system to analyse the flow dynamics and mixing profiles inside the reactor. Materials needed for the test included phenolphthalein solution (pH indicator), sodium hydroxide (NaOH), and hydrochloric acid (HCl). A total of 4 L of NaOH was added to the reactor through inlet port 2 using the pump at a flow rate of 100 mL/hour. Phenolphthalein was added to the reactor via inlet port 1. The solution inside the reactor turned pink, which indicated that NaOH was present, as shown in **Figure 3.8**. A total of 60 mL of phenolphthalein was added.

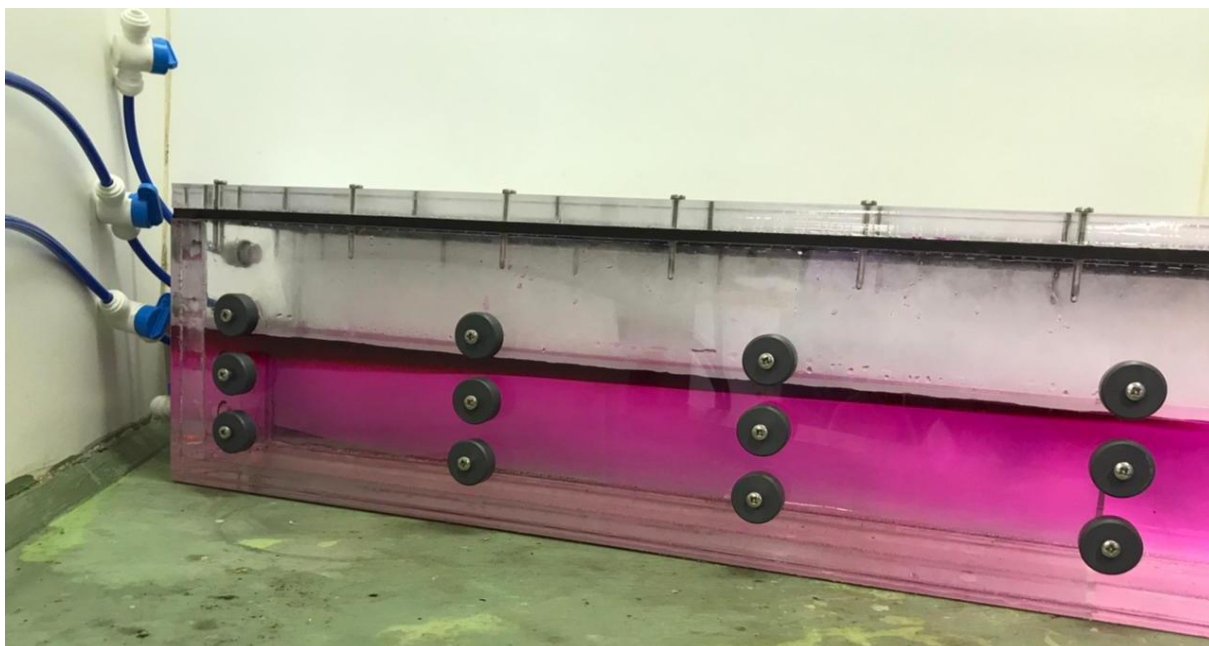


Figure 3.8: The pink colour of the solution in the reactor when phenolphthalein was added

HCl was then added slowly, using inlet port 3, at a flow rate of 60 mL/hour. As neutralisation took place between the HCl and NaOH, the colour of the solution changed from pink to colourless. The pictures were taken in 30-minute intervals and are shown in **Figure 3.9**.

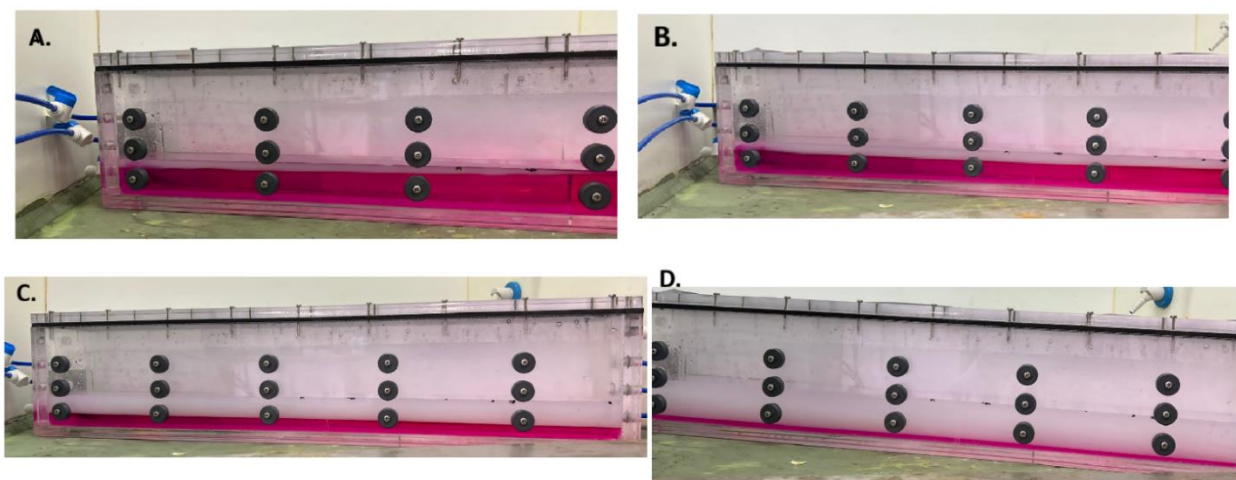


Figure 3.9: The indication of colour changes in the reactor when HCl is added to the solution: A (after 30 minutes), B (after 1 hour), C (after 1 hour 30 minutes), D (after 2 hours)

From the above Figure, it can be seen that the fluid mixing and dispersion were uniform throughout the reactor. The test also demonstrated the reactor's effectiveness in distributing fluids. There were no dead zones or incomplete mixing in the reactor. The test proved that adequate mixing can be achieved in the reactor.

3.4 Operating procedure with AMD

3.4.1 Feed preparation

The AMD was mixed with the inoculum in a 5 L container. Initially, 3 L of AMD and 1 L of inoculum were used for a period of 40 days. Then, the ratio was changed to 3.5 L AMD and 0.5 L inoculum for 30 days, and later to 2.5 L AMD and 1.5 L inoculum for the last 40 days. Additionally, 30 mL of ethanol was added. The mixture was thoroughly stirred to ensure homogeneity before use.

3.4.2 Experimental Setup and Procedure

The experimental setup for the reactor's 100-day operation is shown in **Figure 3.10**.

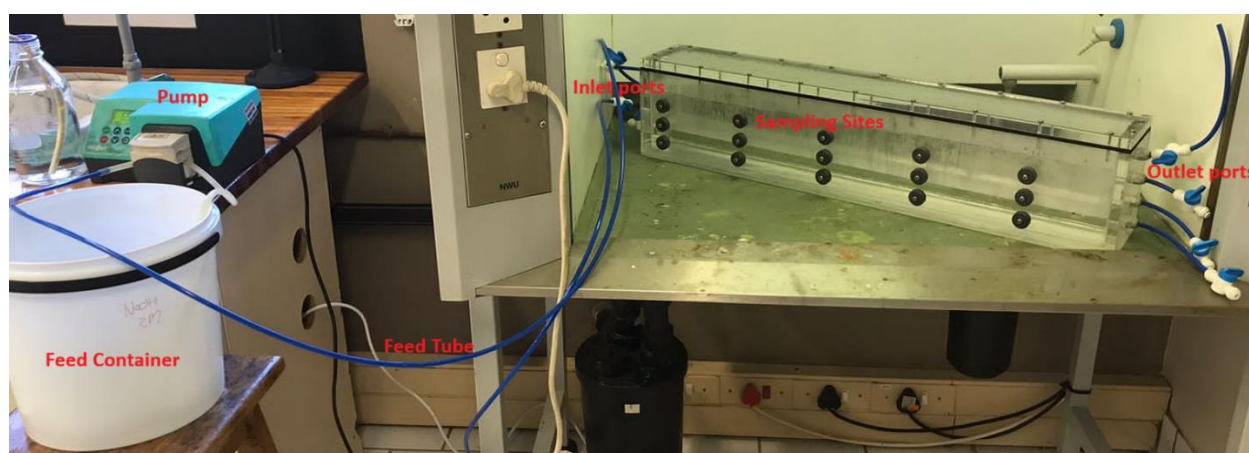


Figure 3.10: Experimental setup for the operation of the bioreactor for 100 days

Before the experiments commenced, it was ensured that all the valves of the inlet and outlet ports were fully closed. The feed tube was connected to the container and the pump. The flow rate was set at 84 mL/hour. The feed tube was placed on inlet port 2, and the valve was opened fully. The pump was started to feed the mixture into the reactor at a fixed flow rate. During this process, another container was used to create an alternative mixture, which was then added to the existing container when all the contents were pumped into the reactor. The reactor's 9 L volume was marked at a height of 113 mm to indicate the working volume. After the reactor was filled to a volume of 9 L, the pump was stopped, and the inlet valve was closed. The biofilm formation was obtained by allowing the reactor to remain uninterrupted for 2 to 3 days. All environmental conditions, including temperature and ventilation, were maintained at constant levels. The formation of the biofilm will be detailed in the results section. Summarised operating conditions for the reactor for period I (40 days), period II (30 days), and period III (40 days) are given in **Table 3-2**.

Table 3-2: Operating conditions for the three periods during which the LFCR was in operation

Condition	Period I	Period II	Period III
AMD volume (L)	6	7	5
Inoculum volume	2	1	3
Ethanol volume (mL)	30	30	30
Reactor liquid volume (L)	9	9	9
Oxygen requirement	Anaerobic	Anaerobic	Anaerobic
Influent pH	5.84	5.13	5.72
Influent Sulphide concentration (mg/L)	55	41	3.4
Influent Sulphate concentration (mg/L)	4179	5050	2761
Temperature (°C)	25	25	25
Hydraulic residence time (HRT) (days)	3	2	3
Sulphate/COD ratio	0.7	0.9	0.6

3.4.3 Sampling procedure

The reactor had 15 sampling ports, but only the middle nine ports were used for sampling to ensure consistent measured parameters throughout the reactor and to prevent dead zones, especially at the top, where a biofilm formed, and at the bottom, where settled solids accumulated. The sampling tools used were 30 mL syringes with hypodermic needles. Samples were taken daily by extracting 30 mL of reactor fluid with a needle into a 50 mL sample bottle. The outlet valve was opened to collect this volume. The rubber seals of the sampling ports were replaced after two days to prevent leaks. Samples were then analysed for the following parameters: pH, sulphate concentration, sulphide concentration, COD, and heavy metal concentration. The parameters were selected to examine the reactor's performance, SRB activity and the usage of the carbon source. Analytical methods adhered to the standard protocols (APHA, 2005).

