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Application of artificial neural network for prediction of sulfidogenic fluidized bed reactor performance and optimization for the co-treatment of acid-mine drainage with liquid pharmaceutical waste

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A thesis submitted to the Faculty of Engineering and the Built Environment, University of the Witwatersrand, Johannesburg, in fulfillment of the requirements for the degree of Doctor of Philosophy

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Co-Supervisor: Prof. B.F. Bakare

2021

Declaration

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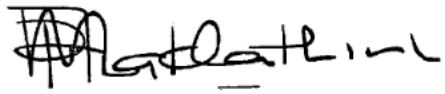

Dedication

Dedicated to myself for the resilience!

This one is for me!

I thank God for the strength, Lord you are worthy!

Abstract

Acid mine drainage (AMD) is one of the most troubling water pollution sources in South Africa due to its history and current mining activities. In reality, South Africa's economy is driven mainly by the mining sector's existence; hence it is crucial to abate the impact and find treatment solutions to manage AMD. On the other hand, there is a growing concern about the pharmaceutical compounds abundantly found in South African natural water bodies. Hospitals are found to be significant contributors among diverse sources contributing to pharmaceutical pollutants in the environment. Currently, hospital wastewater is discharged to the municipal sewer, ending up in Wastewater Treatment Plants (WWTPs), decreasing the organic contaminants' biodegradation process in the treatment plant. Subsequently, the direct discharge of the WWTP treated effluent (with untreated pharmaceuticals) to receiving waterbodies raises a concern about the impact of these compounds' persistent presence in the environment. As such, both acidic metal and sulfate-containing water and pharmaceutical-rich wastewater are toxic to the environment, thus need urgent attention. To date, co-treatment of acid mine drainage and municipal wastewater has been investigated with several advantages; however, the potential removal of pharmaceutical compounds through the biological process has not been explored.

This work proposed, developed, and assessed the sulfidogenic fluidized-bed reactor system for co-treatment of acid mine drainage and isolated stream of hospital wastewater. A laboratory-scale sulfate-reducing fluidized bed reactor at a controlled mesophilic condition of ±28°C examined the biotreatment process of real acid mine drainage and hospital wastewater. In this study, there was no supplement of carbon source during the treatment, but only hospital wastewater (HWW) provided the dissolved organic carbon. The overall removal for the effluent chemical oxygen demand (COD) concentrations and sulfate were 39.5 mg/l and 42 mg/l at a COD/SO_4^{2-} the ratio of 0.68 and hydraulic retention time of 8 h. The overall COD oxidation and sulfate reduction performance achieved an average of 96% and 97%, correspondingly; and recorded the removal efficiencies of 44% naproxen and 55% ibuprofen.

Furthermore, the study evaluated the inhibition kinetics and microbial communities to better understand the diverse species and the reaction mechanisms within the system. The kinetics and

microbiology diversity in the sulfidogenic fluidized-bed bioreactor (at 30°C) for co-treatment of hospital wastewater and metal-containing acidic water were examined. The alkalinity from organic oxidation raised the pH of the effluent from 2.3 to 6.1-8.2. Michaelis-Menten modeling yielded (K_m =7.3 mg/l, V_{max} =0.12 mg/l min^{-1}) in the batch bioreactor treatment using sulfate-reducing bacteria. For COD oxidation, the dissolved sulfide inhibition constant (K_i) was 3.6 mg/l, and the K_i value for H_2S was 9 mg/l. The dominant species in the treatment process belong to the *Proteobacteria* group (especially *Deltaproteobacteria*).

To further explore the co-treatment process, a nanoscale zero valent iron was used to enhance the treatment and monitored through an oxidation-reduction potential (Eh) for 90 days. The removal pathway for the nZVI used co-precipitation, sorption, and reduction. The removal load for Zn and Mn was approximately 198 mg Zn/g Fe and 207 mg Mn/g Fe, correspondingly; achieving sulfate removal efficiency of 94% and organic matter (COD, BOD, DOC), TDN reduced significantly, but ibuprofen and naproxen achieved 31% and 27% removal, respectively. This enriched co-treatment system exhibited a high reducing condition in the reactor, as confirmed by Eh; hence, the nZVI was dosed only a few times in biotreatment duration, demonstrating a cost-effective system. Subsequently, the biological process was run for over 210 days to collect enough data for development of predictive artificial neural network model. The metal concentration removal was more than 99% in effluent for iron and zinc, and precipitated predominantly as FeS, FeS_2 and ZnS. The alkalinity generated by COD oxidation improved the pH of the wastewater considerably when the concentration of feed sulfate was less than 3500 mg/l. At an HRT of 8 h, COD oxidation in the reactor precipitated 1345 mg Fe/l/day, 543 mg Al/l/day and 130 – 170 mg Zn/l/day from acidic wastewater and increased the pH from 2.2 to 6.8, due to the formation of metal sulfide precipitate. The ANN model was successfully developed, and the predicted and actual measured concentrations of the outputs found R-value of 0.97.

To summarize, this research study demonstrated a new application of co-treating AMD with pharmaceutical-rich wastewater using fluidized-bed reactor (FBR). Further to that, the co-treatment was enhanced with nZVI to evaluate the heavy metals that were not adequately removed in prior experiments. Finally, the study developed a performance model which may easily be adopted for process design.

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Without a doubt, God is the 1st on this list because He has been truly amazing. I don't know how I would have done it without you merciful Father. I thank Jehovah El Shaddai for exempting me from the mental ill-health and depression which drew very close to me during this journey.

I thank Professor Mulopo, my principal supervisor, for his indispensable support which made this work a success. I thank Professor Bakare for his efforts for me to complete my study.

I extent my appreciation to Prof Tumba Kaniki, firstly for advising and assisting me to forward my PhD application to the University of the Witwatersrand, and secondly for being my cheer leader. Also, Dr Papi Numbi and Dr Joseph Bwapwa for 'hyping' me every time and calling me a Dr. before I even wrote my first chapter. Your belief in my strengths made me logon in my computer even when I didn't have a sentence to write.

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I like to acknowledge and thank the love of my family – my husband and my daughters for your understanding and your unwavering support during the period of this study. Thank you, my people, - I love you a lot!

This study would not have been completed without the financial support from the Research Directorate Office of Mangosuthu University of Technology. The seed funding scheme is truly appreciated.

List of publications

The work in this thesis or part thereof has been independently reviewed by various engineers and scientists who are field experts. Some of the articles are already available online and others are still under per review. The articles were carefully developed to satisfy the objectives of the thesis, and can be summarized as follows:

Makhathini, T.P., Mulopo, J., Bakare, B.F., **2020**. Possibilities for acid mine drainage co-treatment with other waste streams: A review. *Mine Water and the Environment*. 39, 13–26. https://doi.org/10.1007/s10230-020-00659-w

Makhathini, T.P., Mulopo, J., Bakare, B.F., **2020.** Effective biotreatment of acidic mine water and hospital wastewater using fluidized-bed reactors. *Journal of Water Process Engineering.* 37, 101505. https://doi.org/10.1016/j.jwpe.2020.101505

Makhathini, T.P., Mulopo, J., Bakare, B.F., (**In review**). Performance assessment of sulfidogenic fluidized-bed reactor for co-treating acid mine and pharmaceutical-containing wastewater. *International Journal of Environmental Science and Technology*.

Makhathini, T.P., Mulopo, J., Bakare, B.F., **2021**. Sulfidogenic fluidized-bed bioreactor kinetics and microbial ecology for co-treatment of hospital wastewater and acid mine drainage. *Biotechnology Reports*. 32. https://doi.org/10.1016/j.btre.2021.e00683

Makhathini, T.P., Mulopo, J., Bakare, B.F., **2021**. Enriched co-treatment of pharmaceutical and acidic metal-containing wastewater with nano zero-valent iron. *Minerals*. 11(2), 220. https://doi.org/10.3390/min11020220

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List of acronyms, abbreviations, and symbols

AMD ……. Acid mine drainage
ANN…….……. Artificial neural network
BOD …….…….…… Biological oxygen demand
COD ……..…. Chemical Oxygen Demand
DNA ……..… Deoxyribonucleic acid
DOC ……...……. Dissolved oxygen carbon
FBR ……...……. Fluidized bed reactor
HWW …….…..……. Hospital wastewater
MSE …….…..……. Mean square error
MWW …….…….……. Municipal wastewater
nZVI ……...……. nano-Zero Valent Iron
RSM …….…..…. Response surface methodology
SEM ……...……..…. Scanning electron microscopy
SRB …….……. Sulfate-reducing bacteria
TDN …….…….…… Total dissolved nitrogen
UASB…….……... Upflow anaerobic sludge bed
WWTP …… Wastewater Treatment Plant

CHAPTER ONE

General Introduction

This chapter gives background to the work presented in this thesis. It hopes to clarify the motivation and outline the objectives for the study. As such, the innovation, and contribution of this research to the engineering community are systematically highlighted.

1.1 Background to the Study

Water resources in South Africa are challenged as urbanization and industrialization thrive on contributing to its growing economy (Naik, 2017). Mining is one of the industries that drive South Africa's economy (Naidoo, 2017); however, this industry compromises water quality and ecological protection (Masindi and Muedi, 2018) in the quest for economic growth. The most prominent environmental pollution emanating from mining activities in South Africa and globally is acid mine drainage (Naidoo, 2017). This form of pollution results from the bio-hydrogeochemical hardening of pyrite (Fe_2S) and other reactive sulfide-containing minerals under oxidizing conditions (Akinwekomi et al., 2017; Dold, 2017; Masindi, 2016). The formation of AMD is better summarized by Masindi and Muedi (2018) in the following equations:

$$2FeS_2 + 7O_2 + 2H_2O \rightarrow 2Fe^{2+} + 4SO_4^{2-} + 4H^+ \qquad \textbf{Equation 1-1}$$

Then, sulfide oxidation to sulfate solubilizes the ferrous iron (Equation 1-1), after that oxidized to ferric iron (Equation 1-2):

$$4Fe^{2+} + O_2 + 4H^+ \rightarrow 4Fe^{3+} + 2H_2O \qquad \textbf{Equation 1-2}$$

Sulfur and iron-oxidizing bacteria may catalyze the reactions. Thus Fe(III) produced may further oxidize Fe2S, then reduce to Fe(II) (Equation 1-3):

$$2FeS + 14Fe^{3+} + 8H_2O \rightarrow 16Fe^{2+} + 2SO_4^{2-} + 16H^+ \qquad \textbf{Equation 1-3}$$

Ultimately, equations (1-1 to 1-3) produce a hydrogen ion, thus sustain the solubility of Fe(III); in turn, it has pH $\leq$ 2 with a high content of sulfate (SO_4^{2-}) and metal ions. The low pH of AMD makes it susceptible to hazardous and toxic chemical species due to increased mobility and solubility (Akcil

and Koldas, 2006; Sheoran and Sheoran, 2006). AMD's most visible impact is the precipitation of ferric hydroxide and oxy-hydroxide complexes as an orange coating in water channels around mining sites in South Africa, thus causing degradation in the aquatic environment (Oberholster et al., 2013).

In a quest to combat this pollution challenge, different active and passive remedial strategies have been researched, developed, and commissioned (Akcil and Koldas, 2006; He et al., 2013; Peer et al., 2015; Sheoran and Sheoran, 2006; Strosnider et al., 2011). These strategies are not limited to adsorption (Motsi et al., 2009), neutralization (Igarashi et al., 2020; Lakovleva et al., 2015), ion-exchange (Torres and Auleda, 2013), bio-sorption (Al-Qodah, 2006; Norton et al., 2004), coagulation and precipitation (Oncel et al., 2013; RoyChowdhury et al., 2015). Regardless of these treatment options' success, more consideration is needed before selection, such as cost and toxic sludge production, which further increase the cost of disposal, thus restricting the scope of implementation of most of these methods (Naidu et al., 2019).

Until now, ion exchange and adsorption have demonstrated some limitations regarding selective pollutant elimination, low removal efficacies at high concentration, and conditions for regeneration are often not successful (Torres and Auleda, 2013). For large quantities of water, precipitation strategies have been an excellent treatment option for acid mine drainage, mainly because of its ability to recover metals (Akinwekomi et al., 2017). On the other hand, membrane technology seems to be effective; however, they are energy demanding and produce brine creating another environmental challenge (Simate and Ndlovu, 2014). Practically, these methods actively address the AMD treatment, thus consuming a considerable amount of money, material, and energy resources. Without a doubt, biological processes appear desirable for heavy metal removal from wastewater with technological and economic issues linked to the use of existing technologies (Kiran et al., 2017). These methods are not the best fit for a developing country like South Africa, which is already struggling with its energy reserves and resources. For this reason, researchers must focus considerable attention on passive treatments which are less energy-intensive.

AMD's passive treatment depends on physical, geochemical, and natural biological processes to improve water quality (Skousen et al., 2017). Primarily, passive methods are broadly separated into biological and geochemical systems that often contain inorganic material. Focusing on biological processes, they often require an organic substrate for bacterial sulfate reduction (Strosnider and Nairn, 2010). In particular, sulfate reduction bacteria (SRB)-aided metal precipitation is an attractive choice alternative to other methods (Sahinkaya et al., 2013). The biological process carried out by sulfate-

reducing bacteria under anaerobic conditions has become a suitable alternation for AMD treatment (Kaksonen et al., 2004) with some of the advantages of this bioprocess pathway are alkalinity generation and metal sulfide precipitation (Gallegos-Garcia et al., 2009; Ucar et al., 2011). SRB's ability to produce sulfide and high sulfide propensity to react with metal cations is considered an excellent option for treating water-rich metal and sulfate (Bai et al., 2012). The insoluble metal sulfides generated are separated from the liquid phase through appropriate strategies (Johnson and Hallberg, 2005), making the sulfidogenic treatment of AMD a smart option from an economic and environmental point of view.

AMD's sulfidogenic treatment is a feasible alternative because of its improved quality of sludge and low-cost compared to traditional chemical treatment (Sahinkaya et al., 2013). Even though sulfidogenic remediation is useful for AMD, it is always essential to add an appropriate carbon source electron donor for sulfate elimination (Sahinkaya et al., 2011). Several carbon sources have been researched and efficiently applied to encourage bacterial sulfate reduction like chicken liter, methanol, compost, ethanol, cow and horse manure, and municipal sewage sludge (Cocos et al., 2002; McCullough et al., 2006; Younger et al., 2002). AMD treatment using organic substrate is suitable if the energy source is provided by recycled material or a waste stream, thus becoming a co-treatment strategy. Like wastewater with high organic carbon concentration, alternative substrates are favorable and much better if the other stream (substrate) can be simultaneously co-treated in the process (Makhathini et al., 2020).

AMD and municipal wastewater's co-treatment is well documented, demonstrating success in metal and sulfate reduction (Deng et al., 2016; Deng and Lin, 2013; Johnson and Younger, 2006; Peer et al., 2015; Strosnider et al., 2013). These studies depended on carbon substrate bacterial oxidation required by electron acceptors. Considering the high nutrients and chemical oxygen demand in hospital wastewater compared to municipal wastewater, it is more likely that HWW can be a suitable substrate for passive co-treatment to produce descent sulfate reduction levels (Makhathini et al., 2020). In any case, recently, researchers have detected pollutants like pharmaceutical compounds in South Africa's water systems (Madikizela and Chimuka, 2017). These emerging pollutants extend to a new global water quality challenge, potentially threatening the ecosystem and human health. According to Ngqwala and Muchesa (2020), pharmaceuticals in the aquatic systems have now become a global concern due to their introduction mainly through hospital effluents. Hospital wastewater (HWW) has some similarities in quality to municipal wastewater; however, hospital wastewater is considered a hot spot for pharmaceutical compounds' load (Archer et al., 2017). For this reason, scholars have led the

research community to probe the general practice of discharging HWW into WTTPs (Ort et al., 2010). This practice is common globally in countries like India, Thailand, India, Australia, Egypt, and South Africa (Al Aukidy et al., 2017), to mention a few. Moreover, research indicates that certain pharmaceuticals are susceptible to traditional biological treatment processes used by WTTPs and are thus present in treated effluents (Zupanc et al., 2013). Indeed, it is crucial to consider the disposal, care, and management of HWWs. Nonetheless, this study examines the probability, within the specified context, of co-treating pharmaceutical-containing wastewater with acid mine drainage.

Looking at different reactors that may be suitable for this treatment goal, one of the criteria to consider is the biomass and biomass concentration activity. For an increase in biomass concentrations, biomass retention is applied, necessary for sulfidogenic bioreactors due to the reduced microbial activity growth rate (Sánchez-Andrea et al., 2014). According to Johnson (2000), anaerobic fixed and packed-bed bioreactors are efficient for remediating wastewater. However, they are prone to clogging and channeling, reducing the performance rate (Kolmert and Johnson, 2001). On the other hand, the biomass retention on up-flow anaerobic sludge bed (UASB) reactor depends on granulation (biofilm formation onto other biomass), of which SRB does not granulate as well as methanogenic microorganisms (Weijma et al., 2002). Therefore, UASB is more suitable as a methanogenic reactor than a sulfidogenic reactor; but the methanogenic activity reduces the sulfide yield. Meanwhile, fluidized-bed reactors (FBR) promise success in enhancing the mass transfer of toxic substances and substrate, such as hydrogen sulfide, compared to packed-bed or UASB reactors (Nagpal et al., 2000). Besides, FBR offers improved sulfate reduction rates and supports high biomass, achieving large mass transfer rates and reducing pressure drops (Sahinkaya et al., 2007). Moreover, the FBR recycling stream decreases elevated feed concentrations, rendering FBR an appropriate option for metal and sulfate acidic wastewater treatment (Kaksonen et al., 2003). Against this prevailing context, this study aims to investigate the co-treatment of acid mine water and hospital wastewater using the sulfidogenic FBR.

In conventional strategies of optimization and modeling, one variable or parameter is changed per given time. Consequently, the experimental runs are conducted over a long period and are usually costly due to equipment and chemical purchases. More often, the experiments' results fail to factor all interactions between parameters influencing the process; as such, accurate optimal conditions are unattainable (Shojaeimehr et al., 2014). Nonetheless, several researchers used response surface methodology (RSM) to assess and optimize the water quality in wastewater treatment processes (Agarwal et al., 2016; Jun et al., 2020; Karri and Sahu, 2018). A drawback of RSM is its failure to

integrate parameters that are not controlled easily. However, the artificial neural network (ANN) is a good option for modeling and optimizing parameters by manipulating the network weights to generate the required response (Gadekar and Ahammed, 2019).

In summary, ANNs are data processing networks with integrated neurons set in several layers to any degree of accuracy using a group of independent variables as inputs (Agarwal et al., 2016), not requiring intense knowledge of the physical process. Consequently, the development of a reliable simulative model. Previously, studies comparing ANN with RSM have reported that ANN proves to be superior because it generates the least mean squared error (MSE) and high correlation coefficient values (Dil et al., 2016; Elfghi, 2016; Karri et al., 2018; Lingamdinne et al., 2018). The mentioned ANN strength makes it an excellent choice for modeling biological processes, which are often non-linear and complex (Antwi et al., 2017).

According to Antwi et al. (2017), ANN is even more preferable in biological process modeling since they can be developed with no kinetic parameters, which are often utilized to understand non-linear relationships that exist in a complex process. To date, ANNs have been successfully applied to simulate and predict the performance of biological systems (Jun et al., 2020; Karri and Sahu, 2018; Lingamdinne et al., 2018). Therefore, ANN modeling may help predict contaminants removal in a sulfidogenic fluidized bed reactor co-treating acid mine and pharmaceutical-rich wastewater. Moreover, the model provides an intense understanding of complex interactions between process variables and contaminant removal efficacy, guiding the optimum operation of the FBR. Noticeably, no study could be located employing ANN modeling to predict contaminant removal efficiency in an FBR co-treating AMD and HWW.

1.2 Problem Statement

With much difficulty in prevention strategies, treatment is considered an excellent alternative for AMD remediation, mostly collecting and neutralizing AMD through passive or active treatment methods (Moodley et al., 2018). Passive methods include natural and biological processes to treat AMD, while active treatment provides for the addition of alkaline substrates to neutralize pH for the AMD. The comparison and capabilities of active and passive treatment strategies are widely reported (Hallberg and Johnson, 2005; Simate and Ndlovu, 2014).

Owing to the limitations associated with the use of conventional materials in the remediation technologies like high costs, usage of toxic chemicals, design inaccuracies, poor performance, depletion of natural resources, and generation of more waste; as such, it is crucial to focus the research on the application and use of other waste streams. The effluent that may demonstrate the effectiveness and eliminate some of the limitations listed above could be an attractive option for AMD remediation as long as these effluents' use allows for an overall positive contribution to environmental and economic sustainability.

One waste stream of interest is pharmaceutical-rich wastewater because of the complexity and hazardous nature of this effluent. However, pharmaceutical wastewater is characterized as waste from the pharmaceutical industry and hospitals that contaminates the environment. This study focused on an isolated stream of hospital effluent. Although HWW has some similarities to municipal wastewater quality (El-Gawad and Aly, 2011), it has far more hazardous components (Verlicchi et al., 2012). The majority of South African hospitals discharge their untreated effluent directly to the municipality pipeline, ending in WWTP. Pauwels and Verstraete (2006) warned that discharging hospital effluent into municipal WTTP decreases the biodegradation process of the organic contaminants in WTTP. Moreover, the introduction of pharmaceutical compounds like diclofenac in the anoxic sludge treatment process causes a reduction in gas production. It reduces the denitrifying potential of the microbial community in WWTP (Ozdemir et al., 2015). The treated effluent from WTTP (containing pharmaceuticals) is discharged to water bodies, raising concern regarding the effect of these persisting compounds in the aquatic habitat.

In a quest to treat AMD and HWW as both are problematic in the environment and they pose a threat to human health, it is theorized that a co-treatment of these waste streams could be of great benefit. Research has been done on co-treatment of AMD, and municipal wastewater with much success (Deng and Lin, 2013; Peer et al., 2015; Strosnider and Nairn, 2010), and HWW has some similarities with MWW but much richer. Like in AMD and MWW co-treatment, the HWW will act as a substrate and provide the energy source for bacterial sulfate reduction. It is anticipated that the proposed co-treatment will produce excellent results and further attempt to tackle the issue of pharmaceutical compounds. Additionally, the use of artificial neural network methodologies to predict the biotreatment system's performance for co-treatment of AMD and HWW is not available yet. Therefore, this research seeks to provide new insights in this field through robust investigation and development of an ANN model for prediction purposes.

The following research questions were raised and examined in this research study to prove the hypotheses mentioned above:

1. Would hospital wastewater, therefore, feasibly be used as an energy/carbon source for sulfate-reducing bacteria in AMD treatment while reducing its contaminants?

2. What would be the kinetic model with its associated microbial community supporting the fluidized bed reactor's biological co-treatment?

3. Can artificial neural network techniques be used to predict the biotreatment performance from the fluidized bed reactor?

1.3 Research Aims and Objectives

This study aims to evaluate the feasibility of using hospital wastewater (pharmaceutical-rich) as an energy source to support sulfate-reducing bacteria for co-treatment with AMD in the fluidized bed reactor and subsequently developing a performance model using an artificial neural network.

The specific objectives of this research are:

1. To evaluate the possibility of using hospital wastewater as an organic substrate for AMD treatment by assessing alkalinity production, metal and sulfate reduction, elimination of organic pollutants in the hospital wastewater.
2. To obtain the kinetic parameters, maximum rate (V_{max}), and Michaelis-Menten constant (K_m) for electron donor oxidation through running batch kinetic experiments.
3. To develop an artificial neural network model for performance prediction in the fluidized bed reactor through optimal architecture

1.4 Scope of Research

The research study scope reported in this thesis extends across environmental and energy waste management, introducing an innovative wastewater remedial strategy. This study develops a co-treatment system that seeks to treat two waste streams in a single process. Although there may be challenges with the transportation cost of one stream to a treatment site, this does not fall directly within the scope of the research study in this thesis. This aspect is briefly discussed in chapter two and

in the last chapter to provide direction for extending this work in the future. The sulfidogenic biotreatment demonstrated the advantages of low sludge production, good effluent quality, and high biomass retention. The study uses the application of analytical chemistry, physical chemistry, and process modeling to predict the performance of biotreatment. The developed learned and authenticated artificial neural network model provided excellent fits to the sulfate, COD, naproxen, ibuprofen, and Fe data obtained experimentally. Models built in this study can be used to estimate the HWW and AMD co-treatment sulfidogenic system's efficiency using fluidized bed reactors. Overall, the scope of this research covers the field of environment and energy using many applications of analytical chemistry and physical chemistry techniques.

1.5 The Contribution of Research to the Body of Knowledge

The studies on co-treatment strategies of AMD and other waste streams have been far in between. Most research in this area focused on diluting AMD for alkalinity production but lacked addressing some problematic pollutants, specifically from the substrate stream. There is no available published work regarding the co-treatment of AMD and hospital wastewater using fluidized bed reactors, and it seems this study is amongst the first ones. For the first time, the removal of pharmaceutical compounds was considered in the co-treatment of AMD. In this thesis, new ideas to tackle the challenge of two hazardous waste streams using the bioreactors approach were suggested, developed, and tested. Other contributions to the existing literature in this field including the following:

- A literature review demonstrated that co-treatment of AMD and other waste streams could be efficient when carefully operated. With its limited information on COD/sulfate ratios, water chemistry, and reactor configurations, the published research has not been enough to stimulate ongoing, long-term trials, despite significant potential benefits, i.e., decreased dissolved metal concentrations, increased alkalinity. While credible, the existing studies lack detailed water chemistry and microbial profiling, which would be crucial for the upscaling of equipment and continuous processes. This study introduced a new approach through co-treatment of AMD and hospital wastewater; this approach had not been reported elsewhere in this area of research during the writing of these theses.

- Unlike previous co-treatment reports, this study considered the inhibition effects and associated microbial communities to assess the remedial strategy fully.

- Apart from the bioreactor's regular operation, the research was advanced to its enhancement using nZVI to test where the co-treatment could benefit from new green technologies.

- Development of an optimal artificial neural model to predict the performance of the sulfidogenic fluidized bed reactors for the co-treatment of AMD and pharmaceutical-rich wastewater

For disseminating the information from this study, a few of its findings have been communicated to the scientific community through peer-reviewed journal articles.

1.6 Research Methodology

The study's research methodology is not limited to four main tasks: a review of the literature to identify the study's need; experimental design, run and, data analysis; development of the ANN model; evaluating an enhanced process for better performance; conclusions and recommendation. A brief discussion of these tasks is given below.

1.6.1 A review of literature

The literature was reviewed, (1) to discuss active and passive co-treatment strategies, (2) to examine the effect of mixing ratio in the bioreactor, (3) to discuss the impact of hydraulic retention time, (4) to discuss limitations for co-treating AMD with other waste streams, and (5) to identify future possibilities regarding the co-treatment research. The literature informed the structure and the design of the experimental runs.

1.6.2 The design of experiments

The fluidized bed reactor was used to test the hypotheses. The real hospital wastewater was collected at a district hospital. It was used as a carbon source throughout to treat the actual AMD collected from an abandoned mine in Mpumalanga. Quality determinants used were sulfates, chemical oxygen demand and metal concentration.

1.6.3 Data analysis

To test the hypotheses, a laboratory-scale bioreactor was run under different conditions and data was collected for analysis. As such, the analysis was done to determine the effectiveness of the co-treatment process.

1.6.4 Conclusions and recommendation

After each objective was satisfied by running the experiments for a stipulated time, data was analyzed, and the results were synthesized to draw conclusions and recommendations. The conclusions were drawn from chapters 2 – 4 presented which is aligned to the specific objectives. Then an overall conclusion was made relating to the aim of the study. The recommendations highlight the general applicability and validity aspects of HWW as an energy source to support SRB for AMD treatment.

1.7 Thesis Layout

This work consists of seven chapters, including the current chapter, which presents the general introduction to the background of the work, the problem statement, and the overall objectives of this study. Contribution to existing knowledge in the field is listed, with a mention of published and under-review articles emanating from this study. At the beginning of each chapter, a brief abstract of the key results was obtained, with the conclusions to sum up all the ideas.

In chapter two, a review of literature is presented with a discussion on existing co-treatment strategies, focusing on factors that influence co-treatment feasibility, such as mixing proportions, microbiological elements, reactor design, and mixed water chemistry. The need for research to investigate other streams with the possibility of successful co-treatment with AMD was identified and further emphasized in subsequent chapters. This chapter was published verbatim as a review article in the journal on Mine Water and the Environment.

Chapter three presents the co-treatment strategy methodology for AMD and hospital wastewater operated in a sulfidogenic fluidized-bed reactor. The preliminary investigation of the metal, sulfate, and organic pollutants reduction was reported in this chapter. The feasibility of eliminating some of the anti-inflammatory pharmaceuticals was explored and reported here. This chapter responds to objective one. This chapter was published as a research article in the Journal of Water Process Engineering.

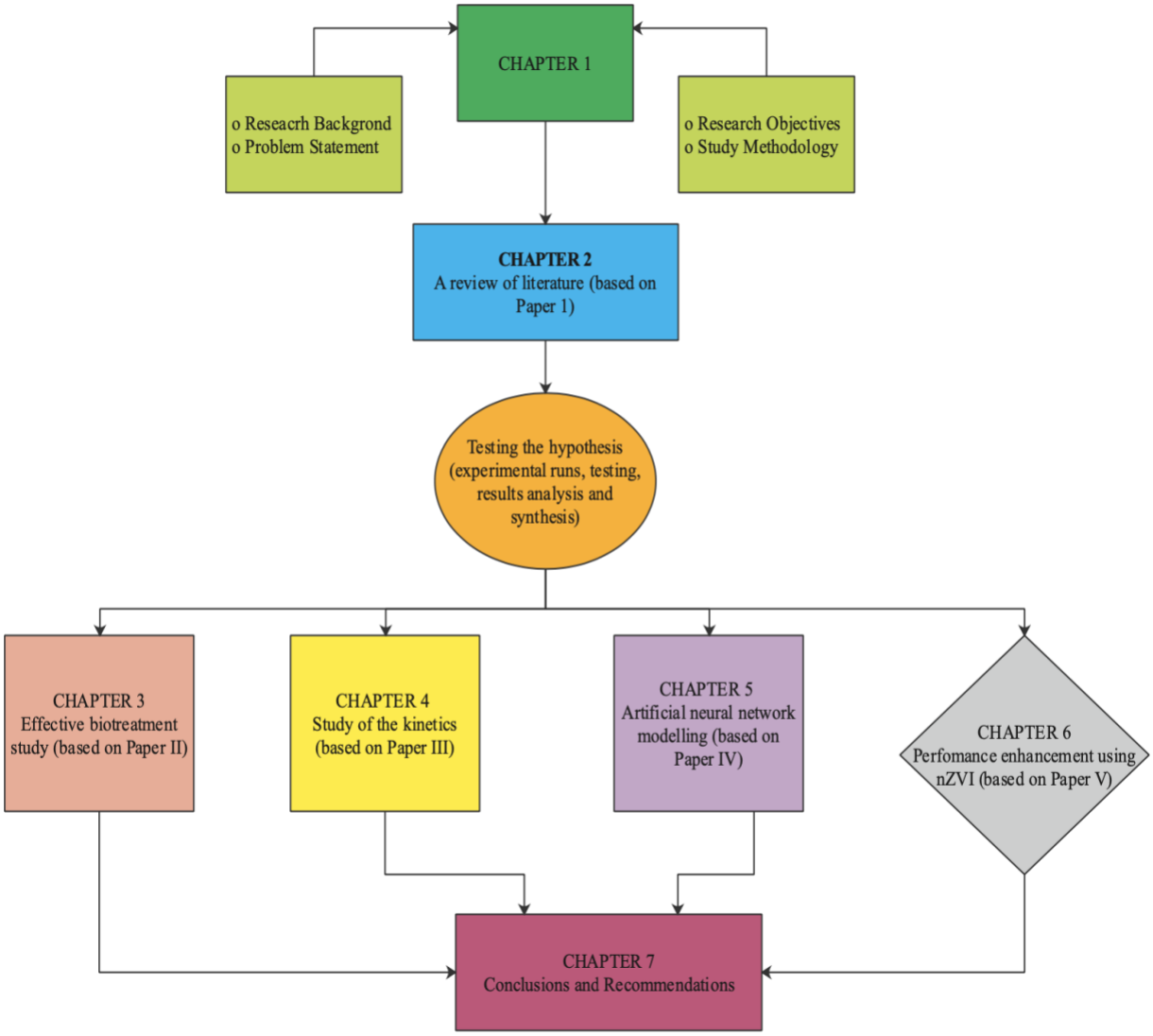

Figure 1.1 The layout of the thesis

Chapter four presents the inhibition kinetics and microbial communities' investigation of the sulfidogenic fluidized bed reactor treatment to understand the diverse species and the system's reaction mechanisms. The alkalinity from organic oxidation Michaelis-Menten modelling is assessed in the batch operated process using sulfate-reducing bacteria. The COD kinetics was used to gain insight into the performance of the process. This chapter responds to objective two. This chapter was published in the journal of Biotechnology Reports.

Chapter five shows the methodology of developing an artificial neural network model to predict the sulfidogenic process using fluidized-bed reactors. The quality parameters considered for the model development were investigated, like COD oxidation, sulfide, and metal reduction. The model showed

confidence in prediction capabilities. This chapter responds to objective three. Also, chapter five is under review in the International Journal of Environmental Science and Technology.

Chapter six, although this chapter is not directly responding to any specific objectives, it is deemed necessary as it expands the knowledge of the proposed co-treatment process. Here the co-treatment is enhanced with zero-valent ion to assess its feasibility for more metal and organic pollutant reduction. This treatment focused on the heavy metals that were not evaluated in previous chapters. This chapter is now published as a research article in Minerals.

The thesis concludes with chapter seven, which gives a summary of the research findings. Significant contributions and breakthroughs to feed on new knowledge are presented in this chapter., with additional recommendations for future research.

1.8 References

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CHAPTER TWO

Possibilities for Acid Mine Drainage Co- Treatment with Other Waste Streams: A Review

The co-treatment of AMD with other common liquid wastes is a promising synergistic approach, fusing characteristics of active and passive AMD treatment for a sustainable remediation strategy. This chapter discusses existing literature on AMD co-treatment approaches, focusing on factors that influence the feasibility of co-treatment, such as mixing proportions, microbiological elements, reactor design, and mixed-water chemistry. Finally, this paper highlights future possibilities, drawing attention to prospects that require exploration. This chapter was peer-reviewed and published in the *Mine Water and the Environment* (see Appendix A1 for the review abstract) in the form of a review article to synthesise the existing information and identify the need for further research.

2.1 General Background

The mining of coal and some metals such as copper, nickel, and gold is correlative with acid drainage challenges that can adversely affect water bodies and the ecosystem at large (Akcil and Koldas, 2006). Moreover, much of the effluent produced by the metal-mining sector contains a sizable number of constituents such as arsenic, cadmium, cyanides, lead, and mercury, with ecological implications and high health risks for both humans and animals (Azapagic, 2004). This effluent is characterised as acid mine drainage (AMD) because it is typically formed after mining exposes sulphide-containing minerals to oxidation reactions. However, the same reactions can occur anywhere pyritic rock and polymetallic sulphides are exposed (Johnson and Hallberg, 2005; Paikaray, 2015). When mining is not involved, the preferred term is acid rock drainage (ARD), and in much of the world, this more general term is used for AMD as well.

There are numerous sulphide minerals that oxidise in a comparable manner to pyrite, discharging sulfate and various metalloids and metals (i.e., Cd, Pb, Fe, As, Al, Cu). The extent of environmental pollution by AMD depends on its pH and composition, which varies according to the geology of the mine site. For example, in South Africa, the average Fe^{2+} concentration in gold mines differs from coal mines, 830 and 2135 mg/L, respectively (Kefeni et al., 2015). Thus, AMD chemistry varies, depending on the sulphide mineral being oxidized and what the acidic wastewater encounters.

For the past couple of decades, there has been an increased interest in using biological processes to treat metal-containing wastewaters because these processes can increase pH and lower metal concentrations (Janssen et al., 2001). Considerable attention has focused on co-treatment options that could reduce cost and energy expenditures by operating a single process rather than running two treatment processes for different waste streams. However, co-treatment application studies have been few and far between.

Factors that have been demonstrated to affect whether co-treatment techniques work include mixing proportions, the range of microbiological elements, reactor design, and mixed-water chemistry. There has been relatively little done on the relationship of microbial communities to co-treatment kinetics, though this is likely important as well. Therefore, there seemed to be a need to collate the published studies and compare their performance to identify further study needs in this fairly young research area, to identify other possible waste streams for co-treatment, to provide a future perspective, and to highlight the type of research that is needed to inspire further exploration of AMD co-treatment as a remedial strategy.

2.2 Outlook on Strategies for Mine Water Co-treatment

The necessity of electron donors for treatment of sulfate-containing wastewater by passive reduction processes is acknowledged (Liamleam and Annachhatre, 2007). Generally, AMD has low concentrations of organics, making sulfate reduction challenging (Mulopo, 2016). For successful co-treatment, one option is to identify waste streams with high concentrations of organics and alkalinity that when mixed with AMD will facilitate biological and physiochemical processes for treating the combined wastewaters. Table 2.1 gives a brief overview of the mechanisms of co-treatment of wastewater and AMD, highlighting examples of potential waste and their compatibility.

2.2.1 Active Co-treatment Strategies

Active treatment of AMD includes adding alkaline reagents to raise the pH and precipitate dissolved metals as hydroxides. The feasibility of using AMD from coal waste dumps as a chemical coagulant for the treatment of grey water was initiated by Pearson and Nesbitt (1974), and then later explored by Rao et al. (1992) using AMD collected from Mattabi Mines Ltd in Canada. The AMD substituted for the widely used $FeCl_3$ coagulant to aid phosphate precipitation to treat wastewater with an average of 45 mg/L suspended solids (SS).

A second experiment examined the possibility of using the same AMD as a chemical coagulant for the treatment of slurries of fine solids. Rao and colleagues replaced an alum coagulant with AMD to treat wastewater with an average of 130 mg/L SS. Ten mL of AMD was mixed rapidly with 100 mL of slurry for five minutes and then allowed to settle. In both experiments, the results showed 40–70% removal of SS and turbidity, with no other noticeable chemical additions. Concentrations of some metals decreased by more than 60%. The acidity of the AMD introduces surface H^+ ions that inhibit coagulation and cation adsorption, whereas calcite supports the neutralisation of H^+ ions. More importantly, the silica and clay minerals in the slurry effectively adsorb the metal ions in the AMD, qualifying this process as a co-treatment remedial strategy. It should be noted, though, that neither of these two studies included data on sulfate reduction, one of the persistent pollutants in AMD. Rao et al. (1992) concluded by highlighting that AMD containing Fe^{3+} may be superior to commercially available $FeCl_3$ as a coagulant due to the presence of Al^{3+} in the AMD. However, the metal levels of the discharge prohibit its overall use without additional treatment.

Another approach is the co-treatment of municipal wastewater (MWW), AMD, and activated sludge. The adsorptive properties of the SS of the MWW, along with its alkalinity, are key aspects that promote the remediation of both streams (Hughes and Gray, 2013b). The activated sludge biomass accelerates metal removal from the AMD and increases the effectiveness of the treatment process. Since metal concentrations in MWW are considerably less than in AMD (Santos and Judd, 2010), the availability of adsorption sites on MWW sludge makes metal sorption possible. Similarly, the high amount of particulates in MWW promotes Fe (oxy)hydroxide precipitation (Johnson and Younger, 2006). The Fe and Al (oxy) hydroxides could enhance nutrient reduction in the MWW by supplying a vacant site for bacterial attachment (Demin and Dudeney, 2003). Additionally, the Fe in the AMD may be an efficient alternative for marketable coagulants (Sandström and Mattsson, 2001).

Table 2.1 Mechanism(s) of wastewater treatment by co-treatment with acid mine waters

Wastewater constituent	Removal mechanism once mixed with AMD	Examples of potential wastewater stream	Literature
Soluble organics COD, BOD, DOC, N (TDN)	Aerobic and anaerobic microbial degeneration	Sewage and green waste	(McCullough et al., 2008a, 2006; McCullough and Lund, 2011; Peer et al., 2015; W. H.J. Strosnider et al., 2013; Younger and Henderson, 2014)
Suspended solids (SS)	AMD can function as a coagulant to enhance removal of SS; Fe^{2+} and Fe^{3+} are co-precipitated and total Fe is reduced to an acceptable level	Sewage and domestic WW Grey water	(Johnson and Younger, 2006; Rao et al., 1992; Roetman, 1932; Younger and Henderson, 2014)
Nutrients: NO_2^- NO_3^- NH_4^+ PO_4^{3-}	• Volatilization of ammonia • Microbial nitrification • Denitrification • Formation of Fe phosphate and its complexation with other chemical precipitates	Raw sewage & activated sludge	(Hughes and Gray, 2013a, 2012; Kumar et al., 2011b; McCullough et al., 2008b; Smyntek et al., 2018; W. H.J. Strosnider et al., 2013; William H. J. Strosnider et al., 2013b)
Ba^{2+} Ra-226 Ra-228 Sr^{2+}	• Precipitation of barite ($BaSO_4$) when mixed with flowback water • Co-precipitation of Ra with $BaSO_4$ resulting in the enrichment of naturally occurring radioactive material	Flowback water produced from shale gas fracturing	(He et al., 2016, 2014b, 2014c, 2013; Kondash et al., 2013; Zhang et al., 2014; Zheng, 2013)
Alkalinity	Alkalinity in the other stream raises the pH of AMD, which promotes metal precipitation and associated adsorption and co-precipitation	Domestic WW & MWW	(Deng et al., 2016; Hughes and Gray, 2013a; Strosnider et al., 2011a)

The characteristics of activated sludge, i.e. the capability of activated sludge to accommodate high loads of sulfates, acidity, and metals, enhance efficient co-treatment of AMD and MWW. Hughes and Gray (2013b) observed that randomly adding AMD barely decreased the sludge oxygen acceptance rate in the sludge biomass from the MMW treatment plant. In addition, acclimatisation of activated mass was detected in three weeks, even though bacteria abundance significantly decreased. In their experimental study, the pH increased from 2.8 to 6.2, demonstrating good performance. The ratio of AMD and MWW was 1:1 and the activated sludge (mixed liquor and SS) concentration was approximately 2.5-4.0 g/L. Furthermore, this study confirmed metal removal rates above 90% for Zn, Fe, Cu, and Al in a batch process with activated sludge from a MWW treatment plant and synthetic AMD (Hughes and Gray, 2013b).

