Laboratory scale testing and modelling of sub-surface wetlands to reduce sulphate

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DEDICATION

“I would like to dedicate this study to Hanri Meyer, my wife, as well as Ewan Meyer, my first-born son”

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ABSTRACT

Water quality impacts related to coal mining are mostly caused by exposing sulphide bearing minerals, such as pyrite, to oxygen and water. The oxidation of sulphide bearing minerals produces acid, metals, trace metals, and sulphate that could impact detrimentally on receiving water courses. Some open cast pits tend to decant if the groundwater level intercepts the surface topography. This study focuses on evaluating the feasibility of biological passive treatment systems, specifically anaerobic bio-substrates, to effectively reduce sulphate from 700 mg/L (decant water) to within acceptable regulatory standards (≤ 250 mg/L), as required by the applicable catchment management agency, the Inkomati Usuthu Catchment Management Agency. The feasibility of using biological passive treatment systems were investigated by means of conducting the following tests on preselected compost and sand substrate mixtures: (1) chemical signature of selected compost, (2) change in dissolved organic carbon concentration over time within 100% compost and 100% sand substrates, (3) constant head permeability testing to measure volumetric flow rate (Q) and hydraulic gradient (i), and calculate hydraulic conductivity (K) as a function of cross-sectional area (A) of flow, (4) calculate dry density (P_b) for each substrate mixture, (5) calculate porosity (n) for each substrate mixture, and (6) conduct tracer testing. These tests were conducted on the following substrate mixtures: 100% compost, 70% compost 30% sand, 50% compost 50% sand, 30% compost 70% sand, and 100% sand. A kinetic model was developed to evaluate sulphate reduction rates as a function of organic carbon (acetate) availability and hydraulic residence time (HRT). The results from the experiments conducted, as well as the kinetic model were used as input to an upscale model, specifically designed for a decommissioned coal mine site that decants. The decant water is characterised by elevated sulphate and metal concentrations. The outcomes from the research conducted indicate that sulphate can effectively be reduced from 700 mg/L to 249,59 mg/L with a minimum HRT of 3,5 days by means of an anaerobic bio-substrate that utilises sulphate reducing bacteria. The lack of readily available acetate can be considered as a limiting factor for optimal sulphate reduction. Anaerobic bio-substrates have a limited lifespan due to the depletion of organic carbon over time. Additional organic carbon will have to be added to ensure sustainable reduction of sulphate. The use of biological treatment systems will require frequent maintenance and monitoring to ensure optimal functionality. Further research is, however, required to validate the findings of the model.

KEY TERMS

Terms	**Definition in context of this dissertation**
Biological passive treatment system (wetlands)	*Refers to a constructed aerobic/anaerobic water treatment system that consists of microbial and/or phytological treatment components.*
Bio-substrate	*Consists of organic material that host sulphate reducing bacteria and provide organic carbon for sulphate reduction to occur.*
Sulphate reducing bacteria (SRB)	*Anaerobic bacteria that facilitate the process of sulphate reduction, and sulphide metal precipitation.*
Substrate mixtures	*Refers to compost and sand mixtures with different sand to compost ratios.*
Hydraulic Conductivity (K)	*Is defined as the volume of water flowing through a cross-sectional area under hydraulic gradient (i).*
Decommissioned mine site	*An area where mining has been completed and all of the operational work has ceased.*
Decant water	*Groundwater that intercepts the surface at a topographical low.*
Anaerobic	*An environment in which microbes require little to no oxygen to thrive.*

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ABBREVIATIONS

Abbreviation	Full name
AP	Acid Potential
AMD	Acid Mine Drainage
CHPT	Constant Head Permeability Test
COD	Chemical Oxygen Demand
CSTR	Continuous Stirred Reactor
DEA	Department Environmental Affairs
DWS	Department Water and Sanitation
EC	Electrical Conductivity
EMPr	Environmental Management Programme
EPA	Environmental Protection Agency
HRT	Hydraulic Residence Time
IUCMA	Inkomati Usuthu Catchment Management Agency
NNP	Net Neutralization Potential
NP	Neutralization Potential
NGA	National Groundwater Archive
NPR	Neutralization Potential Ratio
PFR	Plug Flow Reactor
RCT	Radial Convergent Test
STRM	Shuttle Radar Topography Mission
TDS	Total Dissolved Solids
UTM	Universe Transverse Mercator
WHO	World Health Organization