3.5 Analytical methods

3.5.1 pH measurement

The pH of the influent, reactor samples, and the effluent was measured using a calibrated glass electrode on the Metrohm 744 pH meter.

3.5.2 Heavy metals measurement

The 10 mL samples were filtered and sent for analysis using Thermo Fisher Scientific's 6200 inductively coupled plasma optical emission spectrometry (ICP-OES) to determine the presence of, amongst others, manganese (Mn), iron (Fe), nickel (Ni), copper (Cu), and zinc (Zn). The

removal percentage of the heavy metals can be calculated with **Equation (7)**. The same equation was used to determine the removal percentages of other parameters, such as COD and sulphate.

$$Removal \% = \frac{Initial\ concentration - final\ concentration}{initial\ concentration} \times 100\% \quad (7)$$

3.5.3 COD Measurement

Chemical oxygen demand (COD) was measured using a HI83399 Hanna multiparameter photometer. Samples were filtered to remove solids, and 1 mL of a sample was added to a Hanna reagent vial. The samples were thoroughly shaken to ensure mixing. The sample vials were then placed in a HI839800 Hanna CD digester and heated to 105 °C. After an hour, the samples were removed from the digester and were cooled at room temperature for 20 minutes. The samples were then placed into the Hanna multiparameter photometer to measure COD. The COD indicated the amount of organic content present in the samples.

3.5.4 Sulphide Measurement

The sulphides present in the samples were measured using the Methylene Blue method. This method involves reacting the 10 mL sample with 3 mL of N, N-dimethyl-p-phenylenediamine to produce methylene blue in the presence of ferric chloride. The mixture was measured on a Thermo Scientific Genesys 20 ultraviolet (UV) spectrophotometer to determine the sulphide content in the samples.

3.5.5 Sulphate Measurement

The sulphate present in the samples was measured using Shimadzu UVmini-1240 UV spectroscopy. The 8 mL of samples were filtered to remove solids. In 10 mL vials, the samples were combined with a Hanna sulphate reagent. The mixture was left for 10 minutes, allowing the components to react fully. The UV spectroscopy was calibrated before measuring the sulphates. One of the calibration results is attached in **Appendix C (Analytical methods)**. The sample's sulphate content was analysed using UV spectroscopy. The sulphide production during the BSR process is calculated using **Equation (8)**:

$$Sulphide\ production = Sulphide\ effluent - Sulphide\ influent \quad (8)$$

3.6 Uncertainty and error analysis

The measurement instrument uncertainties for all measurements obtained are given in **Table 3-3**.

Table 3-3: Accuracies and calibration frequency of experimental instruments

Instrument	Measurement parameter	Accuracy	Calibration frequency
Metrohm 744 pH meter	pH	±0.01	Daily
Thermo Fisher Scientific's 6200 ICP-OES	Heavy metals concentration	±0.05 mg/L	Daily
HI83399 Hanna multiparameter photometer	COD concentration	±0.003 Abs	Daily
Thermo Scientific Genesys 20 ultraviolet (UV) spectrophotometer	Sulphide concentration	±2 nm Wavelength ±0.005 Abs	Daily
Shimadzu UVmini-1240 UV spectroscopy	Sulphate concentration	±1 nm Wavelength ±0.005 Abs	Daily

All the instruments were calibrated daily using a certified standard (APHA, 2005). The ICP-OES instrument is reliant on the element's concentration. It has low accuracy, leading to uncertainties in heavy metal concentrations. The Thermo Scientific Genesys has lower accuracy than Shimadzu UV spectroscopy, affecting sulphide concentration calculations.

3.7 Sulphate Composition Modelling

3.7.1 Linear regression

A linear regression was used to model the sulphates in the reactor effluent, thereby determining the reactor's performance. The sulphates were further modelled using other experimentally measured parameters such as influent COD, pH, and sulphides. Before the model was developed, the data was cleaned to remove outliers and improve the model's accuracy. The dependent and independent variables were and are provided in **Table 3-4**.

Table 3-4: Dependent and independent variables of the regression model

Dependent	Independent
Sulphate concentration	COD
	pH
	Sulphide concentration

The performance of the linear regression model was determined using the coefficient of determination (R^2), root mean square error (RMSE) and mean absolute error (MAE). MATLAB

software (R2020a) was used for final calculations. The code developed for modelling is given in **Appendix F (Linear regression)**.

The split ratio for the model was 70% training and 30% testing. The results of this model are discussed in the Results section.

3.7.2 Artificial Neural Network (ANN)

ANN is also used to model the sulphate concentration using other measured parameters. The feedforward ANN was designed with three input layers, one hidden layer (Abiodun *et al.*, 2019; Ge *et al.*, 2017; Uzair & Jamil, 2020; Zhang & Morris, 1998), and one output layer. The design choices are based on literature and are shown in **Figure 3.11**. The number of neurons in a hidden layer was selected based on the error on the test dataset (Basheer & Hajmeer, 2000).

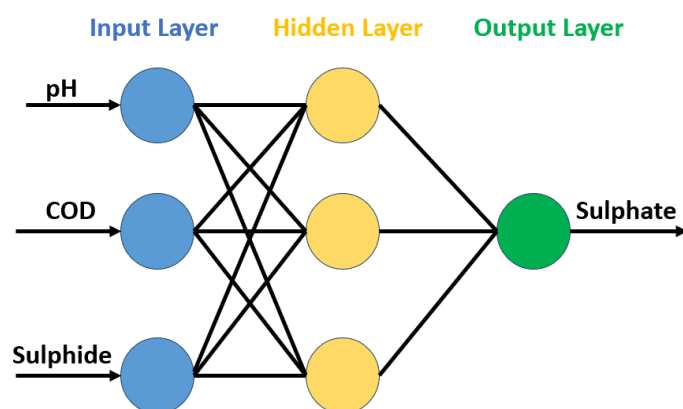


Figure 3.11: The ANN model structure used for modelling the effluent sulphate concentration

The neural network structure, with weight (w) and bias (b), is shown in **Figure 3.12**.

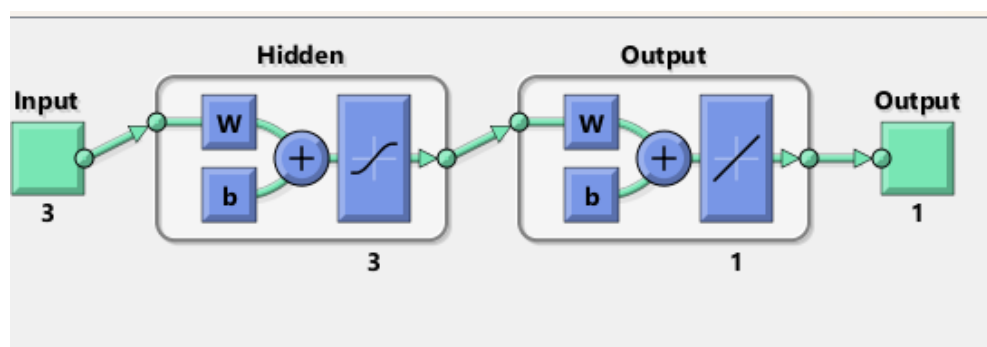


Figure 3.12: The neural network structure that was used to derive the model

The input features were influent COD, pH, and sulphide concentration. The dataset was split into 70% for training and 30% for testing (Bichri *et al.*, 2024; Nguyen *et al.*, 2021). The Levenberg-Marquardt method was used as a training function (Kadnár *et al.*, 2023; Norouzian *et al.*, 2021;

Tosun *et al.*, 2016). Hidden layers applied activation functions such as the hyperbolic tangent (tansig) (Ertuğrul, 2018; Sharma *et al.*, 2017), and the output layer used a linear activation (purelin) (Igwe *et al.*, 2021; Laabid *et al.*, 2023; Seyfi, 2017). The following parameters were used to evaluate model performance: R^2 , RMSE, and MAE. The model results were compared with those of the linear regression model. MATLAB was the software used. The development of the ANN model is illustrated in **Appendix G (Artificial neural network)**. The results are provided in the results section.

CHAPTER 4 RESULTS AND DISCUSSION

4.1 Overview

This section details the linear flow channel reactor (LFCR) performance in treating AMD over a period of 100 days, along with the modelling analyses. The experimental data were collected during the first 40 days of operation, which is Period I; the following 30 days, which is Period II; and the final 40 days, which is Period III. The operating conditions varied throughout the reactor's operation, including hydraulic residence time (HRT), AMD supply and inoculum composition, in addition to the sulphate/COD ratio. Two modelling methods, namely linear regression and artificial neural network (ANN), were used to predict the reactor's performance in sulphate removal. The results highlight key aspects such as biofilm formation, and variations of important parameters in the treated AMD, as well as the patterns observed from modelling the reactor's performance.

4.2 Formation of the Floating Sulphur Biofilm (FSB)

The linear flow channel reactor containing a mixture of AMD, inoculum, and the carbon source (ethanol) was left undisturbed for 3 days to allow the formation of the floating sulphur biofilm (FSB). The biofilm formed at the air-liquid interface in the reactor and functioned as an oxygen barrier, maintaining semi-anaerobic conditions within the reactor. Furthermore, the biofilm trapped elemental sulphur and facilitated sulphur recovery. The biofilm did form on the third day of operation, as demonstrated in **Figure 4.1**. Within the first three days, the biofilm's appearance remained thin, indicating that SRB was still in the early stages of growth. According to Garny *et al.* (2008), the stable attachment of the biofilm was still hindered due to the lower secretion of extracellular polymeric substances (EPS), and the density of the sulphate-reducing bacteria (SRB) was still low at this stage.