Hughes and Gray (2013b) used four different process configurations, with small-scale sequential batch and plug flow reactors, to replicate the co-treatment of AMD and MWW. Process I was a control, with no AMD added. In process II, raw AMD was added to the aeration tanks. In process III, AMD was pre-treated by mixing it with digested sewage sludge, then sent to the sedimentation tank, with the supernatant added to aeration tanks. In process IV, AMD was pre-treated by mixing it with MWW before the aeration tanks. Their study showed that AMD co-treatment with activated sludge produced the best results; Al removal in the second stage of processes II, III, and IV averaged 79.6, 65.3, and 52.2%, respectively. Cu removal efficiency, reported in the secondary stage, was found to be around 60.5%. For processes III and IV, Cu concentrations ranged between 0.04-0.15 mg/L, whereas in the sequential batch reactor, the Cu removal rate in the secondary treatment was reported to be 46.8%.

In the final effluent, Pb concentrations were below the detection limits in processes II, III, and IV. The Pb removal can be attributed to hydroxide precipitation from wastewater and adsorption to sludge biomass (Zhang, 2011). Also, the plug flow reactor showed Zn removal during secondary treatment for processes III and IV (64.9 and 58%, respectively), while process II had low removals, generally less than 10%, compared to the other processes. Similarly, the Zn removal rate was also less than 10% in process II in the sequential batch reactor, while processes III and IV had removal rates of 80.1 and 90.0%, respectively.

Processes III and IV demonstrated similar results in that all metal concentrations in the effluent continued to increase for a lengthy period. There were observable differences in the Al removal rate in process III, as it was much higher in the sequential batch reactor than in the plug flow

process. Also, there was no sulfate removal in the plug flow process; instead, there was an increase in SO_4, with the effluent concentrations in processes II and III exceeding the incoming strength as the experiments approached the end. Some SO_4 removal was observed in the sequential batch reactors at the beginning of the experiments in processes II and III (Hughes and Gray, 2013b).

Additionally, Hughes and Gray (2013b) assessed the sludge biomass. Initially, there was evidence of protozoa in samples from all four reactors. However, their phyla were only determined for the last two days of sampling. Lastly, the samples from the reactor effluent were all found to be net alkaline. Generally, the process I effluent was more alkaline, between 90 and 130 mg/L as $CaCO_3$. Process II generated less than 20 mg/L of alkalinity, while process III generated between 20 and 55 mg/L of alkalinity, and process IV generated among 25 and 75 mg/L, all as $CaCO_3$. A summary of active co-treatment is given in Table 2-2.

Table 2.2 A summary of existing active co-treatment studies highlighting the treatment performance

Reactor system	Mixing ratio	Water chemistry	Treatment performance	Literature
Laboratory-scale plug flow and sequencing batch reactor	2:1 AMD to MWW Residence time ≈ 48 hr	Al and Fe removed as phosphate precipitates and by co-precipitation. Hydroxide precipitation propelled Pb removal from ww. Sulfate can be removed by gypsum precipitation.	Co-treatment improved P removal and sludge settle-ability during MWW treatment. Removal of COD, BOD_5, or TOC was satisfactory	(Hughes and Gray, 2013a, 2013b)

Thus, co-treatment of MWW with AMD using sewage sludge appears to be a viable, innovative remediation strategy, with several practical advantages, including a significant reduction in material and energy costs linked to building and operating AMD treatment systems (Hughes and Gray, 2013a). Hughes and Gray demonstrated metal removal with a pH increase, as well as enhanced phosphate removal and sludge settleability. This should translate into cost savings for chemicals and reduced operating costs.

2.2.2 Passive Co-treatment Strategies

Some passive AMD treatment requires an appropriate organic substrate to provide an electron donor for dissolved oxygen shedding and sulfate reduction, and the bacterially-mediated removal of metals (Younger et al., 2002). On the other hand, conventional MWW treatment often requires an electron acceptor for oxidation of bacteria in carbon substrate, filtration, coagulation, flocculation, and chemicals for pathogen removal. Moreover, municipal influent includes a range of different organic compounds, such as cellulose and sugars (Metcalf and Eddy, 2014). Strosnider et al. (2011a) concluded that the alkalinity achieved by passive co-treatment of MWW, and AMD allows the organic matter and nutrients of the MWW to link homogenously with coagulants and electron acceptors. Strosnider et al. (2011a) highlighted the decontaminating characteristics of AMD in well-controlled conditions, improving water quality with less energy.

Decontamination of domestic sewage with AMD was initially suggested by Roetman (1932) for pathogen elimination, who showed the adverse effects of AMD on faecal indicator bacteria. Joseph and Shay (1952) concluded that the population of *Escherichia coli* quickly deteriorated when subjected to AMD. Rose et al. (1998) established an algae-based system inside waste stabilisation pools that treated richly organic tannery effluent with synthetic AMD, achieving more than 60% metal removal efficiencies. Nonetheless, the mixed system proved to be unsuccessful in a few months as toxic metal levels developed in the algae, even though the short-term performance was good (Van Hille et al., 1999). Van Hille et al. (1999) used MWW to produce an algae-rich stream with an alkaline pH that was subsequently added to the AMD. Johnson and Younger (2006) successfully developed a co-treatment system constructed in a single-stage wetland that produced MWW effluent with a biological oxygen demand of 14 mg/L, but less net-alkaline mine water with 3 mg/L of Fe. In addition, McCullough et al. (2008b) noted the sudden improvement in water quality from an evaporation unit where highly concentrated AMD was added to secondary MWW, even though the study gathered limited data on the reduced faecal count. Strosnider et al. (2013) stressed that the operating conditions of a successful passive co-treatment system must attain a balance between the electron acceptors in the one wastewater and the potential electron donors in the other wastewater.

Metal toxicity must be monitored to sustain various functioning sulfate-reducing microbial groups (Deng et al., 2016) during co-treatment of AMD with another waste stream. Amongst many metals in the AMD, Fe was documented as an inhibitor, causing a reduction in metabolic activity in the

reactor (Utgikar et al., 2002). This inhibition resulted from both iron sulphide formation (Zhang et al., 2009) and the contest for electron donors caused by the Fe^{3+} (Lovley and Phillips, 1986a, 1986b; Van Bodegom et al., 2004). Deng et al. (2016) reported a dual-stage process for co-treatment of liquid MWW with AMD, where they initially mixed the two wastes, followed by a sulfidogenic treatment. This process successfully removed metals, reduced chemical oxygen demand (COD), improved alkalinity, and reduced sulfate in the effluent. A fundamental benefit, amongst many, of co-treatment is the ability of the MWW to act as a neutralising agent for AMD, achieving net-alkaline values. Furthermore, the mixing of municipal sewage and AMD, after it produces net-alkaline values, also accelerates the removal of dissolved metals (Winfrey et al., 2010). A typical experimental setup used by Winfrey et al. (2010) included four replicates of three sequential operational processes. The initial stage of their process was clarification, where the AMD (pH 2.60) and MWW (pH 7.67) were mixed at a 2:1 ratio. The MWW contained various concentrations of faecal and total coliform. The second stage of the process countered the acidity of the AMD, concomitantly producing metal sulphide in the effluent (Younger et al., 2002). The alkalinity in the MWW raised the pH of the AMD, promoting chemical precipitation and co-precipitation. AMD metal concentrations were reduced significantly: Fe from 290 mg/L to 0.18 mg/L, Mn from 55 mg/L to 16 mg/L, and Zn from 390 mg/L to 34 mg/L. All other passive MWW treatment systems, including soil filter and free-water surface, were outdone by this co-treatment method (Winfrey et al., 2010).

A subsequent study by Peer et al. (2015) explored the removal of less commonly addressed metal contaminants with passive co-treatment of AMD and MWW using a multistage batch-reactor. The MWW had an alkalinity of 418 mg/L as $CaCO_3$ and a pH of 9.05, with an equivalence of 38 and 5.6 mg/L of phosphate and nitrate, respectively. Some of the uncommonly addressed metals studied included Ag, Ce, Cr, Gd, and La, which were significantly decreased. The MWW and AMD were allowed to mix, after which solids settled in the clarifier unit. Then, the second process simulated an anoxic limestone drain to generate alkalinity and increase the pH of the AMD, enhancing contaminant precipitation (Younger et al., 2002). In the last unit, an oxidation pond was operated to oxidise and precipitate Fe and Mn. To produce a pH above 4.5, close to that produced in prior passive co-treatment studies (Strosnider et al., 2011a), AMD and MWW were mixed at a 5:1 ratio. The operating conditions for these studies are given in Table 2-5. The overall results demonstrated effective remediation of both streams, especially for the removal of metals typically difficult to address with conventional treatment methods.

Deng and Lin (2013) co-treated AMD and MWW using a dual-stage process that mixed the two wastes before the anaerobic biological treatment process. The entire process lowered metal concentrations by more than 70% and reduced COD and sulfate by more than 80% from the COD/SO_4^{2-} proportion of 0.6–5.4 (Deng and Lin, 2013). Although the proportion of COD and sulfates is emphasised as important, it is difficult to pin down an optimal ratio suitable for co-treatment from the literature, since each study reported different findings. These differences may be due to different carbon sources, sulfate concentrations, and other environmental factors (Lu et al., 2016). The COD/SO_4^{2-} ratio is important because it represents the fraction of electron flow for sulfate-reducing and methanogenic bacteria (Mulopo, 2016). Evidently, some studies do not measure the COD/SO_4^{2-} ratio in the AMD but use the volumetric ratio instead (Tables 2.2 and 2.3). The study by Deng and Lin concluded with a positive performance from a dual-stage treatment (anaerobic) process that treated the MWW and AMD. Subsequently, efficient removal of the COD was achieved by the oxidation of organic matter during the sulfidogenic stage of passive AMD and MWW co-treatment (Deng et al., 2016). These anaerobic and aerobic co-treatment studies are summarized in Table 2.3.

Table 2.3 A summary of existing anaerobic and aerobic passive co-treatment studies highlighting the treatment performance

Reactor system	Mixing ratio	Water chemistry	Treatment performance	Literature
Bioreactor	COD/Sulfate (0.05 – 5.4)	Fe or SO_4^{2-} decreased as COD is oxidised. Further SO_4 reduction results from electron donation from endogenous decay of microorganisms	SRB attached-growth reactor facilitates COD removal while reducing SO_4, increasing pH, and decreasing metal conc.	(Deng et al., 2016; Deng and Lin, 2013; W. H.J. Strosnider et al., 2013)
Bioreactor (three-stage system)	Volumetric ratio 5:2 MWW to AMD 5:1 AMD to MWW	Fe precipitation as hydroxides or phosphate, resulting in concentration decrease. Further Fe reduction from formation of FeS precipitates under the anoxic conditions and high sulphide conc.	Efficient removal of metals and PO_4^{2-}, as well as removal of organic matter (COD, BOD, DOC), nitrogen (TDN) and SO_4^{2-}	(Smyntek et al., 2018; Strosnider et al., 2011b; William H. J. Strosnider et al., 2013a; Strosnider and Nairn, 2010)
Bioreactor (three-stage system)	1:2 AMD to sewage. Residence time of 18 to 42 h	Formation of $FePO_4$ or $Fe(OH)_3$ in initial stage of treatment (aerobic conditions) results in Fe conc. decline. Subsequently, Fe reducing bacteria act on Fe precipitates, freeing dissolved Fe^{2+}	Efficient removal of Al, Mn, and Zn is increased when retention time is extended	(W. H.J. Strosnider et al., 2013)
Bioreactor (three-stage system)	5:1 AMD to MWW Residence time of 48 h	Total As concentration decreased due to sorption to Fe oxyhydroxides. Cd, La, Ce, and Gd removed due to increased pH.	Removal of metalloids supported by ↓ in total metal concentrations throughout system	(Peer et al., 2015)

Table 2.4 A summary of existing field-scale passive co-treatment studies highlighting the treatment performance

Mixing system	Retention time	Water chemistry	Treatment performance	Literature
Addition of AMD to sewage evaporation pond and pit lakes	≈18 months	pH improved from 2. 4 to 7.9. Metal and SO_4 conc. decreased with redox potential down the water column profile, suggesting increased SO_4 reduction rates at deeper lake depths. Decaying algal matter stripped metals from the water column. Algal BOD and sewage decay made hypolimnion anoxic and generated alkalinity by denitrification.	Limited information on Ca, Mg, Na and K concentrations, but Cd and Cr concentration decreased.	(Kumar et al., 2011a; McCullough et al., 2008a, 2008b, 2006)
Full-scale co-treatment of AMD and sewage wetland	≈36 months	For NH_4-N: oxidation to nitrate, with reduction to N2; for Fe: precipitation as ferric hydroxide and ferric phosphate to lower dissolved and total phosphate concentrations.	This co-treatment system demonstrated sustained high rates of pollutant removal	(Johnson and Younger, 2006; Younger and Henderson, 2014)

Table 2.5 A summary of existing flowback water co-treatment with AMD studies highlighting the treatment performance

Mixing system	Retention time	Water chemistry	Treatment performance	Literature
Addition of AMD to flowback water produced from gas production	Barite precipitation is rapid and reaches equilibrium within 30 min. However, celestite precipitation will proceed for more than 24 hr.	The high SO_4 conc. in AMD precipitates high volumes of barium and strontium in produced water.	After precipitation of $BaSO_4$ and Sr, produced water can be reused for hydraulic fracturing	(He et al., 2016, 2014b, 2014c, 2013; Kondash et al., 2013; Zhang, 2015; Zhang et al., 2014; Zheng, 2013)

McCullough and Lund (2011) co-treated pit lake water (pH of 2) with a mixture of primary-treated sewage sludge and green waste (organic garden waste) at a ratio of 1:1 in a controlled microcosm experiment (4.5 L). Two experimental conditions were explored: an organic loading rate of 32:1 and 16:1 pit water: organic matter by mass. This co-treatment technique increased the pH to 5.5 in 145 days and decreased dissolved metal concentrations. The advantage of combining green waste and sewage sludge is that the sludge functions as an instant source of labile organic carbon and micro-nutrients, such as nitrate and phosphate (Kumar et al., 2011b). The water quality improvement was attributed to microbially-mediated sulfate reduction. Moreover, there was evidence of H_2S and NH_3 production and an abundance of total coliforms during the co-treatment process.

A recent study by Smyntek et al. (2018) investigated the rates of iron and sulfate reduction during a start-up anaerobic co-treatment using a process-based kinetic modelling approach. The study, conducted over a 30-day period, employed a mixture of MWW and AMD at a ratio of 5:2. Evidently, they observed similar trends of an instant decrease of Fe concentrations (due to precipitation) as Deng and Lin (2013), Strosnider and Nairn (2010), and Strosnider et al. (2011b; 2013b). However, this co-treatment system resulted in incomplete processing of other components such as nitrogen, sulfate, and organic carbon (Smyntek et al., 2018). Even though successful removal of Fe, Al, and PO_4^{2-} by 96, 100, and 94%, respectively, was observed, there was a smaller decrease in SO_4^{2-}, nitrogen, and organic matter in the reactor: 71%, 60%, 44%, and 33% for SO_4^{2-}, BOD, COD, and total dissolved nitrogen (TDN), respectively.

Despite several years of research investigating alternative co-treatment options for sewage and AMD, including anaerobic digestion (Deng and Lin, 2013) and activated sludge systems (Hughes and Gray, 2012), there is still a dearth of studies investigating full-scale co-treatment. Table 2-4 presents a summary of field-scale co-treatment studies considered for this review. McCullough et al. (2008a) investigated the possibility of mixing sewage with acidic mine pit lake water in field trials. They had observed a rapid decrease in sulfate concentrations for at least a year prior, with the addition of AMD increasing sulfate concentrations and causing gypsum ($CaCO_4$) saturation (McCullough et al., 2008a). Immediately after the addition of the AMD, decaying algal matter was observed, attributed to metals being stripped from the acidic water (McCullough, 2008). Furthermore, the biochemical oxygen demand of the sewage and algae through denitrification resulted in added alkalinity (Abril and Frankignoulle, 2001). Eighteen months later, concentrations

of Zn, Mn, Fe, Cu, Cd, and Fe were below national environmental protection guideline values (McCullough et al. 2008b). A follow-up study determined that fresh MWW was more active in boosting SO_4^{2-} reduction than dried sewage stored for long periods (Kumar et al., 2011a).

Full-scale co-treatment of AMD and sewage was also tested in a constructed wetland system at the Lamesley site in the UK (Younger and Henderson, 2014). This wetland system met all of the regulatory emission limits set by the Environment Agency, performing better than an earlier pilot wetland (Johnson and Younger, 2006). The results demonstrated 66% removal of the Fe, 61% of the SS, 23% of the BOD_5, 40% of the NH_4-N, and 17% of the total phosphate in the sewage (Younger and Henderson, 2014). A drawback for this system, however, was the potential of the high ionic strength of AMD to inhibit vital microbial processes that are important for nutrient removal, particularly PO_4-P and NH_4-N. Strosnider et al. (2011b) suggested probable inhibition of denitrification in mixed sewage and AMD.

2.2.3 Co-treatment of Oil and Gas Wastewater with AMD

The potential environmental impacts of hydraulic fracturing for oil and gas extraction include its effect on groundwater quality, the management of produced or flow back water, and disposal of waste materials that can contain radioactivity and high levels of salinity (Hickenbottom et al., 2013; Kerr, 2010). In Pennsylvania, the proximity of shale gas wells and mining sites gave researchers the innovative idea of using AMD as an alternative source for flow back water reclamation (He et al., 2013; Kondash et al., 2013). Some of the benefits of using AMD for hydraulic fracturing include lessened demand on freshwater sources, reduced freshwater transportation costs, and decreased environmental impacts from the AMD (He et al., 2016). However, the challenge with AMD is its high concentrations of Fe and sulfate. Sulfate can react with Ba, causing severe scaling and Fe can interfere with friction-reducing chemicals (He et al., 2013).

Two published studies were carried out to understand the precipitation of barite ($BaSO_4$) associated with the mixing of AMD and flow back water (He et al., 2014b; Zhang et al., 2014) and to improve the efficiency of solids separation processes (He et al., 2014c, 2014a). These studies found that co-precipitation of Ra with $BaSO_4$ generates Ra solid waste, which far exceeds the disposal limits of technologically enhanced, naturally occurring radioactive material in U.S. (state-dependent) municipal solid waste landfills (Zhang et al. 2014). A subsequent pilot-scale study by He et al.

(2016) explored co-treatment of AMD as make-up water and as a source of chemicals for treatment of real flow back water. Their study attempted to solve the challenges with generation of Ra solid wastes realized from previous studies by optimizing the treatment process to recover the $BaSO_4$ solids to be used as weighing agents in drilling mud. The study used acidic non-treated AMD (pH 2.6), which was rich in both Fe^{2+} and Fe^{3+}. Noticeably, the AMD had a very low sulfate concentration (275 mg/L) compared to the Ba concentration (19,155 mg/L) in the produced water; therefore, Na_2SO_4 was added to the AMD stream prior to pumping it to the pilot-scale system (He et al., 2016). The pilot-scale operation system comprised mixing, flocculation, sedimentation, and filtration. Each experiment ran for 6-8 hr. in steady-state conditions, with constant parameters like influent of Fe, sulfate, and Ba concentrations. Kondash et al. (2013) had reported that the mixing of flow back water and AMD required at least 10 hr. to reach precipitation equilibrium, using conductivity analysis. However, He et al. (2014a) concluded that barite precipitation is fast and can reach equilibrium within 30 min when excess sulfate is added to the flow back water. He and his colleagues concluded that the Fe^{2+} and Fe^{3+} were successful coagulants, assisting in the separation of SS through settling. These studies are summarized in Table 2.5.

Furthermore, they concluded that sludge recirculation can be used to increase the size of barite particles through co-treatment of AMD and flow back water to satisfy the weighing agent specification for drilling fluid. This management approach may counteract the treatment cost and enhance the possibility of flow back water reclamation to reduce environmental impacts.

2.3 Exploring Fluidized Bed Reactors for the Co-treatment Strategy

Lately, studies seem to lean towards process intensification and energy efficiency through process integration (Bello et al., 2017). For example, Kim et al. (2016) reported an integrated anaerobic fluidized bed membrane bioreactor application for wastewater. Another study by Li et al. (2014) reported an energy efficient wastewater treatment process using fluidized bed membrane bio-electrochemical reactor. Also, some studies reported wastewater treatment using FBR applications, including AMD remediation.

Fluidization includes passing wastewater through a static bed of solid particles with superficial velocity to suspend the particles and they seem as fluid as they move. On the other hand, when wastewater is pumped into a static bed at much lower velocity, the solid particles do not move, and the bed remains fixed. However, if the velocity of the wastewater is increased, there is an expansion

of the bed, thus the particles are suspended. A basic concept of FBR in wastewater treatment is shown in Figure 2.1.

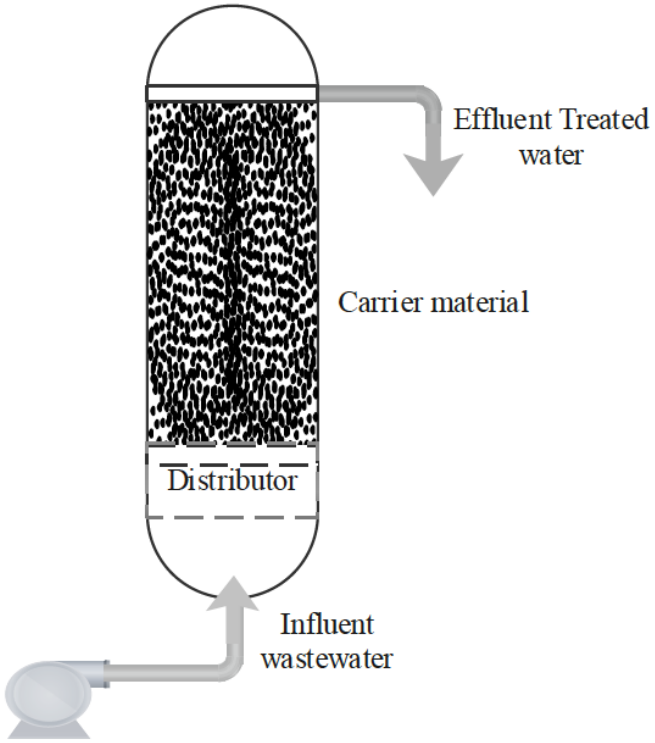

Figure 2.1 A conceptual presentation of a FBR in wastewater treatment

The application of fluidization in wastewater treatment has superior advantages such as high mass transfer rate, efficient mixing and evenly distribution of temperature (Tisa et al., 2014). The operation of fluidized bed reactors uses the principle of fluidization. In many ways, it is comparable to the normally used fixed or packed bed reactors, although packing material becomes fluid because of the force exerted in an upward or downward motion of the wastewater (Dora et al., 2013). The bed expansion capacity is determined by the velocity and density of wastewater, as well as particle size of the carrier material. According to Si et al. (2011) asserts that the superiority of FBR due to its excellent features, it is one of the most reactor systems used in biotechnology applications. Earlier, FBRs application was limited to combustion, coating, drying, catalytic cracking and granulation, then later were introduced to wastewater treatment. However, it is important to note that FBR can be used in advanced oxidation processes, adsorption, and biological treatment. An overview of FBR applications is demonstrated in Figure 2.2, and suggested treatment option for a hybrid co-treatment system of hospital wastewater and acid mine drainage is highlighted.

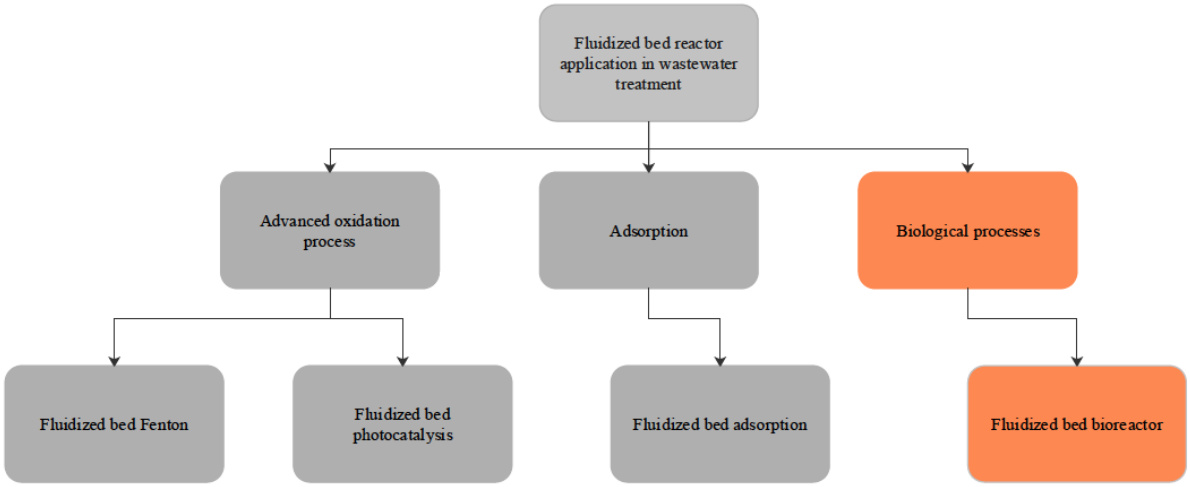

Figure 2.2 A summary of wastewater treatment applications using FBRs

Fluidized bed bioreactor application may be used for both anaerobic and aerobic wastewater treatments. Microorganisms in wastewater may well be attached to the carrier material and be fluidized to keep different phases sufficiently mixed (Kim et al., 2016). The carrier or support material in the bioreactor typically have large specific surfaces, causing it to achieve excellent removal rates in a lesser time compared to traditional biological treatment strategies (Bello et al., 2017). The reason is that fluidization enhances surface contact between pollutants and microorganisms. One advantage for using fluidized bed reactors in that it gives preferential flow paths, thus experiences less clogging as compared to fixed-bed reactors.

Another significant benefit offered by fluidized-bed reactors (FBR) in comparison to UASB or packed-bed reactors is enhancement the mass-transfer of both substrate and toxic products, like hydrogen sulfide (H_2S) (Nagpal et al., 2000). Furthermore, FBR offers better sulfate reduction rates (Somlev and Banov, 1998) and can retain high biomass, achieve significant mass transfer rates, and lower pressure drops. Also, in FBR, the recycle stream reduces high feed concentrations, making FBR more suitable for treating acidic wastewater with metals and sulfate (Kaksonen et al., 2003).

2.4 Discussion

The fundamental objectives of AMD treatment systems require neutralisation of acidity and decreasing the concentrations of sulfate and metals. At the design stage of a passive or active system, fundamental parameters like incoming stream acidity (Zipper and Skousen, 2010) and

metal concentrations (Hedin and Nairn, 1993) must be appropriately considered. The question of how much activated sludge is appropriate must also be addressed in designing co-treatment systems. Furthermore, the success of the co-treatment systems depends on the parameters of the other waste stream, especially its alkalinity. Hughes and Gray (2013b) emphasized alkalinity and the ability to lessen the impact of the other waste stream as critical factors for a successful co-treatment process. Thus, while maintenance requirements and cost are essential factors, they are seldom considered in published studies.

A theoretical advantage of AMD co-treatment with wastewater is that the components are usually in a skewed balance, where, for example, one effluent stream is highly acidic and the other is highly alkaline, yielding neutralisation when the two are mixed (Hughes and Gray, 2013a). For instance, high concentrations of particulates in MWW promote Fe elimination through (oxy)hydroxide precipitation (Johnson and Younger, 2006). The Fe and Al (oxy) hydroxides can then enhance removal of turbidity, SS, and COD in the MWW by adsorption (Demin and Dudeney, 2003; Hughes and Gray, 2013b). In some MWW treatment plants, removal of phosphates is achieved by adding Al or Fe salts, which are naturally present in AMD (Caravelli et al., 2010). These results from Hughes and Gray (2013b, 2013a) proved that the use of AMD sludge as a coagulant is as efficient as the conventional coagulants used in MWW treatment plants.

The most significant factors affecting metal removal are process parameters, e.g. settling time, SS removal, dissolved oxygen concentrations, and hydraulic retention time (Santos and Judd, 2010; Santos et al., 2004; Zipper and Skousen, 2010), and physical and chemical factors, e.g. pH, solubility, particle size, metal concentrations, and temperature (Al-Qodah, 2006; Chang et al., 2007; Crane et al., 2010; Pambrun et al., 2008).

2.4.1 Effect of mixing ratio in the reactor

The mixing ratio of the two streams must be monitored to avoid Fe inhibitive effects on the reactor, especially in biological treatment. While studies have been conducted to obtain the optimum COD to sulfate ratios for AMD treatment with other waste streams under biological conditions, the results are inconsistent. The research of Al-Qodah (2006) reported the optimum performance for a COD/SO_4^{2-} ratio of 5.0, when sludge was used as the organic carbon. In contrast, other studies used natural substrates to support the removal of metals and sulfate in a passive biological process for a COD/SO_4^{2-} ratio less than 1.7 (Choi and Rim, 1991; Mulopo, 2016). The best COD/SO_4^{2-} ratio for effective operation of the passive reactor under biological conditions needs further

evaluation, considering that all the studies produced significant results at different mixing ratios (see Tables 2.2 and 2.3). Other studies mention the mixing ratio of AMD to the other stream, but there is not enough information on why the ratio was selected.

2.4.2 Effect of hydraulic retention time

The hydraulic retention time (HRT) in the reactor affects the quantity of substrate that can be treated per time (Kim et al., 2013) and the amount of contact between substrates and microorganisms, which affects treatment efficiency (Buitrón and Carvajal, 2010; Chang et al., 2000; Kaksonen et al., 2004; Meng et al., 2007). For a stable operation of a co-treatment reactor, there should be a well-maintained balance between sulfate reduction and organic waste degradation, which will largely be determined by the HRT and by the microbial community structure. As such, the biological processes for removal of metals and sulfate reduction demand compacted support to establish a micro-environment that allows the relevant bacteria to survive despite relatively high oxygen concentrations (Bekmezci et al., 2011). The reactor also requires porous media with a large void volume to minimise plugging (Tsukamoto et al., 2004). Eventually, there also needs to be a balance between the surface area and the pore size of the medium to ensure optimum removal of metals and sulfate reduction.

In most studies, metal sulphide precipitation occurs within three to five days (Kaksonen et al., 2003). HRT needs to be controlled or optimized depending on the system. To accelerate the treatment process, the flow rate of the mixed streams should be increased. However, an HRT that is too short will not allow sufficient time for the biological co-treatment process, resulting in biomass escaping from the reactor, while an HRT that is too lengthy will deplete the organic source, stopping the biological process (Klein et al., 2013). The biological treatment processes that treat the AMD and other waste streams require biomass accumulation. The flow rate, loading rate, and type of bacteria involved in the biological treatment determine the biomass characteristics (Rockhold et al., 2002).

HRT is important in both bioreactors and constructed wetlands. Lee et al. (2009) identify hydrology as one of the primary factors in controlling wetland functions, with flow rates being regulated to achieve satisfactory performance. However, the effect of HRT differs between wetlands depending on the dominant plant species and temperature ranges, as these factors can affect the wetland's hydraulic efficiency. Although HRT is a dominant variable that defines the efficiency of metal removal in a wetland co-treatment system, environmental factors of the

geographic area influence the co-treatment as well.

2.4.3 Limitations for co-treating AMD with other waste streams

Co-treatment has been shown to be a cost-effective remedial technique for AMD and other waste streams in tests of various scales (Johnson and Younger, 2006). There are, however, limitations associated with co-treatment. Most documented studies have focused on the batch process with less emphasis on continuous processes (Table 2.6). Moreover, most of these studies are batch studies with short experimental durations.

The literature reviewed in this paper focuses primarily on biological treatment, which is limited by time factors (Santos and Judd, 2010). For such systems, it is important to ensure that microbial communities will not adapt over time into communities that are less favorable for co-treatment. Another drawback is the cost of transporting one of the effluent streams to a centralised treatment facility with another effluent stream. Since mine water is typically formed a considerable distance from areas where other waste streams are situated, pumping costs may offset the anticipated savings from joint treatment. Furthermore, the existing literature shows variable results, depending on the type of wastewaters used for co-treatment, reactor configuration, and retention times. This makes it hard to develop “universal” co-treatment strategies; instead, co-treatment options might need to be optimized on a case-by-case scenario.

Table 2.6 Summary of AMD co-treatment with other waste streams detailing successes and limitations; WW=wastewater, municipal wastewater= MWW; SS=suspended solids, TDN=total dissolved nitrogen, rxn=reaction(s), conc. = concentration(s)

Remedial technique	Co-treating stream	Benefits	Limitations	Future work to be done	References
Passive, anaerobic treatment	Sewage	• Efficient removal of contaminants such as Al, Fe, and PO_4^{3-} • 98% P removal.	• Incomplete processing of some components, e.g., organic C, SO_4^2, and N (TDN)	• Rxn rates and adapted microbial communities have to be investigated.	(Smyntek et al., 2018; Strosnider et al., 2011a; William H. J. Strosnider et al., 2013a; Wei et al., 2008)
	MWW	• Best pH increases and alkalinity generation. • Almost complete nitrate removal with standard retention time • Removes Mn, Pb, Fe, Cd, Al, and As. • Addresses chronic water quality challenges	• Slow Mn removal • Zn removal required more retention time • Removal of other metals like Zn, Ni, and Cu depended on oxidation and limestone units.	• Optimisation study to evaluate loading rates. • Maintenance needs at optimal operating parameters • Investigation of O&M costs is of vital importance. • Sustainability has to be investigated	(Strosnider et al., 2011c; Strosnider and Nairn, 2010; Winfrey et al., 2010) (Deng et al., 2016; Deng and Lin, 2013; Peer et al., 2015; Strosnider et al., 2011b; William H. J. Strosnider et al., 2013a; Winfrey et al., 2010)
Aerobic wetlands and pit lakes	Sewage and AMD	• Significant quantities of Mg, Ca, SO_4^{2-}, and bicarbonate removed • Unexpected Mn removal correlated with SS removal	• Test done in wet season; possibly diluted the sewage • Treated net alkaline secondary MWW effluent with 3 mg/L Fe • Faecal bacteria not assessed	Investigation of associated microbial communities and their adaptation	(Johnson and Younger, 2006; McCullough et al., 2008b)
	Sewage, green waste	• Successful full-scale synergistic wetland treatment • Removal of all key pollutants (i.e., BOD_5, Fe,	• No detailed water chemistry provided • Solid phase sinks for pollutants not identified. Other studies used to predict their fate.	• Investigation of inhibition to microbial communities posed by high ionic strength of AMD • High total coliform count incites an investigation on whether there is a need for	(Younger and Henderson, 2014) (Kumar et al., 2011a; McCullough et al., 2008b, 2008a, 2006)

		NH_4-N, SS) from AMD and raw sewage • Success at field-scale remediating AMD with raw sewage in an evaporation pond	• SO_4^{2-} conc. ↓ when green waste added, evidence of biological SO_4 reduction, with alkalinity ↑	organic decomposition 180 days before primary treatment	
Active treatment	Greywater/ domestic ww	•Successfully replaced $FeCl_3$ coagulant used to treat ww.	•Metals prohibit overall use without pre-treatment	• Investigation of removing metals from AMD prior to use as a coagulant	(Hughes and Gray, 2013a, 2012; Pearson and Nesbitt, 1974; Rao et al., 1992)
Combined active and passive treatment	MWW	•AMD replaced alum to treat WW with 130 mg/L (average) of SS •40 to 70% SS removal •Metal conc. reduced by more than 60% •AMD efficiently neutralised	•No significant decrease in TOC, BOD_5, and COD •Alkalinity addition required?	• Cost implications of pre-treatment to remove metals. Lower AMD to MWW ratios to avoid need for alkali additions	(Hughes and Gray, 2013a, 2013b, 2012)(
Active treatment	Flowback or produced water from gas extraction	Using AMD for hydraulic fracturing reduces pressure on fresh water and mitigates AMD's environmental impact. Fe^{2+} contained in AMD was used as a coagulant to enhance the separation of SS by settling	•Dissolved SO_4^{2-} reacts with Ba; resultant scaling can ↓ well productivity • Fe interferes with cross-linking of gel used to increase fracturing fluid viscosity •$BaSO_4$ formed is <6 μm in size, so can't be used in drilling mud. •Generation of Ra-rich waste	Optimization of the $BaSO_4$ particle size required for barite to be used efficiently as a weighing agent in drilling mud	(He et al., 2014b, 2014c, 2013; Kondash et al., 2013; Zhang and Wang, 2014; Zhang, 2015; Zheng, 2013)

2.5 Future Possibilities and Novel Approach

As emphasised by Neculita et al. (2007), factors vital for a successful AMD passive treatment method using sulfate-reducing bacteria include COD to SO_4^{2-} proportions, the water chemistry of the homogenous mixture, the reactor design, and the range of microbiological species. Although these are supposedly critical factors in the efficiency of the treatment process, there is limited evidence on the bacteriological ecosystem and how molecules are transported between one effluent stream and the other. There is also a need for more information on the bacterial communities, to understand how co-treatment influences bacterial communities over time. As this data is critical for the further development of technological remedial strategies, a more extended period is necessary for testing the feasibility of continuous treatment processes. In addition, there has been too little focus on the mixing ratio of the waste streams, which is critical to circumvent various problems within the reactor. A waste stream that has high COD may benefit from the co-treatment with AMD, for example, since controlled mixing of these two streams may accelerate the elimination of COD while increasing the AMD's pH and lessening its metal and sulfate concentrations.

Moreover, Choi and Rim (1991) concluded that there is a threshold of a 0.67 molar ratio of COD/SO_4^{2-} to remove sulfate from AMD. Since AMD is sulfate-rich, and likely to lack electron donors, there is a need to supplement the remedial process with the required number of electron donors. Thus, the success of co-treatment of AMD with any effluent stream will depend on its potential to act as an electron donor for biological co-treatment processes. There are still unresolved issues facing AMD co-treatment with other wastes. For example, the sludge that is generated could be enriched with various metals; therefore, it is important to consider how the sludge is disposed of or handled thereafter. From this perspective, there remains the possibility of exploring the production of sulfate-based coagulants by selective precipitation of AMD (Menezes et al., 2009) from coal mines. More research is needed on the effectiveness of such sulfate-based coagulants vs. more traditional coagulants in treating MWW. An advantage of producing such a coagulant in an AMD treatment facility is that it would reduce sludge waste challenges while yielding a useful chemical reagent.

Finally, a possible new approach to AMD co-treatment is to combine it with hospital waste, which

is typically alkaline. Hospital wastewaters contain a variety of organic and inorganic constituents including spent solvents, catalysts, additives, reactants, and synthetic pharmaceuticals that are high in COD (Oktem et al., 2008). Finding a solution to this water-pollution problem is a research opportunity that could aid AMD remediation as well, since the characteristics of hospital wastewater meet the criteria for successful co-treatment with AMD, as can be seen by comparing Table 2.7 with Table 2.1.

Table 2.7 Characteristics of hospital wastewater

Characteristics	Range
pH	6.2 – 8.5
COD (mg L^{-1})	48 - 8700
TOC (mg L^{-1})	27 - 190
BOD_5 (mg L^{-1})	20 - 987
Conductivity ($\mu S.cm^{-1}$)	>450
Total nitrogen (mg L^{1})	80 - 177
NH_4^+ (mg L^{-1})	10.1 – 28.9
NO_3^- (mg L^{-1})	0.1 – 0.7
Turbidity (NTU)	5 – 41.7
E. *coli* (PFU/100 mL)	>23000

(García-Muñoz et al., 2017; Hamjinda et al., 2018; Liu et al., 2010; Mesdaghinia et al., 2009; Munoz et al., 2016; Sivakumar, 2014; Varela et al., 2014)

Using fluidised bed reactors could potentially avoid the excessive generation of sludge. The theoretical synergies of co-treatment, such as enhanced removal of nutrients and SS from hospital wastewater, require study. The approach needs to examine these synergies and other aspects of co-treatment, i.e., the ratio of AMD to hospital wastewater for sulfate removal, and the impact of continuous loading of AMD on hospital wastewater, identifying critical advantages and constraints. In this proposed treatment system, it is envisaged that the high alkalinity of the hospital wastewater would neutralize the AMD and that the newly formed iron precipitates would adsorb nutrients from the hospital wastewater.