LIST OF MEASURMENT UNITS

Unit Abbreviation	Description
°C	Degree Celsius
%	Percentage
Eh or pe	Redox potential
g	Gram
g/L/d	Gram per litre per day
g/L	Gram per litre
g/mol	Gram per mole
g/m^2	Gram per square metre
K	Hydraulic conductivity
Kg	Kilogram
Kg/CaCO$_3$/t	Kilogram calcium carbonate per ton
Kg/m^3	Kilogram per cubic metre
L/s	Litre per second
m	Metre
mamsl	Meters above mean sea level
min	Minute
mg/L	Milligram per litre
mg/Kg	Milligram per kilogram
M	Molar mass
Mm^3/a	Million cubic metre per annum
mV	Millivolts
mM	Millimole
mm^2	Square millimetre
mm	Millimetre
mg/s	Milligram per second
m/d	Metre per day
mol/s	Mole per second
m^3	Cubic metre
mg/mol	Milligram per mole
u/L	Microgram per litre
uS/cm	Microsiemens per centimetre
mS/m	Millisiemens per metre

ppm	Parts per million
Psig	Pound-force per square inch gauge
s	Second

CHAPTER 1: INTRODUCTION

1.1. BACKGROUND ON COAL MINING IN SOUTH AFRICA WITH ASSOCIATED WATER IMPACTS

South Africa uses the burning of coal for 77% of the energy generation (Eskom, 2016). The need for energy is still increasing and more coal resources will have to be mined to supply the growing energy demand, unless alternative energy sources are identified. Coal mining is one of the major causes of environmental degradation, specifically water pollution (Tiwary & Dhar, 1994). Mccarthy & Pretorius (2009) provides a brief history on the commencement of coal mining in South Africa: commercial coal mining started in 1864 in the Eastern Cape, 1879 in Vereeniging, and during the late 1880's in Kwazula-Natal. Coal mining only commenced in 1895 in the Witbank coalfields, which were ideally located to supply coal to power stations for energy generation to developing industries. South Africa then became a coal supplier to international coal markets.

Many coal mines were decommissioned over the years as the resource was mined out. The decommissioning of coal mines is accompanied by numerous environmental challenges. One of the major challenges faced by decommissioned sites is impacts on water quality. A serious environmental challenge is the generation of sulphuric acid that occurs as a result of reaction between iron sulphide mineral (pyrite ($FeS2$)) in the coal and host rocks that comes in contact with oxygen and water. During open cast mining, the coal seams and host rock are exposed to the atmosphere, which enables the generation of acid. The produced acid water can dissolve various metals such as iron and manganese. The generated acid and metals could end up in downstream surface and groundwater systems, and impact detrimentally on aquatic ecosystem health. The acidic environment can be neutralized by the addition of lime or associated chemical agents, but one by-product of acid mine drainage is the generation of sulphate that ends up in receiving water systems.

The exact impacts of excessive sulphate in mine impacted water on the environment, humans or animals are still uncertain. The World Health Organization (WHO) recommends that authorities are notified if sulphate concentration exceeds 500 milligrams per litre (mg/L). Some methods do exist for the treatment of sulphate in water, which include: chemical treatment with mineral precipitation, membranes, ion-exchange, and biological sulphate removal (Lorax Environmental, 2003). Decommissioned mines are characterised by very little to no service-infrastructure. The use of conventional active treatment systems, such as reverse osmosis, is, therefore, limited on sites where service-infrastructure is absent. Passive treatment has received more consideration in recent years to act as a potential replacement for active treatment systems. The long term sustainability of passive treatment systems is, however, still to be proven, specifically reducing elevated sulphate concentration to within acceptable levels.