After 40 days, it became evident that the bacteria had grown, as shown by the thicker and more even biofilm in **Figure 4.1**. During this period, the microbial community exhibited active metabolism, which contributed to the formation of a strong EPS that supports a robust biofilm structure with consistent nutrient availability (Marais *et al.*, 2022). This also suggested that the biological sulphate reduction process accelerated, alongside the sulphate-oxidising process.

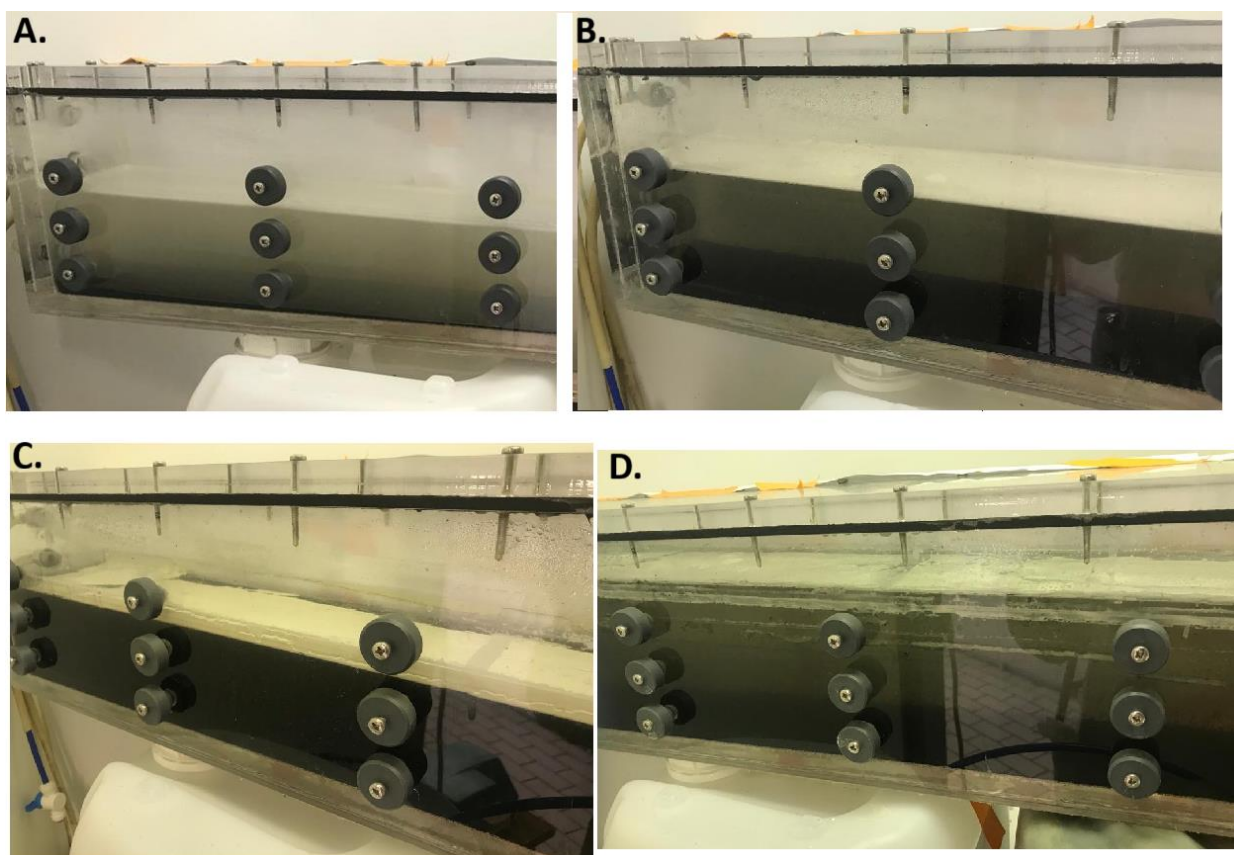


Figure 4.1: The formation and maturing of the biofilm throughout the reactor operation for 100 days. For the first three days (A), for 40 days (B), for 70 days (C), and for the last 90 days (D)

After 70 days, the biofilm appeared significantly thicker and denser, suggesting improved anaerobic conditions. The maturity of this biofilm resulted from the bacteria's highly active metabolism and indicated that the carbon source was consumed more rapidly and efficiently. However, according to van Hille *et al.* (2021), the dense structure raises concerns, such as the onset of mass transfer limitations. After 90 days, it became evident that the biofilm began to degrade and detach. The behaviour is caused by the depletion of the carbon source and increased accumulation of metabolic waste in the biofilm (Gaebler & Eberl, 2018). The biofilm results are vital in the discussion of other findings, such as sulphate removal and sulphide production.

The present study focused on the sulphate reduction stage; however, the distinctiveness of the LFCR depends on the capability to integrate sulphide oxidation with elemental sulphur recovery. Sulphide oxidation proceeded by abiotic mechanism under microaerophilic conditions (Johnson *et al.*, 2021; Pirieh & Naeimpour, 2019; Singh & Chakraborty, 2022), or through biotic mechanism with the involvement of SOB. The formation of the FSB on the air-liquid interface provided a regulated oxygen-restricted zone that benefits partial sulphide oxidation to elemental sulphur.

According to the literature, SOB plays a crucial role in the kinetics of sulphur formation (Alsanea *et al.*, 2023; Cui *et al.*, 2019; Linssen *et al.*, 2023; Martin *et al.*, 2019; Schwarz *et al.*, 2020).

It should be noted that during the reactor operation, light penetration was not a controlled parameter (Egger *et al.*, 2020). Bacteria, such as purple and green sulphur, require light for phototrophic sulphide oxidation (Di Nezio *et al.*, 2021; Kanno *et al.*, 2019; Struk *et al.*, 2023). LFCR was executed under dark conditions, suggesting that SOB, such as Thiobacillus, which thrive in oxygen-limited zones, had a greater effect on sulphide oxidation in the partially aerobic surface layer than photosynthetic sulphur bacteria (Berg *et al.*, 2019; Kanno *et al.*, 2019; Kushkevych *et al.*, 2024).

4.3 The Performance of the LFCR over 100 days

For these results, the main parameters evaluated were influent and effluent pH, COD, sulphate removal, and sulphide production. The results from the nine sampling ports were averaged and are referred to as reactor results. This section evaluates the occurrence of the changes in the parameters during the 100-day period.

4.3.1 Reactor performance based on pH of the treated AMD

The pH was measured regularly for both the influent and effluent, as well as at the sampling ports. **Figure 4.2** reflects the results of the pH levels of the influent, reactor content, and effluent. Based on **Figure 4.2**, the initial pH for the first 40 days was 5.84. The reactor content and effluent pH gradually increased to between 7 and 7.5, and the level was maintained until new feed was added to the reactor. At this stage, the biofilm was still progressing. During period II, the initial pH was 5.13, and as the days passed, the reactor content and effluent pH fluctuated between 6 and 6.5. The feed consisted of AMD volume, which reduced the pH in the reactor. For period III, the initial pH was high, at 5.72, due to the ratio of AMD to inoculum being 5:3. The pH of the reactor content and effluent increased to between 6.8 and 7 and remained there until day 100. According to Sahinkaya *et al.* (2013), the increase in pH can be attributed to the production of bicarbonate during the BRS process.

The pH results of the LFCR are consistent with those of other bioreactors mentioned in the literature in **Table 2-5**, **Table 2-6**, **Table 2-7**, **Table 2-8**, and **Table 2-9**. The pH of the treated wastewater of the other bioreactors was between 6 and 8, which is the same for the current LFCR, but with a maximum pH of 7.5.

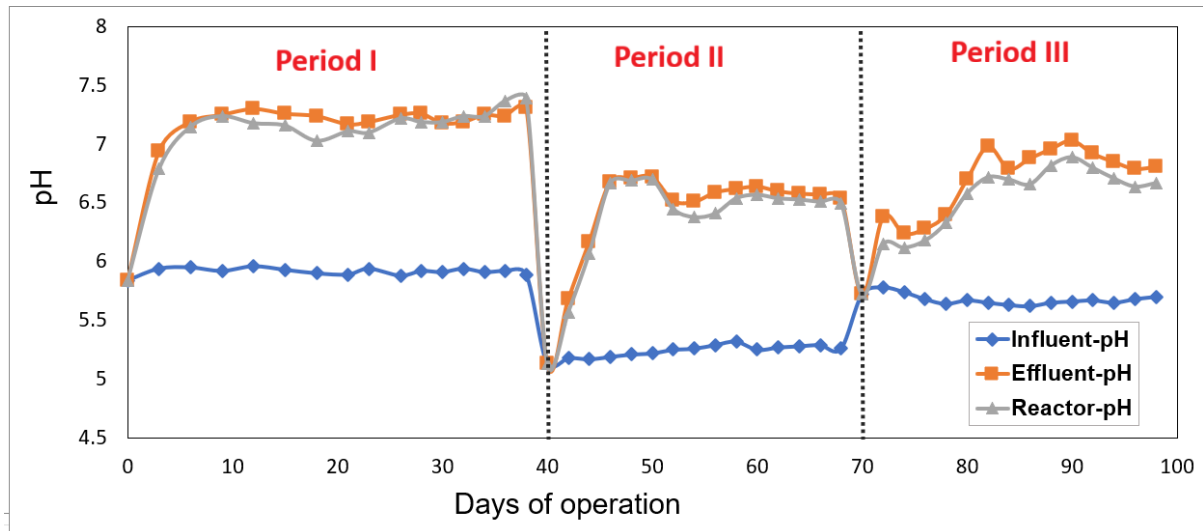


Figure 4.2: The measured pH of the wastewater over the 100 days of reactor operation

For period I, the increase in pH was also due to SRB becoming accustomed to the reactor conditions; as SRB broke down the sulphate, it produced hydrogen sulphide (H_2S), which raised the pH (Austigard *et al.*, 2018). As the biofilm matured, the ions were trapped inside the reactor, and SRB consumed those protons (Cunha *et al.*, 2019). In period II, the low pH was caused by the feed having elevated concentrations of sulphuric acid and dissolved metals, resulting in an acidic environment, as SRB was still adapting to the new acidic conditions (Makhathini *et al.*, 2020). As the bacteria became fully adapted to these conditions, the pH became stabilised. In period III, the mature biofilm enhanced sulphate reduction, maintaining the pH within the neutral range, where the microbial activity was at its highest.