2.6 Conclusion and Recommendations

Research studies and literature from various sources indicate that co-treatment of AMD and other waste streams can be efficient when carefully operated. The published research, with its limited information on COD/sulfate ratios, water chemistry, and reactor configurations, has not been enough to stimulate continuous, long-term trials, despite significant potential benefits, e.g. decreased

dissolved metal concentrations, increased alkalinity. The existing studies, while credible, lack detailed water chemistry and microbial profiling, which would be crucial for the upscaling of equipment and continuous processes. Also, future researchers should always include controlled experiments, to show treatment without AMD addition.

The review could also benefit from the direct comparison of wastewater treatment using conventional methods with co-treatment. It is recommended that researchers not only provide information on metal removal rates, but also the cost of traditional treatment methods for the particular waste stream under consideration, contrasting this with co-treatment data. Although, it is hypothesized in many studies that co-treatment reduces energy costs, the actual cost savings must be demonstrated and documented.

Despite these issues, the success of dual effluent streams treatment is encouraging researchers to extend the approach to more complex waste streams, such as those from the hospital and pharmaceutical industries. Co-treatment of high COD wastewater with AMD has the potential to create a sulfate-reducing water treatment system that should severely reduce the concentrations of most metals in AMD. Biological treatment systems can produce a stable, dense sludge while relying on natural processes to stimulate remediation of AMD. Also, there is still scope for resource recovery and reuse from AMD, which should continue to receive careful attention.

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CHAPTER THREE

Effective Biotreatment of Acid Mine Water and Hospital Wastewater Using Fluidized-bed Reactors

This chapter presents the assessment of a laboratory scale sulfate-reducing fluidized bed reactor for the biotreatment process of real acid mine drainage and pharmaceutical-rich wastewater. In an attempt to substitute the carbon source, the study used hospital wastewater in this regard. In this study, there was no supplement of carbon source during the treatment, but only the hospital wastewater provided the dissolved organic carbon. The process evaluated a biological treatment efficiency in stepwise phases, monitoring the effect of retention time, influent pH, and sulfates. This study is among the first to explore the co-treatment of AMD and HWW, leading to new data contributions in the field of environmental engineering. This chapter was peer-reviewed and published in the *Journal of Water Process Engineering* (see Appendix A2 for the article abstract) in the form of a full-length article to share the results of this study and contribute to the existing information.

3.1 Introduction

Hospital wastewater (HWW) and acid mine drainage (AMD) are amongst typical hazardous waste streams that require treatment to sustain the universal water resource quality. The AMD is a strong, acidic waste stream that is rich in dissolved ferrous, non-ferrous metal sulfates, and salts (Macingova and Luptakova, 2012). Untreated AMD can pollute soil and surface water, resulting in harm against aquatic life, humans, wildlife, and plants (Simate and Ndlovu, 2014). Meanwhile, hospitals generate an average of 750 liters of wastewater each day per bed (Emmanuel et al., 2005). This type of wastewater is rich in pathogenic microorganisms, radioactive elements, discarded and excreted pharmaceutical molecules, and other toxic chemicals (Kanama et al., 2018). However, these pollutants are customarily discarded into the municipal wastewater treatment plants (WWTPs), even though some researchers proposed that wastewater be held where it originates for treatment before being discharged to WWTPs (Ort et al., 2010). As such, once the HWW flows in the WWTP, it forms part of the municipal wastewater (MWW). This practice is common in many

countries worldwide, like Australia, Egypt, India, Japan, Thailand, and South Africa (Al Aukidy et al., 2017). Noticeably, the regulations on pharmaceuticals treatment or their disposal are not as rigorous in developing countries, including South Africa, as compared to developed economies. Hence the current design of wastewater treatment facilities does not provide the capacity to remove these contaminants. More studies are being developed to support the inclusion of emerging pharmaceutical contaminants into water quality legislation in South Africa. In this paper, the HWW is considered an independent stream, before it flows into the WWTP. Acid mine water and MWW are ordinarily addressed independently with active treatment in advanced economies using significant material, financial, and energy resources (Strosnider et al., 2011a).

Various methods are applicable for AMD treatment, mainly, one of which is considered an efficient biological process through microbial treatment with sulfate-reducing bacteria (SRB) (Neculita et al., 2007). Sulfidogenic (passive) treatment of mine water is a feasible alternative because of its improved quality of sludge and low cost compared to traditional chemical treatment (Sahinkaya et al., 2013). Passive treatment of mine water compels primarily adequate organic substrate electron donors to remove dissolved oxygen (DO), and bacterially mediated metal elimination (Younger et al., 2002). While sulfidogenic treatment is useful for AMD, it is always essential to add an appropriate carbon source electron donor for SO_4^{2-} elimination because the dissolved organic carbon matter content is below 10mg/l (Bekmezci et al., 2011). In particular, costly chemicals like ethanol and lactate are often used as a carbon source for SRB (Sahinkaya et al., 2013) for AMD's sulfidogenic biotreatment (Kaksonen et al., 2004a; Zhang and Wang, 2014).

The sulfidogenic process promises many advantages over traditional chemical processes, like lime addition for neutralization. In this process, most metal sulfides are more stable, forming a denser sludge in comparison to the hydroxides produced by chemical treatment. Moreover, the metal sulfides produced in the sulfidogenic process are recyclable (Jalali and Baldwin, 2000). Packed-bed and anaerobic fixed-bed bioreactors are effective in wastewater remediation (Johnson, 2000); however, they are prone to clogging and channeling, which devalue their efficacies (Kolmert and Johnson, 2001). On the other hand, the upflow anaerobic sludge blanket reactors (UASB) encourage the development of granules during the process.

Meanwhile, the methanogenic activity reduces the sulfide yield. A more significant benefit is offered by fluidized-bed reactors (FBR) in comparison to UASB or packed-bed reactors to enhance the mass-transfer of both substrate and toxic products, like hydrogen sulfide (H_2S) (Nagpal et al., 2000). Furthermore, FBR offers better sulfate reduction rates (Somlev and Banov, 1998) and can retain high biomass, achieve significant mass transfer rates, and lower pressure drops. Also, in FBR, the recycle stream reduces high feed concentrations, making FBR more suitable for treating acidic wastewater with metals and sulfate (Kaksonen et al., 2003; Sahinkaya et al., 2007). Against this literature backdrop, this study seeks to use the sulfidogenic FBR to explore the co-treatment of acid mine water and hospital wastewater.

Although ethanol and lactate are suitable substrates in many SRBs, their utilization may lead to increased operating expenses. Alternative substrates, like wastewater with high organic carbon concentration, are therefore beneficial, even better where the other stream can benefit from being co-treated. The co-treatment of municipal wastewater and AMD is well reported, with much success in reducing metal concentrations and sulfates (Deng et al., 2016; Deng and Lin, 2013; Johnson and Younger, 2006; Peer et al., 2015; Strosnider et al., 2011b). For these studies, MWW worked on the premise of carbon substrate bacterial oxidation and pathogen removal, where it requires electron acceptors (Smyntek et al., 2018). Considering the high COD concentration and nutrients in HWW compared to MWW (Table 3.1), it is likely that it could produce a high level of sulfate reduction through a passive co-treatment of the two waste streams (Makhathini et al., 2020). As such, the availability of nutrients and electron donors from HWW could be used to strip off bacterial oxygen, sulfate, and metal concentration reduction, thus producing alkalinity, similar to studies of MWW (Strosnider et al., 2011a, 2011b, 2011c). Even though pharmaceuticals alone pose potential risks to the aquatic environment (Archer et al., 2017), none of the AMD co-treatment strategies with MWW has looked at the pharmaceutical compounds' behavior during the treatment process. However, the pharmaceuticals primary removal mechanism uses biological approaches (Kruglova et al., 2016; Zhao et al., 2015), which is what this study proposes. Thus, it was deemed fitting to extend the co-treatment assessment to the pharmaceutical compounds.

On the other hand, ethanol costs around 0.14 USD/kg (estimated average for the past year, according to ethanol markets) for the sulfate-reducing treatment process. For a 1000 m^3/day

mining effluent with 2000 mg/l SO_4^{2-}, an estimated spend on ethanol is 131, 400 USD. The co-treatment method of two streams of wastewater has a limitation over the expense of delivering one of the streams to a central treatment site (Makhathini et al., 2020). In most cases, mine water typically forms a long distance from the location of hospitals and pumping costs can significantly reduce the investment from the co-treatment system. Irrespective of this setback on co-treatment systems, researchers may still explore opportunities in this regard where the system benefits surpass the impediments.

Against this given context, this paper provides the first attempt to examine the potential of using HWW for AMD treatment in a sulfidogenic FBR and using HWW as a low-cost source of carbon. The objectives of this research study were to determine (1) the practicality of combined treatment of AMD and an isolated stream of hospital wastewater in a sulfidogenic fluidized bed reactor; (2) the effectiveness of SRB (% SO_4^{2-} removed); (3) the removal efficiency of the treatment process (% removal in nutrients, organic constituents' concentrations, and metals); and (4) the efficacy to remove pharmaceutical compounds (naproxen and ibuprofen).

3.2 Materials and Methods

3.2.1 Fluidized bed reactor

In this research study, a 2000 mL fluidized bed reactor (Figure 3.1) was used after being inoculated with anaerobic digester sludge from a WTP. The FBR was packed with Spec Silica Sand (almost 1-1.5 mm), as a biomass carrier in an active bed volume of 1400 mL for the duration of the process. For 36 hours, the reactor was operated on recycle mode to allow for biofilm development, and the bed fluidization (expansion) was 20-25%.

Loading rates and hydraulic retention times were determined using the empty bed volume (Kaksonen et al., 2004a). The reactor recycle flow rate was approximately 1100 ml/min, resulting in the recirculation ratio of 800 - 2200 based on the effluent flowrate. This recirculation ratio supports the complete mixing condition of the reactor contents (Chung et al., 2006; Sahinkaya et al., 2013).

Table 3.1 The quality of the hospital wastewater in comparison to the municipal wastewater

Characteristics	HWW Range	MWW Range
pH	6.0 – 8.1 ± 0.2	7.1 – 7.9 ± 0.6
COD (mg l^{-1})	789– 11788 ± 918	39 – 998 ± 87
BOD_5 (mg l^{-1})	389– 4487 ± 134	127 – 350 ± 18
TOC (mg l^{-1})	31 – 235 ± 67	31 – 73 ± 12
Conductivity (µS.cm^{-1})	>361 ± 114	>98 ± 165
Total nitrogen (mg l^{-1})	45 – 212± 0.2	30 – 101 ± 12
PO_4^{3-} (mg l^{-1})	9.8 ± 0.4	6 – 23 ± 1.5
NH_4^+ (mg l^{-1})	11.5 – 44.9 ± 0.8	20 – 77 ± 23
NO_3^- (mg l^{-1})	0.1 – 0.7 ± 1.0	0.89 – 2.5 ± 0.3
Turbidity (NTU)	6 – 49.3 ± 23	6 – 56.2 ± 17
E. *coli* (PFU/100 mL)	>34000	>27123

A mixture of hospital wastewater from a 900-bed public hospital in KwaZulu Natal, South Africa, and acid mine water was fed to the reactor. The characteristics of hospital wastewater are presented in Table 3.1. Acid mine water was collected twice (between August 2019 and January 2020) from a coal mine area in Mpumalanga, South Africa. Table 3.2 provides the operational conditions of the fluidized-bed reactor from phase I to phase IV

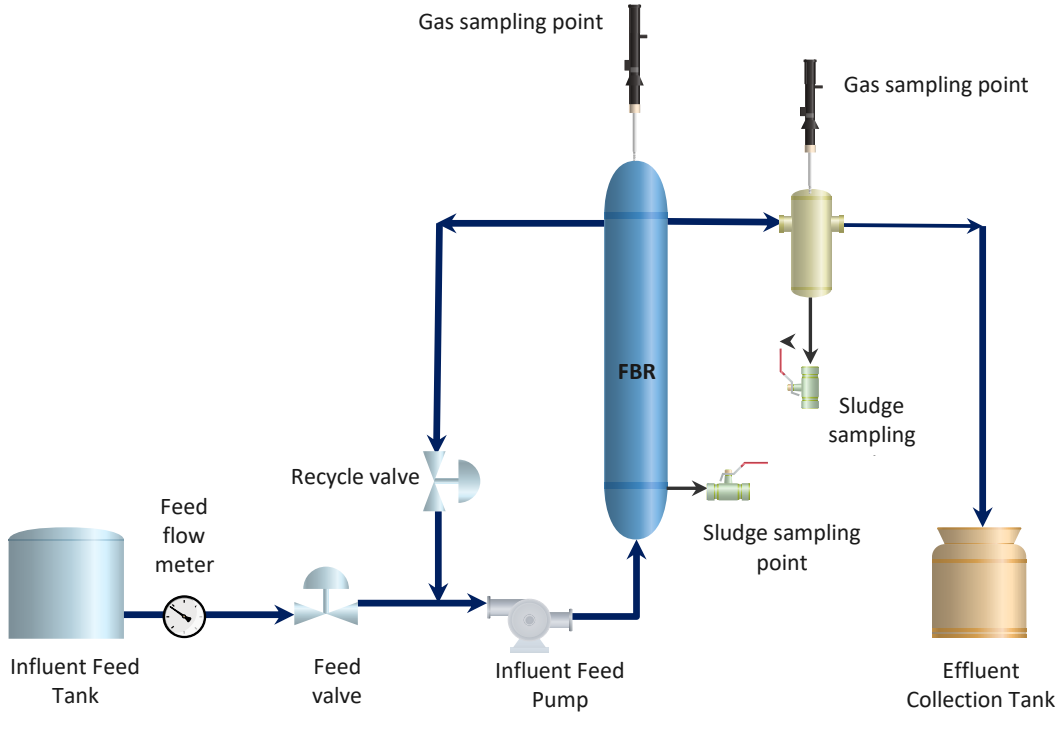

Figure 3.1 The schematic diagram of an FBR biotreatment system

In the initial phase, and the reactor was fed with HWW and diluted real mine water (AMD0) with 2350 mg/l SO_4^{2-} to concentrate the sulfate-reducing bacteria. In the second phase, a mixture of HWW and real acid mine water collected from the first sampling trip was used to feed the reactor for SRB adaptation at a 5:1 volumetric ratio. There was no pretreatment stage in the treatment process before AMD and HWW mixed in Phase I.

The experiment strived to keep the COD /sulfate ratio between 0.68 and 1.39 from phases II to IV, respectively. Since there was no additional carbon source in this experimental setup, the oxygen demand concentration in the HWW was increased by adding a meat extract in phase III. The retention time was maintained at 24 h in phase I, later it was dropped to 16 h in phase II and 8 h during phase III. Then it increased to 24 h in the final phase of the treatment (Figure 3.2) to investigate the robustness of the process. The pump rate was confirmed using the manual method even though the pump recorded the flowrate on the meter.

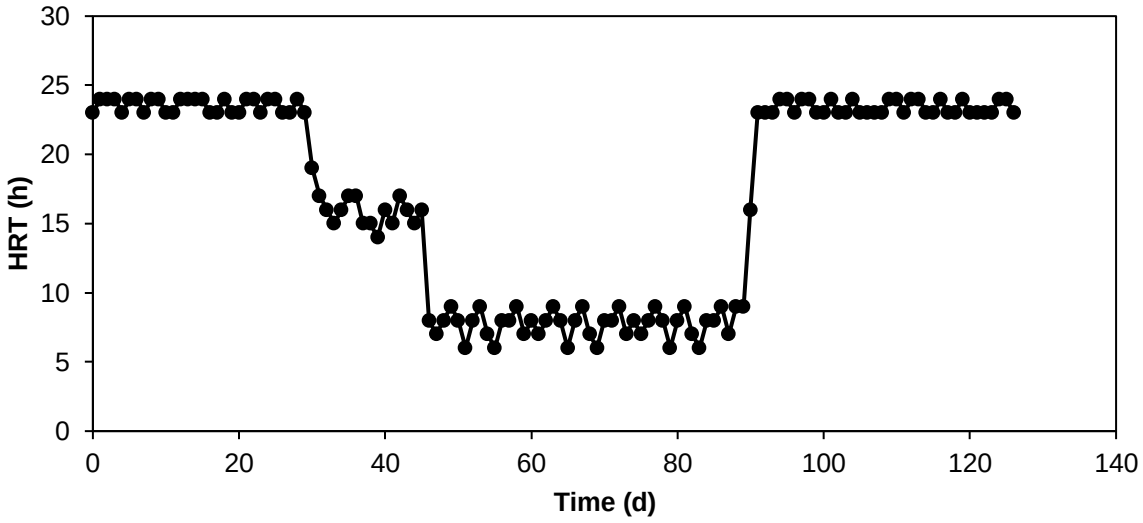

Figure 3.2 Dynamics in the hydraulic retention time in the fluidized volume during the continuous experiments

3.2.2 Analytical procedures

Twice a week, a sample of the influent was taken, and liquid samples were intermittently taken from the reactor recycle flow. Temperature, pH, and conductivity were measured using calibrated PHS-3BW benchtop pH meter, which is maintained as advised by the service provider. The

Thermo Scientific Orion Star T900 auto-titrator was used to determine the alkalinity and acidity of the samples. Using the standard methods, which apply to Hach DR 3900 spectrophotometer the unfiltered samples were analyzed for COD (Rice et al., 2012). In this study, standard methods suggested by Rice et al. (2012) were used to determine the biological oxygen demand (BOD) using a 5-day BOD test. Filtered samples were analyzed for sulfate, sulfide, metals, and nutrients using standard methods stipulated by Rice et al. (2012).

A Hach DR 3900 spectrophotometer quantified nutrients, sulfate, and sulfide. Acid digestion was used to preserve all dissolved samples at pH < 2, using concentrated HNO_3 and stowed at 4^0C pending analysis. Syringe filters (0.45 µm nylon membrane) were used for the preparation of the samples for metal concentration analysis of Mn, Cu, Fe, Zn, Mg, K, and Al before injecting into an (Inductively coupled plasma-optical emission spectrometry) ICP-OES (Varian 720-ES).

Table 3.2 Operation regime of the fluidized bed reactor

Period	**Parameters**						
	Time (days)	**Influent composition**	**Feed (pH)**	**Feed COD (mg/l)**	**Feed Sulfate (mg/l)**	**Ratio**	**HRT (h)**
Phase I	0-30	HWW+AMD0	8.6	3256	2350	1.39	24
Phase II	31-45	HWW+AMD1	9.4	1425	1456	0.98	16
Phase III	46-90	HWW+AMD2	9.1	1452	2177	0.67	8
Phase IV	91-115	HWW+AMD3	8.9	1869	2145	0.87	24

As advised by the USEPA protocol, all analysis was done in duplicates to ensure quality control. The study only reports mean values and standard deviations.

3.2.3 Analysis of pharmaceuticals

All external standard pharmaceutical compounds used in quantification analysis were supplied by Sigma-Aldrich (Germany). Acetone (99.5%), ethyl acetate (>99.9%), acetonitrile (>99.9%), and methyl alcohol (99.5%) were high-pressure liquid chromatography grade solvents sourced from Macron Fine Chemicals, SA. Other reagents used for the analysis were of Analytical grade. The

procedure to quantify ibuprofen and naproxen adopted in this study was adapted from the modification of the methods presented by Madikizela and Chimuka (2017). The procedure is summarized in Figure 3.3.

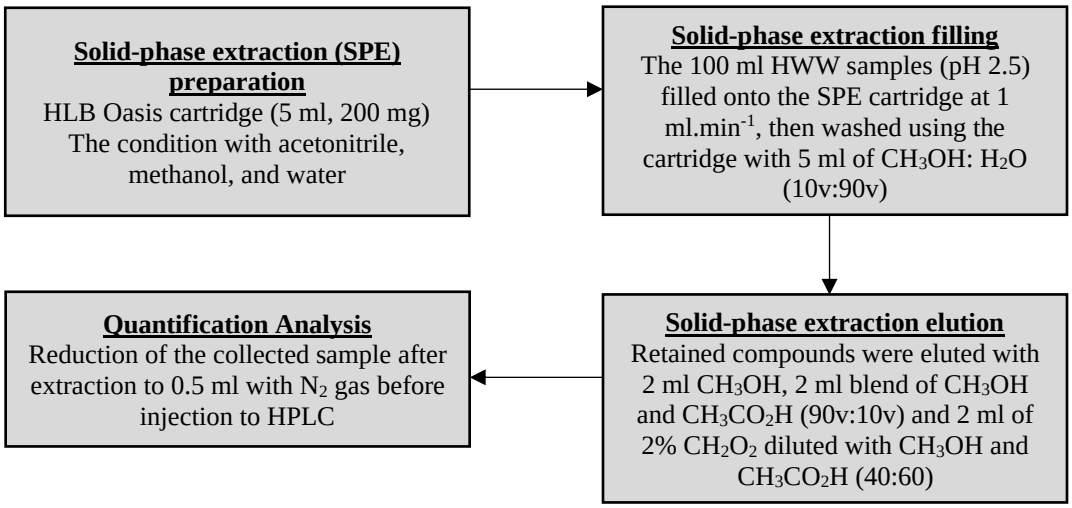

Figure 3.3 The protocol flow diagram which summarizes the steps involved in pharmaceuticals analysis

The method was validated by determining solid-phase extraction efficiency (1µg.L^{-1}), detection limits, and linearity. Detection limits were found three times the standard deviation of four samples (blank), and linearity was based on the coefficient (r^2). Finally, for the effluent results in the control bioreactor were used in comparison to the results found using deionized water (Zupanc et al., 2013). Removal efficiencies from the treatment system were calculated considering the initial concentration of the hospital wastewater sample (P_i), the influent concentration at day 0 (P_0), and the final effluent at day 30 (P_f). The Phase 2 reactor removal percentage was obtained by [P_f - P_0]/ P_i and the overall efficiency using [P_i – P_f]/ P_i. In both cases, P_i is used as a reference to compare and to obtain the overall percentage.

3.3 Results and Discussion

3.3.1 Factors influencing the choice of sulfidogenic bioreactors for metal removal

The sulfidogenic reactor's fundamental goal is to support metal removal through precipitation for metal sulfide formation through the sulfate-reducing bacteria. The fate of the precipitates is a crucial component of metal recovery. For the effective bioreactor design, the above two factors must be considered together with other operational parameters (Table 3.3). The identification of a

suitable sulfidogenic reactor is dependent many design properties, like, HRT, influent pH, metal precipitation, sulfate reduction, type of an electron donor, and cost implications thereof.

Table 3.3 Reactor design properties and their ramifications on sulfate reduction by SRB on sulfidogenic reactors

Operational Parameters	Ramification(s)	References
pH	pH and temperature influence the SRB physiological state and their resistance to metal toxicity	(Garcia et al., 2001; Marchal et al., 2001; Omil et al., 1996)
Temperature	High temperature assists SRB to outperform methanogens	(Dean, 1999; Visser et al., 1993)
Liquid velocity (*V*)	The upward liquid velocity prevents the loss of biomass in the FBR	(Omil et al., 1997)
Sulfide concentration	Higher (liquid or gas phase) sulfide concentration inhibits the growth of SRB	(Omil et al., 1996; Utgikar et al., 2002)
N_2 flushing	Promotes anaerobic conditions and lessen H_2S toxicity	(Marchal et al., 2001)
Hydraulic retention time (HRT)	High HRTs may facilitate SRB inhibition because of sulfide toxicity; consequently, low HRT may result in loss of biomass and affect the sulfate reduction rate	(Fedorovich et al., 2000)
Electron donor source	Metals in AMD have a carbon source deficit which is essential for the growth of microorganisms, then the addition of electron donor is a crucial factor	(Greben et al., 2000; Neculita et al., 2007; Omil et al., 1996)
Metal concentration	Elevated metal concentrations may lead to toxicity	(Hoa et al., 2007; Marchal et al., 2001)
Sulfate concentration in the influent	The high concentration of sulfate may result in inhibition; consequently, the low concentration may outperform SRB	(Omil et al., 1996)
COD/SO_4^{2-} influent ratio	The ratio facilitates the sulfate reduction process	(Henry and Prasad, 2000)
P and N addition	Ideal nutrients levels (C: N: P ratio) supports the growth of SRB; toxicity due to exposure of high ammonia concentrations	(Lens and Pol, 2000)
Oxidation-reduction potential (ORP)	Affects the activity of SRB and competition between SRB and methanogens	(Esposito et al., 2003; Khanal and Huang, 2006; Weijma et al., 2002)

A concise summary of how these operational parameters affect the sulfidogenic treatment process are given in Table 3.3. For example, pH controls the existence of microbial communities in the reactor and influences the metal precipitation; this is the formation of sulfides (Sánchez-Andrea et

al., 2014). The hydraulic retention time controls microbial film activity and the overall performance of the reactor (Rao et al., 2005). The reactor can be designed as either a multistage or a single-stage process. SRB facilitates sulfate reduction through sulfide production in a single stage process, which is the design in this study. Ultimately the metal sulfide in the reactor is produced (Kaksonen et al., 2003). When the reactor is multistage, in the first reactor, sulfate reduction occurs, and the sulfide is diverted to another reactor for metal precipitation as metal sulfides (Sánchez-Andrea et al., 2014).

The current design of a single stage offers merits like lower start-up costs, including no spend on delivering sulfide from one reactor to another. Thus, lowering the inhibition probabilities of sulfide because of metal precipitation. As such, the current design used in this study is less costly and more suitable for a developing country, which might be financially strained.

3.3.2 Effect of hydraulic retention time on COD oxidation and sulfate reduction

The effect of reducing the HRT on the bioreactor for combined HWW and AMD combined treatment is to gradually reduce organic matter in HWW and metal concentration and sulfate in mine water. In the treatment system, the HRT decreased gradually from 24 to 16 h while also maintaining the feed flow rate relatively constant. The influent and effluent COD and sulfate over the 120 days are shown in Figure 3.4.

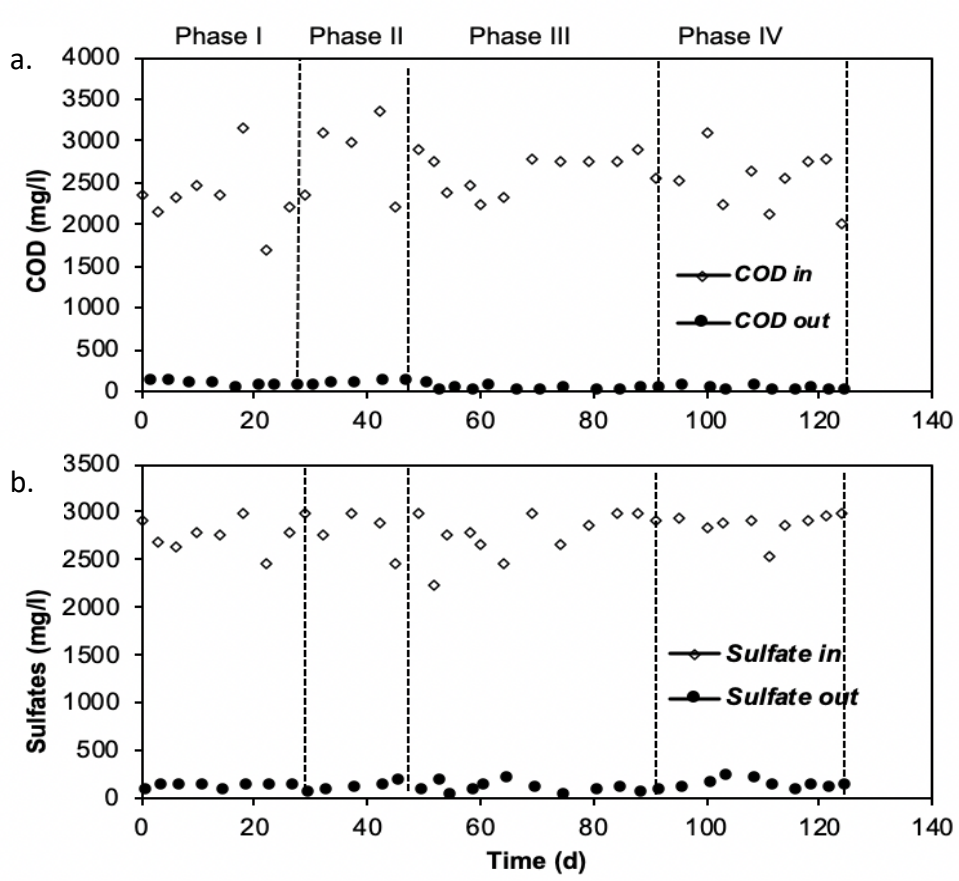

Figure 3.4 Influent and effluent (a) COD and (b) sulfate concentrations in the FBR

The bioreactor achieved high COD and sulfate removal rates, demonstrating the necessary functionality of the sludge used as inoculum from the anaerobic reactors (Bekmezci et al. 2011). During phase I of treatment, the effluent sulfate, and COD concentrations were between 65 - 149 mg/l and 44 - 123 mg/l, correspondingly. The overall COD oxidation and sulfate reduction rates (Equation 3-1 and 3-2) of the FBR in phase I recorded an average of 97 ± 1.0% and 96 ± 1.5%, correspondingly. These removal efficiencies were deemed decent, considering they were achieved during the first 30 days of the treatment.

Figure 3.4 demonstrates the COD oxidation, and sulfate reduction performance was around 1.49 g COD/l/d, and 2.01 g SO_4^{2-}/l/d, correspondingly. Previous FBR studies recorded slightly less performance than the current research, Sahinkaya (2011) documented SO_4^{2-} reduction performance of approximately 4.6 g/l/day in a reactor with ethanol and sulfates (ratio of 0.85 COD/SO_4^{2-}) at 12 h retention time. In another study, Kaksonen (2004b, 2003) recorded the highest sulfate reduction

performance of around 4 g/l/d in a reactor treating synthesized AMD using ethanol as an energy source. Also, a baffled (anaerobic) reactor with ethanol feed treated Zn and Cu in synthesized AMD produced sulfate reduction performance of approximately 3 g/l/day. Even though these studies used ethanol fed FBR, a comparison is made to demonstrate the performance superiority of the co-treatment of AMD and HWW. These results demonstrate great benefits in using another HWW as a carbon source, instead of a fresh feed of ethanol, which may be costly.

On day 32, the retention time was dropped by 33% from 24 h to 16 h, and the feed was now a combined mixture of HWW and AMD1 (real, undiluted mine water) at 5:1 v/v ratio. Following this modification, the effluent sulfate concentration rose somewhat to 207 ± 11 mg/l relating to 91% reduction rates. With a decrease in HRT, it was expected that the sulfate reduction rate would improve, but this study observed results on the contrary. Although this study did not quantify the methane production, it may be possible that methanogens for energy sources were incapacitated at this phase. However, the effluent COD concentration continued to decrease to 23 ± 10 mg/l relating to 99% oxidation efficiency.

On day 46, a fresh stock solution of the combined HWW and AMD_2 at the same volumetric ratio of 5:1 was used, and the HRT further decreased to 8 h. This change did not record any significant effect on the FBR performance with the effluent sulfate and COD concentrations around 67 ± 15 mg/l, and 113 ± 12 mg/l relating to 95% and 90 % reduction rates, correspondingly. The COD oxidation and the sulfate reduction performance is around 2.99 g COD/l/day, and 3.11 g sulfate/l/day. The COD results are compliant with the South African wastewater discharge limit of COD < 75 mg/l.

3.3.3 The production of alkalinity and sulfide in the bioreactor

The remediation began with the chemical process after the aerobic mixing of the HWW and AMD influent samples, and pH levels for both influent and effluent with corresponding sulfide and alkalinity concentrations are provided in Figure 3.5. The process resulted in an increase of 2.39 AMD pH to 6.1 – 7.7 for the duration of all four phases. For the prevention of the precipitation of metal hydroxides in the feed tank, less aeration was used, and also the mixing of AMD and HWW

was done just to maintain the minimum level in the tank. The neutralization of effluent by alkalinity produced through the microbial oxidation of the electron donor (Equation 3-4). The effluent pH levels were around 8.5 ± 0.4 in phase II and phase III but slightly decreased in phase IV to 7.8 ± 0.2 (Figure 3.5). The pH permits dissolved oxygen (DO) suppression through bacterial activity (Strosnider et al., 2011b). The pH results are compliant with the South African wastewater discharge limit of pH 5.5 – 9.5. This process relied solely on HWW as an energy and a carbon source, but it did not realize a higher pH in the effluent than initially anticipated.

Comparably, the effluent alkalinity was about 1450 ± 103 mg $CaCO_3$/l and 1080 ± 121 mg $CaCO_3$/l in first two phases, meanwhile, the alkalinity lessened to 780 ± 34 mg $CaCO_3$/l and 450 ± 21 mg $CaCO_3$/l phase III and final phase, correspondingly. The results show that the decrease in HRT from 16 to 8 h significantly decreased the alkalinity production in phase III. Even after the HRT was ramped back up to 24 h, the alkalinity production continued to decrease in the last phase of treatment. These results could be attributed to sulfate reduction performance in the final phase of the treatment, which produced less pH than in period III, resulting in the development of metal sulfide precipitates. The reactions for the sulfide production and metal precipitation can be commonly articulated as follows (Neculita et al., 2007):

M^{3+} + **organic compound** → $\boldsymbol{M^{2+} + HCO_3^- + CO_3 + H^+}$ **Equation 3-1**

$\boldsymbol{SO_4^{2-} + 2CH_2O \rightarrow H_2S + HCO_3^- + CO_2 + H^+}$ **Equation 3-2**

$\boldsymbol{M^{2+} + H_2S \rightarrow MS + 2H^+}$ **Equation 3-3**

$\boldsymbol{HCO_3^- + H^+ \rightarrow CO_2 + H_2O}$ **Equation 3-4**

where M represents the metal and CH_2O is an electron donor (organic matter in HWW)

The sulfide production revealed a parallel movement with alkalinity on the effluent (Figure 3.5). The dissolved sulfide in phase I averaged 350 ± 75 mg/l, which reduced to 167 ± 33 mg/l once the retention time was dropped from 24 to 16 h, and the feed COD was maintained at a range of 1450 ± 134 mg/l. Nonetheless, the sulfate reduction efficiencies in phase III and the final phase were almost the same, and the dissolved sulfide concentration lessened to 127 ± 32 mg/l because of sulfide precipitation of the new feed of metals. The mean BOD_5, although measured latterly of

phase II and phase IV, it had significantly reduced to 234 ± 43 mg/l and 167 ± 58 mg/l, correspondingly.

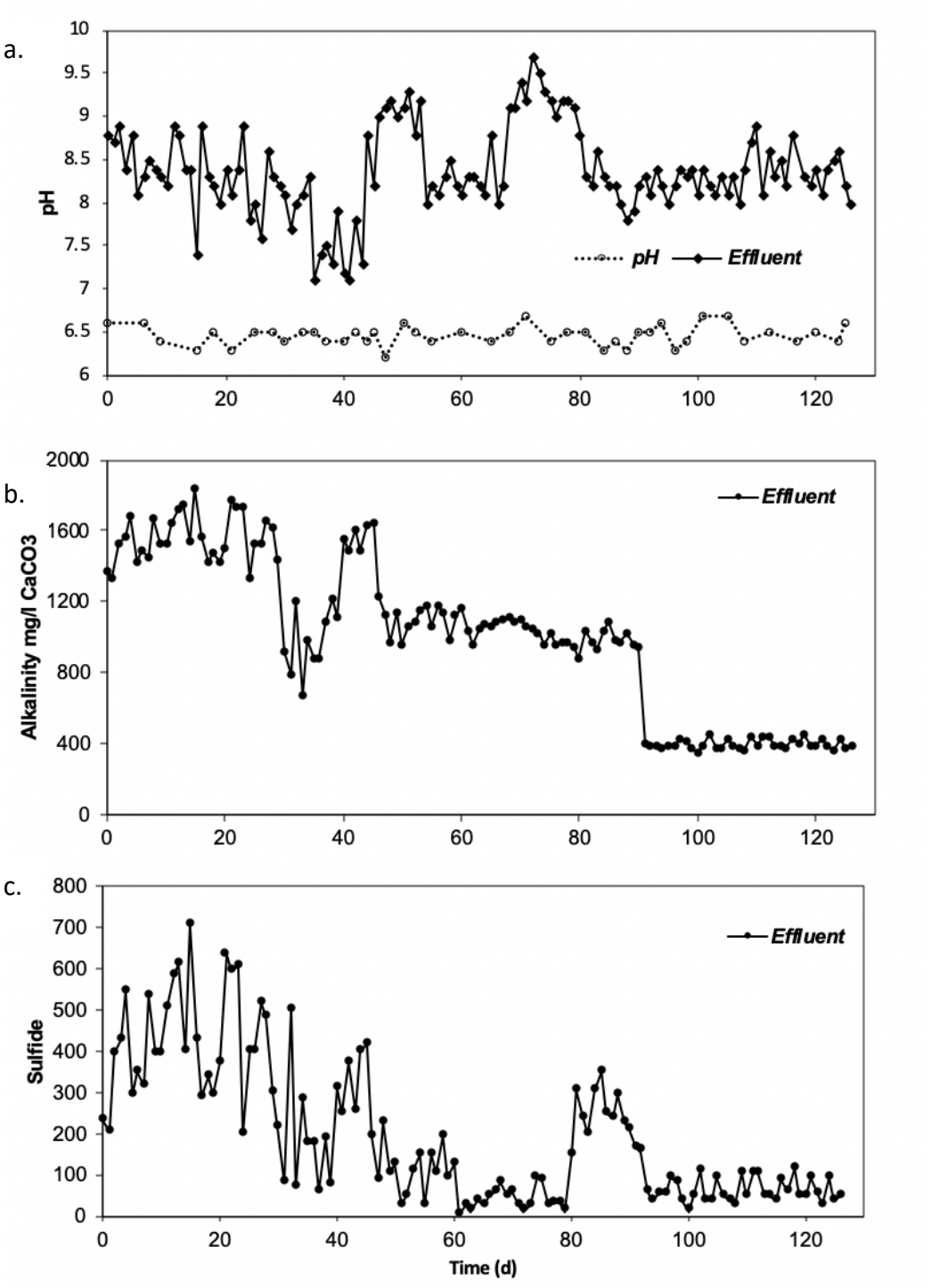

Figure 3.5 Effluent dissolved (c) sulfide and (b)effluent alkalinity concentrations, and (a) influent and effluent pH during the treatment process in the FB

3.3.4 Metal reduction in the bioreactor

Total metal concentrations from the influent and effluent are given in Table 3.4. The soluble effluent concentration of Cu was remarkably low, but the removal efficiency was high at 99%. On the other hand, effluent concentrations of soluble Fe were significantly high and could be attributed to the FeS solubility product (Sahinkaya et al., 2011), and the existence of organic matter in HWW, which further enhanced the solubility of FeS. Iron was removed in the FBR as Fe_2S was formed and combination with abiotic and biotic organic ligands (Strosnider and Nairn, 2010), which likely decreased dissolved Fe in the reactor.

Table 3.4 Total metal concentrations (mg/l) in four operational phases of the FBR from influent and effluent

Period	Days		Mn	Cu	Fe	Zn	Al	Mg	K
Total metals in influent (mg//)									
1	0 – 30	Conc.	21	48	112	34	12	109	1.23
		Stdev.	0.1	3.4	17	1.1	1.3	11	0.11
2	31 - 45	Conc.	18	55	101	26	18	87	19.23
		Stdev.	0.3	9.4	11	0.4	2.2	4	2
3	46 - 90	Conc.	17	41	89	29	19	79	18.1
		Stdev.	0.2	2.1	7	0.2	1.2	4	9
4	91 - 115	Conc.	15	44	87	21	11	54	12
		Stdev.	2.1	1.1	12	1.1	1	8	4
Total metals in effluent (mg//)									
1	0 - 30	Conc.	<0.02	1.02	1.9	0.2	2.1	33.2	0.91
		Stdev.	<0.02	0.1	1.3	0.01	0.09	2.5	1.9
2	31 - 45	Conc.	<0.05	1.12	<0.02	0.7	1.8	2.3	9.9
		Stdev.	<0.05	0.4	-	0.3	0.01	0.02	2.3
3	46 - 90	Conc.	<0.05	0.6	<0.02	0.81	<0.05	<0.01	-
		Stdev.	-	0.1	-	0.01	-	-	-
4	91 - 115	Conc.	<0.05	<0.05	2.1	<0.05	<0.05	<0.05	-
		Stdev.	-	-	0.3	-	-	-	-

Subsequently, in Phase III and IV concentration of effluent Fe in soluble form averaged 9 mg/l and 0.66 mg / l, equivalent to 93% and 99% soluble Fe elimination, correspondingly. As shown in Table 3.4, total effluent Fe concentration in phase III and phase IV were <0.02 mg/l and 2.1 ± 0.3 mg/l, relating to 99.8% and 99.2% removals, correspondingly. Fe increased in the final phase of treatment from a concentration of less than 0.02 mg / l in phase III to 2.1 mg/l can be attributed to the decreased sulfide concentration, as shown in (Figure 3.5). Also, the increase in pH from a mean

of 2.8 to 8 caused remaining Fe^{3+} precipitation by oxyhydroxide formation because F^{3+} is rapidly removed from a solution in waters with a pH > 4 (Younger et al., 2002) (Equation 3-5). Then Fe^{2+} oxidation occurrence may be related to low DO concentrations.

$$Fe^{3+} + \textbf{organic compound} \rightarrow \boldsymbol{Fe^{2+} + HCO_3^- + CO_3 + H^+} \qquad \textbf{Equation 3-5}$$

Although the dominant Zn concentration was significantly lower than Cu, in Phase II and Phase IV, the total effluent Cu concentration was smaller than zinc. Zn was removed from the AMD, and the HWW mixture was also removed as Zn sulfide was formed, and complex biotic and abiotic organic ligands present in HWW could have served as the primary Zn removal reactions (Norton et al., 2004; Strosnider and Nairn, 2010). Since the Fe oxyhydroxides were present, it is more likely that sorption occurred because Zn has a high affinity for Fe oxyhydroxides, especially at pH around 7 (Buitrón and Carvajal, 2010). Although this study uses HWW as a carbon source, a similar trend was observed where landfill leachate was used as an energy source (Sahinkaya et al., 2013, 2011). Metal removal efficiencies were all above 98%, which demonstrates that HWW may be used in the sulfidogenic bioreactor as an energy and carbon source. In this regard, the hospital wastewater is also benefiting from this combined treatment system.