1.2. PROJECT SUBSTANTIATION

Further research into sustainable biological passive water treatment of mine impacted water is still required. This is specifically the case for the removal of sulphate from mine impacted water at decommissioned mine sites. Water related impacts will persist even after mining has ceased. Environmental legislation in South Africa supports sustainable rehabilitation of disturbed areas. The Mineral and Petroleum Resources Development Act 28 of 2002, section 43 (1) states (South Africa, 2002):

"The holder of a prospecting right, mining right, retention permit, or mining permit remains responsible for any environmental liability, pollution, ecological degradation, pumping and treatment of extraneous water, and the management and sustainable closure thereof, until the Minister has issued a closure certificate".

The Mineral and Petroleum Resources Development Act 28 of 2002, section 43 (5) also states (South Africa, 2002):

"No closure certificate may be issued unless the department has confirmed that the provisions pertaining to health and safety and management of pollution to water resources and compliance to the condition of the environmental authorisation have been addressed…"

For mines in South Africa to attain a closure certificate, sufficient rehabilitation of disturbed areas must be conducted. The use of conventional water treatment methods, such as reverse osmosis, is impractical on decommissioned sites. Alternative water treatment methods that require less service-infrastructure and maintenance or monitoring must be investigated to manage and treat mine impacted water in order to prevent impacts on the receiving environment at mine sites where operations have ceased.

1.3. PROBLEM STATEMENT

The inspiration and initiative to focus on the use of biological passive treatment systems to reduce sulphate concentration in mine impacted water developed from a case study where water is decanting from a backfilled rehabilitated coal pit into the natural catchment. The decant water has a pH of near neutral (7.5), with elevated concentration of sulphate, aluminium, manganese, and iron. The sulphate concentration in the water ranges between 550-850 mg/L, with catchment management agencies requiring a sulphate concentration of ≤ 250 mg/L before discharged into the natural catchment. Hydro-geochemical modelling was conducted to determine the potential impact of the decant water on the receiving environment. The outcome from the model presents that the decant water will impact detrimentally on receiving water courses, if no mitigation measures are implemented. The site has been decommissioned in 2001, and the mining company would like to apply for mine closure. The concentration of water quality constituents and the availability of service-infrastructure are of such nature that active water treatment technologies would not be a feasible solution, therefore, the potential to use biological passive treatment.

1.4. GAPS IN LITERATURE

Research has been conducted on the use of biological passive treatment systems in reducing sulphate from mine impacted water. Uncertainty, however, still exists whether these systems can be used as a long-term solution for effective and sustainable sulphate removal. The gaps specifically relate to flow conditions that can be expected under constant head conditions in organic substrates, as well as the suitability of such substrates to host sulphate reducing bacteria for the purpose of reducing sulphate in mine impacted water. The initial use of biological passive treatment systems was for the removal of metals from acid mine water by means of metal sulphide precipitation, and not for sulphate reduction. Intensive research on sulphate reduction by using biological passive treatment systems only occurred in the last two decades. The long-term sustainability of biological passive treatment systems is still to be proven.

1.5. RESEARCH QUESTION

This research questions include the following:

1. Which substrate mixture will be suitable to use within the anaerobic component of the passive treatment system? This specifically relates to evaluating the impact of different compost and sand mixture ratios on flow parameters and hydraulic residence time (HRT).

2. Will organic carbon be readily available to support effective biological sulphate reduction?

1.6. RESEARCH AIMS AND OBJECTIVES

The aims of this project are to evaluate whether an anaerobic bio-substrate could be produced for potential colonisation of SRB, to model the sulphate reduction that could potentially be obtained through the bio-substrate, and to upscale the results to field conditions for potential use of a treatment wetland at a decanting coal mine. Additionally, the aim of the model is to reduce sulphate concentration in mine impacted water from 700 mg/L to ≤ 250 mg/L (as required by the Inkomati Usuthu Catchment Management Agency (IUCMA)).

The specific objectives of this study include the following:

- To develop a fundamental understanding of the processes required for effective microbial sulphate reduction within an anaerobic bio-substrate.
- Conduct predictive kinetic modelling to evaluate the importance of organic carbon on sulphate reduction rate.
- Coupling kinetic and hydraulic data to evaluate the feasibility of an anaerobic bio-substrate as a sustainable water treatment solution, specifically for reducing sulphate concentration in mine impacted water at a decommissioned coal mine.