4.3.2 Heavy Metals Removal

The concentration of heavy metals in the effluent was measured over the first 40 days. The results are shown in **Figure 4.3**. It is worth noting that it took 15 days to observe the decrease in the heavy metal concentrations.

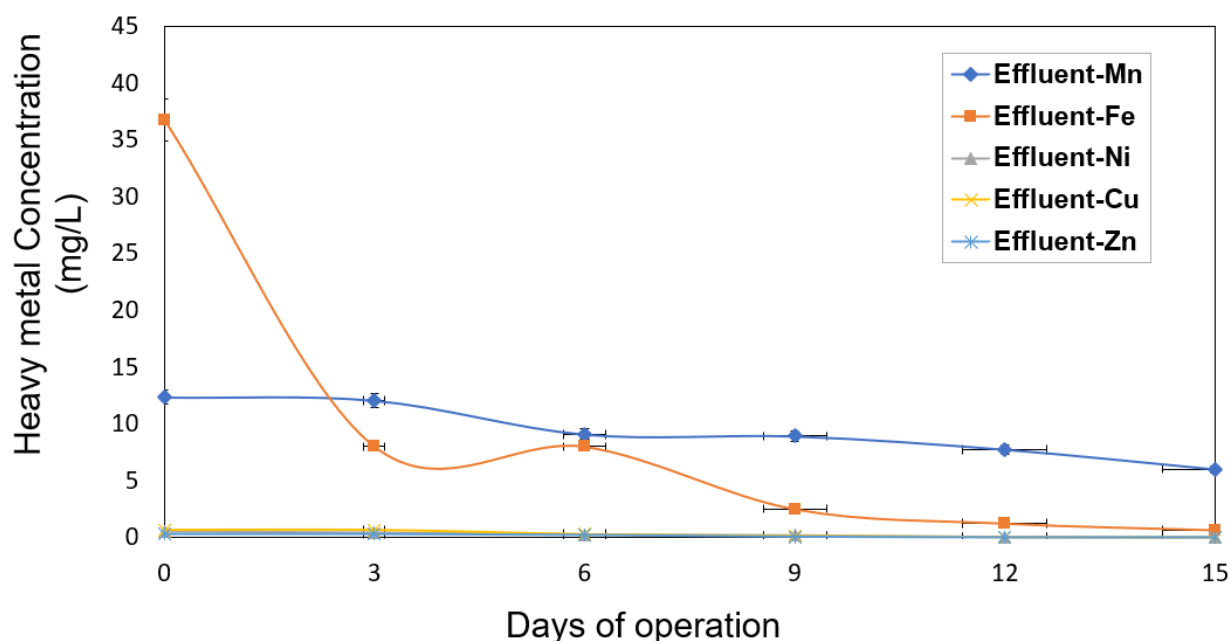


Figure 4.3: The measured heavy metals of wastewater over the 15 days of reactor operation

Initially, the concentrations of nickel (Ni), copper (Cu), and zinc (Zn) were 0.9 mg/L each. After 15 days, the metals were removed entirely from the effluent. According to Ayach *et al.* (2024), heavy metals such as Ni, Cu, and Zn readily attached to the microbial surfaces. Because of precipitation reaction with the produced H_2S , insoluble metal sulphides were formed. Another reason could be that SRB may use these metals as micronutrients (Bratkova *et al.*, 2018). Iron (Fe) initially had a high concentration of 37 mg/L, which decreased rapidly until day 6, after which it increased gradually, then decreased again to 1 mg/L, a suitable level for the effluent. The initial drop in Fe is due to its precipitation as ferric hydroxide under anaerobic conditions, with the final decrease in Fe attributable to the high microbial activity of SRB, resulted in the final precipitation of Fe. Manganese (Mn) was initially measured at 12 mg/L, and subsequently it decreased to 7 mg/L over time. Because the Mn level remained high, further measurements of the effluent Mn concentration were subsequently taken. The slow removal of Mn is attributed to its high solubility and its requirement for conditions with elevated pH (Yadav & Jamal, 2015). During this period, SRB microbial activity continued to increase, which prevented it from breaking down or precipitating all the Mn.

4.3.2.1 Mn removal over a period of 60 days

The Mn concentration in the effluent was monitored again for period I and period II, with the results shown in **Figure 4.4**. The removal of Mn was not feasible within the 60-day measurement, compared to the other metals. Normalised Mn concentrations were calculated and are presented in **Appendix E (Normalised Mn and sulphide concentrations)**.

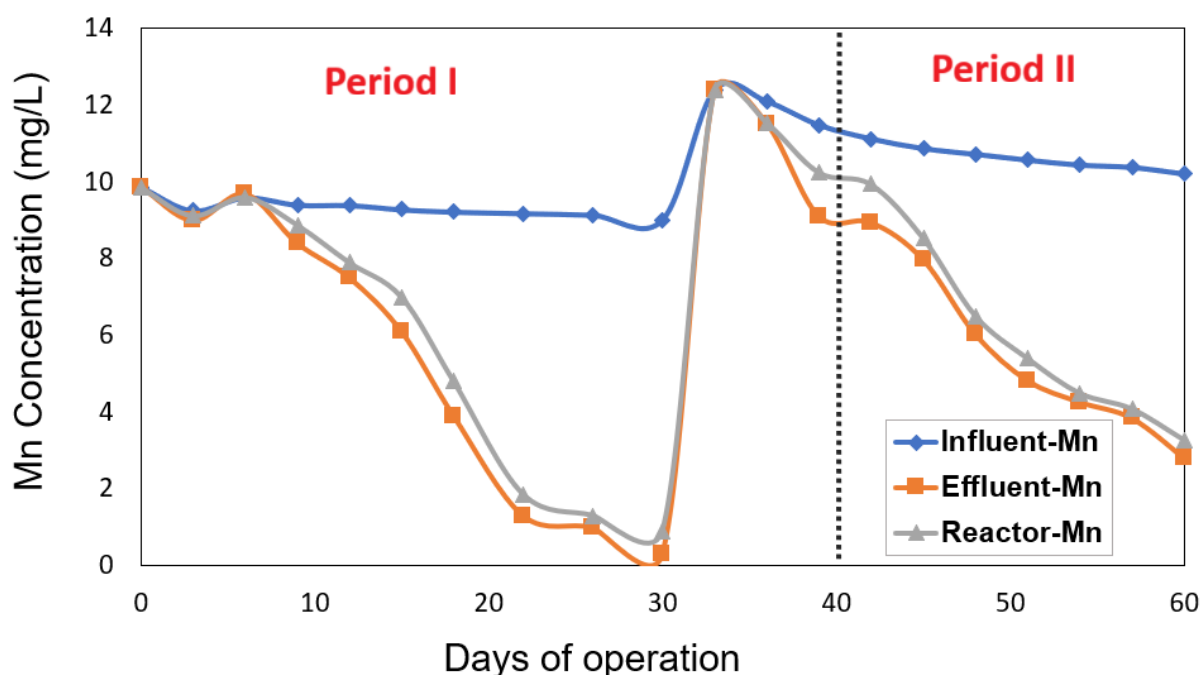


Figure 4.4: The measured manganese (Mn) concentration of wastewater over the 60 days of reactor operation

The initial influent Mn concentration was 10 mg/L. The effluent concentration sharply declined from day 5 to day 30, reaching 0.1 mg/L. During this period, the precipitation of Mn as MnS increased due to heightened SRB activity, which nearly raised the pH to neutral levels. Additionally, the biofilm was developing, which subsequently enhanced the Mn removal (Tobiason *et al.*, 2016). A sudden increase in Mn levels to 12 mg/L occurred between days 30 and 37, following the addition of AMD to the reactor. The addition of AMD lowered the pH, possibly causing MnS to dissolve from the biofilm (Afsar *et al.*, 2022). The addition of AMD introduced acidic conditions and metal toxicity, negatively affecting SRB activity. In period II, the Mn concentration began to decrease rapidly again, but only to 2.8 mg/L, which is suitable for treated acidic wastewater. As SRB adapted to the new conditions, microbial activity increased, triggering further precipitation of dissolved Mn. As the biofilm matured, it was assumed that metal dissolution would not occur when adding AMD.

4.3.3 COD reduction of the treated AMD

The COD in the influent, effluent, and reactor content was monitored over the 100 days of reactor operation. The results are shown in **Figure 4.5**.

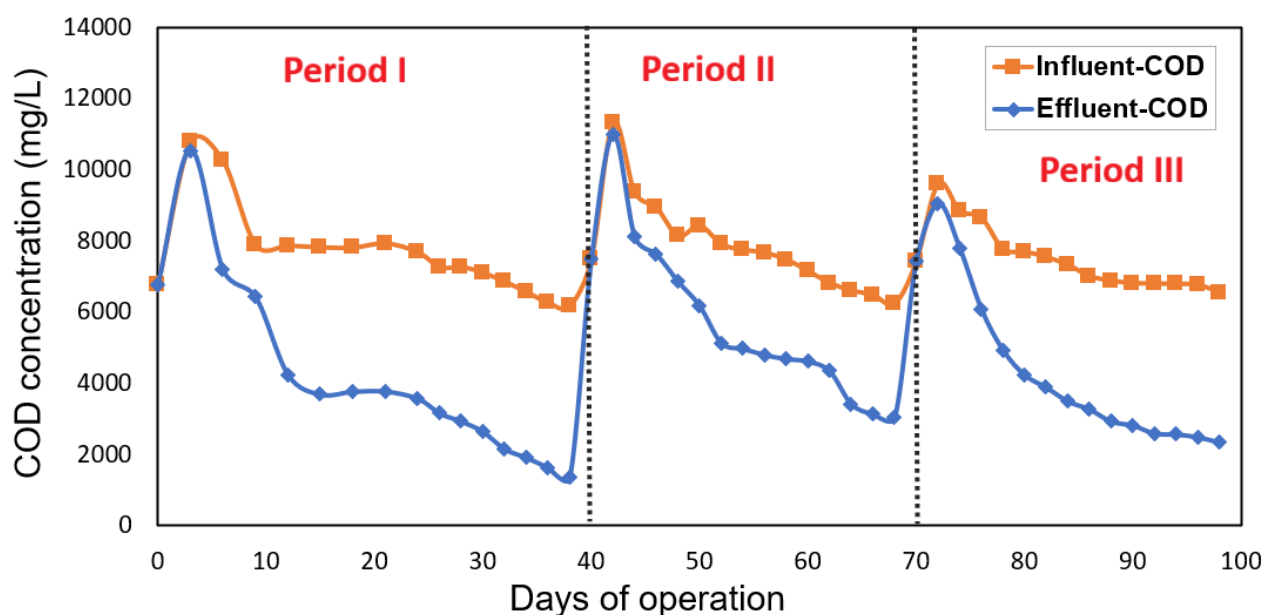


Figure 4.5: The measured COD in the influent, effluent and reactor over the period of 100 days

During period I, the initial effluent COD was 6800 mg/L, but it rose sharply to 11500 mg/L after day 3. The COD spike is attributed to the delay in SRB degrading the organic matter due to its adaptation to the reactor conditions; additionally, the organic matter started to solubilise rapidly (Cunha *et al.*, 2019). Once SRB adapted to the reactor conditions, it began to consume the carbon source efficiently, which reduced the COD and led to the production of sulphide. The effluent COD decreased rapidly over the days, reaching 560 mg/L by day 38, resulting in 95% COD removal.