Manganese oxidation and hydrolysis were feasible since the pH went up to 6.98 simultaneously as the Fe concentration dropped to less than 4 mg/l. Although Mn only changed towards the end of the treatment process when Fe removal was above 95%, considering that F^{2+} reduces the oxidized form of manganese (Watzlaf et al., 2004). When Al is combined with organic matter while reacting with dissolved organic carbon, it precipitates to form phosphates. As the pH increased in phase III, the Al solubility decreased, then most likely it formed insoluble unstructured $Al(OH)_3$ (Younger et al., 2002).

While the downflow fluidized-bed reactor (DFBR) demonstrated improved recovery of metals as metal sulfides (Gallegos-Garcia et al., 2009) compared to other FBR studies, the current study still achieved much higher removal rates at greater than 99% for all metals. Moreover, most studies presented in Table 3.5 use commercial electron donor, like ethanol (Gallegos-Garcia et al., 2009; Janyasuthiwong et al., 2016; Sahinkaya et al., 2007) and lactate (Bekmezci et al., 2011; Sahinkaya

and Gungor, 2010; Ucar et al., 2011; Villa-Gomez et al., 2011). Other studies that used recycled streams just like the current study could not achieve the sulfate reduction rate above 90% (Lakaniemi et al., 2010; Somlev and Banov, 1998). Overall, the study achieved < 0.3 mg/l for all metals, and the reduction rates are in compliance with the South African wastewater discharge limits of Fe <0.3 mg/l, Mn< 0.1mg/l, Zn<0.1mg/l.

Table 3.5 Comparison of sulfidogenic reactor systems for treating acidic wastewater with metal contamination

Reactor type	Wastewater type	Reactor volume	Electron donor source	Enrichment culture	HRT	SO_4^{2-} % removal	Metals % removal	Reference
IFBR	Synthetic AMD	2.5 l	Ethanol	SRB	24 h	>50%	>90%	(Janyasuthiwong et al., 2016)
Sulfidogenic FBR	AMD with Fe, Al, Ni, Cu, Pb, Mn, and Zn	0.3 l	Ethanol	SRB	12 and 24 h	90%	94% of Mn, 99.9 % of Fe, Al, Pb, Cu and Zn	(Sahinkaya et al., 2011)
Three-stage sulfidogenic FBR	Synthetic AMD with Cu and Fe	0.6 and 0.25 l	Ethanol and lactate	SRB	24 h	60-90%	>90%	(Ucar et al., 2011)
Sulfidogenic FBR	Synthetic AMD	0.6 l	Landfill leachate and ethanol	Sulfate-reducing sludge from sulfidogenic ABR	15-28 h	>90%	80-99.9%	(Sahinkaya et al., 2013)
FBR	Wastewater containing Zn and Fe	0.5 – 1.3 l	Lactate and ethanol	SRB	16 h	65% and 81%	>99% on both cases	(Kaksonen et al., 2004a, 2003)
DFBR	Synthetic AMD with Cd, Fe, and Zn	2.5 l	Ethanol	SRB	24 – 48 h	41%	>99%	(Gallegos-Garcia et al., 2009)
DFBR	Wastewater with Zn and Cu	20 l	Lactate	SRB	na	40 -80%	>90%	(Sahinkaya and Gungor, 2010)
UFBR	Wastewater with Zn and Cu	20 l	Lactate	SRB	na	60-86%	>99%	(Sahinkaya and Gungor, 2010)
FBR	Wastewater with Cd	na	Lactate and yeast extract	SRB	8 h	na	99%	(Ma et al., 1997)

Reactor type	Wastewater type	Reactor volume	Electron donor source	Enrichment culture	HRT	SO_4^{2-} % removal	Metals % removal	Reference
Inverse fluidized-bed reactor (IFBR)	Synthetic AMD with Cd, Cu, Pb, and Zn	5 l	Sodium Lactate	Anaerobic sludge from activated sludge digester	24 h	68-88%	97.9% Cd, 98.4% Cu, 96% Pb and 96.5% Zn	(Villa-Gomez et al., 2011, 2015)
FBR	Synthetic AMD	0.625 l	Soluble reed canary grass hydrolysate	Harvest from sulfidogenic ethanol-lactate fed FBR	6 -16 h		>99%	(Lakaniemi et al., 2010)
Sulfidogenic FBR	Acidic wastewater with Fe	0.425 – 0.625 l	Lactate and ethanol	Sulfate reducing bacteria	24 h	Approx. 50%	>99%	(Sahinkaya et al., 2007)
FBR		7.5 l	Lactate	Methanogenic granular sludge from an anaerobic digester	24 h	35 - 95%	na	(Papirio et al., 2013)
FBR	Wastewater with As, Cd, Cu, Ni, Pb and Zn	6.6 l	Molasses	Sulfate reducing bacteria	6.8 h	65% and 81%	60% Cd, 93.3% Cu, 90% Pb, 76.7% Zn 93.0% Ni, 95.% As and 60% Cd	(Somlev and Banov, 1998)
Sulfidogenic FBR	Acidic mine water from an abandoned mine	2 l	COD in hospital wastewater	Anaerobic sludge from activated sludge digester	6 – 24 h	>90%	>99%	Current study

3.3.5 Assessment of pharmaceuticals

In anaerobic processes, some researchers demonstrated the effects of biodegradation of certain pharmaceuticals (Carballa et al., 2008; Chen et al., 2014; Musson et al., 2010). It is well understood that traditional biological treatment processes in WWTPs are not effective in eliminating certain pharmaceuticals (Tiwari et al., 2017; Zupanc et al., 2013). The requirement is for the development of advanced biological treatment systems; thus, this study took an opportunity to assess whether its unconventional biological treatment affected selected pharmaceutical compounds. Only two pharmaceutical compounds were measured to narrow the scope of research, that is, naproxen, and ibuprofen. The existence of naproxen and ibuprofen in water bodies is recorded in developed economies including Europe (Samaras et al., 2011; Vergeynst et al., 2015; Zhao et al., 2009). In Spain mainly, naproxen and ibuprofen were found in drinking water (Carmona et al., 2014; Rodil et al., 2012). Also, in South African rivers, naproxen and ibuprofen have been detected, amongst others (Madikizela and Chimuka, 2017; Matongo et al., 2015a, 2015b). Thus, drawing interest in these compounds as they seem to be more persistent in most parts of the world. Firstly, the analytical approach was validated by solid-phase extraction (SPE) performance determination, which was found to be higher than 80% for both naproxen and ibuprofen compounds, and the linearity was found to be $r^2 \geq 0.97$. The bioreactor effluent is comparable to deionized water when looking at the SPE output and linearity. The results of the validation are set out in Table 3.6.

Table 3.6 Results of analytical method validation

Pharmaceutical matrix	**Effluent**		**Deionized**	
	Naproxen	**Ibuprofen**	**Naproxen**	**Ibuprofen**
SPE efficiency (%)	80-86	80-84	85	83
Detection limit	1-5	0.2-3	1	0.3
r^2	≥0.97		≥ 0.97	

The efficiencies in pharmaceuticals removed from the FBR samples demonstrate that there was no significant decrease in effluent concentrations across all phases of treatment. The median removal efficiency of naproxen was about 56% at the end of the 4th phase, but the ibuprofen removal was about 44% at the same phase.

Table 3.7 The efficiency of removal of selected pharmaceuticals at different FBR treatment phases expressed as average removal ± standard deviation

	Removal (%)			
	I	**II**	**III**	**IV**
Ibuprofen	19 ± 9	21 ± 15	51 ± 3	56 ± 7
Naproxen	26 ± 8	30 ± 3	34 ± 5	44 ± 9

The data in Table 3.7 demonstrate the increase in pharmaceutical removal efficiencies as the treatment process progressed. Although the main factor is yet realized, it may be that the biomass produced in the FBR consists of microorganisms that are using pharmaceuticals as organic substrates. At the moment, there is less evidence in the literature supporting the biofilm development process as an advantage to the removal of pharmaceuticals, but in this research, the observation confirms that the biofilm favors the removal of naproxen and ibuprofen.

3.4 Conclusions

This study shows that hospital wastewater and acid mine water fed in the sulfidogenic FBR co-treatment process removed COD from HWW then removed metals, sulfate, and acidity from acid mine drainage. The highest removal for COD and sulfate concentrations were between 39.5 mg/l and 42 mg/l at 0.68 COD/SO_4^{2-} ratio and retention time of 8 h. The overall COD oxidation and sulfate reduction performance recorded an average of 96% and 97%, correspondingly. The sulfide concentration averaged 235 mg/l, and the alkalinity generated in sulfidogenic organic from HWW neutralized the AMD. The metal removal rates documented at a retention time of 8 h were higher than 98%. The study achieved average removal higher than 55% for ibuprofen and 44% for naproxen. Although this was not the study's primary objective, it was necessary to document the possibility of removing the most persistent pharmaceuticals in HWW using a non-conventional treatment system. In the future, different configurations of the bioreactor may be necessary to check the effects of variables like temperature. The results are valuable to extend knowledge on AMD co-treatment options with the hospital, pharmaceutical, and municipal wastewater.

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CHAPTER FOUR

Sulfidogenic Fluidized-bed Bioreactor Kinetics for Co-treatment of Hospital Wastewater and Acid Mine Drainage

A passive co-treatment of acid mine drainage and hospital wastewater previously demonstrated a promising bioremediation viable approach for both toxic streams. The study of inhibition kinetics and microbial communities is essential to understand better the diverse species and the reaction mechanisms within the system. The kinetics and microbiology diversity in the sulfidogenic fluidized-bed reactor (at 30 °C) for co-treatment of hospital wastewater and metal-containing acidic water were examined. The alkalinity from organic oxidation raised the pH of the effluent from 2.3 to 6.1-8.2. Michaelis-Menten modeling yielded (K_m =7.3 mg/l, V_{max} =0.12 mg/l min^{-1}) in the batch bioreactor treatment using sulfate-reducing bacteria. For COD oxidation, the dissolved sulfide inhibition constant (K_i) was 3.6 mg/l, and the K_i value for H_2S was 9 mg/l. The dominant species in the treatment process belong to the *Proteobacteria* group (especially *Deltaproteobacteria*). The ibuprofen and diclofenac compounds achieved the highest removal rates in the bioreactor of 58.6% and 52.3%, respectively; while ketoprofen and naproxen of 41.9% and 46.6%, respectively. The findings in COD kinetics, sulfate-reducing bacteria abundance, and selected pharmaceutical concentration reduction provide insight into this co-treatment process's capability. This chapter was peer-reviewed and published in the *Biotechnology Reports* (see Appendix A4 for the article abstract) in the form of a full-length article to share the results of this study and contribute to the existing information.

4.1 Introduction

Treatment of wastewater from mineral processing and mining still demands an alternative to predictable chemical treatments, which is usually expensive. Amongst, alternative treatment options are the sulfate-reducing bioreactors (Kaksonen et al., 2004a) and co-treatment processes. The process of bioremediation is reliant on hydrogen sulfide (Equation 4-1) using sulfate-reducing bacteria (SRB) in an anaerobic environment.

$$\mathbf{SO_4^{2-} + 2CH_2O\ (electron\ donor) \rightarrow H_2S + 2HCO_3^-} \qquad \textbf{Equation 4-1}$$

Then, alkalinity production (Equation 4-2) through the electron donor's oxidation while precipitating metal sulfide.

$$HCO_3^- + H^+ \rightarrow CO_2 + H_2O \qquad \textbf{Equation 4-2}$$

Usually, the bioremediation process option requires an electron donor for SRB, which becomes a drawback for the process as it involves high operational costs. Hence, it is crucial for researchers to continuously seek ways of replacing commercial electron donors with low-cost electron donor options. The treatment option may include mixing acid mine water with another stream like fermentation industry wastewater or landfill leachate to benefit the mixed streams regarding pollution reduction. For example, Roetman (1932) was the first to propose to mix acid mine waster and municipal wastewater to reduce pathogens in sewage. After that, more studies were developed to assess the practicability of the co-treatment approaches in AMD remediation and reduction of organic matter from wastewater (Deng and Lin, 2013; Hughes and Gray, 2013; Strosnider et al., 2011a). These studies demonstrated improvements in water quality for metal, organics, and nutrients removal with elevated pH and alkalinity.

On the other hand, pharmaceuticals are increasingly detected in surface waters, and drinking water as not all are removed in traditional wastewater treatment plants (Agunbiade and Moodley, 2016). Hospital wastewater can be considered one of the point sources of pharmaceuticals, and separate treatment of this waste stream is of interest. One of the treatment options of pharmaceuticals in wastewater is degradation through biological remediation, including bioreactors. To date, only one bacterial strain, which degrades ibuprofen and uses ibuprofen as a carbon and energy source, has been described, although less is confirmed by the bacteria that degrade these compounds and their underlying biodegradation mechanisms (Langenhoff et al., 2013). Diclofenac is biologically degradable, yet the bacteria responsible are unidentified.

The ability to directly utilize hospital wastewater is not a common feature of the SRB. The combination of acid mine water in hospital wastewater treatment promises great environmental merits compared to traditional activated sludge processes achieved by combining the two stream's water chemistry. For example, a high sulfate concentration in AMD can function as an SRB electron-acceptor to oxidize organic compounds Deng et al. (2016) in HWW under anaerobic

conditions. In this case, the active treatment method, which requires energy consumption in wastewater treatment plants, is eliminated (Strosnider et al., 2013b). Also, biological sludge production is reduced significantly under anaerobic conditions (Neculita et al., 2007).

The biological approach of treating AMD is derived from various microorganisms' potential to generate alkalinity, eliminate the metals, and then reverse the reactions responsible for AMD formation (Johnson and Hallberg, 2005). The available carbon and electron source for SRB can support the biological approach utilizing sulfate reduction. Wastewater treatment processes that utilize sulfate-reducing bacteria include reactive barriers, wetlands, and bioreactors (Neculita et al., 2007). Unlike other bioreactors, fluidized bed reactors (FBRs) are superior in terms of less clogging or channeling, excellent treatment efficiencies, and a low probability of shock loads (Kiran et al., 2017a).

A fluidized bed reactor achieves better SO_4^{2-} reduction rates and greater carrier surface area than anaerobic filter reactor (Gallegos-Garcia et al., 2009). In an FBR, carrier material assists with settable biomass through biofilm development in comparison to granulation in an up-flow anaerobic granular sludge bed (UASB) (Sánchez-Andrea et al., 2014). Moreover, effluent recycling, similar to the extended granular sludge bed (EGSB) reactor, results in the carrier material's fluidization. The SO_4^{2-} reducing FBRs for rehabilitating metal-containing acidic wastewater using sand as a carrying medium has been used (Kaksonen et al., 2006). The reduction of biological sulfate is performed under mesophilic conditions (25–35°C), even under thermophilic conditions (35–70°C) (Sahinkaya et al., 2007). The thermophilic process for reducing SO_4^2 is suitable for treating reasonably warm metal-containing water, such as the wastewater from the pulp and paper industry. As such, this study uses hospital wastewater, so it is fitting to assess the biotreatment at mesophilic conditions.

Attempts were made to establish biological treatment options with both acid mine water and hospital wastewater remediation but as independent streams. An earlier reported AMD co-treatment process from an abandoned mine and an isolated stream of hospital wastewater, mixing the streams then pumped to a sulfidogenic bioreactor, showed effective reduction rates of COD, sulfate, metals, and selected pharmaceutical compounds (Makhathini et al., 2020). This research

is focused on sulfide kinetics, iron inhibitory effects, and the fluidized-bed reactor's microbial ecology. The study presents the first attempt to assess the degradability of the selected anti-inflammatory pharmaceutical compounds in the sulfidogenic FBR co-treatment system, that is, ibuprofen, diclofenac, ketoprofen, and naproxen.

4.2 Materials and Methods

4.2.1 Sampling

Acid mine water was collected at the abandoned mining site in Mpumalanga Province, South Africa. Hospital wastewater (HWW) was collected at the effluent point from a public hospital in Kwa Zulu Natal, South Africa. A sampling of HWW was carried out at regular intervals from August 2019 to January 2020, collecting 500 ml per time and contained in 4°C cooled acid-washed bottles to check the variability of constituents in HWW samples in this site. During the sample collection trips, on-site measurements of pH, temperature, and electrical conductivity were tested. Municipal wastewater samples are collected from a southern Durban treatment plant, in South Africa, for the same duration as the HWW. Even though this study did not use the MWW in the treatment process, but the samples of MWW were analyzed to draw comparisons on the COD concentrations and nutrients to support the study hypothesis. Key constituents in HWW, MWW, and AMD samples are shown in Table 4.1.

Table 4.1 Key constituents in acid mine drainage, hospital wastewater and municipal wastewater

Characteristics	HWW Range	MWW Range	AMD
pH	6.1 – 8.3 ± 0.4	7.1 – 7.9 ± 0.6	2.34
Alkalinity	412 ± 45	371 ± 23	0
COD (mg l^{-1})	136 – 15788 ± 116	39 – 998 ± 87	22
DOC (mg l^{-1})	45 – 235 ± 102	31 – 73 ± 12	<1
TDN (mg l^{-1})	212 – 1445 ± 12	145 – 350 ± 18	<1
H_2S	0.092 ± 1.1	0.023 ± 0.1	<0.001
SO_4^{2-} (mg l^{-1})	34 – 49 ± 1.8	21 – 66 ± 0.4	3212
PO_4^{3-} (mg l^{-1})	11.6 - 37 ± 0.4	6 – 23 ± 1.5	<0.2
Cl^- (mg l^{-1})	145 – 22 ± 0.5	120 – 17 ± 23	<3
Fe (mg l^{-1})	<0.03	<0.03	1305
Mn (mg l^{-1})	<0.07	<0.1	102
Al (mg l^{-1})	0.11	<0.11	218
Cu (mg l^{-1})	<0.01	<0.2	25
Zn (mg l^{-1})	<0.01	<0.01	96

4.2.2 The biotreatment protocol

A fluidized-bed reactor was used for this treatment system with 2000 ml (Figure 4.1). Each FBR was inoculated with 200 ml of anaerobic sludge harvested from the Durban South Basin wastewater treatment plant, after packing with silica sand (Spec Silica Sand, SA: medium loading) 20% fluidization rate for biofilm development. Also, the study observed one reactor with HWW only and another with AMD only for control experiments. The control bioreactors were only sampled on the last day of the experiment. The biological treatment experiments were run for six months at approximately 30˚C using a heating element to control the temperature, where physiochemical, nutrient, organic carbon parameters, and metals were continuously monitored. During the initial phase of the process and the loading, experiments took 60 days to enrich the SRB. On day 180, sludge was sampled for chemical element analysis. The control experiments were run in parallel reactors to assess the abiotic process contribution to sulfate and COD removal.

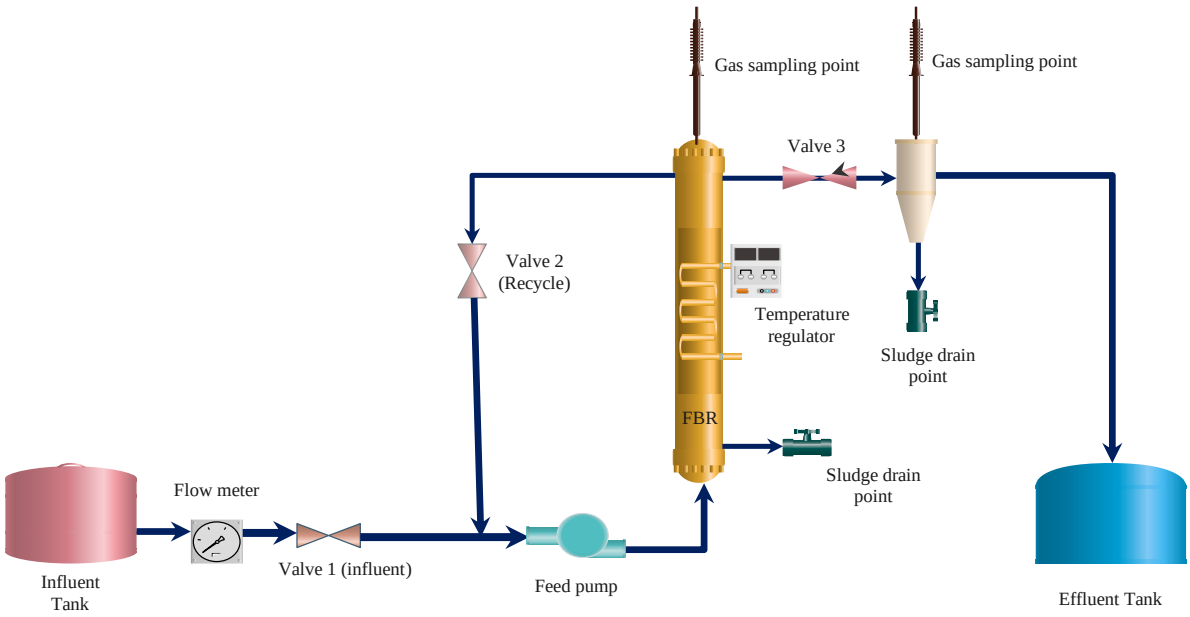

Figure 4.1 Configuration of the fluidized-bed reactor

The FBR efficiency was evaluated with experiments on continuous flow where the HRT and influent pH were decreased, but the metals and HWW concentrations were used in their pure form as measured from the source. However, a stage where the COD of the HWW was low, and meat extract was added to increase the concentration to maintain the COD/SO_4^{2-} ratio as such influent flow rate was varied depending on the targeted HRT value. Meanwhile, batch kinetics experiments were carried out in intermittent stages during the biotreatment processing. To find the kinetic factors (V_{max} and K_m) for the oxidation of COD in HWW. When the reactor was running on continuous mode, it was continually fed at HRT of 24 h with HWW only, until the next batch experiment was carried out. This procedure ensures the cleanout of excess H_2S accumulated through the batch experiments and retains constant biomass in the FBR (Kaksonen et al., 2006). During each batch experimental run, the influent flow was stopped, and the FBR was controlled in a recycling operation. In each batch experiment's initial and final period, sulfide and sulfate samples were collected for analysis.

4.2.3 Analysis

Measured temperature, pH, and conductivity using a calibrated PHS-3BW benchtop pH meter maintained as recommended by the service provider. The T900 auto-titrator Thermo Scientific Orion Star was used to measure the acidity and alkalinity of influent and effluent samples. The standard techniques, undiluted samples were measured for COD (Rice et al., 2012) applicable to Hach DR 3900 spectrophotometer. For this analysis, a 5-day BOD test was used to assess the biological oxygen demand (BOD) using existing protocol suggested by Rice et al. (2012). Metals, sulfide, sulfate, and nutrients were analyzed with the conventional methods defined by Rice et al. (Rice et al., 2012). The hydrogen sulfide concentrations were determined using the USEPA Method 8131 (methylene blue) on a Hach DR 3900 (USEPA, 2014). Samples were quantified for $SO_4^{2-}$and PO_4^{3-} by a Hach DR 3900 spectrophotometer using a standard method. Upon filtering through 0.45 microns of nylon, all dissolved metal samples were preserved by acid digestion with concentrated HNO_3 and kept at 4°C until analysis was completed. An ICP-Optical Emission Spectrometer (Variation 720-ES) was used to measure metal concentrations of Mn, Cu, Fe, Zn, and Al.

As advised by Sharp et al. (1995), all samples were filtered through 0.45-micron membrane filters for total dissolved nitrogen (TDN) and dissolved organic carbon (DOC) and deposited in 50 ml glass vials with membrane caps at -17°C. Samples were liquefied at about 4°C inside the refrigerator before analysis (Rice et al., 2012). In addition, analyzes for alkalinity, sulfide, TDN, COD, DOC, BOD, metals, and concentrations of anions were conducted in duplicates to ensure quality control, as required by the USEPA protocol.

4.2.4 Kinetics modeling for COD oxidation

The COD oxidation rates were consistent with the total amount of biomass in the bioreactor. Estimation of maximum reaction rate (V_{max}, mg/l) and constant of Michaelis-Menten (K_m, mg/l) (Equation 4-3) using the Lineweaver-Burk transformation equation (Kaksonen et al., 2006; Maillacheruvu and Parkin, 1996):

$$\frac{1}{V} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}} \times \frac{1}{S} \qquad \textbf{Equation 4-3}$$

where S is the substrate concentration, COD (mg/l), and V is the rate of reaction (mg/l min). The inhibition constants (K_i) were estimated through Fe concentrations using the noncompetitive (Equation 4-4) inhibition model (Maillacheruvu and Parkin, 1996) for H_2S and DS.

$$\frac{V_{max} \times S}{(K_m + S) \times \left(1 + \frac{1}{K_i}\right)} \qquad \textbf{Equation 4-4}$$

Where I= concentration of inhibitor (mg/l), and constant inhibition of Ki (mg/l). The model was fitted to the data to estimate K_i, $V_{max,}$ and K_m using a non-linear least-squares optimization subroutine, MATLAB_R2020a (see Appendix B1 for the code).

The dissolved (total) sulfide concentration was used to calculate the undissociated hydrogen sulfide concentration from the dissociation constant (K_{a1}) of H_2S using (Equation 4-5).

$$\boldsymbol{H_2S} = \frac{\boldsymbol{DS}}{\boldsymbol{1 + 10^{pH - pK_{a1}}}} \qquad \textbf{Equation 4-5}$$

where pK_{a1} is -log K_{a1}.

4.2.5 Microbiological analyses

The microbial FBR communities were evaluated using clone libraries and denaturing gradient gel electrophoresis (DGGE) of the polymerase chain reaction of 16S rRNA genes (Kaksonen et al., 2004b). The dsrA gene associated withSO_4^{2-} reduction was quantified by polymerase chain reaction (qPCR) analyses (Vasquez et al., 2018). A 16S rRNA-gene sequencing has identified anaerobic bacteria (Kaksonen et al., 2004b).

4.2.6 Ecotoxicological analysis

Toxicity was determined by standard toxicity tests, *Vibrio fischeri* (ISO, 1998), and *Daphnia Magna* (ISO, 1996). The first test (*D. Magna*) was conducted using neonates hatched at 20-22°C for 72 h, under illumination conditions. Each sample was analyses using replicates, ten neonates were used in each dilution with *D. Magna*, and dilutions were incubated at approximately 20°C. Then intermittently between 24 and 48 h in the incubator, the organisms were measured. Instead, with V. *fischeri*, each sample dilution's osmolality was corrected to achieve a 2% saline. Each sample dilution was replicate-tested, and the sample concentration was assigned as EC50 at 15°C exposure after 30 minutes, resulting in a 50 percent bioluminescence inhibition. According to the Sprague and Ramsay (Sprague and Ramsay, 1965) equation, the units of toxicity were determined for each sample.

$TU = (EC_{50})^{-1} \times 100$

4.2.7 Pharmaceutical analysis

Sigma-Aldrich (Germany) supplied ibuprofen (>98%), naproxen (98%), ketoprofen and diclofenac sodium salt used for quantification analysis. Ethyl acetate (>99.9%), methyl alcohol (99.5%), acetonitrile (> 99.9%), and acetone (99.5%) were solvents of high-pressure liquid chromatography grade from Macron Fine Chemicals, SA. Analytical grade was also used for specific reagents. The solid-phase extraction cartridges used were 5 mL (200 mg mass) Oasis HLB. The protocol adopted in this study to measure ibuprofen, diclofenac, ketoprofen, and naproxen was adapted following the modification of the methods proposed by Agunbiade and Moodley (Agunbiade and Moodley, 2016). Figure 4.2 provides an overview of the technique

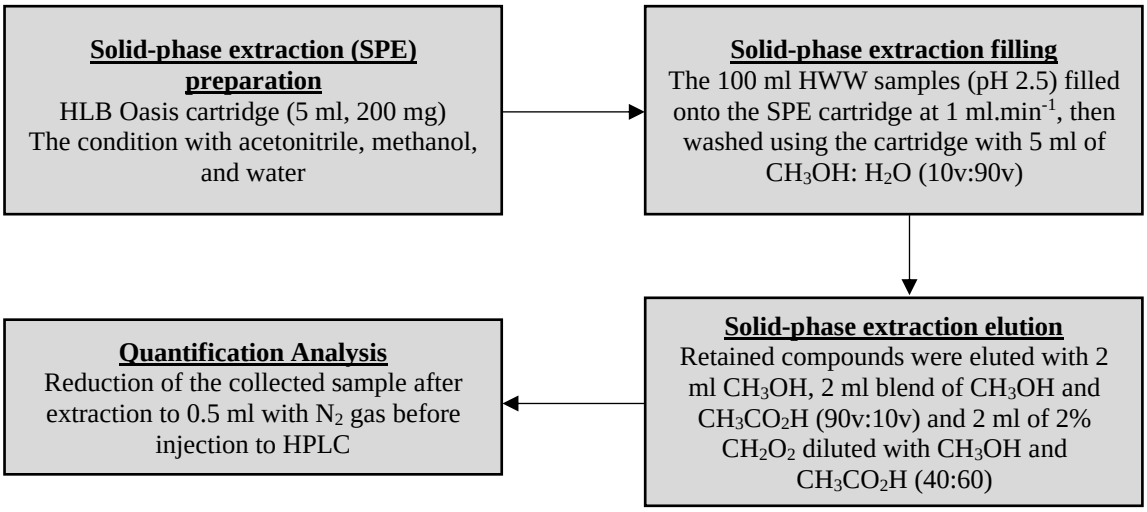

Figure 4.2 The protocol flow diagram which summarizes the steps involved in pharmaceuticals analysis

The process has been validated by evaluating the efficiency of solid-phase extraction (1µg. l^{-1}), the detection limits, and linearity. Detection limits found three times the average four-sample deviation (blank), and linearity was based on the coefficient (r^2). Finally, effluent tests in the bioreactor system were used instead of deionized water (Zupanc et al., 2013). Considering the initial concentration of the hospital wastewater sample (C_i), the influential concentration at day 0 (C_0), and the final effluent at day 30 (C_f), disposal efficiencies from the treatment method were determined. The percentage of phase 2 reactor elimination was obtained by $[C_f\text{-}C_0]/C_i$ and the overall output using $[C_i - C_f]/C_i$. For all cases, C_i is used as a guide for comparison and the average amount for receiving.

4.3 Results and Discussion

4.3.1 Co-treatment evaluation

After aerobic mixing of the samples, the remediation HWW and AMD started with the chemical phase, leading to an increase of 2.5 AMD pH to 6.1–8.2 (Figure 4.3a). After a few hours of mixing, precipitates of metal hydroxides emerged in all reactors due to the coagulant properties of metal cations (Rao et al., 1992). The dissolved oxygen and alkalinity on the experienced a changed as

time passed, which is aligned to the chemical reactions related to aerobic mixing preceded by SO_4^{2-} reduction (Pearson and Nesbitt, 1974). The initial phase of aerobic mixing resulted in net alkaline conditions after mixing, and even in the second phase of biological treatment, further alkalinity was produced. A slight pH decreased from 7.19 ± 0.10 to 7.10 ± 0.02 between 4 and 6 h HRT in the treatment system, then recovered to 7.23 ± 0.14 by 20 h HRT (checked by one-way ANOVA, where $F = 6.11$ $P<0.001$). This trend was expected to show in earlier days, which could have been attributed to metal hydroxides.

Nonetheless, the pH recovery increase is attributed to much bicarbonate production by reducing sulfate. The pH trend followed the alkalinity production from 479 ± 11 to 498 ± 8 mg/l from day HRT 15, and 20 h, after that remained almost the same for 24 h HRT. The production of alkalinity may be attributed to the biotic reduction of sulfate to hydrogen sulfide, thus generating bicarbonate ions (Johnson and Hallberg, 2005).

Nitrogen and organic carbon concentrations were almost certainly influenced by precipitation in the initial phase of the experiment. An overall observation of BOD, DOC, and TDN proved a decrease over the 180 days duration. It is most likely that the bacterial SO_4^{2-} reduction facilitated the efficiency of processing the BOD and DOC (Strosnider et al., 2013b). The HWW control sample demonstrated about 28% and 37% decrease in DOC and COD, in that order. The inorganic nitrogen in the reactors was introduced from the HWW sample in ammonia form and measured a 9-28% NH_4^+ decline at the end of the 180-day treatment period. The DOC concentrations followed the Fe concentration decline trend, apart from 16 h HRT, where there was a slight increase to 110 ± 11 mg/l (Figure 4.3b). Nitrate and nitrite concentrations were recorded to be reasonably low at $\leq$0.1 mg/l and $\leq$ 5µg/l, in that order.

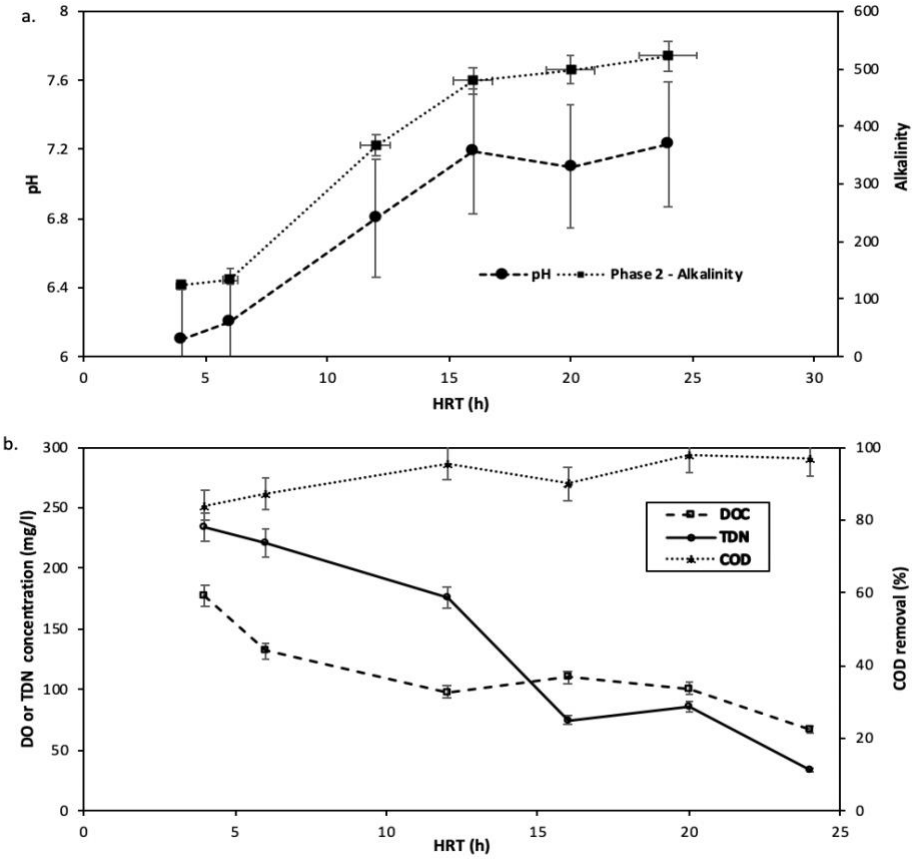

Figure 4.3 (a) pH and alkalinity dynamic response over a 180-day co-treatment period, at different HRT (b) Total dissolved nitrogen (TDN), DOC and COD concentrations at the end of day 180-day treatment period, at different HRT

The initial phase of the experiment, where HWW and AMD were aerobically mixed, Al and Fe recorded an efficient removal from the rest of the metals. In distinctive reactors, as per AMD/HWW mixing ratio, the concentrations of metals differed as such. Compared to control samples, the combined treatment of HWW and AMD demonstrated outstanding reduction rates, (Al: > 99%, Fe: > 98%, Mg: 44-65%, Mn>87%, and Zn: 52-79%) which is the highest removal recorder rate of manganese thus far from co-treatment studies. Further reduction of Al and Fe in biological treatment is most likely facilitated by AMD's combination with organic binding molecules (Younger and Henderson, 2014). On 12 h HRT (Figure 4.4), the treatment measured an increase in Fe concentration, which may well be due to microbial Fe decrease of $FePO_4$ and $Fe(OH)_3$ (Strosnider et al., 2013b). Iron sulfide precipitated due to the sulfate-reducing environment dominant at this experiment (Kiran et al., 2017b). Lastly, metal biosorption to the organic molecules related to the metabolism mechanism may cause metal removal (Deng et al.,

2016; Peer et al., 2015; Strosnider et al., 2013b, 2013a, 2011b). The high COD removal rate and decrease in TDN and BOD confirm the removal of dissolved nitrogen and organic carbon during the 180-day treatment period, only a small amount of concentration remained at the end of the treatment.

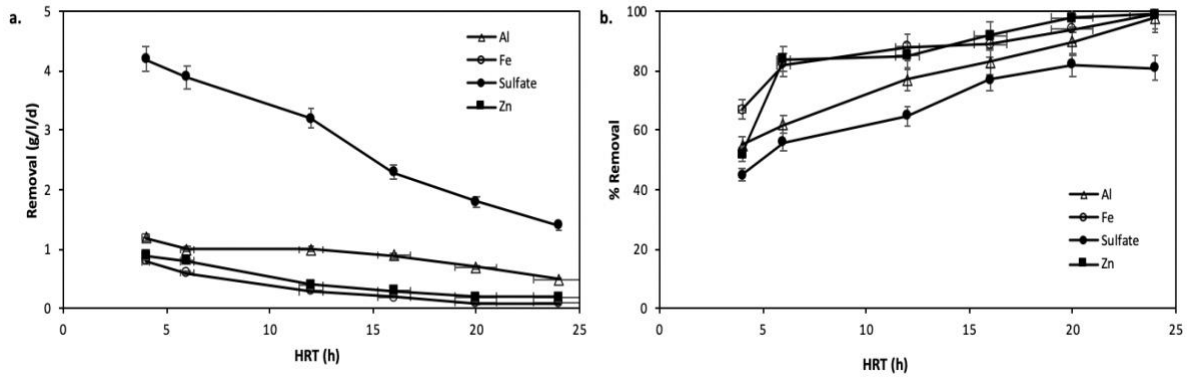

Figure 4.4 The effects of hydraulic retention time on (a) amount of sulfate removed and (b) removal rate of sulfate, Al, Fe and Zn in the FBR.

4.3.2 Modeling and COD oxidation kinetics

The COD, BOD, and TDN concentration decrease were accompanied by biomass accumulation in the reactors during the 180-day treatment period. The total biomass quantity in the reactor was approximately 3.8 ± 0.81 g during the oxidation period. On average, 87 – 90% of the biomass was attached to the silica sand material, 7 – 8% on metal precipitates not attached to the carrier material, and 4 – 5% may be found in the effluent stream (Bekmezci et al., 2011).

Figure 5a and b demonstrate the impact of concentrations of dissolved sulfide and hydrogen sulfide on COD oxidation levels. The Michaelis-Menten (K_m) constant and maximum oxidation of COD (V_{max}) were assessed as 42 mg/l and 0.12 mg/l min, respectively (Figure 5a and b). The K_m was valued was less compared to the study that used MWW as a source of SRB (Deng et al., 2016), where an addendum to Deng et al. (Deng et al., 2016) study was published in 2018, with K_m changed to 6220 mg/l but still higher than the other studies that used pure SRB cultures (Ingvorsen et al., 1984). This current study achieved a K_m value comparable to *Desulforhabdus amnigenus* and *Desulfobacca acetoxidans* culture treatment, where acetate was used as an electron donor (K_m = 35), although at a slightly higher temperature of 37˚C (Elferink et al., 1998), suggesting a

reasonably good co-treatment process. Most published studies (Table 4.2) presented V_{max} and K_m values for either acetate or ethanol oxidation at mesophilic SRB cultures. As such, it is difficult to make a direct performance comparison with the current study.