In period II, the initial effluent COD was 7800 mg/L, which spiked to 11750 mg/L on day 43. The rise was caused by the addition of a carbon source along with AMD and the inoculum. The spike in COD resulted from adding fresh carbon and the slow uptake of carbon matter by SRB as they were still acclimatising to the new reactor conditions. Once SRB began to adapt, the COD degradation gradually increased. The effluent COD then decreased sharply to 2600 mg/L after day 68, resulting in a 77% COD removal. However, the low removal percentage is attributed to the introduction of acidic conditions, resulting in incomplete acclimation of SRB (Bratkova *et al.*, 2018).

In period III, the initial COD was 8000 mg/L. After the feed mixture was added to the reactor, the COD slightly increased to 9800 mg/L. The moderate spike was due to the lower ratio of AMD to inoculum, which had lower acidity compared to the feed mixture added during period II. The microbial activity began to increase over time, attributed to balanced reactor conditions. It then gradually decreased throughout this period, reaching 2200 mg/L, and achieved a 78% reduction in COD. The removal percentage is slightly higher than in period II; however, due to the ageing biofilm, the BSR process was not as effective as in period I.

The South African standard for the COD is less than 125 mg/L for wastewater treatment plants and less than 5000 mg/L for industrial effluent (Africa, 1998). The final COD of the effluent after period I, II and III did not meet the wastewater COD standard, although the BSR process met the industrial effluent standard. The residual COD consists mostly of the biodegradable fraction, which can be further degraded. To meet the wastewater discharge standard (<125 mg/L), a polishing stage is required to further treat the wastewater. Options to be considered include aerobic post-treatment reactors, trickling filters, membrane bioreactors, or constructed wetlands. It is important to consider cost, footprint and performance when selecting the polishing stage.

4.3.4 Sulphate removal of the treated wastewater

Throughout the 100 days of reactor operation, the sulphate levels in the influent, reactor content, and effluent were measured. The results are shown in **Figure 4.6**. For period I, the initial effluent sulphate concentration was 4179 mg/L. Over time, the sulphate levels in the reactor and effluent decreased, reaching 25 mg/L after day 38. SRB adapted to the reactor conditions, leading to an exponential growth, which increased the conversion of sulphate to sulphide and resulted in a 99% reduction in sulphate. In period II, the initial effluent sulphate concentration was 5050 mg/L due to the addition of AMD/inoculum at a ratio of 7:1 into the reactor. The sulphate levels in the effluent and reactor dropped to 380 mg/L after day 68, achieving a sulphate removal efficiency of 92%. The addition of the feed mixture resulted in a higher sulphate load, stressing SRB bacteria. Adding less SRB worsened the situation. Despite harsher conditions, SRB recovered and began converting sulphate to sulphide.

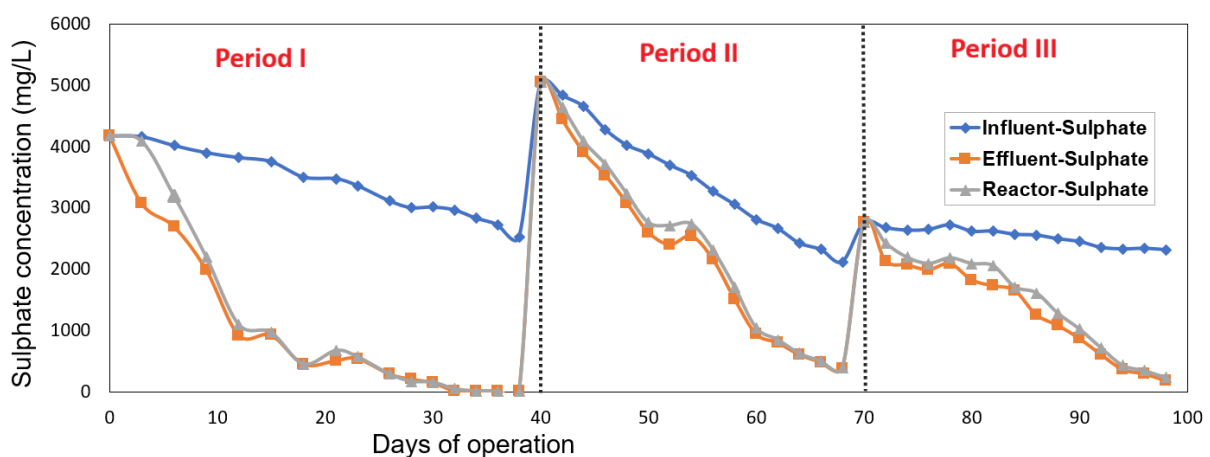


Figure 4.6: The measured sulphate in the influent, effluent and reactor over the period of 100 days

For period III, the initial effluent sulphate concentration was 2761 mg/L due to the addition of AMD/inoculum at a ratio of 5:3 to the reactor. The effluent and reactor sulphate levels decreased sharply to 175 mg/L, resulting in a sulphate removal efficiency of 94%. The higher inoculum

addition increased the density of SRB, making it easier for SRB to adapt to a lower sulphate load (Jiménez-Rodríguez *et al.*, 2010; Melgaço *et al.*, 2020; Teiri *et al.*, 2016). The increased microbial activity resulted in stable, improved performance compared to period II, although it remained lower than in period I, which may be due to the biofilm starting to degrade or sloughing off.

4.3.5 Sulphide production in the treated AMD

The sulphides in the influent, reactor content, and effluent were measured during the reactor's 100-day operation. The results are shown in **Figure 4.7**. Normalised sulphide concentrations were calculated and are presented in **Appendix E (Normalised Mn and sulphide concentrations)**. In period I, the initial effluent sulphide concentration was 55 mg/L. The sulphide levels in the reactor and effluent increased over the days until reaching 240 mg/L after day 32, resulting in a sulphide production of 195 mg/L. The production was due to the BSR process, where more sulphates were converted into sulphide. SRB exhibited high microbial activity due to adaptation to the reactor conditions. From day 33 to day 40, however, the sulphide content decreased to 45 mg/L before the addition of the feed mixture. The primary reason for the drop can be attributed to sulphate-oxidising bacteria (SOB), which oxidised sulphide to elemental sulphur (Marais *et al.*, 2022; Masindi *et al.*, 2015). The decline in sulphide may also be due to precipitation with Mn forming MnS (Marais, 2022). Another possible reason is the escape of sulphide gas from the reactor.

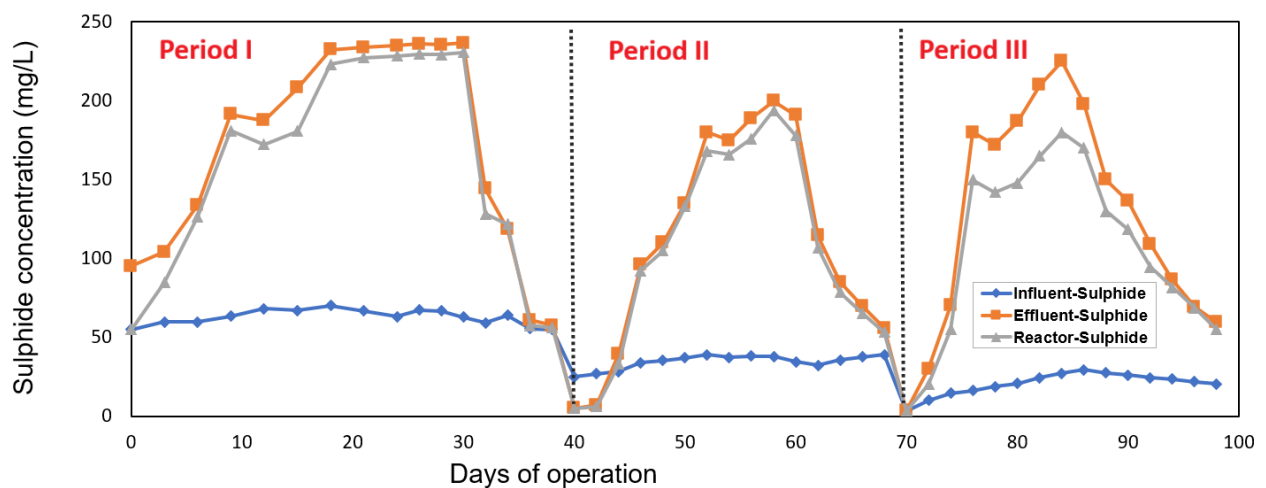


Figure 4.7: The measured sulphide in the influent, effluent and reactor over the period of 100 days

For period II, the influent and effluent sulphide concentration was 41 mg/L due to the addition of the feed mixture. The effluent concentration began to increase rapidly from day 42 to day 58, reaching a maximum of 185 mg/L, which corresponds to 144 mg/L of sulphide production. Sulphide production was slow during this period as SRB needed to adapt to the new conditions in the reactor. However, the sulphide concentration also dropped quickly during this time, falling

to 47 mg/L after day 68. For period III, the influent and effluent sulphide concentration was 3.4 mg/L, again due to the addition of the feed mixture. The effluent concentration increased rapidly from day 72 to day 87, reaching a maximum of 235 mg/L, corresponding to 232 mg/L of sulphide production. Nevertheless, the sulphide concentration also declined swiftly to 48 mg/L during this period. The degradation of the biofilm may have caused some sulphide to escape (Sarti *et al.*, 2011).

4.3.6 Effect of varying HRT and sulphate/COD ratio on the reactor performance

The effects of the HRT and the sulphate-to-COD ratio on the reactor's performance were evaluated by varying these parameters. The results are provided in **Table 4-1**.

Table 4-1: The effect of varying the HRT and sulphate/COD ratio on the reactor performance

Period	HRT (days)	Sulphate/COD Ratio	Sulphate Removal (%)	COD Removal (%)	Sulphide Production (mg/L)
I	3	0.7	99	95	195
II	2	0.9	92	77	144
III	3	0.6	94	78	232

Reducing the HRT from 3 days to 2 days meant there was always a feed addition after 2 days, which decreased the contact time between the carbon source and SRB. As a result, SRB did not fully utilise the carbon source (Farajzadehha *et al.*, 2012; Reyes-Alvarado *et al.*, 2017). The increase in the ratio indicated a limitation of the carbon source due to the addition of more AMD. SRB activity was restricted because of the limited availability of the carbon source (Hu *et al.*, 2015; Nurandani *et al.*, 2018). The combined effect of these two factors impacted the BSR process. Microbial activity recovered after increasing the HRT, allowing longer interaction between the carbon source and SRB. Reducing the sulphate/COD ratio meant more carbon source was available for SRB, which enhanced SRB activity and improved performance (Mannucci *et al.*, 2014; Subtil *et al.*, 2012).

4.4 Summary

Period I exhibited an excellent reactor performance, characterised by a neutral pH, the removal of heavy metals, sulphate, and COD, as well as the production of sulphide. Period II showed the poorest performance, attributed to the increased addition of AMD and changes in HRT, as well as the sulphate/COD ratio. In period III, performance improved well due to the addition of more inoculum, which increased the density of SRB. The conditions during period I are suitable for AMD treatment. bed and performed similarly to other bioreactors, such as fixed-bed, fluidised-

bed, and batch reactors. Compared to other LFCR studies, such as (Marais *et al.*, 2022), (Mooruth, 2013), and van Hille *et al.* (2019), the newly designed LFCR performed relatively better.

4.5 Modelling of sulphate in the LFCR

4.5.1 Linear regression modelling

The linear regression model was derived using the dependent and independent variables as discussed in section 3.7.1, where the sulphate concentration was the dependent variable and other parameters, such as pH, COD, and sulphide, were the independent variables. The code, derived in MATLAB, is given in **Appendix F (Linear regression)**. The dataset was split into 70% training and 30% testing sets. The results of this code are discussed in this section.

4.5.1.1 Model performance

The performance of the model was evaluated based on the coefficient of determination (R^2), root mean square error (RMSE) and mean absolute error (MAE) for both the training and test datasets. The results are given in **Table 4-2**.

Table 4-2: The results of the linear regression model for predicting the reactor performance

Parameters	Training data	Test data
R^2	0.83	0.42
RMSE	589	735
MAE	425	541

The training R^2 indicates that the model explains 83% of the variance in effluent sulphate concentration in the training dataset. The test R^2 was lower than the training R^2 , which implies that the model does not generalise data accurately. The training set showed a strong fit compared to the testing set. Because there were few data points, it is possible that the model might have overfitted. The linear regression may not have captured the non-linearities of the BSR process. The high RMSE and MAE of the test data indicate a decrease in predictive accuracy and weaker generalisation. The model is less robust and cannot capture complex BSR relationships. Some new features, such as the addition of the carbon source and HRT, might need to be considered.

4.5.1.2 Scatter plot of actual data vs predicted data

The scatter plots of the actual effluent sulphate concentration and the predicted sulphate concentration for both training and testing data are provided in **Figure 4.8**. The plots relate to the

model performance parameters, where the training data set fits on the line of best fit. However, the test data does not fit properly on the line of best fit.

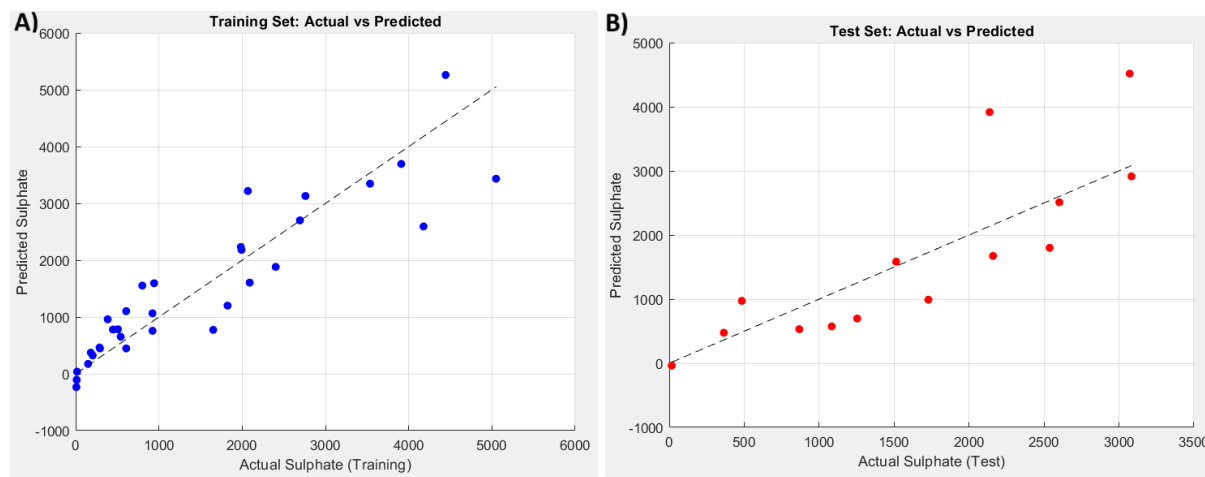


Figure 4.8: The scatter plot of the predicted and actual sulphate concentration for the training data (A) and test data (B)

The test data scatter plot indicated significant residual errors, suggesting poor accuracy in predicting the effluent sulphate concentration. The predictions from linear regression deviate significantly from the actual values, which is evidenced by high RMSE and MAE values. The performance is poor compared to studies that reported R^2 values greater than 0.8, such as Okeke *et al.* (2022), Aparna and Swarnalatha (2023), Rahmat *et al.* (2022), and Mutai *et al.* (2022). The BSR process is non-linear; thus, linear regression does not accurately reflect what occurs during the process. Some new variables, such as influent flow rate, can be measured and added to the model to enhance its performance.

4.5.2 Artificial neural network (ANN) modelling

The ANN model was derived using the dependent and independent variables as discussed in section 3.7.2. Where the sulphate concentration was the dependent variable, other parameters, such as pH, COD, and sulphide, were the independent variables. The code derived in MATLAB is presented in **Appendix G (Artificial neural network)**. The dataset was split into 70% training and 30% testing sets. The model employed a single hidden layer with three neurons.

4.5.2.1 Model performance

The performance of the ANN model was evaluated based on the R^2 , RMSE, and MAE for training and test data sets. The results are given in **Table 4-3**.

Table 4-3: The results of the ANN model for predicting the reactor performance

Parameters	Training data	Test data
R^2	0.93	0.75
RMSE	389	483
MAE	228	305

The training R^2 indicates that the model accounts for 93% of the variance in effluent sulphate concentration in the training dataset and demonstrates an excellent fit and strong learning of the data by the model. The test R^2 suggests better generalisation, with the ANN model capturing the complex interactions and nonlinear relationships of the BSR process (Reyes-Alvarado *et al.*, 2017; Sinharoy *et al.*, 2020; Yao *et al.*, 2024). There is also moderate overfitting. The training and test RMSE indicate that the predictive accuracy has been significantly improved. The hidden layer used enabled the model to have a flexible function prediction (Arce-Medina & Paz-Paredes, 2009; Sahinkaya, 2009). The low MAE indicates the increased robustness of the model. The ANN model is less sensitive to outliers and reduces deviations.

4.5.2.2 Scatter plot of actual data vs predicted data

The scatter plots of the actual effluent sulphate concentration and the predicted sulphate concentration for both training and testing data from the ANN model are provided in **Figure 4.9**. The plots relate to the model performance parameters, where the training data set fits the line of best fit, and also the test data fits properly on the line of best fit.

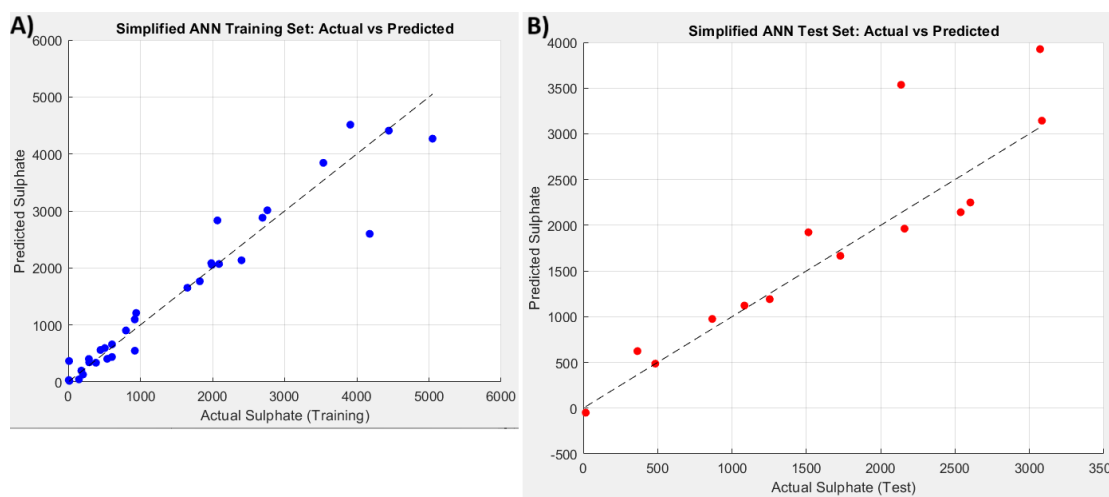


Figure 4.9: The scatter plot of the predicted and actual sulphate concentration for the training data (A) and test data (B)

The test data scatter plot indicated only a few residual errors, demonstrating good accuracy in predicting effluent sulphate concentration using ANN. The ANN predictions closely match the actual values, indicated by the lower RMSE and MAE values compared to linear regression. Similar performance has been reported in other studies, such as Türkmenler and Pala (2017), Alsulaili and Refaie (2021), (Ibrahim, 2023), (Ramya & Srinath, 2024), and (Çimen Mesutoğlu & Gök, 2024), all of which reported R^2 values exceeding 0.85.