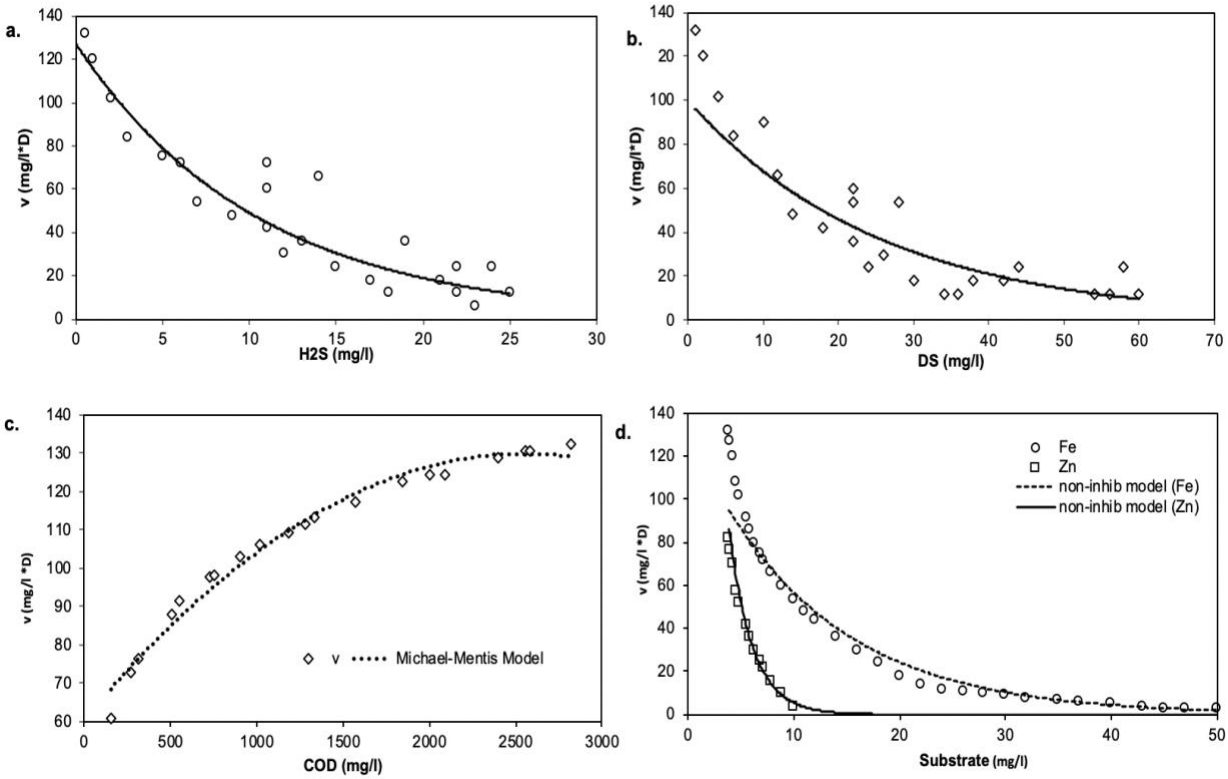

Figure 4.5 The effect of (a) hydrogen sulfide (H_2S) and (b) dissolved sulfide (DS) in the batch reactor experiments. The noncompetitive inhibition model fit is represented by the curves. Michaelis-Menten and inhibition model for sulfidogenic COD oxidation rate (*V*), and (b) Fe and Al non-competitive inhibition model

The sulfide inhibition of a culture of enrichment that reduces sulfate was well established in a noncompetitive inhibition model (Figure 4.5c). The correlation between sulfide concentration and COD use was not linear, and the use of metals (Fe and Zn) with examined concentrations was not completely inhibited. COD substrate use is correspondingly impaired at concentrations of zinc and iron greater than 8 and 24 mg/l (Figure 4.5d). The dissolved sulfide inhibition constants (K_i) were calculated to be 3.6 mg/l for the associated growth, and the related K_i value was 9 mg/l for H_2S.

Table 4.2 Estimated kinetic parameters for COD oxidation by sulfate-reducing enrichment cultures

Source of inoculum	Temp. (°C)	Carbon source	K_m (mg/l)	Reference
AMD and MWW mixture	20	AMD and MWW	4.3	(Deng et al., 2016)
SRB and methanogens	30	Acetate, butyrate	9.5	(Omil et al., 1998)
Enriched SRB culture	31	Acetic acid	5.9	(Kaksonen et al., 2006)
Granular sludge	35	Acetate	2.7-3.5	(Kaksonen et al., 2003)
Desulforhabdus amnigenus	37	Acetate	35	(Elferink et al., 1998)
Desulfobacter postgatei	30	Lactate	3.8-4.5	(Ingvorsen et al., 1984)
Desulfobacca acetoxidans	35	Acetate	35	(Elferink et al., 1998)
Granular sludge (mining)	35	Ethanol	4.3-7.1	(Kaksonen et al., 2003)
AMD and HWW mixture	34	HWW	7.3	This current study

4.3.3 Factors affecting the COD oxidation kinetics

This study realized an optimal pH range of 6.1–8.2 for sulfate-reducing bacteria in AMD/HWW reactor co-treatment. One of the critical factors in microbial reactions is redox potential for controlling the outcome of key chemical elements like sulfur and iron. The findings agreed with Smyntek (Smyntek et al., 2018) that the biochemical process in this experiment comprises microbially mediated oxidation with Fe^{3+} and sulfate of the labile fraction of the organic matter. Dissolved oxygen was measured to be <0.01mg/l, and the oxidation-reduction potential measured in a range of -65 to -198 mV. Under these conditions, iron occurs as Fe^{2+} and sulfur as S(II), leading to iron sulfide formation.

The precipitation of zinc and phosphates was observed in this study following a similar removal system trend in wastewater treatment (Sahinkaya et al., 2011; Strosnider et al., 2013a). Iron concentrations decreased during the initial phase of the experiments after HWW and AMD were

mixed in the reactor, confirming Fe precipitation with phosphate or hydroxides, similar to several studies' observation (Deng and Lin, 2013; Strosnider et al., 2013b). The iron (III) reducing bacteria contending with SRB may have been caused by iron the inhibitive effect demonstrated within high Fe concentrations. Deng et al. (Deng et al., 2016) found a similar inhibitive effect trend of Fe(III) for co-treatment with MWW and AMD. For this current work, in the experiment, the total iron exhibited a significant decrease from 44 to <±2 mg/l between day one and day 30 (One-way ANOVA, $F=19.21$ $P<0.001$). The effluent samples from the reactors could not measure copper and aluminum, but manganese and magnesium were measured at <2 ± 0.7 mg/l. Hence there is no report on these chemical elements and were excluded from the inhibitive model. The AMD/HWW mixing ratio was controlled in the first phase of treatment to prevent SRB's inhibitive consequences.

4.3.4 Toxicity assessment

The toxicity results on both standardized tests with V. *fischeri* show EC_{50} (at 5 min) is higher than 50% of effluent for all samples. This result demonstrates that the assay (at 5 min) is non-toxic. Nevertheless, the results for EC_{50} (at 30 min) were different from EC_{50} (at 5 min), where the results were more significant than 3 TU. The concentrations ranged from 4.4 – 5.8, which shows that the FBR's effluent toxicity on V. *fischeri* is similar to municipal wastewater toxicity.

On the other hand, toxicity EC50 values obtained from D. *magna* biological assays were higher than 2 TU but still categorized as low toxicity acute risk. The concentrations ranged from 15–112 TU for EC_{50} on the effluent. The toxicity of effluent from the FBR could hurt the aquatic organisms. These toxicity values do not correlate with the NH_4^+ values (34±12mg/l) measured in the FBR effluent samples; thus, a few areas of concern were identified. Since the HWW is mixed with the AMD (highly acidic), It may result in the development of intermediate degradation agents with higher polarity and toxicity than the original compounds (Jelic et al., 2012).

During the ecotoxicity testing process, the effluent sample pH was corrected to ±7.4 using NaOH, causing some uncertainty to the final results. On the contrary, some aqueous-phase degradation studies have observed a similar trend, where the toxicity increased at the end of the treatment for

some micropollutants, like carbamazepine and ibuprofen (Jelic et al., 2012). As such, in this process, ibuprofen was present, with its removal assessed.

4.4.4 The microbial diversity in the fluidized-bed reactor

The bacterial communities were maintained for over 120 days in the FBR, then characterized by culture-independent molecular methods (Kaksonen et al., 2004a) and the development of bacterial culture (Kaksonen et al., 2004b). A 16S rRNA gene and dsrA gene-based clone library was used to assess the microbial diversity, and results suggested that most functional group diversity in the FBR was sulfate reducers and fermenters. In the fermentative group, sulfate-reducing bacteria species were abundant (>90% related) *Desulfobacter*, *Desulfococcus*, *Desulfomona*, *Desulfomicrobium*, *Desulfovibrio* (Rampinelli et al., 2008), and *Syntrophobacter*. Also, a few other bacterial species were present (>94% related) in the domain of *Nitrospira*, *Clostridia*, *Crenarchaeota*, and *Thermodesulfobacteria* (Rabus et al., 2006). Church et al. (Church et al., 2007) found the involvement of SRB communities like *Desulfosporosinus*, *Bacillus*, and *Clostridium* in SO_4^{2-} reduction process. Another study by Hiibel et al. (2011) compared the microbial diversity in two field-scale pilot SRB bioreactors used to treat acidic water. The results demonstrated a 60% reduction in SO_4^{2-} from one reactor. Moreover, the study identified *Desulfovibrio* taxa, and the clone library confirmed that the enhanced SO_4^{2-} reduction was due to the availability of SRB (*Desulfovibrio*, *Desulfobulbus*, and *Desulfosporosinus*). Elsewhere, different organic reactive mixtures were assessed on AMD remediation with consideration of HRT on performance, organic substrates, and microbial communities (Vasquez et al., 2018). These studies found that organic mixtures achieved >70% SO_4^{2-} reduction, and >99% Fe and Zn removal. The dominance of the microbial community was from the SRB belonging to genera *Desulfobacter*, *Desulfomona*, *Desulfobulbus*, *Desulfomicrobium*, *Desulfovibrio*, *Desulfococcus*, and other fermentative groups.

The *Clostridia* can convert trichloroethene to ethane, but also capable of fermenting organics to sugars in anaerobic digesters (Palatsi et al., 2010). *Clostridium* species' presence indicates that the biotreatment system can treat high salinity wastewater (Deng et al., 2016). The other strains were affiliated with *Prolixibacter bellariivorans* with more nitrate-reducing bacteria, which can grow

at very low temperatures, <4°C (Holmes et al., 2007). These species' existence confirms that the sulfidogenic FBR is capable of treating acidic, sulfate, and nutrient-rich wastewater at mesophilic conditions.

The DGGE evaluation was conducted to assess the dynamics in the microbial communities of the FBR. The average anaerobic microbial diversity was observed not to yield the pure strains of the significant genotypes in the DGGE study of the *Magnetobacterium* genera (Kaksonen et al., 2004b). The reason could be the challenge in achieving pure strains of anaerobes during the FBR enrichment stage. It is advised that culturing and molecular methods are combined to assess more species in the sulfidogenic FBR microbial community (Kaksonen et al., 2004b). However, the FBR had a large number of sulfate-reducing species of various genera.

4.3.5 Mass balance of COD and sulfur

Estimated SO_4^{2-} reduction rates based on COD oxidation were higher than the measured rates, which agrees with Sahinkaya et al. (2007), that some of the electrons were most likely not utilized for SO_4^{2-} reduction. During continuous experimental mode, sulfate reduction and COD oxidation decreased dissolved sulfide concentration at HRT less than eight hours. The sulfate, Fe and Zn reduced mass quantities by oxidized COD were calculated 1.2 g SO_4^{2-}/g COD, 6 g Zn/g COD, and 9.5 g Fe/g COD. At an HRT of 12 h, about 540 mg/l/d Fe and 490 mg/l/d Zn concentrations of effluent dissolved Fe and Zn remaining under 0.01 mg/l in the effluent stream. Fermentative reactions influenced some of the electron flow; detected *Clostridium* confirms this as a microbial community member in the FBR. Also, there was less evidence of methanogenesis assays in the microbial community, which confirms no methane production in the FBR. Mass balance evaluation estimated approximately 50±9% of total sulfur, of which 10±3% is dissolved sulfur in the sludge, and approximately 50% of sulfur can be accounted for in metal precipitation.

4.3.6 Pharmaceutical removal

The analytical method was validated by the determination of SPE efficiency, which was higher than 80% for all compounds, and the linearity was calculated to be $r^2 \geq 0.96$. The removal efficiencies for all anti-inflammatories group assessed did not demonstrate excellent results at the

end of treatment (Table 4.3). In a sulfidogenic treatment system, it was expected that the microorganisms capable of degrading some organic compounds could do the same for the selected pharmaceuticals. For example, ibuprofen has shown bacteria degradation in some studies (Langenhoff et al., 2013; Nguyen et al., 2014).

Table 4.3 Pharmaceuticals' concentration in the influent and effluent samples of the FBR

Compounds	Influent		Effluent		% Removal
	Concentration ($ng\ g^{-1}$)	Relative Std. Deviation %	Concentration ($ng\ g^{-1}$)	Relative Std. Deviation %	
Diclofenac	277	4.12	132	0.91	52.3
Ibuprofen	203	13.3	84	0.68	58.6
Ketoprofen	39.1	5.87	22.7	1.05	41.9
Naproxen	28.1	1.12	15	0.21	46.6

The ibuprofen and diclofenac compounds achieved the highest removal rates in the sulfidogenic FBR of 58.6% and 52.3%. An anaerobic analysis on a laboratory scale showed 30-60% ibuprofen elimination under anoxic conditions and greater than 75% diclofenac degradation (Carballa et al., 2007). As such, the results of this study are not far off from what other studies have achieved. Moreover, diclofenac is one of the most used anti-inflammatories and has proved to be less removed in traditional WWTP (Verlicchi et al., 2012). In previous studies, WWTP achieved a 50-65% removal of ketoprofen and naproxen (Kimura et al., 2007). Unfortunately, this study did not achieve comparable removal results demonstrated by these studies. Table 4-3 shows the removal rate for ketoprofen and naproxen of 41.9% and 46.6%, respectively.

4.4 Conclusions

The study provides important information regarding the performance of sulfidogenic bioreactors treating acidic metal-containing water and hospital wastewater. This work demonstrates the possibility of an SBR reactor for synchronized decreasing concentrations of COD, metals, sulfate, and selected anti-inflammatory pharmaceutical compounds in wastewater. At an HRT of 12 h, about 540 mg/l/d Fe and 490 mg/l/d Zn were precipitated with effluent soluble Fe and Zn concentrations remaining below 0.01 mg/l in effluent stream. The wastewater pH was increased

from 2.5 to 8.2 during the co-treatment. Michaelis Menten constants (K_m) for COD oxidation found in the batch FBR were 7.3 mg/l. The maximum oxidation velocity (V_{max}) was found to be 0.12 mg/l min. The dissolved sulfide inhibition constants (K_i) were 3.6 mg/l for the associated growth, and the related K_i value was 9 mg/l for H_2S. The microbial community provided insights into the main microbes in biological treatment. The dominant species in the treatment process belong to the *Proteobacteria* group (especially *Deltaproteobacteria*).

4.5 References

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CHAPTER FIVE

Performance Assessment of Sulfidogenic Fluidized-bed Reactor for Co-treatment of Acid Mine and Pharmaceutical-containing Wastewater

This chapter presents a process performance study of sulfidogenic co-treatment of real acid mine and hospital wastewater under mesophilic operated fluidized-bed-reactor. The hospital wastewater that is enriched with the organic matter was used as the primary source of carbon for sulfate reduction bacteria. The biotreatment was tested for its robustness since it used samples from real wastewater streams which were collected at different times, which shocked the systems. The ANN model was successfully developed, and the predicted and actual measured concentrations of the outputs found an R-value of 0.97. The extension of the measured targeted pollutants in this study beyond just the normal wastewater quality routine checks in WWTPs include measurement of non-steroidal anti-inflammatory pharmaceuticals like naproxen and ibuprofen. This study provides major contribution to the existing knowledge in the field of environmental engineering. This chapter is currently under peer-review as a research article in *International Journal of Environmental Science and Technology*.

5.1 Introduction

In the past several years, the prevalence of pharmaceuticals in the aquatic environment has attracted considerable attention among these emerging contaminants. Detectable pharmaceutical concentrations have been identified in effluents and natural waters of South African wastewater treatment plants (Madikizela and Chimuka, 2017; Matongo et al., 2015a) and some parts of the world, like Europe (Carmona et al., 2014; Vergeynst et al., 2015). Most pharmaceuticals are discharged to the sewer from hospital wastewater and domestic setting through excretion of human waste, ultimately these are received by the wastewater treatment plant (WWTP) at low concentrations. Current WWTPs are not equipped to manage these emerging constituents and hence their capacity to treat pharmaceuticals is not inherently strong. The pharmaceutical groups that are more prevalent in environmental samples around the world include anti-inflammatory drugs, steroids, antibiotics, and anti-retro virials (Matongo et al., 2015b). These emerging pollutants present a new worldwide water quality problem with potentially severe repercussions for human health and the ecosystem.

Due to the extremely polar nature of anti-inflammatory drugs such as ibuprofen, diclofenac, and naproxen, they are highly likely to survive the WWTP and persist in aquatic water yet causing an environmental challenge. As such, technologies which promise to degrade these pollutants are becoming increasingly valuable to research. There are several methods such as biological process (Chelliapan and Sallis, 2013), advanced oxidation process (Segura et al., 2013), bio-electrochemical system (Yun et al., 2017), and membrane processes (Huang et al., 2018) have been applied to degrade pharmaceutical concentration. Undoubtedly, the biological processes are likely superior in treatment of pharmaceuticals, largely because they are economically viable and ecologically friendly (Huang et al., 2018). Upon narrowing down the scope of the treatment process, this paper focuses on the biological treatment technology for pharmaceuticals. Most published literature states that the anaerobic mechanism is more effective with less energy usage for high-strength pharmaceuticals, as seen in Table 5.1. Nevertheless, certain serious challenges such as slow-growth rates of anaerobic micro-organisms, long start-up times, and poor biomass retention typically affect the anaerobic wastewater treatment process for pharmaceuticals. In addition, the pollutants were also significantly below the discharge level in the anaerobic system effluent. For further treatment, therefore, post units are typically needed.

On the other hand, acid mine drainage (AMD) in the mining industry worldwide represents a challenging environmental issue also, extremely toxic to aquatic organisms. AMD is generated by metal sulfides' biological oxidation to metal sulfate in mine waste, given its strong acidity and metal and sulfate (Huang et al., 2018). In addition, under anaerobic conditions, by sulfur-reducing bacteria that cause air pollution, sulfates can be converted to sulfides. Different methods apply to treatment with AMD, one is considered to be an effective biological process via microbial sulfate-reducing bacteria treatment (Neculita et al., 2007). For its enhanced sludge consistency and low cost relative to conventional chemical treatment, mine water treatment in sulfidogenic conditions is a viable alternative (Sahinkaya et al., 2013). Mineral water treatment primarily requires sufficient organic substrates to remove metal and reduce the dissolved oxygen (DO) concentration (Younger and Henderson, 2014). Even if sulfidogenic treatment is effective for AMD, an acceptable electron donor from the carbon source is required for SO_4^{2-} removal and is often necessary for success of the treatment strategy.

Table 5.1 The summary of biological treatment technologies for pharmaceuticals wastewater

Process Technology	Influent and operation condition	Process performance	References
UASR	4 d HRT, Tylosin: 20 – 200 mg/l, COD: 7000 ± 800 mg/l, OLR: 1.86 kg.COD.m^{-3}.d^{-1}	Tylosin: 95% COD: 70-75%	(Chelliapan and Sallis, 2013)
Activated sludge process	SS: 160-220 mg/l, COD: 240-410 mg/l, VSS/SS: 76%-72%, NH_4^+ -N: 30-50 mg/l, T(°C):10-18	SS: 87-98% BOD: 88-98% COD: 88.8-93.5% NH_4^+-N: 69-73.34%	(Wang et al., 2020)
UASB	COD: 4016-13093 mg/l, amoxicillin: 69.2 -105 mg/l, APA (6): 143-315 mg/l	COD: 52.2% 6-APA: 26.3% Amoxicillin: 21.6%	(Chen et al., 2011)
(ABR-BASR)	ABR aureomycin: 3.3 mg/l, 2.5 d HRT, COD: 14119 ± 3367 mg/l, BASR HRT: 8.3 h, ampicillin: 1.0 mg/l	Ampicillin: 42.1% COD: 97.8% Aureomycin:31.3%	(Zhou et al., 2006)

"Anaerobic baffled reactor-biofilm airlift suspension reactor" – ABR-BASR; "Up-flow anaerobic stage reactor" - UASR

Considering the quality of hospital wastewater which is high in the organic matter with pharmaceutical compounds and the AMD is proposed to employ a co-treatment strategy for these two waste streams. Hospital wastewater is regarded as a good-cost carbon source for the sulfidogenic treatment of AMD. AMD and hospital wastewater are toxic streams that are harmful to the environment, as such, the treatment strategy could yield remediation for both wastes simultaneously maybe environmental and economic benefits.

The biological interactions in the co-treatment system of AMD and any other wastewater stream are complex in nature and many variables affect the fluidized bed reactor (FBR) performance. However, the biodegradation correlation between contaminants and variables in the co-treatment is still not well understood. There are studies that have proposed to discuss the performance of sulfidogenic FBR processes, but there is no evidence of any study that proposes to predict the co-treatment process. Consequently, there is a need to supplement and explain the experiments. As such, the neural network backpropagation (BPNN) algorithm is a successful and effective tool for defining the performance measures and corresponding interactions (Huang et al., 2018). BPNN has demonstrated many advantages over current models, such as the probability of adapting BPNN

to dynamic and non-linear systems, and the potential of eventually replacing experiments with forecasts.

Within this context, a fluidized bed reactor in a laboratory scale was used for cotreatment of real mine and hospital wastewater (HWW). The objectives of this work were to (1) investigate the removal efficiency of the fluidized-bed reactor under sulfidogenic conditions for the simultaneous remediation of AMD and HWW, and (2) develop the prediction model of the FBR outputs' quality from the co-treatment system. As it stands in the published literature, this is the first study using BPNN modelling of AMD and HWW co-treatment in a sulfidogenic bioreactor to predict performance. The results provide a better overall understanding of the biotreatment technology with the removal of the selected pharmaceuticals in an FBR.

5.2 Materials and Methods

5.2.1 Sampling and wastewater characteristics

Acid metal-containing water was collected at an unused mine in the Province of Mpumalanga, South Africa. Hospital Wastewater (HWW) was collected from a public hospital in Kwa Zulu Natal, South Africa. From August 2019 to March 2020, a sampling of HWW was conducted at regular intervals. On-site measurements of pH, temperature, and electrical conductivity have been tested during sample collection trips. For the same length as the HWW, municipal wastewater samples are obtained from a treatment plant in South Durban, in South Africa. While this analysis did not use MWW in the treatment process, in order to support the research hypothesis, MWW samples were analyzed to draw parallels on the COD concentrations and nutrients, and this data is found in Makhathini et al. (Makhathini et al., 2020).

Table 5.2 Characteristics of the wastewater streams and operating conditions of the bioreactor

Parameters	Phase I	Phase II	Phase III	Phase IV
Hospital wastewater				
pH	6.0 – 8.5 ± 0.2	6.1 – 8.0 ± 0.4	6.4 – 8.3 ± 0.4	6.2 – 8.3 ± 0.2
TDN (mg/l)	144 ± 11	876 ± 121	1128 ± 111	954 ± 132
COD (mg/l)	4953 ± 123	4352 ± 187	2595 ± 223	2786 ± 325
DOC (mg/l)	55 ± 13	231 ± 11	142 ± 17	67 ± 16
H_2S	0.022 ± 0.4	0.092 ± 0.1	0.11 ± 0.3	0.12 ± 0.5
NH_4^+-N (mg/l)	14.5 ± 0.9	17.5 ± 0.3	13.6 ± 0.6	43.5 ± 0.9
PO_4^{3-} (mg/l)	9.1 ± 0.4	7.7 ± 0.7	7.1 ± 0.3	9.3 ± 0.2
Cl^- (mg/l)	123 ± 21	154 ± 11	143 ± 18	128 ± 23
Acid mine drainage				
pH	2.34 ± 0.7	2.13 ± 0.4	2.23 ± 0.5	2.31 ± 0.4
SO_4^{2-} (mg/l)	3213	3987	3876	3217
Al (mg/l)	265	213	215	177
Zn (mg/l)	96	54	66	85
Mn (mg/l)	112	154	134	145
Fe (mg/l)	1302	1291	1354	1678
Cu (mg/l)	25	36	76	23
Operating parameters				
Treatment day	0 - 45	45 - 90	91 – 120	121 - 210
HRT (h)	24	16	8	24
Temperature (°C)	± 30	± 30	± 30	± 30
COD/SO_4^{2-} ratio	1.54	1.09	0.67	0.87

5.2.2 The bioreactor operational settings

A fluidized-bed reactor with a working volume of 2000 mL was subculture with an anaerobic digester sludge harvested in a wastewater treatment plant. The bioreactor, as shown in Figure 5.1

was operated under anaerobic conditions, immediately after being inoculated and packed with silica sand at a 20% fluidization rate to develop the biofilm. The sludge residence time was maintained between 8 and 10 days for the duration of the treatment. As proposed earlier, hospital wastewater provided a source of electron and carbon to convert SO_4^{2-} in AMD to H_2S and to oxidize organic matter.

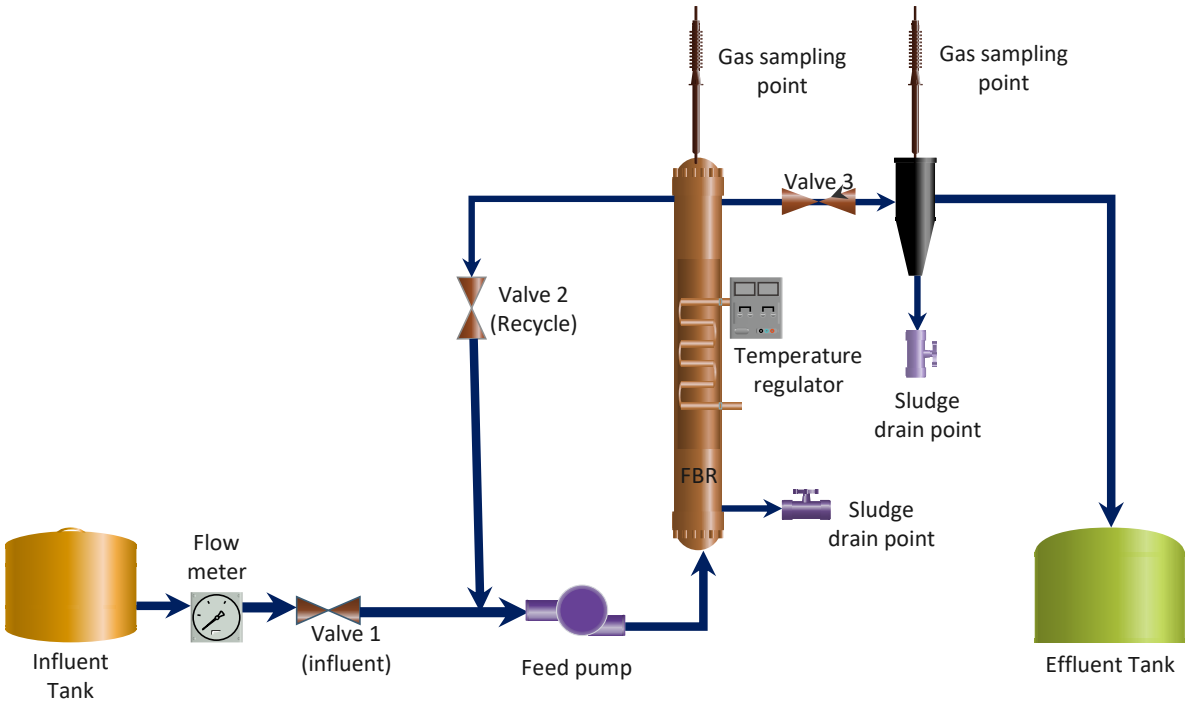

Figure 5.1 The schematic diagram of the bioreactor setup

The reactor efficiency was assessed for 210 days at various influential metals, SO42- and organic loads to measure the robustness of the biotreatment (Table 5.2). During the start-up phase, the reactor was fed with hospital wastewater only dosed with 3000 mg/l of sulfate to enhance the SRB growth. The reactor was run under recycled mode for 21 days, thereafter, phase I (1 – 45 days) of treatment began and the reactor performance was assessed as fresh loadings of metals, sulfate and organics were fed into the reactor (Table 5.2). The recycle stream was also used during the transition stage between one phase to the next, this was done to stabilize the reactor after each change. The bioreactor was fed with HWW and AMD at 5:2 v/v but carefully monitoring the COD/SO_4^{2-} ratio. The FBR operating conditions are given in Table 5.3 for easier understanding of

the process. The water level adjuster was used to keep the level steady in the reactor, also to capture any sludge and sand that may escape from the reactor. The bioreactor influent and effluent were sampled every other day to measure suspended solids, sulfate, sulfide, COD, alkalinity, naproxen and ibuprofen.

Table 5.3 The fluidized-bed reactor operating parameters and variables

Operating Parameters/Variables	Process values	Rationale and performance observation
Carrier material (silica beads)	1-1.5 mm	Proved to have good biomass attachment capabilities with varying reactor operating conditions
Electron donor source	COD in HWW	Electron donors from HWW could be used to strip off bacterial oxygen, sulfate, and support metal concentration reduction, thus producing alkalinity
COD/SO_4^{2-} ratio	0.5–1.39	For a complete sulfate reduction, the reactor thrived to operate at an average of 0.67, a theoretical (g COD/g SO_4^{2-}).
pH	2.8 – 8.3	The pH improved after the acid mine drainage was introduced to HWW
Temperature	± 30^0C	Even though higher temperature enhances the SRB to outperform methanogens, but energy saving was more important in this process.
Influent velocity	37.7 – 92 m/h	There was no bed expansion observed at these flow rates, hence were selected as an operating range
Hydraulic retention time	4 – 24 h	At lower HRTs the reactor achieved higher alkalinity, however, could not sustain the effluent quality for longer period

5.2.3 Analytical techniques

Metals, sulfide, sulfate, and nutrients were analyzed using the conventional Rice et al. (2012) methods. Concentrations of hydrogen sulfide on a Hach DR 3900 were determined using USEPA Method 8131 (methylene blue) (USEPA, 2014). For SO_4^{2-} and PO_4^{3-} samples were quantified using a standard method using a Hach DR 3900 spectrophotometer. Upon filtering through 0.45 microns of nylon, the acid digestion with concentrated HNO_3 retained all dissolved metal samples. The metal concentrations of Zn, Al, Cu, Fe, and Mn were measured using the ICP-Optical Emission Spectrometer (Variation 720-ES).

Samples were filtered with membrane (0.45-micron) filters for DOC and TDN as advised by Sharp et al. (1995), and deposited in 50 ml glass vials with membrane caps at -17°C. Until study, samples were liquefied inside the refrigerator at around 4°C (Rice et al., 2012). In addition, analyzes were

performed in duplicates for alkalinity, sulfide, TDN, COD, DOC, BOD, metals, and anion concentrations to ensure quality control, as required by the USEPA protocol.

5.2.4 Pharmaceutical analysis

Sigma-Aldrich (Germany) supplied ibuprofen (> 98%) and naproxen (98%) used in quantification analysis. Methyl alcohol (99.5 percent) Ethyl acetate (>99.9 %), Acetone (99.5 %), and acetonitrile (99.9%), are Macron Fine Chemicals, SA high pressure liquid chromatography solvents. The solid-phase extraction cartridges used were 5 mL (200 mg mass) Oasis HLB. The procedure adopted in this study for the calculation of ibuprofen and naproxen was adapted following modification of the approach suggested by Agunbiade and Moodley (2016). The analysis protocol is summarized in Figure 5.2.

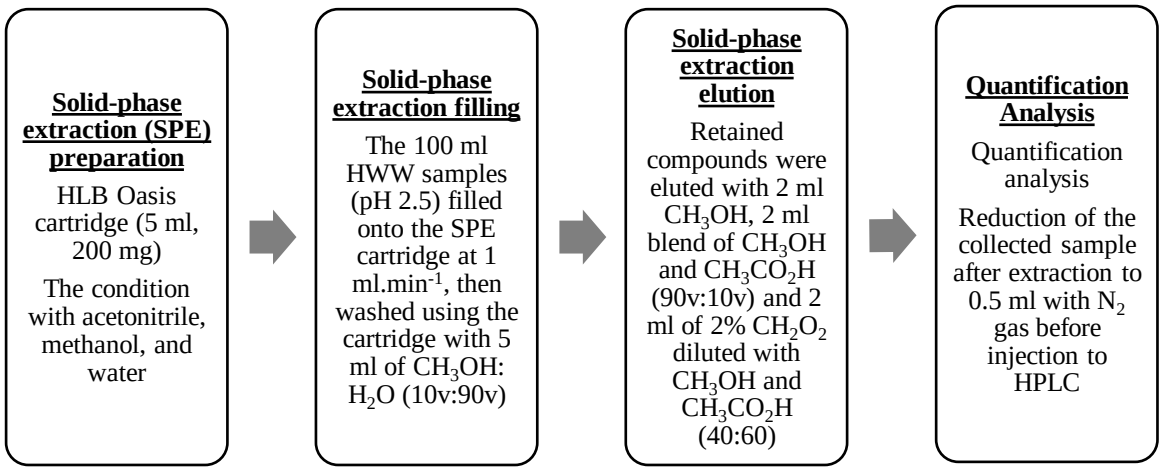

Figure 5.2 The overview of the selected pharmaceuticals analysis technique flow diagram

A (100 mg/l) buffer solution was made in acetonitrile containing all of the target compounds, using an HPLC instrument, the solutions were analyzed. Thereafter, the detection limit, quantification limit, and linearity limit were determined. The precision and consistency of the analytical method were calculated using deionized water, which was spiked with naproxen and ibuprofen compounds at the 50 and 5 µg/l concentrations. The concentrations were spiked based on the previously recorded levels of naproxen and ibuprofen found in WWTPs in South Africa (Madikizela and Chimuka, 2017). In these experiments, the target compounds were identified at low µg/l level in

wastewater. Prior to quantification of HPLC, the optimized solid phase extraction method was used for the extraction and pre-concentration of target compounds.

5.2.5 Model development using BP algorithm

A commercially available software program, MATLAB neural network toolbox R2020a, was used to implement artificial neural networks. The ANN architecture was built on two hidden layers, and an output layer as shown in Figure 5.3, and trained with the algorithm of backpropagation (BP). The input variables comprised of treatment time, sulfate, COD, pH, NH_4^+-N, TDN, Fe, Zn, ibuprofen and naproxen. The sulfate, COD, ibuprofen, and naproxen removal efficiencies were configured as forecast variables in the output nodes. Here, the performance of fourteen back-propagation algorithms was compared to select the best fitting algorithm, as shown in Table 5.4. The evaluation was based on the regression coefficient and the mean square error (MSE) comparing the measured output and modeled output data. The hidden layer raw data were distributed using a nonlinear tan sigmoid learning algorithm in the hidden layers plus in the output layer. The model used Levenberg-Marquardt 'trainlm' training algorithm which recorded the best regression coefficient (R^2). The regression coefficient and mean squared error between actual data recorded during the experiment and predicted figures were described as Equation 5-1 and 5-2. The data learned through random data division (*dividerand*) using *nntraintool*. Hidden layer with separate target neurons was achieved on trial and error with mean square error of 0.001. The accuracy of the model was predicted by the mean square error (MSE) computed by Equation 5-2 (Huang et al., 2018):

$$\boldsymbol{R^2} = \frac{\boldsymbol{N\sum_{i=1}^{N} y_{pre}y_{act} - (\sum_{i=1}^{N} y_{act}) \times (\sum_{i=1}^{N} y_{pre})}}{\sqrt{\left[\boldsymbol{N\sum_{i=1}^{N} y_{act}^2 - \left(\sum_{i=1}^{N} y_{act}\right)^2}\right] \times \left[\boldsymbol{N\sum_{i=1}^{N} y_{pre}^2 - \left(\sum_{i=1}^{N} y_{pre}\right)^2}\right]}} \qquad \textbf{Equation 5-1}$$

$$\boldsymbol{MSE} = \boldsymbol{\frac{1}{N}\sum_{i=1}^{N}(y_{act} - y_{pre})^2} \qquad \textbf{Equation 5-2}$$

for which N is the data points' value, y_{pre} is the value expected, while y_{act} is the actual value of the experiment. Following the selection of the best fit model algorithm, all parameters remained constant while the optimization of the number of neurons was tested, as shown in Table 5.5.

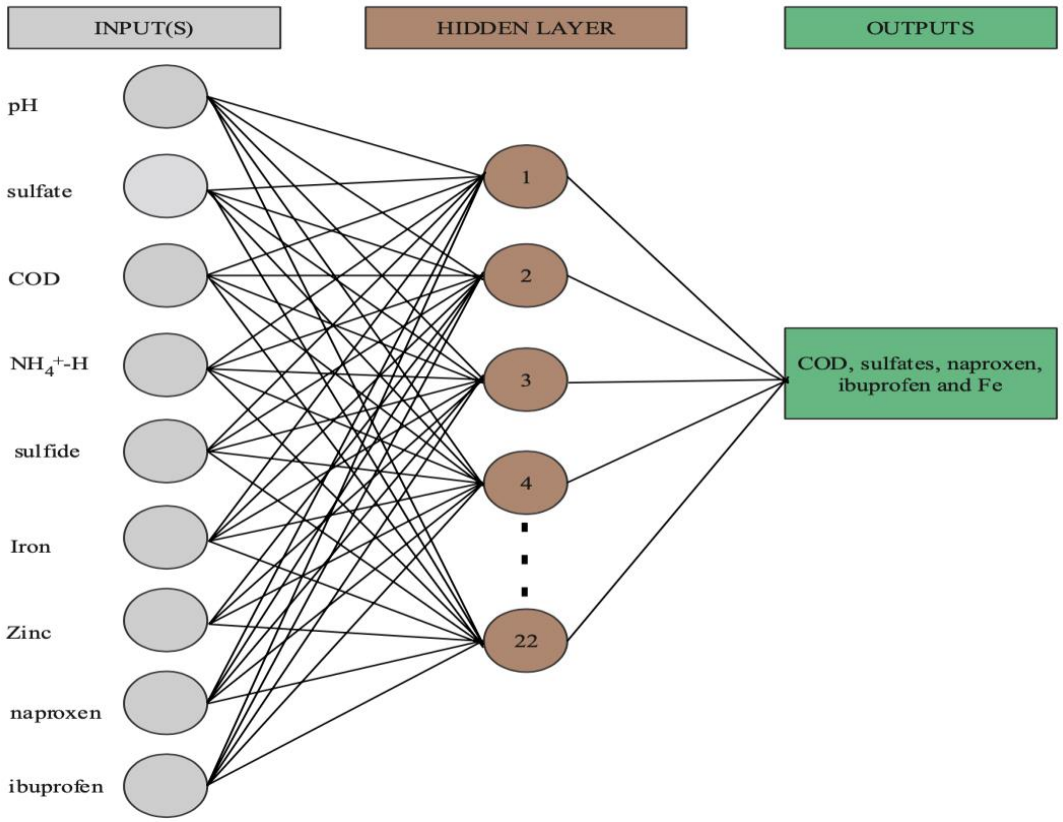

Figure 5.3 ANN architecture structure

5.3 Results and Discussion

5.2.4 Alkalinity, pH and sulfide in the FBR

The pH and alkalinity are the critical features influencing anaerobic system's stability (Huang et al., 2018). Effluent neutralization by alkalinity formed by the electron donor's microbial oxidation (Equation 5-3). The effluent pH levels in phase II and phase III were around 6.8 ± 0.4 but marginally decreased to 6.2 ± 0.2 in phase IV (Figure 5.4). The pH enables suppression of dissolved oxygen (DO) by bacterial activity (Strosnider et al., 2013). This process depended mainly on HWW as a source of energy and carbon but failed to achieve a better effluent pH than originally anticipated, in fact, achieved less pH level as the previous study of HWW and AMD (Makhathini et al., 2020). In the previous study, the range for the feed COD was between 1425 to 3246 mg/l compared to the current study which has higher feed COD of 2786 to 4953 mg/l.

$$2CH_2O + SO_4^{2-} \rightarrow 2HCO_3^- + H_2S \qquad \textbf{Equation 5-3}$$

$$M^{2+} + H_2S \rightarrow MS + 2H^+$$ **Equation 5-4**

For which CH_2O is the organic compound and M denoting the metal in AMD

Table 5.4 Analysis of back-propagation algorithms in a sulfidogenic fluidized-bed reactor to predict effluent naproxen, ibuprofen, sulfate, COD, and Fe

Back	COD (mg/l)			Fe (mg/l)			Sulfates(mg/l)			Naproxen (μg/l)			Ibuprofen (μg/l)		
Prop. Alg.	Train value	Valid. value	Test value	Train value	Valid. value	Test value	Train value	Valid value	Test value	Train value	Valid value	Test value	Train value	Valid value	Test value
Trainrp	0.94	0.89	0.82	0.96	0.91	0.92	0.95	0.92	0.88	0.98	0.94	0.96	0.92	0.91	0.90
Trainscg	0.99	0.90	0.92	0.93	0.96	0.89	0.94	0.98	0.97	0.93	0.93	0.96	0.98	0.92	0.81
Trainlm	0.98	0.97	0.99	0.99	0.98	0.99	0.99	0.99	0.99	0.98	0.97	0.96	0.99	0.98	0.97
Traincgf	0.96	0.88	0.89	0.92	0.91	0.93	0.94	0.92	0.93	0.92	0.92	0.93	0.91	0.92	0.89
Traingdm	0.97	0.91	0.94	0.94	0.94	0.97	0.92	0.89	0.91	0.93	0.94	0.95	0.94	0.95	0.94
Traincgp	0.99	0.93	0.93	0.91	0.94	0.95	0.91	0.94	0.97	0.92	0.89	0.92	0.98	0.97	0.93
Traingdx	0.96	0.94	0.91	0.95	0.83	0.96	0.92	0.93	0.99	0.90	0.91	0.98	0.93	0.95	0.91
Traincgb	0.94	0.95	0.96	0.98	0.81	0.89	0.89	0.88	0.89	0.92	0.96	0.97	0.92	0.94	0.96
Trainbfb	0.94	0.92	0.97	0.92	0.94	0.92	0.95	0.96	0.98	0.93	0.98	0.98	0.97	0.93	0.97
Trainbr	0.99	0.93	0.92	0.93	0.92	0.88	0.92	0.95	0.93	0.95	0.93	0.92	0.92	0.90	0.92
Traingd	0.92	0.95	0.99	0.94	0.95	0.93	0.90	0.94	0.92	0.93	0.97	0.97	0.95	0.99	0.99
Trainr	0.98	0.94	0.96	0.93	0.91	0.81	0.98	0.92	0.93	0.94	0.88	0.81	0.96	0.98	0.96
Trainoss	0.93	0.93	0.91	0.91	0.88	0.94	0.97	0.91	0.93	0.90	0.82	0.82	0.90	0.95	0.91
Traingda	0.91	0.91	0.95	0.95	0.87	0.95	0.93	0.94	0.96	0.93	0.93	0.92	0.91	0.93	0.95

Table 5.5 A comparison of neuron numbers using R values for training, validation, and test for predicting effluent naproxen, ibuprofen, sulfate, COD, and Fe from a sulfidogenic fluidized reactor

No.	COD (mg/l)			Fe (mg/l)			Sulfates (mg/)			Naproxen (µg/L)			Ibuprofen (µg/L)		
of Neurons	Train value	Valid values	Test values	Train values	Valid values	Test Values	Train values	Valid values	Test values	Train values	Valid values	Test values	Train values	Valid values	Test Values
5	0.78	0.82	0.88	0.93	0.92	0.94	0.95	0.92	0.88	0.92	0.91	0.81	0.92	0.97	0.92
13	0.81	0.90	0.92	0.88	0.91	0.92	0.93	0.96	0.93	0.90	0.93	0.92	0.89	0.65	0.89
15	0.94	0.91	0.94	0.91	0.94	0.95	0.92	0.94	0.96	0.93	0.96	0.95	0.92	0.77	0.83
18	0.92	0.93	0.97	0.87	0.98	0.97	0.94	0.95	0.97	0.96	0.92	0.96	0.93	0.89	0.89
22	0.99	0.99	0.99	0.98	0.98	0.98	0.99	0.99	0.98	0.97	0.98	0.99	0.98	0.97	0.97
25	0.96	0.94	0.93	0.92	0.93	0.93	0.91	0.94	0.92	0.93	0.91	0.91	0.91	0.83	0.91
30	0.91	0.93	0.93	0.93	0.98	0.92	0.92	0.93	0.99	0.93	0.95	0.92	0.94	0.93	0.94
33	0.89	0.91	0.94	0.97	0.81	0.94	0.91	0.83	0.89	0.94	0.90	0.94	0.96	0.91	0.95
36	0.93	0.92	0.92	0.91	0.92	0.89	0.93	0.95	0.81	0.98	0.94	0.91	0.93	0.94	0.98
40	0.78	0.97	0.91	0.94	0.95	0.91	0.96	0.95	0.87	0.89	0.88	0.89	0.90	0.88	0.81
44	0.92	0.95	0.99	0.98	0.97	0.96	0.93	0.88	0.96	0.88	0.82	0.80	0.96	0.77	0.83

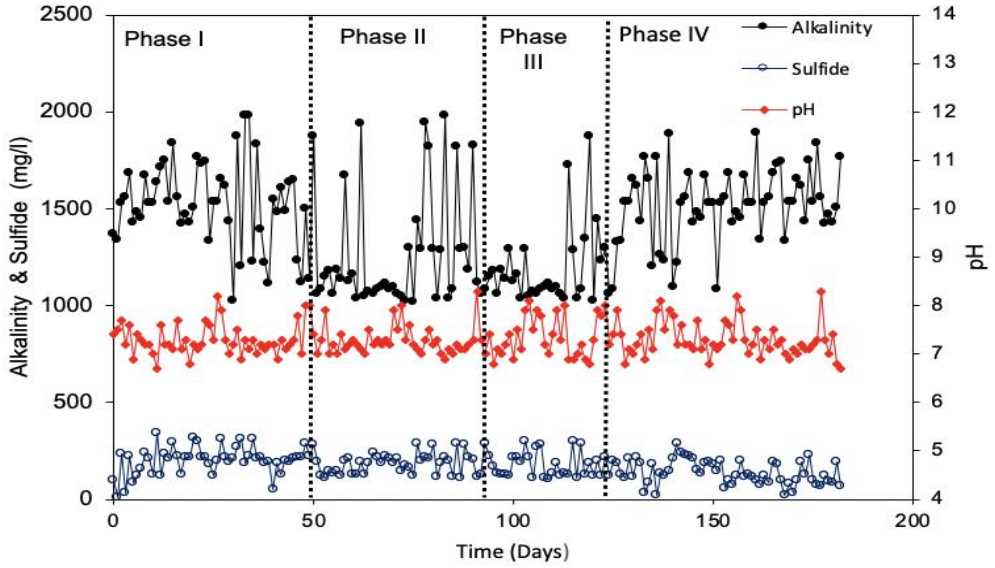

Figure 5.4 The alkalinity, pH and sulfide of the FBR during the biotreatment different phases

The effluent alkalinity was comparatively approximately 1618 ± 218 mg (in $CaCO_3$ per liter) in Phase I, where else the alkalinity further decreased to 1121 ± 21 mg $CaCO_3$/l 1189 ± 211 mg $CaCO_3$/l in the phase II and III, then increased again 1556 ± 10 mg $CaCO_3$/l in the final phase. Results show that the decrease in HRT in phase III alkalinity production from 16 to 8 h had moderately decreased. In the last step of therapy, alkalinity production recovered and increased slightly when the HRT was increased to 24 h. These findings may be due to the success of SO_4^{2-} reduction at end of the phase IV, however yielded less pH than all treatment phases, subsequent the production of precipitated metal sulfide.