4.5.2.3 Summary

The ANN performance was considerably better than the linear regression. The test R^2 of the ANN was greater than that of linear regression. The RMSE and MAE of ANN were lower than those of linear regression indicating fewer residual errors, and the predicted sulphate concentration is close to the actual concentration. The results are similar to those found in other studies, such as Zare Abyaneh (2014), Norouzian *et al.* (2021), Reddy and Malathi (2022), and Doicin *et al.* (2024), which clarified that ANN outperformed linear regression. The studies established that the R^2 of ANN was greater than that of linear regression indicating that the ANN provides more precise and consistent predictions for non-linear processes with a large data set.

In addition to superior statistical performance, the ANN captured key aspects of the BSR process dynamics. The input variables, such as COD, pH, and sulphide, enabled the ANN to model microbial activity patterns: COD indicates electron donor utilisation for sulphate reduction, pH controls microbial metabolism, and sulphide acts as both a possible inhibitor and a product. The hidden layer achieved a nonlinear mapping, allowing the model to effectively replicate saturation behaviour at high COD concentrations and indicate inhibitory effects at elevated sulphide concentrations; these capabilities were not fully captured by linear regression. This indicates that the ANN's predictions are biologically coherent and consistent with established process behaviour. Additionally, the reduced sensitivity to outliers indicates robustness under fluctuating operating conditions, making the ANN a valuable tool for monitoring and managing sulphate-reducing bioreactors.

CHAPTER 5 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

This section presents conclusions derived to address the study objectives. The biological sulphate reduction (BSR) process occurred in a newly designed linear flow channel reactor (LFCR), operated for 100 days. The development and maturity of the floating sulphur biofilm (FSB) were assessed, where the biofilm continued to thicken for 70 days. Between days 73 and 100, the biofilm began to degrade, which affected the BSR process. For heavy metal removal, there was an efficient removal of heavy metals, including Ni, Cu, and Zn. The Fe was also completely removed after day 10. However, Mn was persistent; its levels remained at 7 mg/L due to the conditions that persisted after day 10. However, after 40 days, 92% of the Mn was removed from the effluent. The effect of the AMD/inoculum ratio on the reactor performance was observed. As the ratio increased, the BSR process slowed because the activity of sulphate-reducing bacteria (SRB) decreased, as evidenced in period II, wherein the pH was below 7, where the sulphate removal was under 95%, the COD reduction was below 80%, and the sulphide production was less than 150 mg/L, lower than in period I. The higher ratio slightly improved the results in period III, shortening the HRT from 3 days to 2 days and increasing the sulphate/COD ratio from 0.7 to 0.9, which also had a positive effect on reactor performance. Overall, most contaminants were removed from the effluent.

Modelling the reactor's performance, using linear regression revealed lower accuracy, overfitting, and poor generalisation, as indicated by an R^2 value of 0.42 for the test dataset, a high RMSE of 735, and an MAE of 541. The artificial neural network (ANN) model outperformed the linear regression by fully capturing the BSR process dynamics and understanding its nonlinearities. The ANN's performance on the test data yielded an R^2 of 0.75, an RMSE of 483, and an MAE of 305, suggesting that both models can be used to predict effluent sulphate levels; however, the ANN is the superior choice.

5.2 Recommendations

Recommendations are divided into recommendations for implementing a BSR process and recommendations for future work.

5.2.1 Recommendations for BSR process

There is a need for a pilot plant to treat AMD continuously under practical operating conditions. The use of organic carbon sources, such as cheese whey and chicken manure, can improve the performance of the biological sulphate reduction (BSR) process. Future studies should conduct microbial analysis, quantify sulphur recovery efficiency and oxygen transfer dynamics, as these parameters are critical to optimising LFCR operation and realising its potential as both a treatment and resource recovery technology. Future research should address several limitations identified in the study, including microbial community analysis, quantitative sulphur mass balances, gas-phase monitoring (H_2S), alkalinity balance, and investigations into scale-up implications, all of which are essential to assess the feasibility of LFCR technology under industrial conditions.

5.2.2 Recommendations for future work

Future studies should include improved analytical methods to measure sulphate and sulphide to enhance the accuracy of results for modelling. For modelling, longer reactor operation is required to have more datasets. Methods such as ridge and lasso can be used to capture the BSR process performance better for regression. Feature selection can be improved by adding new features that capture the process dynamics. Non-linear regression modelling techniques should be considered for the study. The comparison of model performance should be performed using the errors of the uncertainty of experimental measurements.

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APPENDIX A (EXAMPLE OF A SINGLE NODE)

The neuron consists of two inputs (y_1 and y_2), the weights are w_1 and w_2 and the bias (b) given by **Equations (9), (10), and (11)**.

Multiplication:

$$y_1 \times w_1; y_2 \times w_2 \quad (9)$$

Sum:

The weighted sum is given by z .

$$z = y_1 \times w_1 + y_2 \times w_2 + b \quad (10)$$

Transfer function:

z is passed through a transfer function such as the Sigmoid function:

$$\sigma(z) = \frac{1}{1+e^{-z}} \quad (11)$$

APPENDIX B (SAFETY DATA SHEETS)



CO2.pdf



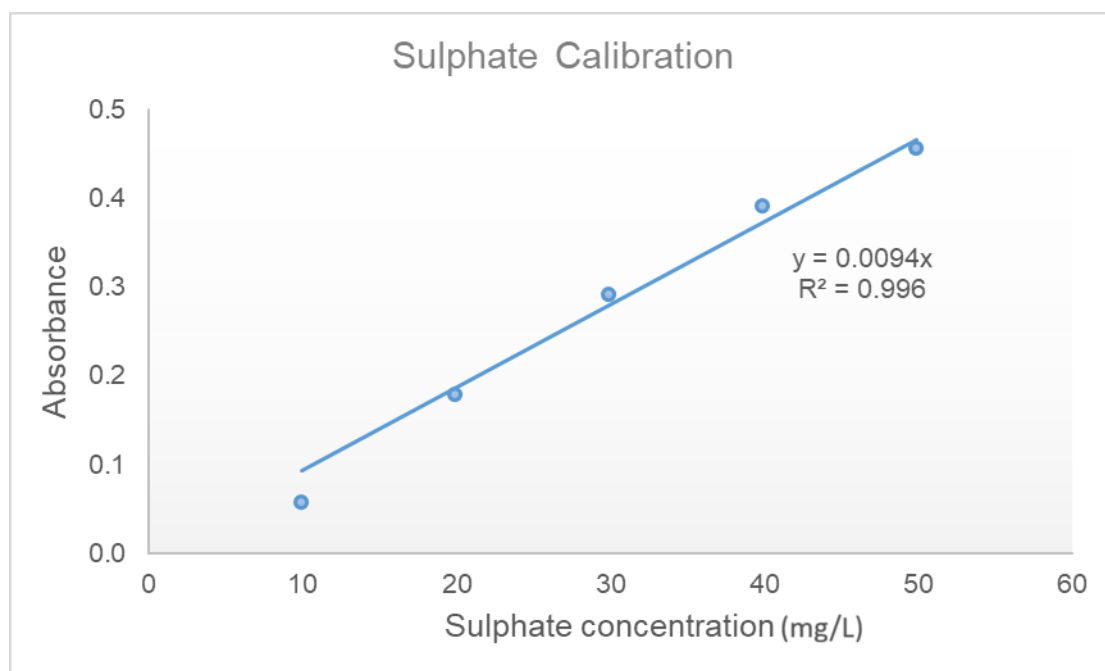
H2S.pdf



Sulphate.pdf

APPENDIX C (ANALYTICAL METHODS)

UV Spectroscopy calibration results are given below:



Name	Dilution	Absorbance	[Concentration (mg/L)]	Final [conc] (mg/L)
Influent (SA)	100	0.407	41.783	4178.3
Effluent (EffL)	100	0.380	39.243	3924.3
Port 2 sample	100	0.394	40.889	4088.9
Port 3 sample	100	0.392	40.308	4030.8
Port 4 sample	100	0.384	39.777	3977.7

APPENDIX D (EXPERIMENTAL RESULTS)

Sulphate/COD ratios of the feed.