The development of sulfide showed a parallel movement at the effluent with alkalinity (Figure 5.4). Phase I dissolved sulfide averaged 270 ± 34 mg/l that decreased to 96 ± 33 mg/l once the retention time reduced from 24 to 16 h and the influent oxygen demand was sustained at a maximum of 4953 ± 124 mg/l. However, the efficiencies of SO_4^{2-} reduction in the final phase, and the concentration of dissolved sulfide decreased to 93 ± 12 mg/l due to sulfide precipitation of phase IV feed metal concentration. The average BOD_5 (not shown in Figure 4) had decreased significantly from 643 ± 76 mg/l in phase I to 121 ± 58 mg/l in phase IV.

5.3.2 Hydraulic retention time effect on dissolved metals

Continuous flow studies (Bekmezci et al., 2011; Makhathini et al., 2020) assessed the effectiveness of SO_4^{2-} reducing bioreactor for efficient elimination of metals, acidity, and SO_4^{2-} from the waste stream. During the initiation period and first loading experiments (21 days), the hospital wastewater functioned as a carbon source for SO_4^{2-} reduction, it is considered a suitable substrate for enhancing SRB growth (Makhathini et al., 2020). In this analysis, at an HRT of 16 h for the development and conservation of one FBR culture, the original HWW feed was slowly replaced by a mixture of AMD and HWW over 60 days. The electron donor was never supplemented with any other substrate, however, at the beginning of Phase II, meat extract was added into the HWW, since the COD was less than expected. During this alteration in the HWW, 57–68% of the DOC was absorbed through SO_4^{2-} reduction with DOC elimination of 77-94%. A summary of metal removal is given in Table 5.6.

Table 5.6 Metal concentration removal in the FBR

Days	Treatment period	Al (mg/l)	Zn (mg/l)	Fe (mg/l)
Influent concentration				
0 – 45	Phase I	263 ± 1.9	93 ± 1.1	1302 ± 21
45 – 90	Phase II	217 ± 2.2	53 ± 0.8	1276 ± 23
91 – 120	Phase III	215 ± 12	66 ± 0.5	1353 ± 12
121 – 210	Phase IV	167 ± 18	84 ± 0.1	1678 ± 43
Effluent concentration				
0 – 45	Phase I	1.9 ± 0.8	0.7 ± 0.2	2.1 ± 1.1
45 – 90	Phase II	± <0.02	-	1.8 ± 0.9
91 – 120	Phase III	± <0.02	-	1.1 ± 0.3
121 – 210	Phase IV	± <0.02	± <0.05	± <0.05

The FBR's robustness was tested by adding the fresh feed from field trips, which calculated parameters at different points, simulating a real environment. At an HRT of 8 h, COD oxidation in FBR precipitated 2345 mg Fe/l/day, 543 mg Al/l/day, and 130 – 170 mg Zn/l/day from acidic wastewater and increased the pH from 2.2 to 6.8. As a consequence, the soluble Fe, Al, and Zn in

the effluent recorded less than 0.2 mg/l. The performance of the FBR regarding the acid neutralization and metal precipitation compared favorably with the performance achieved with the previous studies on sulfidogenic reactor configurations (Table 5.7). In past studies, high levels of metal removal from AMD were achieved with SRB mechanisms which increased the retention of biomass with flocculants (Peer et al., 2015), wastewater neutralization was chemically enhanced, or metal precipitation (Strosnider et al., 2013). Precipitation of metal sulfide and neutralization of the pH are interdependent, as acidity is created by the precipitation reaction. In this research analysis, the efficacy of the SO_4^{2-} reducing bioreactors in precipitating Zn, Al, and Fe from AMD and producing alkalinity to neutralize the effluent stream has been documented.

Table 5.7 The sulfidogenic reactor techniques for treatment of acidic mine water

Form of wastewater	Volume of the reactor	Source of carbon	Microbiology development	$\%SO_4^{2-}$ removal	% Metal removal	Reference
Acid mine with Zn, Mn, Al, Fe, Cu, Al, and Pb	0.3 liter at 12 and 24 h HRT	Ethanol	SRB	90%	99.9 % of Fe, 94% of Mn, Zn, Al, Cu and Pb	(Sahinkaya et al. 2011)
Acid mine (synthesized) with Fe and Cu	0.6 and 0.25 l at 24 h	Ethanol and lactate	SRB	60-90%	>90%	(Ucar et al. 2011)
Synthetic AMD	0.6 liter at 15 – 28 h HRT	Ethanol and landfill leachate	SO_4^{2-} reducing sludge from sulfidogenic ABR	>90%	80-99.9%	(Sahinkaya et al. 2013)
Acid mine (synthesized) Pb, Cu, Cd and Zn	5.1itre at 24 h HRT	Sodium Lactate	Sludge from anaerobic sludge digester	68-88%	98.4% Cu, 96.5% Zn, 96% Pb, and 97.9% Cd	(Villa-Gomez et al. 2015)
Wastewater from an un-operational mine	2 l at 6 – 24 h HRT	Chemical oxygen demand from HWW	Sludge from anaerobic sludge digester	>90%	>99%	(Makhathini et al. 2020)
Acidic wastewater with Fe	0.425 – 0.625 l at 24 h HRT	Lactate and ethanol	SO_4^{2-}reducing bacteria	Approx. 50%	>99%	(Sahinkaya et al. 2007)
Acidic wastewater with Fe	7.5 l at 42 h HRT	Lactate	Methanogenic granular sludge from an anaerobic digester	35 – 95%	Not available	(Papirio et al. 2013)
Wastewater from an operational mine	2 l at 8 – 24 h HRT	COD in hospital wastewater	Sludge from sulfidogenic bioreactor		>99%	Current study

Zinc was observed to completely inhibit sulfate-reduction in the concentration range of 25–60 mg/l (Sahinkaya et al., 2011). In the present study, the FBR, which reduces sulfate, was able to treat wastewater with 95 mg Zn/l, leading to effluent of only about 0.1 mg Zn/l (Kaksonen et al., 2004). Having a high recycling proportion in the FBR dilutes feeds on metal and acidity concentrations. Bacteria were exposed to concentrations of Zn near those of the effluent in the completely mixed FBR and thus was accomplished wastewater treatment with usually inhibitory metal concentrations and pH values reached.

5.3.3 The oxidation of COD and reduction of sulfates

The COD and sulfate concentrations of the influent and effluent were assessed to demonstrate the performance of the FBR. The COD removal in the FBR was attributed to biological degradation. The influent COD concentration fluctuated between 1645 and 10326mg/l from phase I to phase IV since the quality of the real HWW was unsteady. The FBR effluent COD concentration fluctuated between 102 and 398 mg/l and displayed an upward trend as the HRT increased. Correspondingly, the performance of COD elimination ranged from 96.0% to 88.1% with an average of 92.0 ± 0.5%, as seen in Figure 5. Nearly all of the effluent COD concentration was higher than 100 mg/l, the COD removal efficiency was lower than Hu et al. research (Nagpal et al., 2000), which reported the total COD removal rate of 97.4 percent with the effluent COD retained below 100 mg/l, the lower efficiency presumably attributable to large quantities of antibiotics in HWW being recalcitrant to biodegradation (Martin-Laurent et al., 2019). The presence of antibiotics in the environment, even at low concentrations, will prevent bacterial growth by disrupting the synthesis of the bacterial cell wall and inducing antibiotic-resistant bacteria (Huang et al., 2017).

$$SO_4^{2-} + 2CH_2O \rightarrow H_2S + HCO_3^- + CO_2 + H^+ \qquad \textbf{Equation 5-5}$$

$$M^{2+} + H_2S \rightarrow MS + 2H^+ \qquad \textbf{Equation 5-6}$$

HRT 's effect on the efficiency of fluidized-bed treatment of acidic wastewater was studied by slowly decreasing HRT from 24 h to 6.0 h, while maintaining constant feed concentrations

(Kaksonen et al., 2004). The decline in HRT resulted in a decrease in the amount of dissolved oxygen used and a decrease in sulfate reduction along with a decrease in dissolved sulfide concentrations and alkalinity in the FBR effluent Equation 5-4 and 5-5. HWW consumption and precipitation efficiency (percentage) of Zn and Fe remained high until poor performance occurred at the lowest HRTs.

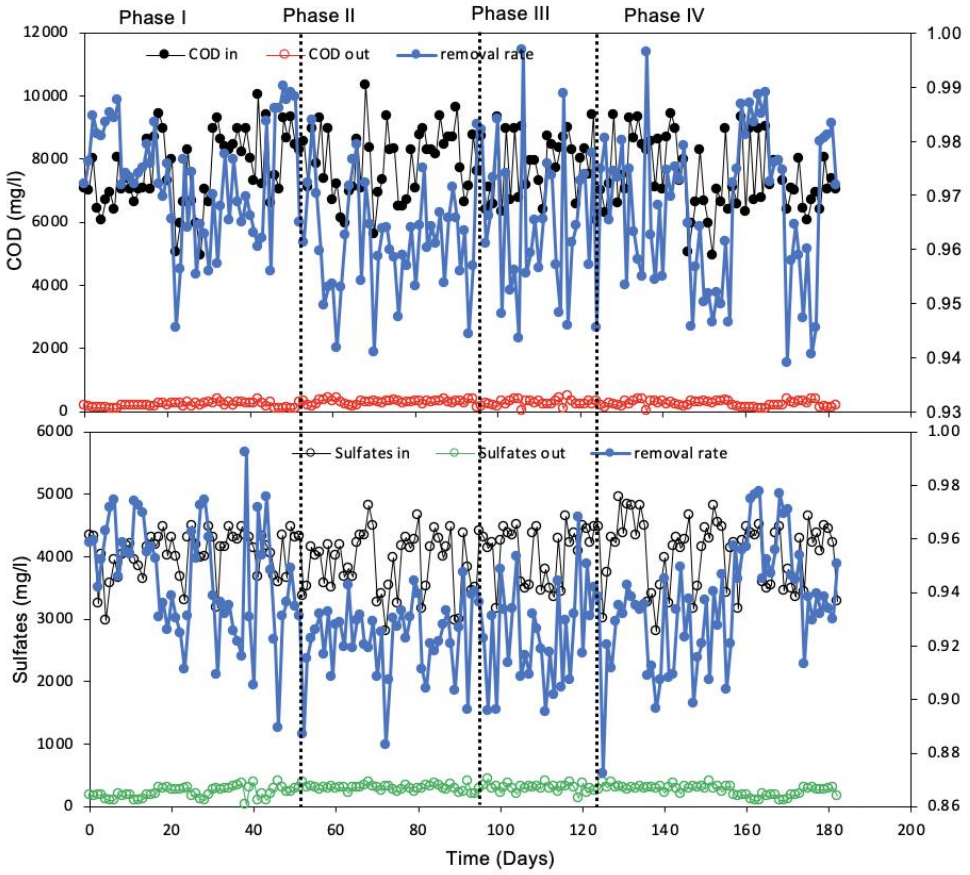

Figure 5.5 The COD and sulfate influent and effluent concentration, and indicating the removal rate

The degradation of sulfate, oxidation of the substrates, precipitation of metals, effluent sulfide, pH, and alkalinity decreased during an intentional overloading on day 130. The FBR cycle was recovered in a few days after the output loss by changing the FBR liquid pH to 7 and halting feed for 1 day. For a duration of over 16 HRTs, the FBR effectively precipitated metals at HRT down to 6.0 h, but a further decrease in HRT resulted in a poor performance (and these results were not recorded). The corresponding efficiency of metal removal was more than 99.9 percent and concentrations of effluent Zn and Fe below 0.15 mg/l of these conditions, COD oxidation was

incomplete, and sulfide was produced in the FBR. The activity of the FBR at shorter HRTs (at the corresponding loading rate) is restricted by sulfide output, since the output of excess alkalinity by COD oxidation is required for the neutralization of wastewater (Kaksonen et al. 2004).

5.3.4 Biomass yield

At the end of the experiment, the biomass distribution within the reactors was observed. Biomass was distributed uniformly throughout the FBRs fluidized volume. The differences in volatile suspended solids and suspended solids concentrations were assumed to present reactor activity in Figure 5.6. The VSS/SS ratio was about 0.53 between days 7 and 45 when the AMD was not added to the system yet. By adding the AMD, which has Fe, Zn and Al of 1302, 96 and 265 mg/l, the VSS/SS ratio decreased to approximately 0.18 due to precipitated metal in the reactor as time increased in the reactor.

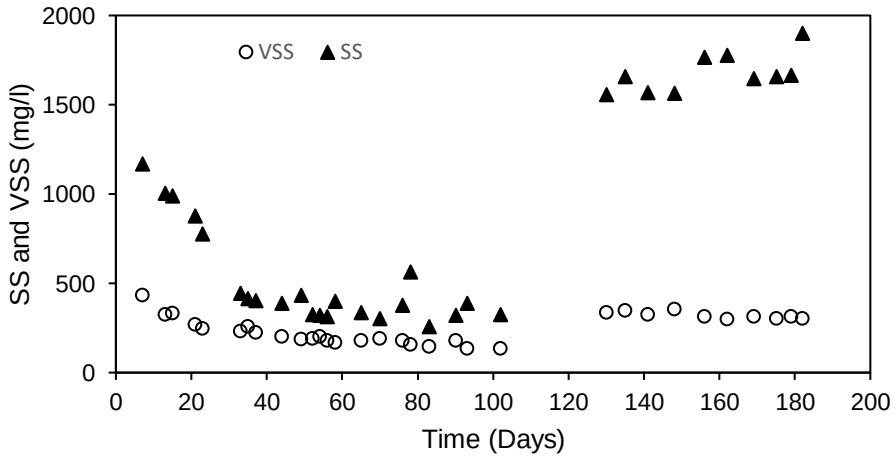

Figure 5.6 The variation of suspended solids and volatile suspended solids in the FBR

The biomass yield of about 2.4–4.4% (g/g COD oxidized) in continuous flow sulfate-reducing reactors was much lower than previously reported for pure cultures of SRB (Kaksonen et al., 2006). The difference in yield coefficient (0.18–0.22 mg VSS/mg reduced sulfate) at different concentrations of sulfate was insignificant, and the average yield coefficient was reduced by 0.198 ± 0.07 mg VSS/mg. Yet, the biomass yield of 4.1–6.2% (g/g sulfate reduced) was achieved and still below the reported by Kaksonen et al. (2006). Papirio et al. (2013) reported reduced VSS/mg sulfate of 0.053–0.074 mg in mesophilic fluidized bed reactors. Whilst Sahinkaya et al. (2007)

reported about 0.1 mg of VSS / mg sulfate reduced in ethanol-fed FBRs and worked at 8 and 65°C in another analysis. A relatively higher value in the present study is due to low sludge production in the FBR compared to attached growth reactor systems.

5.3.5 Pharmaceutical's removal

Four anti-inflammatory pharmaceuticals were tracked during the treatment period, these pharmaceuticals were of most interest as they are evidently persistent in South African water streams (Madikizela and Chimuka, 2017; Matongo et al., 2015a) and also in advanced economies like Europe (Carmona et al., 2014; Vergeynst et al., 2015). The analytical method was validated with a determination of the efficiency of solid-phase extraction (SPE) found to be higher than 84% for both all measure pharmaceutical compounds and r^2 was greater than 0.98.

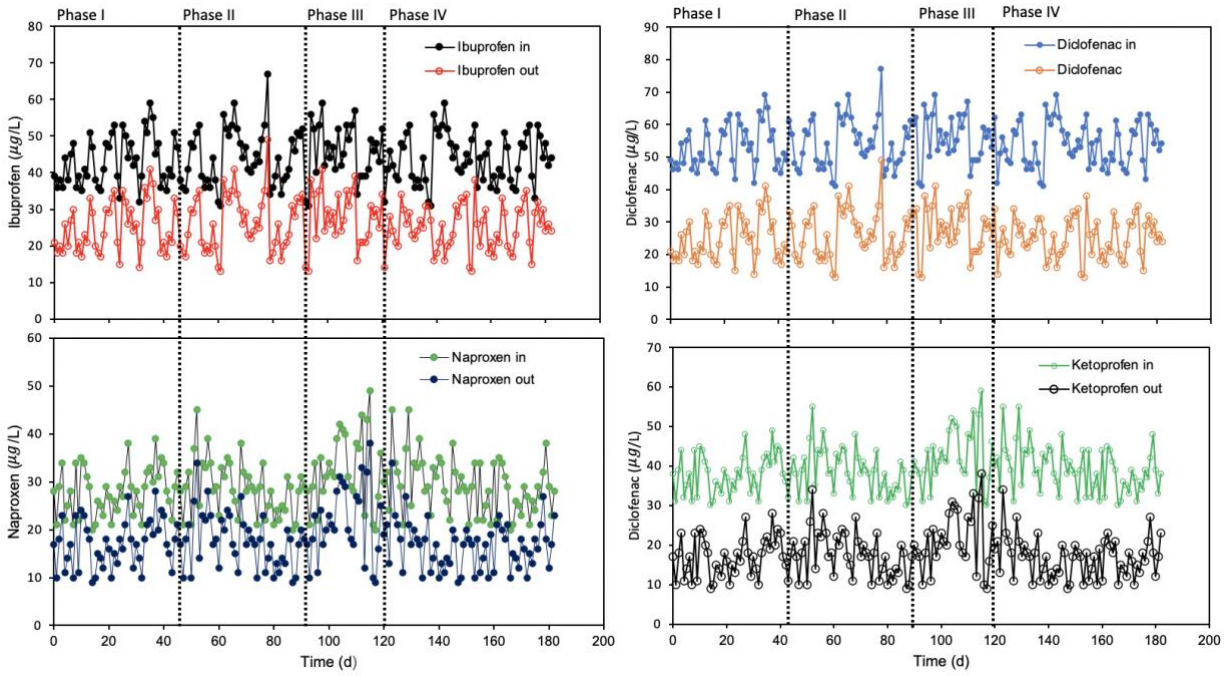

Figure 5.7 The influent and effluent pharmaceutical concentration in the influent and effluent

The pharmaceuticals, naproxen, and ibuprofen removal seem not to be influenced by the HRT, as shown in Figure 5.7. During the biotreatment Phase III, the concentration of naproxen increases in the inlet stream but also the concentration on the effluent stream increased. The removal rate achieved 57% and 50% for ibuprofen and naproxen, respectively. An anaerobic analysis on a laboratory scale showed 30-60% ibuprofen elimination under anoxic conditions, which is in

agreement with the results from Carballa et al. (2008). Additionally, diclofenac is one of the most used anti-inflammatories and has demonstrated less removal in conventional WWTP (Kimura et al., 2007). In previous studies, WWTP achieved a 50-65% removal of ketoprofen and naproxen (Kimura et al., 2007). Regrettably, these experiments have not shown comparable findings in the removal.

5.3.6 Model evaluation

The applicability of the artificial neural network to predict the performance of the FBR based on SRB biological co-treatment of AMD and HWW was assessed. The data collected from day 1 to day 77 of the biotreatment were used as the training value of the model for pharmaceuticals, sulfate, and COD. The model showed that ANN can successfully predict the levels of Fe, naproxen, ibuprofen, sulfate and COD concentration at fluctuating feed composition, then optimize the FBR operational conditions. Figure 8 shows that the predicted values are very close to the actual experimental values. In this way, it is easy to predict the ratio of HWW to AMD, where HWW acts as a carbon source used by sulfate-reducing bacteria or methanogenic microorganisms. Since the sulfate concentration in the effluent is one of the targets in the model, the flow of organic matter to reduce sulfate can be predicted and thus the biotreatment can be optimized as such. This study used about 83% of COD for sulfate reduction. There was no significant methane production detected.

The studies resulted in significant sulfate reduction using mesophilic conditions under SRB, but this is amongst the first studies that model the results from a biotreatment system of AMD and HWW. Moreover, the sulfide measurements in the effluent stream confirm the efficient metal removal capability. Figure 5.9 shows the training validation and test MSE values for the targeted data of COD, sulfate, naproxen, ibuprofen, and Fe. Levenberg-Marquardt algorithm with 22 neurons was used in the prediction model. Since the quality of the influent to the bioreactor varies tremendously with time, thus causing a shock load in the reactor, it may be necessary that the ANN model predicts the effluent's quality after that. The prediction model gives the process team time to respond to the expected system shock, thus making decisions to prevent environmental pollution. Even though the neutralization process is not desirable because of the costs involved,

the ANN prediction model could trigger a need for it as a deterrent measure during an upset process period.

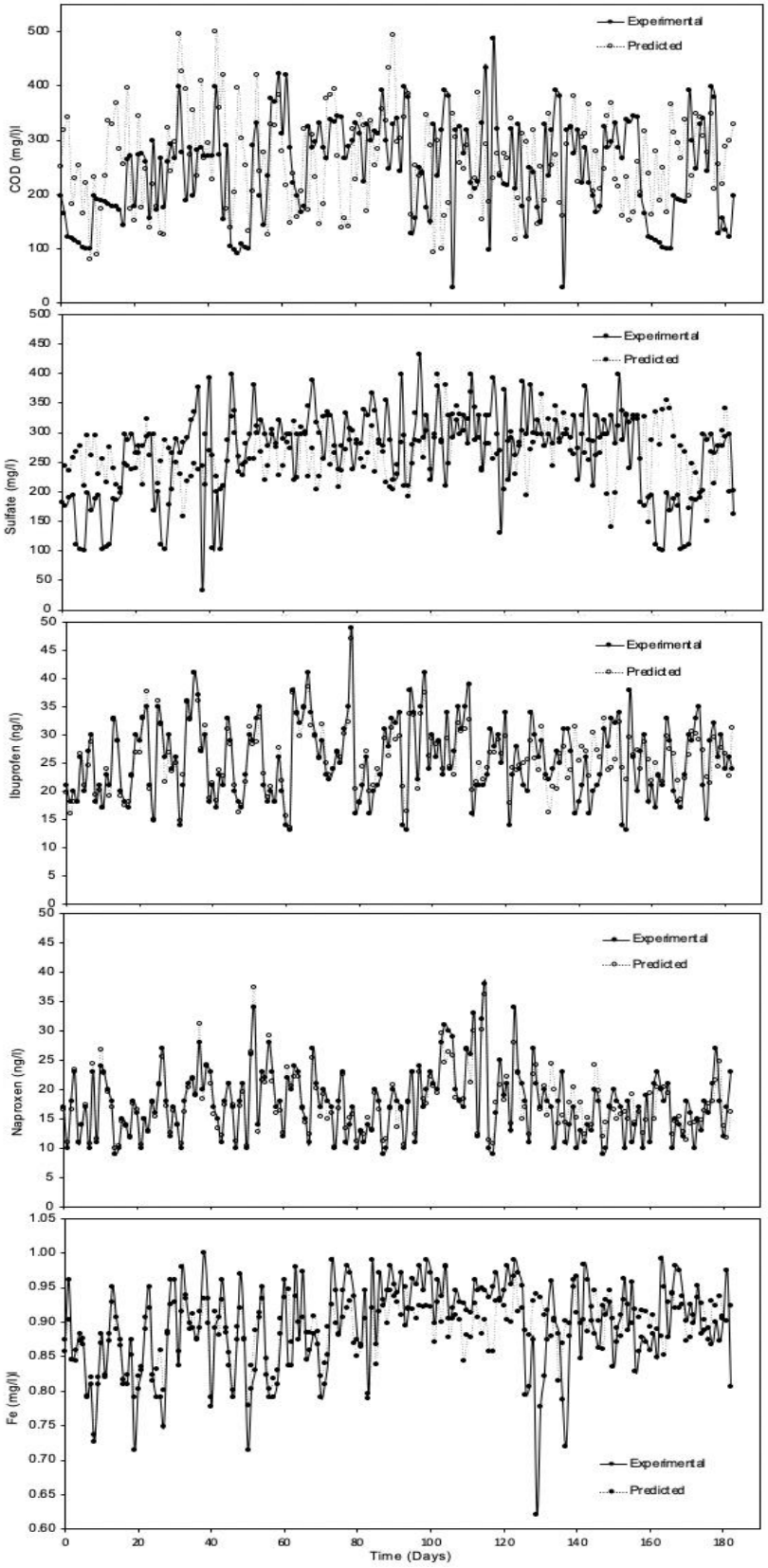

Figure 5.8 Measured and the neural network prediction of fluidized-bed reactor effluent parameters

The test and the validation set errors are only 0.02% apart which demonstrates any significant change in the fitting scale. The *R* values were recorded as 0.98 and 0.96 for test and validity, and the predicted output *R* was found to be 0.97.

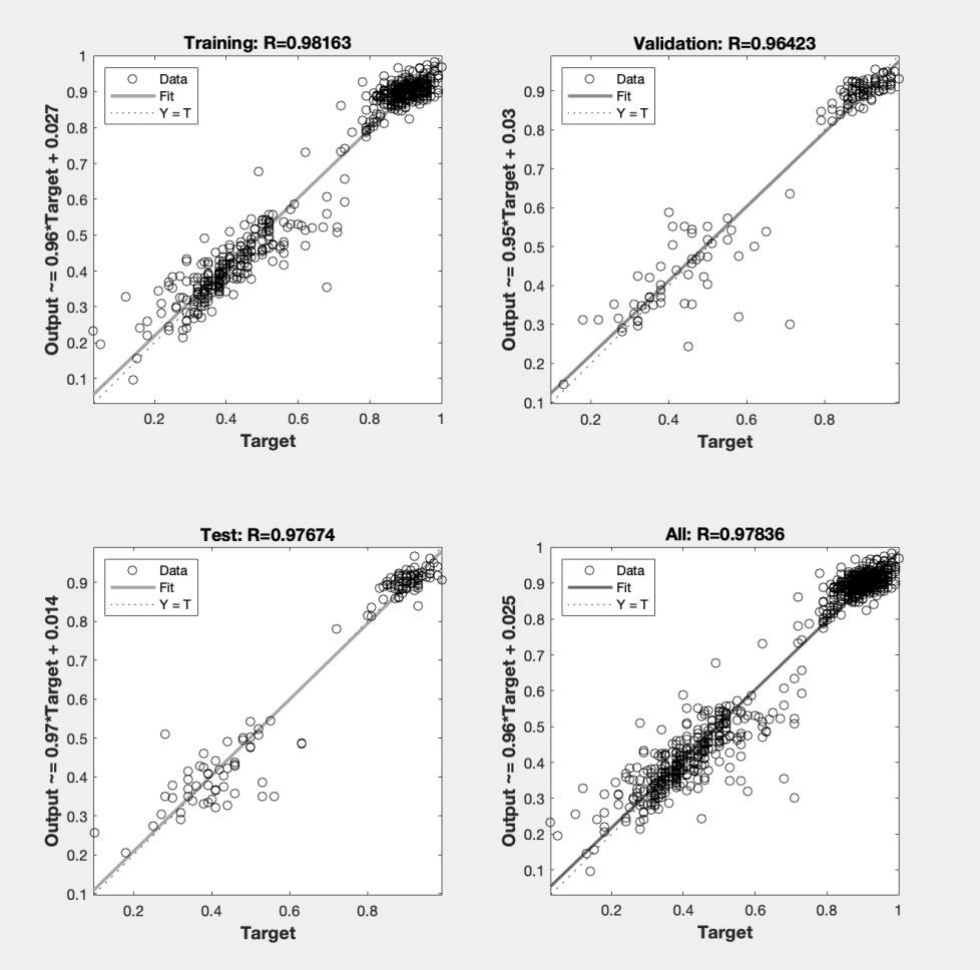

Figure 5.9 Training, validation, and test MSE and linear regression in all targets for Levenberg-Marquardt algorithm

5.4 Conclusions

In the analysis, an FBR was fed with acidic metal-containing water and pharmaceutical-rich wastewater, to enrich the SRB activity in the treatment system. Efficiency in sulfate removal was more than 94 percent at 3987 mg/l concentration of feed sulfate. The efficiency of COD removal at 4953 mg/l of feed COD concentration exceeded 94%. The successful reduction of COD concentration was attributed to oxidation. Therefore, organic oxidation is the rate-limiting step in

the sulfidogenic treatment of wastewater-containing metal and sulfate. The metal removal was more than 99.9 percent and concentrations of effluent Al, Zn, and Fe below were 0.15 mg/l of these conditions, COD oxidation was incomplete, and sulfide was produced in the FBR. The alkalinity produced by COD oxidation considerably increased the pH of wastewater when the concentration of feed sulfate was less than 3500 mg/l. At an HRT of 8 h, COD oxidation in FBR precipitated 1345 mg Fe/l/day, 130 – 170 mg Zn/l/day, and 543 mg Al/l/day from acidic wastewater and increased the pH from 2.2 to 6.8, due to the formation of metal sulfide precipitate. Overall, this sulfidogenic biotreatment demonstrated the advantages of low sludge production, good effluent quality, and high biomass retention. The developed, trained, and validated model of the artificial neural network provided excellent fits to the sulfate, COD, naproxen, ibuprofen, and Fe data obtained experimentally. Models developed in this study can be employed to predict the effluent quality of the HWW and AMD co-treatment sulfidogenic system using fluidized bed reactors, for the purpose of protecting the environment. Even though these results were obtained from laboratory-scale experiments but may be useful to the process engineering team for design initiatives in the wastewater treatment plant. The knowledge of physiochemical processes and other outcomes of water quality in this work bears viability for up-scale co-treatment system which may benefit the municipality.

5.5 References

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CHAPTER SIX

Enriched co-treatment of pharmaceutical and acidic metal-containing wastewater with nano zero-valent scale

This chapter presents a technique of a nano zero-valent iron (nZVI)-enriched co-treatment for AMD and pharmaceutical-rich wastewater where it is evaluated for simultaneous removal of various heavy metal ions, organic pollutants, sulfates, the efficiency of the treatment system, and separation of reaction products in the fluidized-bed reactor. The biotreatment process is monitored through an oxidation–reduction potential (Eh) for 90 days. This enriched cotreatment system exhibited a high reducing condition in the reactor, as confirmed by Eh; hence, the nZVI was dosed only a few times in biotreatment duration, demonstrating a cost-effective system. This chapter contributes new data for an nZVI enriched treatment strategy of two combined waste streams and for the first time the system measures the pharmaceutical compounds in wastewater. As a result, a major contribution to existing knowledge in the field of environmental engineering. This chapter has been peer-reviewed and published as a research article in *Minerals* journal (see Appendix A3 for the article abstract).

6.1 Introduction

Acidic mine water resulting from the open mine tailings often contains sulfates and a complex mixture of heavy metal ions. These mine tailings are characterized to have a high concentration of sulfide containing minerals, such as pyrite (FeS_2). As a result, pyrite‘s bio-hydro-geochemical weathering with other sulfide containing minerals in oxidizing conditions produces acid mine drainage (Masindi et al., 2017). According to Masindi and Muedi (2018), the formation of acid mine drainage (AMD) may be represented as:

$$\mathbf{2FeS_2 + 6O_2 + 4H_2O \rightarrow 2Fe^{2+} + 4SO_4^{2-} + 8H^+} \qquad \textbf{Equation 6-1}$$

Through sulfide oxidation to sulfate solubilizes the Fe^{2+} Equation (6-1), then oxidized to Fe^{3+} Equation (6-2):

$$4Fe^{2+} + O_2 + 4H^+ \rightarrow 4Fe^{3+} + 2H_2O$$ **Equation 6-2**

Microorganisms, such as iron-oxidizing bacteria, can be catalyzed by microorganisms that draw energy from oxidation reactions. Then Fe^{3+} produced may also oxidize FeS_2 and also be reduced into Fe^{2+} Equation (6-3):

$$2FeS + 14Fe^{3+} + 8H_2O \rightarrow 16Fe^{2+} + 2SO_4^{2-} + 16H^+$$ **Equation 6-3**

Generally, acid mine drainage contains metal concentrations that pose a risk to the environment and is strongly acidic. In South Africa, the mining industry is one of its economy's driving industries (Naidoo, 2017); on the other hand, this industry's impact on the environment through the generation of acid mine drainage (AMD) is a call for concern. More so, a crisis in abandoned mining sites is the less attention given to remediate AMD, resulting in the significant environmental problem in South Africa (SA) and globally. AMD remediation for heavy metal removal includes adsorption, biosorption, ion-exchange, filtration, precipitation, and stabilization (Masindi and Muedi, 2018).

AMD is not the only challenging water pollutant in SA; pharmaceutical compounds originating from hospital wastewater (HWW) are frequently found in natural water bodies and increasingly become a persistent pollutant requiring attention. Hospital wastewater is among the commonly found effluent in urban water streams, mainly pharmaceutical, toxic chemicals, and pathogens as dominant pollutants (Gumbi et al., 2017; Madikizela and Chimuka, 2017). Regrettably, all these pollutants are typically pumped to the municipal wastewater treatment plants (WTTPs), regardless of the call to hold the wastewater on-site for treatment before pumping to WTTPs (Chonova et al., 2016). The on-site wastewater retention and prior treatment of HWW may help treat most hazardous pollutants before discharge to the natural environment. The pharmaceutical compounds' chemical and physical properties resulting in their polarity in nature hence resistant to degradation processes in WTTPs. Hence an urge to find new treatment strategies that are more environmentally friendly and minimize acute health threats from HWW. Noticeably, the regulation on pharmaceuticals disposal or their treatment is not as stringent in emerging economies, including SA, in comparison to developed countries. Hence the current design of wastewater treatment facilities do not bear the capacity to remove pollutants like pharmaceuticals. To date, more

methodologies have been investigated to support the integration of growing pharmaceutical pollutants into South Africa's water quality legislation.

A plan to employ a cotreatment strategy for simultaneous remediation of AMD and HWW is proposed after briefly providing the background of these two hazardous pollutants. This proposal is an extension of published studies that cotreated AMD and municipal wastewater, achieving success in reducing metal concentration and sulfates (Johnson and Younger, 2006; McCullough et al., 2008; Strosnider et al., 2011a). In these studies, municipal wastewater (MWW) offered carbon substrate bacterial oxidation to reduce the metals, while pathogens are removed from it as well (Smyntek et al., 2018). The hospital wastewater is much richer in nutrients and organic carbon, making it slightly superior to municipal wastewater, hence promises higher alkalinity production. In the studies by Strosnider et al. (2011a, 2011b; 2013), where MWW was cotreated with AMD, the organic matter from MWW was used to strip-off bacterial oxygen thud reduction in metal concentration and sulfate. As an alternative, an aerobic process is preferred to remove high strength organic matter. This cotreatment strategy's efficacy is better described in Makhathini et al. (2020a); however, the heavy metals such as Pb, As, and Mn were not documented as they experienced a low removal rate in this system. As such, the cotreatment needs to be enhanced to assess its efficacy under the presence of nZVI, specifically to assess the heavy metals.

According to Dong et al. (2019), the nZVI promises a beneficial influence on sulfate-reducing bacteria's development and activity. As such, it is expected that the wastewater under hypoxic groundwater environment, the Fe^0 will be oxidized to ferrous iron, thus increasing the pH value of the treatment system through the discharge of OH^- ion (Xiao et al., 2013). Kumar et al. (2015)assert that the alkaline conditions emanating from Fe^0 oxidation are favorable for developing sulfate-reducing bacteria, and hydrogen and Fe^{2+} may act as energy sources SO_4^{2-}-reduction by sulfate-reducing bacteria. Moreover, Fe(II) has a beneficial effect in hydrogenase synthesis, which is intricate for the sulfate-reducing bacteria (SRB) metabolism process of electron transfer and mass transport (Guo et al., 2017). Generally, the SRB systems are known to take a long time for treatment because of lacking the energy source; however, to increase the remediation efficacy, Fe^0 introduction could assist in speeding up the SRB process (Guo et al., 2017).

Therefore, the SRBs‘ possibility to gain from interacting with nZVI for heavy metals’ removal and stabilization of precipitates is to be tested in this treatment system.

Notably, the main reason for using fluidized bed reactors for this system rather than packed-bed or anaerobic fixed-bed bioreactors is their superiority in retaining the biomass and achieving large mass transfer with less pressure drop. While anaerobic fixed-bed and packed-bed bioreactors have demonstrated good remediation performances for wastewater (Kolmert and Johnson, 2001), yet they are likely susceptible to blockages and channeling, which lessen their efficacy (Gallegos-Garcia et al., 2009). Furthermore, Sahinkaya et al. (2013) and Kaksonen et al. (2003) agree that the fluidized bed reactor (FBR) recycling stream decreases concentrations in the feed, thus rendering these bioreactors most fitting for acid mine drainage. However, just like most treatment systems, the cotreatment of two wastewater streams has shortcomings for one effluent‘s transportation cost to the shared facility (Makhathini et al., 2020b). Generally, AMD is formed some distance away from the urban area where hospitals might be situated; perhaps pumping cost may equip them with the cotreatment system‘s savings. However, some authors have constructed a wetland to treat hospital wastewater (Laber et al., 1999), as such, with an abundance of literature on wetlands from the treatment of AMD (Allende et al., 2014; Johnson and Younger, 2006; Sheoran and Sheoran, 2006; Younger and Henderson, 2014), this gives hope that such cotreatment strategy may be possible to set up.

This work reports on the application of nZVI as an enhancing reagent in the cotreatment of AMD and HWW using fluidized-bed reactors for the removal of heavy metals and toxic pollutants. A 2000 mL FBR was fed with nZVI slurry and operated continuously at a flow rate of 1.5–3.0 m^3/h. The nZVI slurry was fed from the top of the column while the effluent‘s feed was from the bottom, hence the slurry‘s suspension. The main objective was to assess the treatment process‘s efficiency while treating the real stream of AMD with a high content of heavy metals and sulfates, together with a real stream of HWW with a high content of organic matter, including the pharmaceuticals. Furthermore, the study seeks to contribute to AMD remediation literature using cotreatment strategies, thus guiding future design.

6.2 Materials and Methods

6.2.1 Materials and initial experiments

nZVI supplied by Romachem (SA) with a specific surface area recorded as approximately 20 m^2/g. Initially, to prepare the nZVI slurry, 250 g of nZVI particles were mixed to 1000 mL of deionized water to check the slurry‘s consistency. After this, the preparation was done to fill a 5000 mL container at a final *w/w* Fe to water ratio of 0.25 (Ribas et al., 2017). The Fe^0 content in the slurry was measured to be 93%, based on the amount of hydrogen gas emitted when iron nanoparticles react at a volumetric volume of 60% with the addition of sulfuric acid (Liu et al., 2005). There was no activation procedure investigated to enhance the reactivity of surface nZVI particles before application. The slurry was then continuously stirred at ±20°C while monitoring electrode potential (E_h) and pH using oxidation–reduction potential and pH electrodes. Later after the E_h of the slurry was observed to have stabilized using the oxidation–reduction. Sampling was done at 60 min intervals, where at least 20 mL samples were withdrawn then filtered using 0.2-micrometer membrane filters.

The AMD samples were collected from an abandoned coal mine in Mpumalanga, South Africa. To investigate the nZVI concentration effect, batch experiments were set up in five 650 mL glass bottles sealed with plastic caps with a 5:2 *v/v* of HWW to AMD mixture (pollutant characteristics listed in Table 6.1) and an nZVI slurry prepared as the different mass dosage of nZVI between 0.5 and 12 g/L. Each mixture in a batch reactor of AMD and HWW was dosed with a different slurry concentration.

An additional two bottles without nZVI were also set up in parallel to the five bottles as control experiments. The batch reactors were agitated at room temperature on a shaker at 280–310 rpm. A selected number of heavy metals like Pb, Zn, and Mn, which were not previously removed, were of interest here; hence they were measured using Inductively Coupled Plasma – Optical Emission Spectrometry ICP-OES (Varian 720-ES). After optimizing the slurry concentration, the slurry concentration was kept between 0.5 and 2.5 g/L. The fresh slurry was only made twice in the biotreatment duration since there was less demand for it (more details are given in the discussion session).

Table 6.1 Characterization of the acidic mine water samples

Parameters	Acidic Mine Water	Hospital Wastewater
pH	±2.1–3.8	±6.2– 7.9
COD (mg/L)	±98	±156–1234
PO_4^{3-}	±11–31	±9.8–14
NH_4^+	±34–87	±34–87
Total nitrogen (mg/L)	±21–123	±45–223
Aluminum (mg/L)	±275	<2
Potassium	±34	<1
Iron (mg/L)	±5329	±12
Sodium (mg/L)	<0.05	-
Calcium (mg/L)	±210	<6
Copper (mg/L)	±112	±56
Zinc (mg/L)	±145	±23
Manganese (mg/L)	±113	-
Magnesium (mg/L)	±243	±29
Sulfate (mg/L)	±4900	±134
Turbidity (NTU)	±46– 55	±11– 52
Conductivity (µS/cm)	>82	>413
Naproxen (µg/L)	-	2.39 ± 0.75
Ibuprofen (µg/L)	-	8.72 ± 0.98

6.2.2 Fluidized-bed reactors

An existing laboratory-scale fluidized bioreactor was converted to an nZVI reactor (Figure 6.1). The reactor used an effective bed volume of 1400 mL, which was packed with silica sand as a biomass carrier; further details on the rector specification are discussed on Makhathini et al. (2020a). As part of the initialization stage, the bioreactor was on recycle operation for 24 h, and the bed fluidization was kept approximately 15–25% after the biofilm was developed.

The hydraulic retention time (HRT) was not rigorously varied since the previous study had given some insight into its effect (Makhathini et al., 2020a). This study gradually reduced the HRT from 24 to 16 then later to 8 h while maintaining the feed rate relatively constant. The mixed stream of pump 1 and pump 2 delivery lines were only sampled four times with only a few contaminants measured, for example, COD, pH, Zn, Pb, Mn, sulfates (Table 6.2). The pharmaceuticals were not measured in the reaction chamber, and the assumption was that there is no change in the initial values from HWW.

Table 6.2 Measurements of acid mine drainage (AMD) and hospital wastewater (HWW) at the mixing point of 2:5 *v/v*.

Parameter	HWW + AMD
Zinc (mg/L)	138 ± 11.2
Manganese (mg/L)	102 ± 7.8
Lead (mg/L)	114 ± 10.1
SO_4^{2-} (mg/L)	5 210 ± 12.3
COD (mg/L)	1523 ± 20.9
pH	± 6.8–8.1

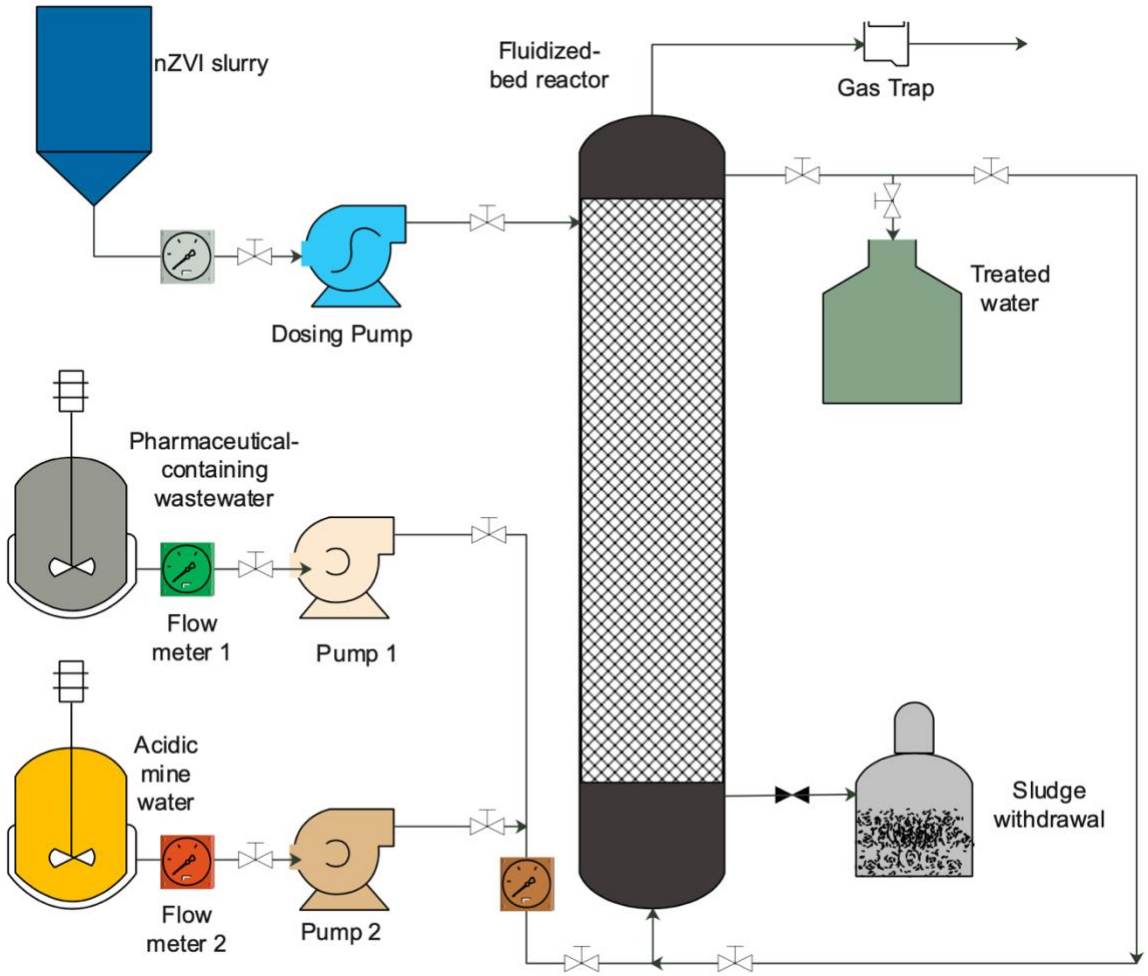

Figure 6.1 The experimental se-up for the fluidized bed reactor

AMD and pharmaceutical-containing wastewater composite sample at a ratio of 2:5 was pumped to the reactor; simultaneously, the slurry of nZVI was pumped at 350 mL/min. The strength of reduction circumstance generated by Fe^0 oxidation may support SRB development, which may already exist by adding highly organic pharmaceutical-containing wastewater.

6.2.3 Analytical procedures

All samples collected from the field we filtered with 0.4 microfilters before analyses. Using the calibrated PHS-3BW pH meter maintained as recommended by the supplier, the temperature, conductivity, and pH were measured. To specify the alkalinity and acidity of the samples, the auto-titrator Thermo Science Orion Star T900 (Thermo Fisher Scientific Inc., Massachusetts, USA) was used. The unfiltered samples were analyzed for COD using the APHA (2012) standard methods

applicable to the Hach DR3900 spectrophotometer (Hach Company, Colorado, USA). For NH_4-N, sulfide, sulfate, and metals, filtered samples were analyzed by APHA (2012) standard methods.

A Hach DR3900 spectrophotometer was used to quantify the COD, sulfate, and sulfide. For sulfide measurement, each sample cell was filled with 10 mL of deionized water. Then 10 mL of sample was added to a second sample cell, mixed carefully to prevent sulfide loss. A 0.5 mL sulfide reagent one was pipetted to each sample cell, then swirled carefully. Then 0.5 mL of sulfide reagent two was added to each cell; again, the sample cells were then inverted to mix and then left to react for five minutes. After the time-lapse, the blank cell was used to reset the instrument (DR3900), then the cell with the sample was read.

For COD concentration measurement, the DRB200 (Hach Company, Colorado, USA) was prepared by preheating the temperature to 150°C. While waiting for the instrument to get ready, a 100 mL sample was homogeneously mixed in a blender for 30–60 s. Using the TNT plus vials, 0.3 mL of sample was pipetted inside then the vial was inverted several times for proper mixing. The vials were then left in a closed preheated DRB200 reactor for 2 h. After the lapse, the vials were taken out of the reactor, cooled to room temperature then inserted in the cell holder to produce a reading. The dissolved organic carbon (DOC) was measured using Hach standards methods.

Each sample cell was filled with 10 mL of sample for sulfate measurements; then, a SulfaVer 4 powder pillow was added to dissolve the powder completely. After this, the cell was left to complete the reaction for five minutes. After the time-lapse, the blank sample cell was inserted in the cell holder of the DR3900 to reset the instrument, and then the prepared sample was inserted in the cell holder to read the results.

The BOD measuring system required 300 mL incubation bottles and a nitrifying inhibitor (N-Allylthiourea). About 40–100 mL volume of sample was added into the incubation bottles, then few drops of ATH are added to the sample, after which the BOD system was placed for five days in an incubator at 20°C. After five days, the dissolved residual oxygen was measured for all analyzed samples to estimate the BOD.

The total dissolved nitrogen (TDN) measurements were initiated by preheating the DRB200 reactor to 103°C. A total nitrogen persulfate reagent powder pillow was added to two high range total nitrogen hydroxide digestion reagent vials. After this, 0.5 mL of sample was added to one of the vials, and the other vial, which was 0.5 mL of deionized water, was added. The prepared vials were put in the reactor for 30 min; after time elapsed, vials were cooled down to room temperature before second total nitrogen (TN) reagent A was added. A 3-min reaction took place, then TN reagent B was added, allowing a further 2-min for the reaction. On both the prepared sample and on the blank, a 2 mL TN reagent C was added. A 5-min reaction time was allowed then samples were read on the spectrophotometer.

For all dissolved samples, the pH was kept at <2 using concentrated HNO_3, then stored at 4°C until use. Syringe filters with a 0.45 nylon membrane were used to prepare the samples for metal concentration analysis of Al, Fe, Cu, Mg, Pb, Mn, and Zn before injecting into ICP-OES (Varian 720-ES, Varian Inc., Palo Alto, CA, USA). The analyses were performed in duplicate, as recommended by the USEPA protocol.

6.2.4 Pharmaceutical analysis procedure

Ibuprofen (98%) and naproxen (98%) were purchased from Sigma-Aldrich. In contrast, HPLC-grade ethyl acetate (>99.6%) and HPLC–methanol (>99%) was supplied by Macron Fine Chemicals used in the quantification of pharmaceutical compounds in the wastewater samples. Hydrochloric acid (37%) and hexane (approx. 60%) were purchased from Merck (SA). A flow rate of 1 mL/min was set for the mobile phase at acetonitrile to formic acid ratio of 3:2, v: v.

A solution was made in acetonitrile containing 100 mg/L concentration for the targeted compounds, using the high-performance liquid chromatography (HPLC) (Perkin Elmer, Wellesley, USA) instrument. The detection limit, quantification limit, and linearity limit were determined during this test. The method validation was tested using a deionized water sample mixed with naproxen and ibuprofen at an instant addition of 50 to 5 microliter concentration, in that order. The concentrations were determined from known values from SA WWTPs. Before each sample quantification, the solid-phase extraction (SPE) method was used to preconcentrate the sample. The method is described, and its results are presented in Section 6.6.

6.2.5 Sludge characterization

The sludge samples were prepared for analysis using developed methods (Newbury and Ritchie, 2015; Utgikar et al., 2001). The sample was withdrawn at the bottom of the reactor at the end of the treatment period. The sludge was stopped from further reacting by adding 2% glutaraldehyde solution for approximately 15 min. After this, the loose sludge was filtered using a vacuum filter with 0.5 μm pores. The filtered residue was maintained under vacuum at −70 °C for at least three days. Before measuring SEM, the samples were allowed to warm to ambient conditions. Each sample was prepared in triplicate to ensure consistency and to evaluate the reliability of the results. Characterization of the sludge used for immobilizing the biological treatment biomass was carried out (Janssen et al., 2001) a field emission gun (FEG) of the Thermo Fisher Nova NanoSEM (Thermo Fisher Scientific, Waltham, USA). The EDS analysis was performed using the Oxford X-ma detector with Oxford INCA software (version 4.0, Oxford Instruments, UK).

6.3 Results and Discussion

There are influential factors that may drive metals concentrations and the growth of sulfate-reducing bacteria in the sulfidogenic environment for this cotreatment system with nZVI. As such, the results are presented to assess the optimum reaction conditions and influential factors thereof. These factors include alkalinity, sulfide, organic ions, are given below.

6.3.1 Batch experiments

Since this study continues from the published work of Makhathini et al. (2020a), there was no need to assess treatment process feasibility. However, nZVI in the biotreatment was to be explored for its possibility in this treatment system. The batch experiments were then conducted to evaluate the nZVI removal viability in the sulfidogenic bioreactor treating AMD and pharmaceutical-containing wastewater. The nZVI was tested for the removal of Pb(II), Mn(II), and Zn (II) ions simultaneously with sulfates as well and was found to be successful in this regard. The removal efficiencies recorded well over 90% of Mn, 92% of Zn, 94% of Pb, and 80% of sulfates in 60 min (Figure 6.2).

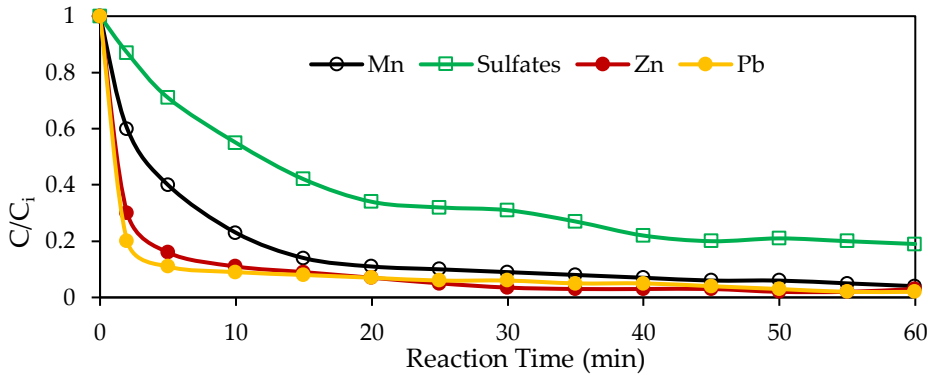

Figure 6.2 Removal of heavy metals from a mixture of acid mine drainage and pharmaceutical-containing wastewater at an initial pH of 5.6, using nano zero-valent iron (nZVI).

Guo et al., (2017) warn that the presence of high nZVI concentration may potentially trigger oxidative stress on bacterial development. In agreement, the study of Kumar et al. (2015) stated that when the nZVI dosage exceeded 1.0 g in a liter, the SO_4^{2-} the decline was significantly inhibited. While Velimirovic et al. (2015) suggest that the microbial community activity can be hindered if the addition of nZVI concentration exceeds 0.05 g/L. In contrast, Kirschling et al. (2010) demonstrate that a sufficient concentration of nZVI could stimulate SRB development, and it is helpful to eliminate pollutants. Since this current study sought to reduce the heavy metals and the sulfates, it is for this reason that the optimization of the nZVI dosage was initiated (Figure 3).

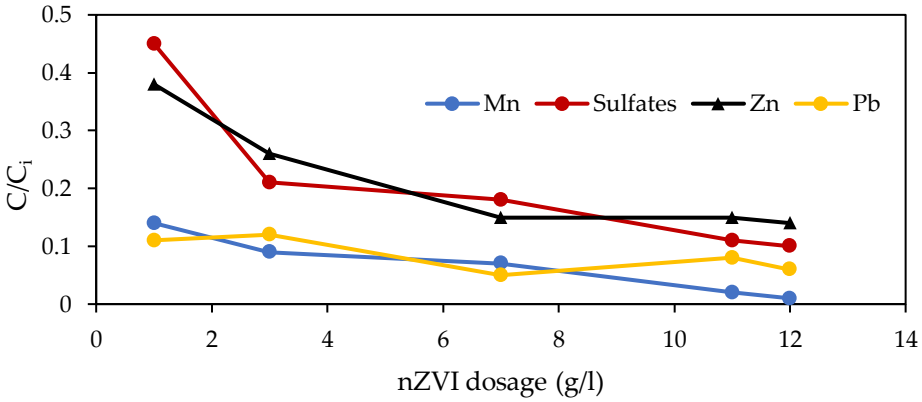

Figure 6.3 The dosage of nZVI with time testes over 24 h.

6.3.2 Reaction mechanism

Organic carbon, mainly from the HWW, serves as the energy source for the sulfate reduction and removing metals from AMD by SRB in the bioremediation process. However, in the nZVI with

HWW + AMD treatment configuration, hydrogen and Fe(II) ions originating from Fe^0 oxidation may also act as an energy source for the sulfate reduction (Velimirovic et al., 2015) in a shorter time. In a case where heavy metals are involved, as is in this study, several pathways of their removal are suggested by Dong et al. (2019) as biosorption, co-precipitation, reductive, and sulfide precipitation (Figure 6.4).

The SRB cell membrane and extracellular synthetic polymers will directly touch the heavy metals (Venzlaff et al., 2013) and adsorb them by physical or chemical adsorption to the nanoparticle membrane (Liu et al., 2018). The ferrous ions released from Fe^0 corrosion may react to produce iron sulfides, which are otherwise relatively effective adsorbents for heavy metals (Zhang et al., 2017).

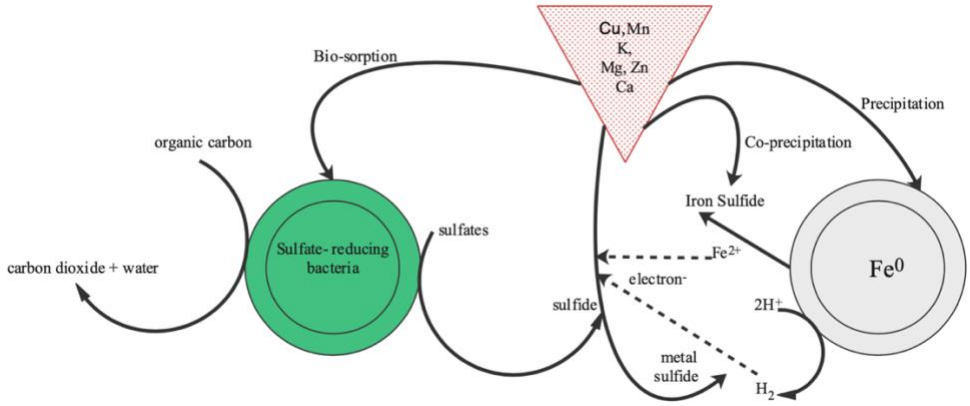

Figure 6.4 The elimination pathways of heavy metals in the (HWW+AMD)–nZVI system.

For the reductive precipitation, as a reducing agent, nZVI reduces certain heavy metals like Pb^{2+}, Zn^{2+}, and Mn^{2+}, then develops insoluble metal precipitates, disintegrate heavy metals, and mitigate environmental hazards (Liu et al., 2017). The SO_4^2 can also be decreased by SRB metabolism to the sulfide, which may oxidize to the sulfide precipitates with metal ions (Bai et al., 2012). Notably, the above removal pathways occur concurrently. However, the nZVI remedial strategy‘s primary removal pathway is reductive precipitation and adsorption, a critical elimination pathway in the SRB treatment process (Ravikumar et al., 2018).

6.3.3 Influence of pH and the source of carbon

As mentioned earlier, this study used hospital wastewater as the organic substrate for SO_4^{2-} and microbial growth, basically using the following reaction Equation (6-4):

$$\mathbf{organics(COD)} + \mathbf{SO_4^{2-}} + \mathbf{H_2O} \rightarrow \mathbf{H_2S} + \mathbf{2HCO_3^-} \qquad \textbf{Equation 6-4}$$

COD mass for desulfurization was based on the stoichiometry of 1 g of SO_4^2 be reduced, 0.67 g of COD is desired (Mulopo, 2016); thus, the alkalinity of approximately 1.042 g is formed. The SRB development is optimal at a pH of 6.5 but still favorable from 5–9 (Dong et al., 2019). In this study, the pH was at an average of 6.4, at least for phases II and III. In phase I, the pH was averaging at 6.9; thus, the metal removal was rapid. The addition of Fe^0 raises the alkalinity value within a turn, improves the activity development of SRB. The response in alkalinity and pH is presented in Figure 6.5. Moreover, after phase I, which was mainly bacterial acclimation and reaching sorption capacity, from phase II, reducing conditions caused by high SRB start to increase the alkalinity, proving metal sulfide precipitation (Márquez-Reyes et al., 2013; Song et al., 2012).

When Kumar et al. (2015) examined the impact of oxidation–reduction potential and the pH on the strength of Zn(II) precipitates in the ZVI and sulfate-reducing bacteria with ZVI systems. They found that column experiments signified Zn precipitates‘ strength as less sensitive to ORP change but significant sensitivity to pH change. Sulfide concentration was recorded as low as <0.05 mg/L for most of phases II and III. Simultaneously, the alkaline production increased during phases II and III, which could be attributed to a reduction in sulfate concentration.

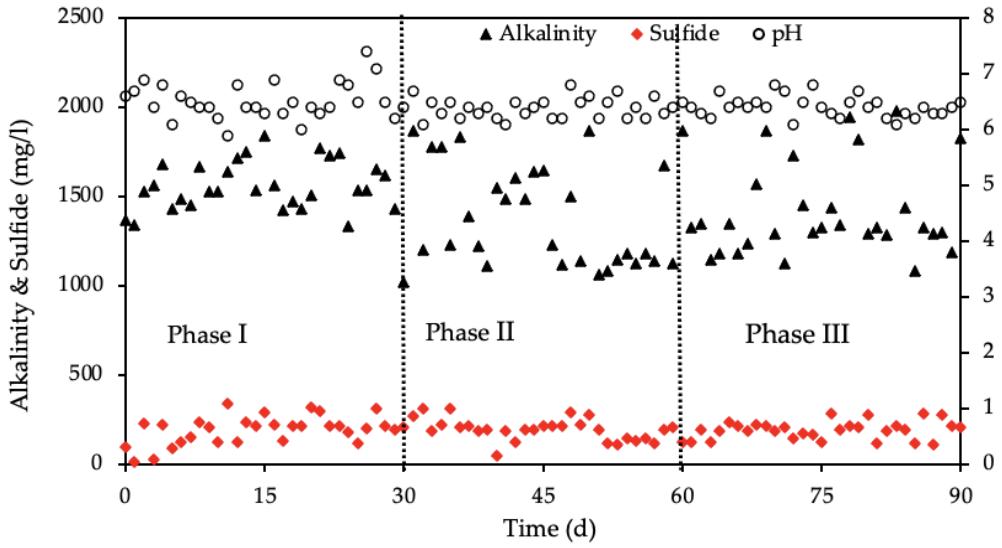

Figure 6.5 Alkalinity, pH, and sulfide response with time in the fluidized bed reactors.

The initial pH on SO_4^{2-} reduction and final pH indicates that Fe^0 positively affects pH and may increase it to support the SRB activity. In Fe^0 enhanced processes, like this current study, alkalinity may be generated by anoxic corrosion of F^0 Equation (6-5) and SO_4^{2-} Equation (6-6) since both equations use hydrogen ions.

$$\mathbf{Fe^0 + 2H_2O \rightarrow Fe^{2+} + H_2 + 2OH^-} \qquad \textbf{Equation 6-5}$$

$$\mathbf{SO_4^{2-} + 4H_2 + H^+ \rightarrow HS^- + 4H_2O} \qquad \textbf{Equation 6-6}$$

Nitrogen and organic carbon concentrations were almost certainly influenced by precipitation in phase I of the treatment system. An overall observation of BOD, DOC, and TDN proved a decrease over the first 30 days, phase I (Figure 6.6). It is most likely that the bacterial SO_4^{2-} reduction facilitated processing of the BOD and DOC (W. H.J. Strosnider et al., 2013). In the FBR, the inorganic nitrogen was introduced from the HWW sample in ammonia form; at the end of phase II, 9–28% NH_4^+ was measured. The DOC concentrations were evidently following the metal concentration decline trend. Nitrate and nitrite were recorded to be reasonably low at ≤0.1 mg/L and ≤5 g/L, in this order.

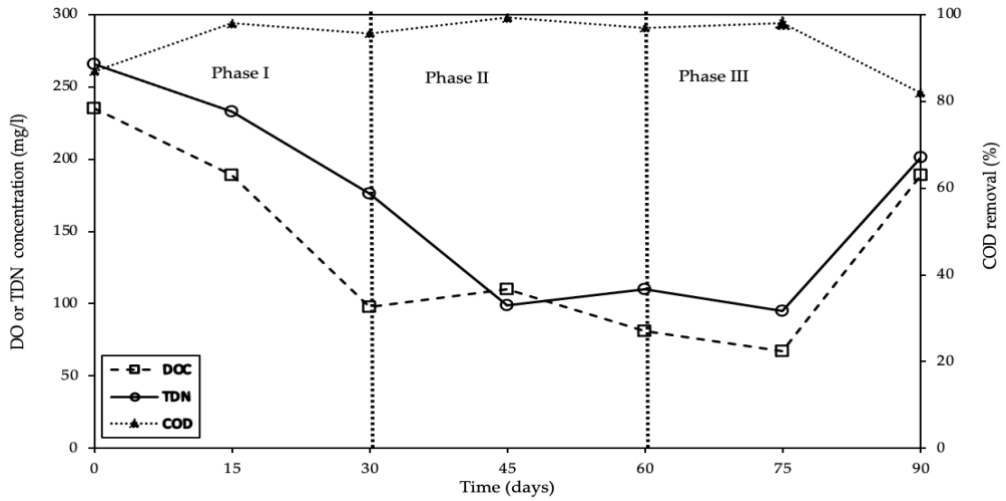

Figure 6.6 Total dissolved nitrogen (TDN), dissolved organic carbon (DOC) and organic matter (COD) concentrations at different phases of treatment.

6.3.4 Effects of COD and sulfates

Throughout the first phase of treatment, there was a substantial reduction of sulfates and COD concentrations (Figure 6.7). Although the removal of metals and phosphates was high, 88% PO_4^{3-}, sulfate, and other organic matter were moderately reduced. Smyntek et al. (2018) highlighted that SO_4^{2-} reduction rates are likely to be affected by establishing the microbial ecosystem or lack thereof. Evidently, during phase I, the FBR was not afforded enough time to develop a microbial ecosystem, which may have resulted in complete SO_4^{2-} reduction (Deng et al., 2016). In addition, there is well-documented literature supporting the fact that sulfate reduction is dependent on COD, and the ratio of SO_4^{2-}/COD changes with the type of organics available (Neculita et al., 2007; Sánchez-Andrea et al., 2014).

The second phase of treatment confirmed the bioreactor‘s reducing setting was given by redox potential measurements of −101 to −190 mV depending on the COD level on the FBR. Since the hospital wastewater samples had higher total suspended solids (TSS) than mine water samples, 478 and 2 mg/L, respectively, TSS significantly increased in the FBR during phase I. However, in phase II there was a reduction in TSS to the range of 5–24 gm/L. On the contrary, the total dissolved solids (TDS) decreased s phase I due to subsequent phase I formation of precipitates in the FBR. After this, there was no significant reduction of TDS in phase II observed.

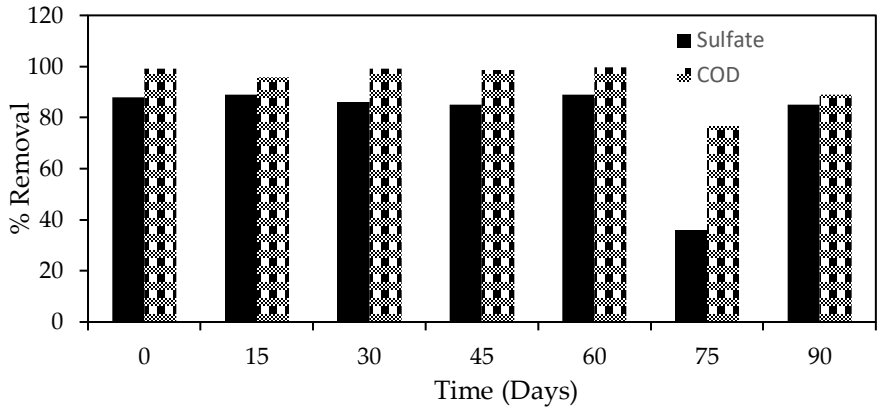

Figure 6.7 The removal rate for sulfate in comparison to COD.

6.3.5 Reactor monitoring using E_h

The nZVI was rapidly mixed using the laboratory mixer before its use to reduce conditions by even dispersing the resin in water. As such, the E_h in the FRB reduces below −310 mV instantly after the introduction of nZVI. There was no agitation in the FBR; influent was fed from the bottom, stabilizing fluidization and even dispersion on nZVI in the reactor. The control of the bed volume was carefully controlled by adjusting the influent while optimizing the dosage on nZVI (Figure 2). The Eh of the reactor contents was also used to monitor the FBR treatment performance, making necessary adjustments to nZVI dosage and the influent feed. It was evident that an automatic control strategy to regulate the nZVI and feed pump responding to Eh value could have made the treatment system more stable. This system's manual operation may compromise the reactor's performance, as there are times when the time elapsed without any response from the pumps, whereas the Eh was way below −200 mV. Li et al. (2017) advised monitoring redox-potential in the nZVI enhanced reactor promises a quick, consistent, and efficient approach to managing the treatment performance. Consequently, by managing the treatment system, there may be less need for nZVI and, therefore, lower the cost of treatment (Babel and Kurniawan, 2003).

6.3.6 Metal concentration removal

Two key factors may decide the toxic effects on metals for a biological treatment process that depends on SRB: the type of metals and their associated concentration. As such, the treatment process' performance is assessed by Fe^{2+}, Zn^{2+}, and Mn^{2+} removal, as highlighted in Table 6.3. The average concentration of Zn(II) was lowered from 144 to 0.55 mg/L and Mn(II) from 113 to 0.21 mg/L in the final reactor discharge, achieving an average of more than 99.8% removal

efficiency. The results suggest that during phase I, the metal removal may be attributed to sorption on organic waste and precipitation of hydroxides and carbonates (Kieu et al., 2011).

Table 6.3 Metal concentrations removal and standard deviation (SD).

Period	Days		Pb	Cu	Zn	Fe	Ca	Mn
Total metals in influent (mg/L)								
I	0–30	Conc.	33.12	112	421	3,112	212	355
		SD	0.11	1.1	3.4	17	1.3	0.1
II	31–60	Conc.	18.1	59	81	589	119	117
		SD	9	0.2	2.1	7	1.2	0.2
III	61–90	Conc.	12	21	44	187	211	45
		SD	4	1.1	1.1	12	1	2.1
Total metals in effluent (mg/L)								
I	0–30	Conc.	12.1	1.02	0.2	11.9	2.1	<0.02
		SD	1.9	0.1	0.01	1.3	0.09	<0.02
II	31–60	Conc.	9.9	1.12	0.7	17	1.8	<0.05
		SD	2.3	0.4	0.3	1.4	0.01	<0.05
III	61–90	Conc.	-	<0.05	<0.05	19	<0.05	<0.05
		SD	-	-	-	1.1	-	-

For the whole of 90 days experimental run, the influent concentration of Mn, Fe, and Zn was spiked up and at times exceeded 350, 5,000, and 420 mg/L, respectively. The spike was done deliberately to shock the system for simulation of the field experience. Regardless of these simulated unsteady state conditions, the effluent was consistently achieving less than 2 mg/L concentration for all metals, like K, Na, Ca, and Mg. These results demonstrate the reliability of the treatment process. Table 3 demonstrates that Pb, Mn, and Zn were removed 96%, >80%, and 92%, respectively, in the first treatment phase. This performance may be due to mine water-binding with organic molecules (Younger et al., 2002; Younger and Henderson, 2014). On the other hand, the sulfates were reduced by 70% in phase I, which is lower than the previous studies published for similar FBR application in the absence of nZVI (Makhathini et al., 2020a). At the end of phase III, the

increase in Fe could well be due to microbial Fe decrease of $FePO_4$ and $Fe(OH)_3$ (Clyde et al., 2016; W. H.J. Strosnider et al., 2013). FeS precipitated due to the sulfate-reducing environment that is dominant in this period (Kiran et al., 2017). Lastly, metal biosorption to the organic molecules related to the metabolism mechanism could be the reason for metal removal (Deng et al., 2016; Peer et al., 2015; William H. J. Strosnider et al., 2013).

Having incorporated nZVI as an enhancement tool for removing a wide variety of contaminants from chemical solutions; however, the current study focused on evaluating material efficiency for the bioremediation process of two real streams. The performance of nZVI for pollution prevention for groundwater depends on bioremediation as a parallel method to hinder metalloid and metal discharge (Li et al., 2017). Nonetheless, reactions that are microbially facilitated with Fe may assist and prevent removal reactions. Like, ferric ion decreasing microorganisms, it may lessen most high-valence metal pollutants, i.e., Cr, over intended enzymatic decrease and through unintended reduction catalyzed by biogenic ferrous ion (Crane and Scott, 2012).

6.3.7 Pharmaceutical compounds assessment

The chromatograms generated for the separation of naproxen and ibuprofen compounds are shown in Figure 8. These pharmaceutical compounds were separated using the HPLC column (C18) with the resolution values above 1.4. As described by Madikizela and Chimuka (2016), the sensitivity of the method was measured by the limit of quantification (LOQ) and limit of detection (LOD).

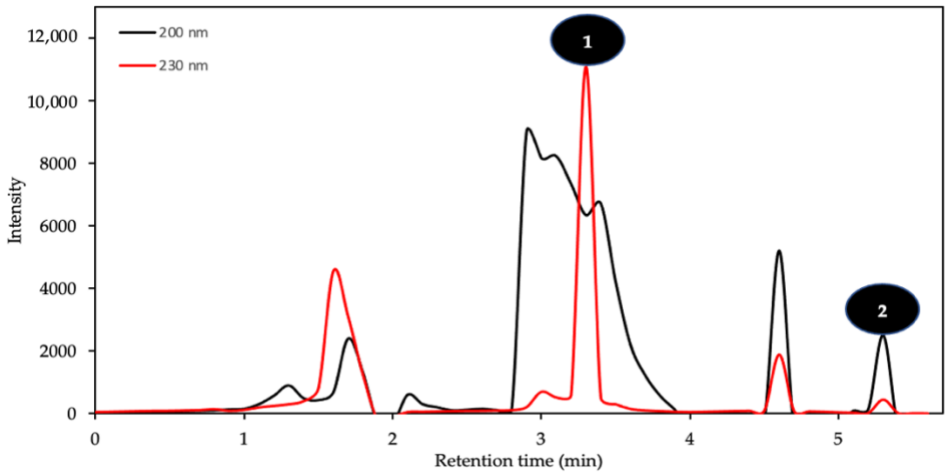

Figure 6.8 Naproxen (1) and ibuprofen (2) chromatograms 1 × 10^3 μg/L at 200 and 230 nm.

The results shown in Table 4 demonstrate that this method may be applied to target low levels of compounds, i.e., μgL^{-1}. Published studies quantified the same compounds using HPLC and diode array detection (Agunbiade and Moodley, 2014; Amdany et al., 2014; Madikizela and Chimuka, 2016). Solid-phase extraction (SPE) efficiency validated the analytical method, which was higher than 80 percent for naproxen and ibuprofen compounds. The linearity was found to be $r^2 \geq 0.97$, which demonstrates the acceptable method accuracy. The values of the SPE efficiency are given in standard deviation to ensure method precision further. When looking at the SPE production and linearity, the bioreactor effluent is identical to deionized water. Table 6.4 sets out the results of the validation.

Table 6.4 Analytical method validation.

Pharmaceutical matrix	**Effluent**		**Deionized**	
	Ibuprofen	**Naproxen**	**Ibuprofen**	**Naproxen**
SPE efficiency (%)	82 ± 3.4	81 ± 8.7	83	84
Detection limit	1.2–1.9	0.2–1.1	0.3	0.2
Quantification limit	0.5–1.4	2.3–3.7	0.6	1.2
r^2	0.98	0.99	0.99	0.99

The pharmaceutical compounds like ibuprofen and naproxen are in the class of nonsteroidal anti-inflammatory drugs (Madikizela and Chimuka, 2017). These organic compounds are acidic and polar; naproxen and ibuprofen have a pKa value of 4.2 and 4.9, in that order (Dahane et al., 2013). These compounds‘ polarity nature makes it easier for them to escape the wastewater process and contaminate the receiving waters.

Pharmaceutical removal was not as successful as was initially anticipated; however, Figure 6.9 demonstrates a progressive increase in the removal as time lapsed. These results are worse than the previous study on cotreatment of AMD and HWW, but with no nZVI as an enhancing reagent. In this previous study, ibuprofen and naproxen achieved 51% and 34% removal, respectively, at

the end of 90 days (Makhathini et al., 2020a). In this current work, the maximum removal achieved for ibuprofen is 33%. Again, the main factor for this low removal is not clearly understood yet, and it could be that biomass in the FBR consists of microorganisms that are utilizing pharmaceuticals as organic substrates.

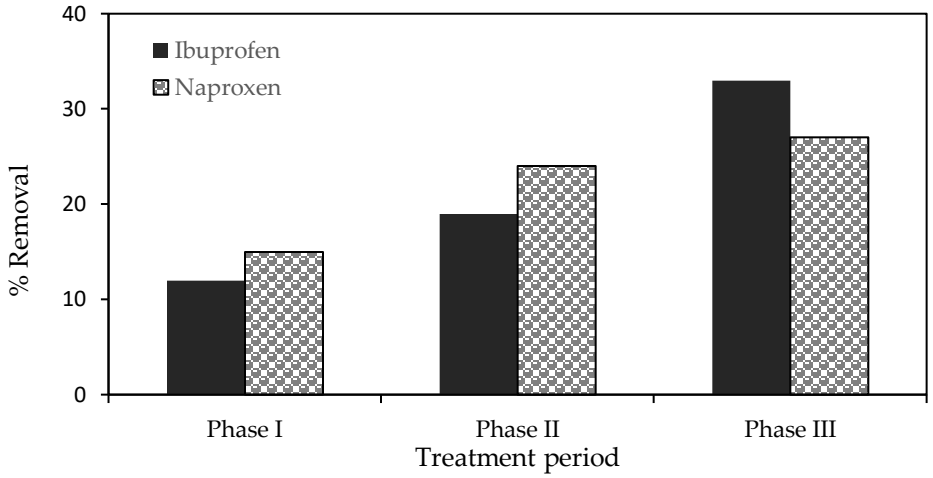

Figure 6.9 The final removal for naproxen and ibuprofen at the end of each treatment period.