Days	Ratio
0	1.6
3	2.6
6	2.6
9	2.0
12	2.1
15	2.1
18	2.2
21	2.3
23	2.3
26	2.3
28	2.4
34	2.3
36	2.3
38	2.4
40	1.5
42	2.3
44	2.0
46	2.1
48	2.0
50	2.2
52	2.1
54	2.2
56	2.3
58	2.4
60	2.5
62	2.6
64	2.7
66	2.8
68	2.9
70	2.7
72	3.6
74	3.4
76	3.3
78	2.9
80	2.9
82	2.9
84	2.9
86	2.7
88	2.8
90	2.8
92	2.9
94	2.9
98	2.8

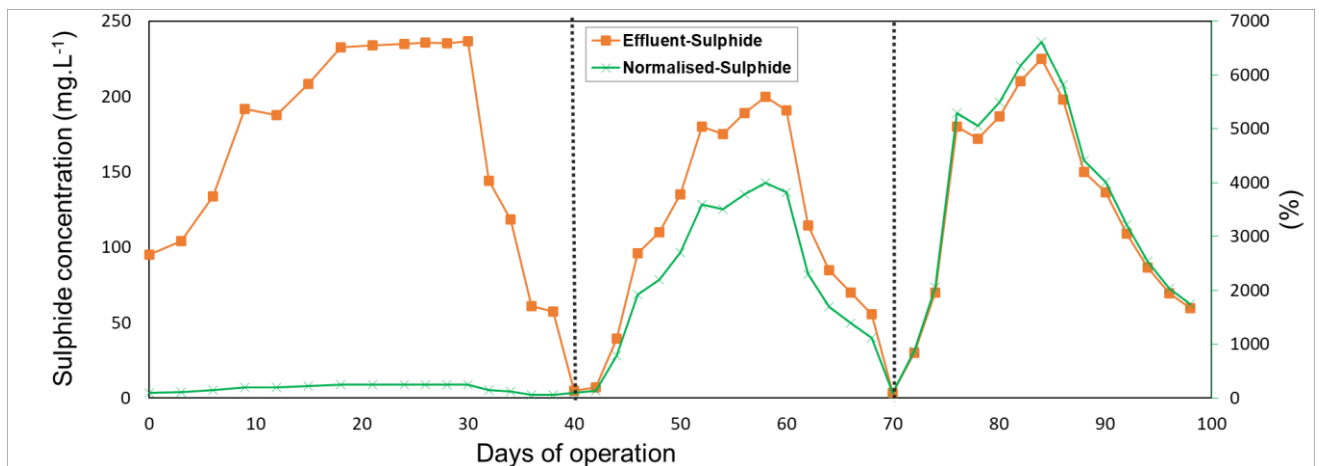
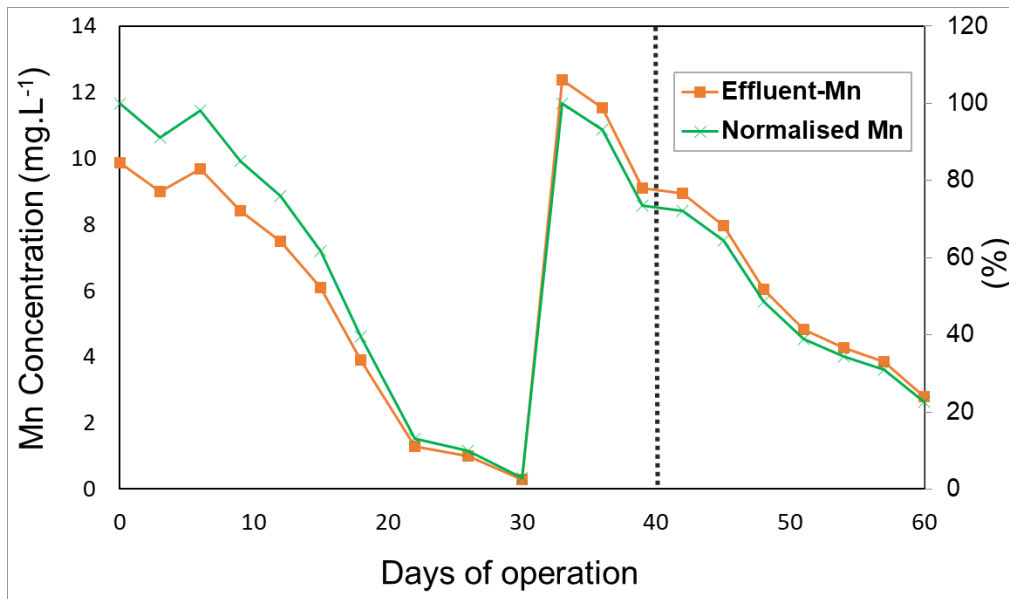
APPENDIX E (NORMALISED MN AND SULPHIDE CONCENTRATIONS)

The normalised concentrations were calculated using **Equation (12)**.

$$\text{Normalised Conc (\%)} = \frac{C_t}{C_0} \times 100 \quad (12)$$

Where C_t is the concentration at time t , and C_0 is the initial concentration.

The comparison between the normalised Mn and sulphide concentrations and the effluent concentrations.



APPENDIX F (LINEAR REGRESSION)

```
data = readtable('C:\Users\F-ENG Student\Documents\00001-Finances\Modelling Sheet.xlsx', 'PreserveVariableNames', tr

% Step 2: Define variables
Y = data(:, 'Sulphate conc');
X1 = data(:, 'pH values');
X2 = data(:, 'COD conc');
X3 = data(:, 'Sulphide conc');

% Combine independent variables
X = [X1, X2, X3];

% Add intercept term
X = [ones(size(X,1),1), X];

% Split ratio
splitRatio = 0.7;

% Randomize indices
n = size(X,1);
rng(1); % For reproducibility
idx = randperm(n);

% Split data
trainIdx = idx(1:round(splitRatio*n));
testIdx = idx(round(splitRatio*n)+1:end);

X_train = X(trainIdx, :);
Y_train = Y(trainIdx);

R2_train = 1 - sum((Y_train - Y_train_pred).^2) / sum((Y_train - mean(Y_train)).^2);
rmse_train = sqrt(mean((Y_train - Y_train_pred).^2));
mae_train = mean(abs(Y_train - Y_train_pred));
```

```

mae_train = mean(abs(Y_train - Y_train_pred));

% Display training metrics
disp('Training R-squared:');
disp(R2_train);

disp('Training RMSE:');
disp(rmse_train);

disp('Training MAE:');
disp(mae_train);

X_test = X(testIdx, :);
Y_test = Y(testIdx);
|
% Train model
[b, ~, ~, ~, stats] = regress(Y_train, X_train);

% Predict
Y_train_pred = X_train * b;
Y_test_pred = X_test * b;

% Evaluate
R2_test = 1 - sum((Y_test - Y_test_pred).^2) / sum((Y_test - mean(Y_test)).^2);
disp('Test R-squared:');
disp(R2_test);

% Plot training results
figure;
scatter(Y_train, Y_train_pred, 'b', 'filled');
hold on;
plot([min(Y_train), max(Y_train)], [min(Y_train), max(Y_train)], 'k--');
xlabel('Actual Sulphate (Training)');

```

```

xlabel('Actual Sulphate (Training)');
ylabel('Predicted Sulphate');
title('Training Set: Actual vs Predicted');
grid on;

% Plot test results
figure;
scatter(Y_test, Y_test_pred, 'r', 'filled');
hold on;
plot([min(Y_test), max(Y_test)], [min(Y_test), max(Y_test)], 'k--');
xlabel('Actual Sulphate (Test)');
ylabel('Predicted Sulphate');
title('Test Set: Actual vs Predicted');
grid on;

% Calculate RMSE and MAE for test set
rmse = sqrt(mean((Y_test - Y_test_pred).^2));
mae = mean(abs(Y_test - Y_test_pred));

% Display results
disp('Test RMSE:');
disp(rmse);

disp('Test MAE:');
disp(mae);

fprintf('Regression Equation:\n');
fprintf('Sulphate conc = %.4f + %.4f*pH + %.4f*COD + %.4f*Heavy metals\n', b(1), b(2), b(3), b(4));

```

APPENDIX G (ARTIFICIAL NEURAL NETWORK)

```
data = readtable('C:\Users\F-ENG Student\Documents\00001-Finances\Modelling Sheet.xlsx', 'PreserveVariableNames', tr

% Step 2: Define variables
Y = data(:, 'Sulphate conc');
X1 = data(:, 'pH values');
X2 = data(:, 'COD conc');
X3 = data(:, 'Sulphide conc');

X = [X1, X2, X3]';

% Prepare target
Y = Y';

% Split data
splitRatio = 0.7;
n = size(X,2);
rng(1);
idx = randperm(n);

trainIdx = idx(1:round(splitRatio*n));
testIdx = idx(round(splitRatio*n)+1:end);

X_train = X(:, trainIdx);
Y_train = Y(:, trainIdx);

X_test = X(:, testIdx);
Y_test = Y(:, testIdx);

% Create a simple feedforward network with 1 hidden layer
net = feedforwardnet(3); % 3 neurons in hidden layer

% Set transfer functions
net.layers{1}.transferFcn = 'tansig'; % Hidden layer
net.layers{2}.transferFcn = 'purelin'; % Output layer

% Training settings
net.trainFcn = 'trainlm'; % Levenberg-Marquardt
net.divideParam.trainRatio = 1.0;
net.divideParam.valRatio = 0.0;
net.divideParam.testRatio = 0.0;
net.trainParam.showWindow = false;

% Train network
net = train(net, X_train, Y_train);

% Predict
Y_train_pred = net(X_train);
Y_test_pred = net(X_test);

% Evaluate
R2_train = 1 - sum((Y_train - Y_train_pred).^2) / sum((Y_train - mean(Y_train)).^2);
rmse_train = sqrt(mean((Y_train - Y_train_pred).^2));
mae_train = mean(abs(Y_train - Y_train_pred));

R2_test = 1 - sum((Y_test - Y_test_pred).^2) / sum((Y_test - mean(Y_test)).^2);
rmse_test = sqrt(mean((Y_test - Y_test_pred).^2));
mae_test = mean(abs(Y_test - Y_test_pred));

% Display metrics
fprintf('Training R²: %.4f\n', R2_train);
fprintf('Training RMSE: %.4f\n', rmse_train);
fprintf('Training MAE: %.4f\n', mae_train);

fprintf('Test R²: %.4f\n', R2_test);
fprintf('Test RMSE: %.4f\n', rmse_test);
fprintf('Test MAE: %.4f\n', mae_test);
```

```

% Plot results
figure;
scatter(Y_train, Y_train_pred, 'b', 'filled');
hold on;
plot([min(Y_train), max(Y_train)], [min(Y_train), max(Y_train)], 'k--');
xlabel('Actual Sulphate (Training)');
ylabel('Predicted Sulphate');
title('Simplified ANN Training Set: Actual vs Predicted');
grid on;

figure;
scatter(Y_test, Y_test_pred, 'r', 'filled');
hold on;
plot([min(Y_test), max(Y_test)], [min(Y_test), max(Y_test)], 'k--');
xlabel('Actual Sulphate (Test)');
ylabel('Predicted Sulphate');
title('Simplified ANN Test Set: Actual vs Predicted');
grid on;
view(net)

```