6.3.8 Sludge management with nZVI

First, degradation efficiency for nZVI from the FBR is assessed by the ratio of heavy metals to the amount of nZVI dosed in the system. The pharmaceuticals removal was not reported here in the sludge as they showed less to no removal within this system. The removal load for Zn and Mn was approximately 144 mg Pb/g Fe, 198 mg Zn/g Fe and 207 mg Mn/g Fe, correspondingly. By the end of the 90 days treatment period, the combined load of metal ions removed for nZVI was estimated >600 mg metals/g Fe, which is higher than other reports on resins usually used for heavy metal separation.

As seen in Figure 10e, an elevated concentration of toxic elements was detected, which shows that nZVI is a flexible material for metal extraction (Li et al., 2017). However, the image of the morphology in the sludge was different than in other studies that used SRB and nZVI (Fichtel et al., 2012; Zacarías-Estrada et al., 2020) in that it was not rod-shaped. It was expected that there would be small particles on the surface of the cells, which are called extracellular biofilms. However, not identifying the extracellular polymeric substance responding to toxic compounds

may be due to less availability of microorganisms (Redmile-Gordon and Chen, 2017) at the end of the treatment. As such, bacterial biofilms act as nucleation sites for sulfide mineral precipitation (Labrenz et al., 2000); however, in this study, the SEM was only done at the end of phase III (at day 90), which may mean there were no longer available sites for precipitation. In addition, the inadequate removal of naproxen and ibuprofen could be attributed to no available nucleation sites.

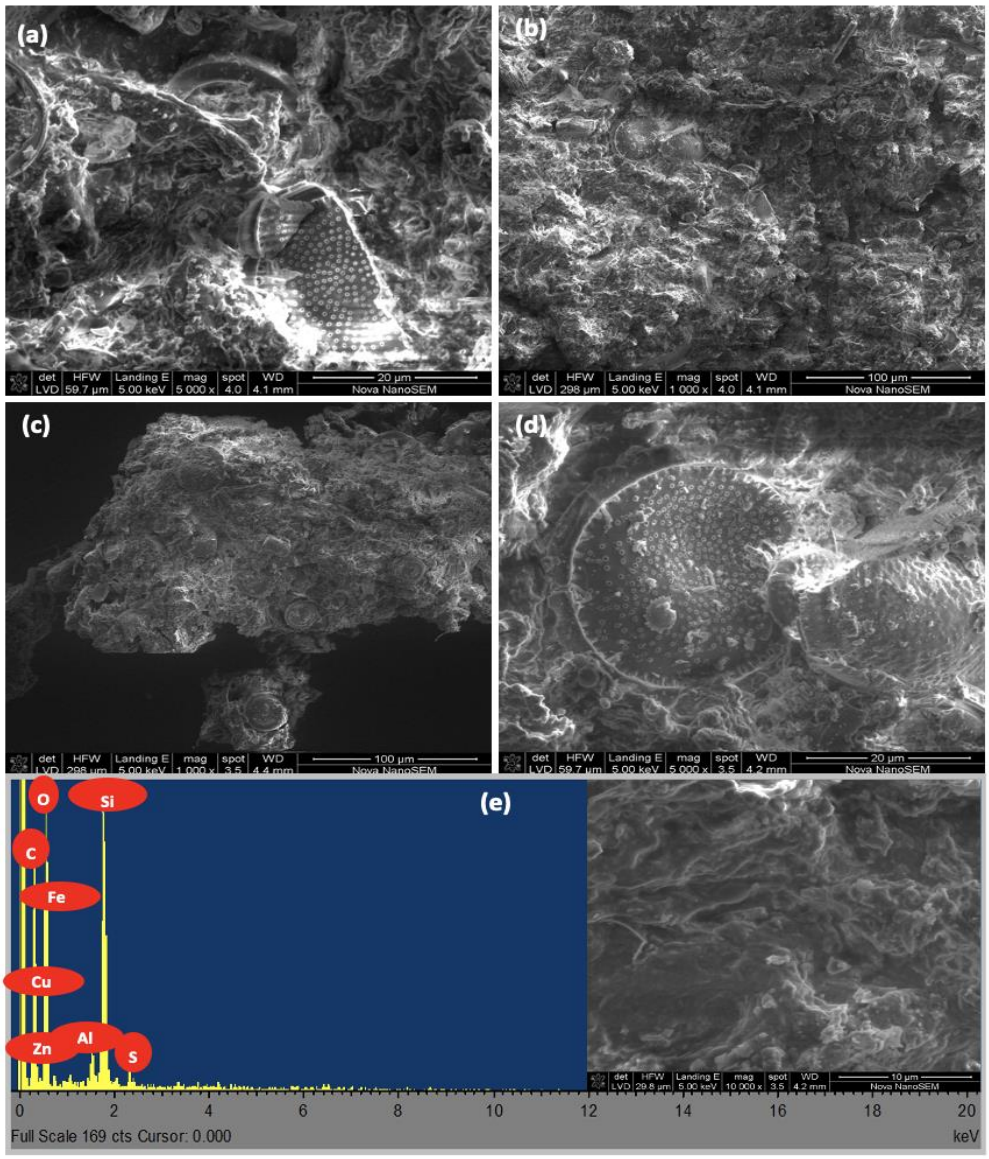

Figure 6.10 SEM photomicrograph of the sludge sample from the fluidized-bed reactor (FBR) (a,b**) at 45 days; (**c,d**) at the end of 90 days and chemical element spectrum of the sludge (**e**) with an EDS scan inserted.**

Since the study could not assess the solids recirculation of the spent nZVI because of its attachment to the silica sand, the hydraulic retention time was kept between 6 and 8 h. All the initially detected elements in the mine water samples were also found in the sludge (Figure 6.10). Both SEM and EDS analysis was performed to further characterize the sludge; when performed alone, the EDS has received an unwelcome reputation as a "semiquantitative" technique (Newbury and Ritchie, 2015). Furthermore, Figure 10a, d illustrates the SEM photomicrograph image of the sludge harvested in the FBR at the end of phase III. The EDS qualitative analysis highlighted signals C, O, Al, Fe, Mn, and Si on the solid phase, in addition to small quantities of Zn in a range of 0.1–1%. These results are slightly different from Kumar et al. (2016), who used zero-valent iron to enhance sulfate reduction, but permeable reactive barriers. Silicon originates from the silica sand used as biomass at the initial stage of the treatment.

6.3.9 Advantage of using nZVI

One of the critical factors when choosing a treatment option after efficacy is the cost. The nZVI specifically is easily re-usable in the treatment process, then the dosage is reduced, which minimizes the consumption of fresh material. The reaction times are shortened, where nZVI is used to enhance the treatment process. Moreover, the process is simple in terms of monitoring since there are a few parameters to control. However, for a developing country like South Africa, this technology is yet to gain traction. It is an active treatment, energy-intensive since the mixing of nZVI powder makes slurry before use. South Africa is still struggling with energy reserves; this method may still be challenging to employ commercially. Even though some researchers assure that the effluent quality may offset the treatment cost (Li et al., 2017).

6.3.10 The overall pollution reduction in AMD

Regarding the treatment system performance, the sulfidogenic fluidized bed reactors removed over 85% of each metal measured in the initial samples before treatment (Figure 6.11). The iron removal rate was consistent if not superior with other SRB reactors (Johnson and Hallberg, 2005; Papirio et al., 2013; Sahinkaya et al., 2013; Utgikar et al., 2002), and achieved an average of 97%. At the observed removal rates, the Fe concentrations meet the water quality objectives. The removal of Mn was observed to be 90%, and this result was satisfactory since the manganese retention was evident in the FBR during phase I and phase II of treatment. As observed by Clyde et al. (2016), the retention could have resulted from Mn sorption in the FBR.

The removal mechanism may also be attributed to $MnCO_3$ and said not completely to remove manganese from the solution (Hallberg and Johnson, 2005). For copper, the removal rate was 88% in the FBR and may be considered a low removal compared to the other pollutants. However, the results are similar to the bench-scale study using SRB done by Clyde et al. (2016) since copper was retained in the FBR throughout the treatment process. In terms of zinc removal, the average was found to be 92%. The result was satisfactory and better than a pilot-scale study done by Jarvis et al. (2014). The Zn result is somewhat pH-dependent; it has been reported that zinc removal increased with elevated pH > 4 (Mayes et al., 2009).

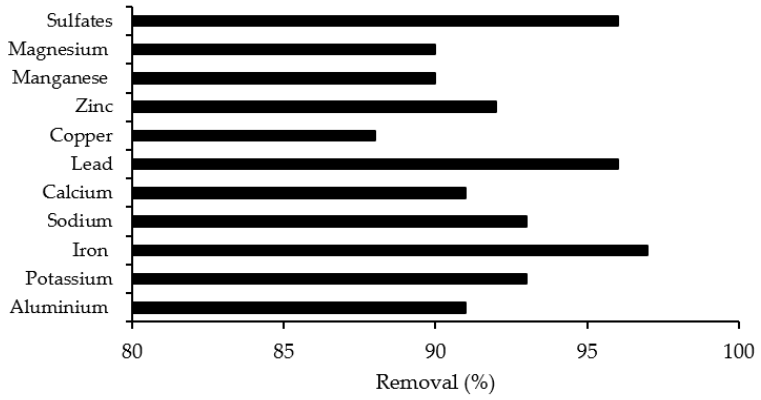

Figure 6.11 The average percent removal for pollutants, specifically in the FBR, over the treatment period.

Overall, the treatment process observed that the SO_4^{2-} concentration was gradually reducing every day, which was expected as per the study hypothesis. The effluent results showed an average of 96% decrease throughout the treatment process, with an average area of removal rate of 7 $g/m^2/d$. This system proved to be highly effective with sulfate removal, one of the persistent pollutants in mine water.

6.4 Conclusions

The degradation of pharmaceuticals in wastewater has recently received considerable attention as a potential solution to the growing demand for strict effluent standards. This work demonstrated nanoscale zero-valent iron‘s efficacy as an enhancing element for remediating real mine water and pharmaceutical-containing wastewater from a hospital. The laboratory-scale fluidized-bed reactor proved to simultaneously remove metal ions, like Mn and Zn, from wastewater while achieving a

significant reduction of SO_4^{2-} and organic matter (COD, BOD, DOC), TDN. In terms of the treatment performance, effluent pH values were generally higher than the FBR influent. The generation of alkalinity corresponding to sulfate reduction in the sulfidogenic reactor demonstrates that SRB could maintain biological activities. The cotreatment in FBR could be used to treat pharmaceutical wastewater containing nonsteroid inflammatory compounds and COD reduction of over 90%, suggesting biomass had acclimated to the compounds. The overall treatment effectiveness for AMD was found to be at an average of 92.5%. Since the COD degradation is directly affected by the complexity and variability of the hospital wastewater, long HRT in the FBR may lessen the effects. The reactor contents 'redox potential was used to monitor the FBR treatment performance, making necessary adjustments to nZVI dosage and the influent feed. For the future, it is recommended that an automatic control strategy to regulate the nZVI and feed pump responding to E_h value be used to make the treatment system more stable. An investigation of the nZVI activation procedure may be necessary to enhance the reactivity of its surface-passivated particles in order to explore pharmaceutical removal in that case further. Finally, building from the work of Laber et al. (1999) where they constructed a wetland to treat hospital wastewater, with such rich literature on wetlands from the point of treating AMD (Allende et al., 2014; Johnson and Younger, 2006; Sheoran and Sheoran, 2006; Younger and Henderson, 2014), this work contributes to the possibility of amalgamating the two-waste streams in one wetland.

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CHAPTER SEVEN

Overall Conclusions and Recommendations

The purpose of this chapter is to consolidate the research findings reported in this work. The conclusions are based on the positive or negative results obtained when testing the hypotheses. Most importantly, pay attention to the addition of knowledge in the specific field of acid mine remediation. Furthermore, this chapter highlights the direction for future research in this field.

7.1 Overall Conclusions

Recalling the literature review which has shown that the sulfidogenic treatment of AMD is a possible alternative because of its improved quality of sludge and low running cost compared to traditional chemical treatment strategies; however, for the sulfidogenic process to be effective for AMD remediation, an additional carbon source is necessary.

Consequently, the overall aim of this work was to evaluate the feasibility of using hospital wastewater (pharmaceutical-rich) as an energy source to support sulfate-reducing bacteria for co-treatment with AMD using a fluidized bed reactor, subsequently developing a performance model for this process.

In order to assess this prospect, the study's objectives were defined as:

- To evaluate the possibility of using hospital wastewater as an organic substrate for AMD treatment, by assessing alkalinity production, metal and sulfate reduction, elimination of organic pollutants in the hospital wastewater.
- To obtain the kinetic parameters, maximum rate (V_{max}) and Michaelis-Menten constant (K_m) for oxidation of electron donor through running batch kinetic experiments.
- To develop an artificial neural network model for performance prediction in the fluidized bed reactor through optimal architecture

7.1.1 Evaluation of using HWW as carbon source for AMD co-treatment

The literature review has shown that commercial substrates used in SRBs like ethanol and lactate have a potential of increasing the operating costs. Hence alternative substrates, like wastewater with high organic carbon content may be suitable and more beneficial if wastewater can be co-treated in the process. An advantage of this co-treatment system is the mutual benefit for both streams being treated at the same time, thus reducing the energy and operational cost for treatment of each individual stream. Existing studies have demonstrated the co-treatment of municipal wastewater and AMD as potential success for reducing metal concentrations and sulfates. Having shown in recent literature that pharmaceutical compounds pose a potential risk to the aquatic environment, none of the AMD co-treatment strategies have extended their investigation to the pharmaceutical removal rates during the treatment process.

As such, this was identified as a gap in the literature and the study proposed to use hospital wastewater, which is rich in organic carbon, nutrients and pharmaceuticals. The hospital wastewater was identified as a great potential to be used as an organic substrate for co-treatment of AMD. The co-treatment was tested in FBRs since they have been proven to generate less sludge over other reactors. This strategy was found to be effective for generation of alkalinity, removal of metal concentrations and sulfates from AMD. As for removal of pharmaceuticals, this method did not achieve high removal rates, as initially anticipated. The treatment strategy seemed to have limitations since the inhibition of further removal was not established.

In the treatment system, the HRT decreased gradually from 24 to 16 h while maintaining the feed flowrate relatively constant. With a decrease in HRT, it was expected that the sulfate reduction rate would improve, but this study observed results on the contrary. Although this study did not quantify the methane production, it may be possible that methanogens for energy sources were incapacitated in the bioreactor at this stage. The results found that the decrease in HRT from 16 to 8 h significantly decreased the alkalinity production. Even after the HRT was ramped back up to 24 h, the alkalinity production continued to decrease in the last phase of treatment. These results could be attributed to sulfate reduction performance in the final phase of the treatment, which produced less pH than phase III, resulting in the development of metal sulfide precipitates. On the

other hand, the sulfide production revealed a parallel movement with alkalinity on the effluent. The results confirm the importance of controlling the hydraulic retention time during treatment.

7.1.2 Assessment of batch experiments to evaluate kinetic parameters (V_{max} and K_m) for oxidation of electron donor

Almost with certainty, nitrogen and organic carbon concentrations were influenced by precipitation in the initial phase of the experiment. An overall observation of BOD, DOC, and TDN proved a decrease over treatment period. It is most likely that the bacterial SO_4^{2-} reduction facilitated the efficiency of processing the BOD and DOC. The DOC concentrations followed the Fe concentration decline trend, apart from 16 h HRT, where there was a slight increase. The high COD removal rate and decrease in TDN and BOD confirm the removal of dissolved nitrogen and organic carbon, only a small amount of concentration remained at the end of the treatment.

The COD, BOD, and TDN concentration decrease were accompanied by biomass accumulation in the reactors during the 180-day treatment period. The sulfide inhibition of a culture of enrichment that reduces sulfate was well established in a noncompetitive inhibition model. The correlation between sulfide concentration and COD use was not linear, and the use of metals (Fe and Zn) with examined concentrations was not completely inhibited.

One of the critical factors in microbial reactions is redox potential for controlling the outcome of key chemical elements like sulfur and iron. The results showed that the biochemical process in this experiment comprises microbially mediated oxidation with Fe^{3+} and sulfate of the labile fraction of the organic matter. The precipitation of zinc and phosphates was observed in this study following a similar removal system trend in wastewater treatment. Iron concentrations decreased during the initial phase of the experiments after HWW and AMD were mixed in the reactor, confirming Fe precipitation with phosphate or hydroxides. The iron (III) reducing bacteria contending with SRB may have caused by iron inhibitive effect demonstrated within high Fe concentrations.

The dissolved sulfide inhibition constants (K_i) were 3.6 mg/l for the associated growth, and the related K_i value was 9 mg/l for H_2S. The microbial community provided insights into the main microbes in biological treatment. The dominant species in the treatment process belong to the

Proteobacteria group (especially *Deltaproteobacteria*). The study provides important information regarding the performance of sulfidogenic bioreactors treating acidic metal-containing water and hospital wastewater. This work demonstrates the possibility of an SBR reactor for synchronized decreasing concentrations of COD, metals, sulfate, and selected anti-inflammatory pharmaceutical compounds in wastewater.

7.1.3 Development of artificial neural network for FBR performance prediction

Biomass was distributed uniformly throughout the FBRs fluidized volume. The differences in volatile suspended solids and suspended solids concentrations were assumed to present reactor activity. This study presents a relatively higher value compared to existing studies due to low sludge production in the FBR compared to attached growth reactor systems. The applicability of the artificial neural network to predict the performance of the FBR based on SRB biological co-treatment of AMD and HWW was assessed. The model showed that ANN can successfully predict the levels of Fe, naproxen, ibuprofen, sulfate, and COD concentration at fluctuating feed composition, then optimize the FBR operational conditions.

The study concludes with the predicted values are very close to the actual experimental values. In this way, it is easy to predict the ratio of HWW to AMD, where HWW acts as a carbon source used by sulfate reducing bacteria or methanogenic microorganisms. Since the sulfate concentration in the effluent is one of the targets in the model, the flow of organic matter to reduce sulfate can be predicted and thus the biotreatment can be optimized as such. This study used about 83% of COD for sulfate reduction. There was no significant methane production detected. Models developed in this study can be employed to predict the effluent quality of the HWW and AMD co-treatment sulfidogenic system using fluidized bed reactors, for the purpose of protecting the environment.

7.1.4 Evaluating the performance of an enhanced co-treatment with nZVI

The degradation of pharmaceuticals in wastewater has recently received considerable attention as a potential solution to the growing demand for strict effluent standards. This work demonstrated nanoscale zero-valent iron‘s efficacy as an enhancing element for remediating real mine water and

pharmaceutical-containing wastewater from a hospital. The laboratory-scale fluidized-bed reactor proved to simultaneously remove metal ions, like Mn and Zn, from wastewater while achieving a significant reduction of SO_4^{2-} and organic matter (COD, BOD, DOC), TDN. In terms of the treatment performance, effluent pH values were generally higher than the FBR influent. The generation of alkalinity corresponding to sulfate reduction in the sulfidogenic reactor demonstrates that SRB could maintain the biological activities. The cotreatment in FBR could be used to treat pharmaceutical wastewater containing nonsteroid inflammatory compounds and COD reduction of over 90%, suggesting biomass had acclimated to the compounds.

The overall treatment effectiveness for AMD was found to be at an average of 92.5%. Since the COD degradation is directly affected by the complexity and variability of the hospital wastewater, long HRT in the FBR may lessen the effects. The reactor contents 'redox potential was used to monitor the FBR treatment performance, making necessary adjustments to nZVI dosage and the influent feed. For the future, it is recommended that an automatic control strategy to regulate the nZVI and feed pump responding to E_h value be used to make the treatment system more stable. An investigation of the nZVI activation procedure may be necessary to enhance the reactivity of its surface-passivated particles in order to explore pharmaceutical removal in that case further.

7.1.5 Potential for real application

This study has brought forward some important information for possible application for AMD and hospital wastewater co-treatment. The limitation for real application is the pumping cost of one waste stream to the common site for treatment. However, until an actual cost estimation study is performed, it cannot be said with certainty that pumping costs would offset the environmental benefits of this co-treatment.

7.2 Recommendations and direction for future research

Studies involving co-treatment of AMD with other waste streams has been few and far in between and has not been fully exploited. As such the knowledge and findings gathered from this study proposes the following future research directions:

- The study evaluated the feasibility of using pharmaceutical-rich wastewater as a substrate to support SRB in the treatment of AMD. The study extended its evaluation to the pharmaceutical compounds' removal efficiencies, to harness more benefits of the co-treatment strategy. The pharmaceutical removal pathway could not be attributed to specific reasons, although probable factors were highlighted since there is limited information on the removal mechanisms. Therefore, the removal mechanism for the pharmaceuticals in this regard needs to be thoroughly investigated.

- The initial evaluation of the co-treatment process was determined by analysis of metals, sulfates, nutrients and pharmaceuticals (naproxen, ibuprofen and diclofenac). Though these compounds are important as primary indicators or measures of successful treatment strategy for water quality, further research needs to be carried out to investigate the co-treatment on other pharmaceuticals.

- The complex nature of the co-treatment process makes it difficult to fully understand the pathway for removal and the limitations associated with the strategy. As such, more work needs to be done in this area to dissect the pathways and provide concrete information relating to the removal strategy. The suggested work should include the microbial community study to understand all aspects involved.

- The understanding of the pathways will also assist in selection of the variables and parameters that will form part of the inputs in model development.

Appendix A - Copies of research output (Publications)

A1. Paper I

Mine Water and the Environment
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REVIEW

Possibilities for Acid Mine Drainage Co-treatment with Other Waste Streams: A Review

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Abstract
The co-treatment of AMD with other common liquid wastes is a promising synergistic approach, fusing characteristics of active and passive AMD treatment for a sustainable remediation strategy. This paper discusses existing literature on AMD co-treatment approaches, focusing on factors that influence the feasibility of co-treatment, such as mixing proportions, microbiological elements, reactor design, and mixed-water chemistry. Finally, this paper highlights future possibilities, drawing attention to prospects that require exploration.

Keywords Metal removal · Neutralisation capacity · Remediation

Introduction

The mining of coal and some metals such as copper, nickel, and gold is correlative with acid drainage challenges that can adversely affect water bodies and the ecosystem at large (Akcil and Koldas 2006). Moreover, much of the effluent produced by the metal-mining sector contains a sizable number of constituents such as arsenic, cadmium, cyanides, lead, and mercury, with ecological implications and high health risks for both humans and animals (Azapagic 2004). This effluent is characterised as acid mine drainage (AMD) because it is typically formed after mining exposes sulphide-containing minerals to oxidation reactions. However the same reactions can occur anywhere pyritic rock and polymetallic sulphides are exposed (Johnson and Hallberg 2005; Paikaray 2015). When mining is not involved, the preferred term is acid rock drainage (ARD), and in much of the world, this more general term is used for AMD as well.

There are numerous sulphide minerals that oxidise in a comparable manner to pyrite, discharging sulphate and various metalloids and metals (i.e. Cd, Pb, Fe, As, Al, Cu). The extent of environmental pollution by AMD depends on its pH and composition, which varies according to the geology of the mine site. For example, in South Africa, the average Fe^{2+} concentration in gold mines differs from coal mines, 830 and 2135 mg/L, respectively (Kefeni et al. 2015). Thus, AMD chemistry varies, depending on the sulphide mineral being oxidized and what the acidic wastewater encounters.

For the past couple of decades, there has been an increased interest in using biological processes to treat metal-containing wastewaters because these processes can increase pH and lower metal concentrations (Janssen et al. 2001). Considerable attention has focused on co-treatment options that could reduce cost and energy expenditures by operating a single process rather than running two treatment processes for different waste streams. However, co-treatment application studies have been few and far between.

Factors that have been demonstrated to affect whether co-treatment techniques work include: mixing proportions, the range of microbiological elements, reactor design, and mixed-water chemistry. There has been relatively little done on the relationship of microbial communities to co-treatment kinetics, though this is likely important as well. Therefore, there seemed to be a need to collate the published studies and compare their performance to identify further study needs in this fairly young research area, to identify other possible waste streams for co-treatment, to provide a future perspective, and to highlight the type of research that is

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A2. Paper II

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Effective biotreatment of acidic mine water and hospital wastewater using fluidized-bed reactors

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ABSTRACT

A laboratory-scale sulfate-reducing fluidized bed reactor at a controlled mesophilic condition of ± 28 °C examined the biotreatment process of real acid mine drainage and hospital wastewater. In an attempt to substitute the carbon source, the study used the hospital wastewater in this regard. In this study, there was no supplement of carbon source during the treatment, but only the hospital wastewater provided the dissolved organic carbon. The process evaluated a biological treatment efficiency in stepwise phases, monitoring the effect of retention time, influent pH, and sulfates. The overall removal for the effluent COD concentrations and sulfate were between 39.5 mg/l and 42 mg/l at COD/SO_4^{2-} ratio of 0.68 and hydraulic retention time of 8 h. The overall COD oxidation and sulfate reduction performance of the reactor was recorded as an average of 96 % and 97 %, correspondingly. The sulfide concentration averaged 235 mg/l, and the alkalinity generated in sulfidogenic organic from hospital wastewater neutralized the acid in mine water. The metal removal efficiencies documented at HRT of 8 h, were all above 98 %. The biological treatment further recorded the removal efficiencies of 44 % for naproxen and 55 % for ibuprofen pharmaceutical compounds as an additional advantage to the system.

1. Introduction

Hospital wastewater (HWW) and acid mine drainage (AMD) are amongst typical hazardous waste streams that require treatment to sustain the universal water resource quality. The AMD is a strong, acidic waste stream that is rich in dissolved ferrous, non-ferrous metal sulfates, and salts [1]. Untreated AMD can pollute soil and surface water, resulting in harm against aquatic life, humans, wildlife, and plants [2]. Meanwhile, hospitals generate an average of 750 L of wastewater each day per bed [3]. This type of wastewater is rich in pathogenic microorganisms, radioactive elements, discarded and excreted pharmaceutical molecules, and other toxic chemicals [4]. However, these pollutants are customarily discarded into the municipal wastewater treatment plants (WWTPs), even though some researchers proposed that wastewater be held where it originates for treatment before being discharged to WWTPs [5]. As such, once the HWW flows in the WWTP, it forms part of the municipal wastewater (MWW). This practice is common in many countries worldwide, like Australia, Egypt, India, Japan, Thailand, and South Africa [6]. Noticeably, the regulations on pharmaceuticals treatment or their disposal are not as rigorous in developing countries, including South Africa, as compared to developed economies. Hence the current design of wastewater treatment facilities does not provide the capacity to remove these contaminants. More studies are being developed to support the inclusion of emerging pharmaceutical contaminants into water quality legislation in South Africa. In this paper, the HWW is considered an independent stream, before it flows into the WWTP. Acid mine water and MWW are ordinarily addressed independently with active treatment in advanced economies using significant material, financial, and energy resources [7].

Various methods are applicable for AMD treatment, mainly, one of which is considered an efficient biological process through microbial treatment with sulfate-reducing bacteria (SRB) [8]. Sulfidogenic (passive) treatment of mine water is a feasible alternative because of its improved quality of sludge and low cost compared to traditional chemical treatment [9]. Passive treatment of mine water compels primarily adequate organic substrate electron donors to remove dissolved oxygen (DO), and bacterially mediated metal elimination [10]. While sulfidogenic treatment is useful for AMD, it is always essential to add an appropriate carbon source electron donor for SO_4^{2-} elimination because the dissolved organic carbon matter content is below 10 mg/l [11]. In particular, costly chemicals like ethanol and lactate are often used as a

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A3. Paper III

minerals

MDPI

Article

Enriched Co-Treatment of Pharmaceutical and Acidic Metal-Containing Wastewater with Nano Zero-Valent Iron

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Abstract: Among traditional hazardous waste sources, pharmaceutical-containing wastewater and acidic mine drainage need treatment to preserve the expected water supply quality. A nano zero-valent iron (nZVI)-enriched treatment of these two streams is evaluated for simultaneous removal of various heavy metal ions, organic pollutants, sulfates, the efficiency of the treatment system, and separation of reaction products in the fluidized-bed reactor. The reactor packed with silica sand was inoculated with sludge from an anaerobic digester, then 1–3 g/L of nZVI slurry added to cotreat a hospital feed and acid mine wastewater at 5:2 *v*/*v*. The biotreatment process is monitored through an oxidation–reduction potential (Eh) for 90 days. The removal pathway for the nZVI used co-precipitation, sorption, and reduction. The removal load for Zn and Mn was approximately 198 mg Zn/g Fe and 207 mg Mn/g Fe, correspondingly; achieving sulfate (removal efficiency of 94% and organic matter i.e., chemical oxygen demand (COD), biological oxygen demand (BOD), dissolved organic carbon (DOC), total dissolved nitrogen (TDN) reduced significantly, but ibuprofen and naproxen achieved 31% and 27% removal, respectively. This enriched cotreatment system exhibited a high reducing condition in the reactor, as confirmed by Eh; hence, the nZVI was dosed only a few times in biotreatment duration, demonstrating a cost-effective system.

Keywords: zero-valent iron; cotreatment; acidic mine drainage; fluidized-bed reactor

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1. Introduction

Acidic mine water resulting from the open mine tailings often contains sulfates and a complex mixture of heavy metal ions. These mine tailings are characterized to have a high concentration of sulfide containing minerals, such as pyrite (FeS_2). As a result, pyrite's bio-hydro-geochemical weathering with other sulfide containing minerals in oxidizing conditions produces acid mine drainage [1]. According to Masindi and Muedi [2], the formation of acid mine drainage (AMD) may be represented as:

$$2FeS_2 + O_2 + 2H_2O \rightarrow 2Fe^{2+} + 4SO_4^{2-} + H^+ \quad (1)$$

Through sulfide oxidation to sulfate solubilizes the Fe^{2+} Equation (1), then oxidized to Fe^{3+} Equation (2):

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

Microorganisms, such as iron-oxidizing bacteria, can be catalyzed by microorganisms that draw energy from oxidation reaction. Then Fe^{3+} produced may also oxidize FeS_2 and also be reduced into Fe^{2+} Equation (3):

A4. Paper IV

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Sulfidogenic fluidized-bed bioreactor kinetics for co-treatment of hospital wastewater and acid mine drainage

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ABSTRACT

A passive co-treatment of acid mine drainage and hospital wastewater previously demonstrated a promising bioremediation viable approach for both toxic streams. The study of inhibition kinetics and microbial communities is essential to understand better the diverse species and the reaction mechanisms within the system. The kinetics and microbiology diversity in the sulfidogenic fluidized-bed reactor (at 30 °C) for co-treatment of hospital wastewater and metal-containing acidic water were examined. The alkalinity from organic oxidation raised the pH of the effluent from 2.3 to 6.1–8.2. Michaelis-Menten modeling yielded (K_m =7.3 mg/l, V_{max} = 0.12 mg/l min^{-1}) in the batch bioreactor treatment using sulfate-reducing bacteria. For COD oxidation, the dissolved sulfide inhibition constant (K_i) was 3.6 mg/l, and the K_i value for H_2S was 9 mg/l. The dominant species in the treatment process belong to the *Proteobacteria* group (especially *Deltaproteobacteria*). The ibuprofen and diclofenac compounds achieved the highest removal rates in the bioreactor of 58.6% and 52.3%, respectively; while, ketoprofen and naproxen of 41.9% and 46.6%, respectively. The findings in COD kinetics, sulfate-reducing bacteria abundance, and selected pharmaceutical concentration reduction provide insight into this co-treatment process's capability.

1. Introduction

Treatment of wastewater from mineral processing and mining still demands an alternative to predictable chemical treatments, which is usually expensive. Amongst, alternative treatment options are the sulfate-reducing bioreactors [1] and co-treatment processes. The process of bioremediation is reliant on hydrogen sulfide (Eq. (1)) using sulfate-reducing bacteria (SRB) in an anaerobic environment.

$$SO_4^{2-} + 2CH_2O(\text{electrondonor}) \rightarrow H_2S + 2HCO_3^- \quad (1)$$

Then, alkalinity production (Eq. (2)) through the electron donor's oxidation while precipitating metal sulfide.

$$HCO_3^- + H^+ \rightarrow CO_2 + H_2O \quad (2)$$

Usually, the bioremediation process option requires an electron donor for sulfate-reducing bacteria (SRB), which becomes a drawback for the process as it involves high operational costs. Hence, it is crucial for researchers to continuously seek ways of replacing commercial electron donors with low-cost electron donor options. The treatment option may include mixing acid mine water with another stream like fermentation industry wastewater or landfill leachate to benefit the mixed streams regarding pollution reduction. For example, Roetman [2] was the first to propose to mix acid mine waster and municipal wastewater to reduce pathogens in sewage. After that, more studies were developed to assess the practicability of the co-treatment approaches in acid mine drainage (AMD) remediation and reduction of organic matter from wastewater [3–5]. These studies demonstrated improvements in water quality for metal, organics, and nutrients removal with elevated pH and alkalinity. As such, this study hypothesized that any other waste stream that has elevated concentration of COD and nutrients is likely to produce higher sulfate reduction in a passive co-treatment with AMD.

On the other hand, pharmaceuticals are increasingly detected in surface waters, and drinking water as not all are removed in traditional wastewater treatment plants [6]. Hospital wastewater can be considered one of the point sources of pharmaceuticals, and separate treatment of this waste stream is of interest. One of the treatment options of pharmaceuticals in wastewater is degradation through biological remediation, including bioreactors. To date, only one bacterial strain, which

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Appendix B – MATLAB Code

B1. Kinetics Experiments

```
load KineticsPartII.mat



plot(COD,v,'o')
xlabel('COD (mg/L)',"FontSize",11)
ylabel('v (mg/l*D)',"FontSize",11)
title('COD vs Velovity',"FontSize",12)

% Calculate unkown coefficients in the model using
nlinfit
model=@(b,x) b(1).*COD./(b(2)+COD);
initialguess=[1 1];
[beta,R,J,CovB,MSE]=nlinfit(COD,v,model,initialgu
ess);
% 95% CI of coefficients
```

```
betaci=nlparci(beta,R,J);
% Plot the nonlinear fit with the raw date
plot(COD,v,'o')
hold on
x=linspace(0,20,1000);
f=beta(1).*x/(beta(2)+x);
plot(x,f,'-')
xlabel('COD (mg/l)',"FontSize",11)
ylabel( 'v (mg/l*D)',"FontSize",11)
legend('Data Points','Nonlinear Fit','Best')

% Make a residual plot
clf
plot(x,0,'-',COD,R,'rp')
xlabel('COD (mg/l)',"FontSize",11)
ylabel('Residuals',"FontSize",11)
```

```
title('Residual Plot',"FontSize",11)

% Compute the r^2 value
ssresnonlin=sum(R.^2);
sstotnonlin=sum((v-mean(v)).^2);
rsqrnonlin=1-(ssresnonlin/sstotnonlin);

% Plot the transformed data, it should be linear
clf
plot(1./COD,1./v,'o')
xlabel('1/[COD] (l/mg)',"FontSize",11)
ylabel('1/v (l/mg/D)',"FontSize",11)
title('Linearweaver-Burk Plot (1/v vs
1/[COD]',"FontSize",12)

% Set up fittype and options
```

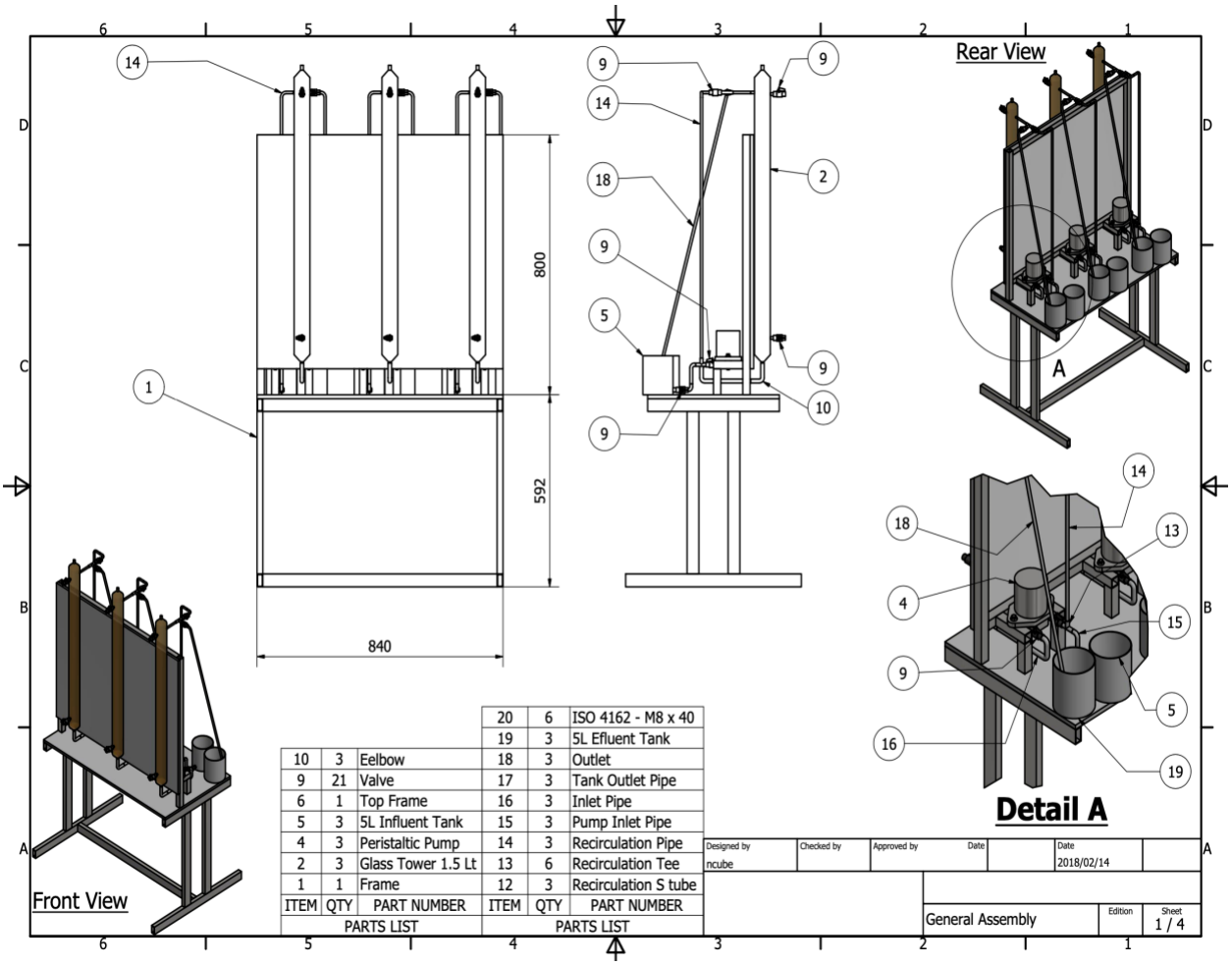
```
fitobject = fittype('poly1');
options = fitoptions (fitobject);
options_Lower = [-Inf -Inf];
options_Upper = [Inf Inf];

% Fit model to date and find 95% confidence
bounds
[xData, yData] = prepareCurveData(1./COD,1./v);
[fitresult,gof]= fit(xData,yData,fitobject,options);
p=polyfit(1./COD,1./v,1);

% Plot linear fit
x=linspace(0,0.5,1000);
line=p(1).*x+p(2);
hold on
plot(x,"LineStyle","-")
```

```
% Make residual plot
Rlin=(1./v)-((p(1).*(1./COD)+p(2)));
clf
plot(1./COD,Rlin,'rp')
hold on
plot(x,0,'-')
xlabel('1/[COD] (l/mg)',"FontSize",11)
ylabel('Residuals',"FontSize",11)
title('Residual Plot',"FontSize",12)

% Determine constants form linear fit
Vmax=1/(p(2));
Km=Vmax.*p(1);

% Determine the r^2 value
```

```
ssreslin=sum(Rlin.^2);
sstotlin=sum((1./v-mean(1./v)).^2);
rsqrlin=1-(ssreslin/sstotlin);

% F-test between nonlinear and linear models
F=ssreslin./ssresnonlin;
df=length(v)-2;
P=1-fcdf(F,df,df)
```

Appendix C – Fluidized bed reactor design

C1 – General Assembly



ITEM	QTY	PART NUMBER
10	3	Eelbow
9	21	Valve
6	1	Top Frame
5	3	5L Influent Tank
4	3	Peristaltic Pump
2	3	Glass Tower 1.5 Lt
1	1	Frame

PARTS LIST

ITEM	QTY	PART NUMBER
20	6	ISO 4162 - M8 x 40
19	3	5L Efluent Tank
18	3	Outlet
17	3	Tank Outlet Pipe
16	3	Inlet Pipe
15	3	Pump Inlet Pipe
14	3	Recirculation Pipe
13	6	Recirculation Tee
12	3	Recirculation S tube

PARTS LIST

C2 – FBR Picture 1

C2 – FBR Picture 2