1.7. CHAPTER OUTLINE

This thesis consists of the following chapters:

Chapter 1 Introduction: The project is introduced by firstly focusing on the background of coal mining in South Africa, together with a brief discussion on the associated water quality impacts. The importance of this research project is highlighted by means of the problem statement, objectives, and research question.

Chapter 2 Literature overview: The study was inspired by a decommissioned coal mine, where water from a backfilled coal pit is decanting into the natural catchment. The decanting water contains an elevated sulphate concentration, which needs to be reduced to within regulatory standards. The decommissioned site has little to no service-infrastructure, and treatment systems utilising advanced technologies, such as reverse osmosis, will not be feasible. This scenario inspired further research to be conducted on the use of microbes to remove sulphate from mine impacted water. A detailed literature study was conducted focusing on the processes involved during biological passive treatment, specifically anaerobic sulphate reduction within an anaerobic bio-substrate. The literature study was conducted firstly by focusing on the current situation in South Africa, specifically with regard to the impacts of coal mining on surface and groundwater quality. The literature study provides a broad overview of current biological technologies used to improve water quality. A number of design factors are highlighted that must be considered during the design phase of a biological passive treatment system. This chapter is concluded by focusing on the hydraulic parameters that should be considered during the design of such a system.

Chapter 3 Methodology: Research gaps were identified after the literature study was conducted. This chapter provides a chronological approach to addressing the gaps identified. Focus is placed on the methodology applied to design the experiments. An overview is provided of the kinetic model development, as well as the design of the upscale model.

Chapter 4 Experimental design and setup: This chapter focuses on the experimental designs, setups and execution of each experiment. Multiple factors play a role during biological sulphate reduction, but only a few fundamental components were selected for further investigation.

The following experiments were conducted:

- Evaluating the suitability of selected compost to be used an organic carbon source. This was conducted by means of determining the chemical signature of the selected compost.
- Determining whether the selected compost would be an appropriate substrate to host sulphate reducing bacteria, specifically whether dissolved organic carbon will be readily available to sulphate reducing bacteria.
- Evaluating the hydraulic properties of different compost and sand mixture ratios that could potentially be used as a substrate material in the anaerobic bio-substrate treatment component. This was conducted by means of a constant head permeability test.
- Calculating the dry density for each of the substrate mixtures.
- Calculating the porosity for each of the substrate mixtures as a function of dry density and relative density.
- Conduct tracer testing to calculate hydraulic residence time (HRT), as well as estimate seepage velocity and porosity from radial convergent test (RCT).

Chapter 5 Numerical modelling: This chapter focuses on the numerical modelling component of this study. Kinetic data was obtained from literature to assess sulphate reduction rates as a function of acetate availability and HRT. PHREEQC model code was used to simulate conditions within an anaerobic bio-substrate.

Chapter 6 Results and discussion: The results obtained from the experiments conducted, as well as the results obtained from the predictive modelling by means of PHREEQC model code, are presented in this chapter. A detailed discussion on the results obtained is provided. Statistical methods were used to critically discuss the results obtained. An error analysis was also conducted to calculate the precision of the measurements taken.

Chapter 7 Case study: This chapter provides the case study of this research project. A site was investigated where mine water is decanting into the natural catchment. The mine water contains elevated metal and sulphate concentrations. Emphasis is, however, only placed on sulphate reduction within an anaerobic bio-substrate. The results from the kinetic model, as well as the experiments are used as input to an upscale model design, which entails the design of a site specific biological passive treatment system with emphasis on the anaerobic bio-substrate.

Chapter 8 Conclusion and further research: This chapter concludes the findings of this research project. A discussion on further research is also provided.

CHAPTER 2: LITERATURE OVERVIEW

2.1. THE MANAGEMENT OF ACID MINE DRAINAGE

South Africa is a water stressed country and sustainable water management has become a key strategic issue. Mining activities are unfortunately associated with environmental impacts, such as acid mine drainage. The perception is that acid mine drainage is mostly associated with gold mines, while research and on-site evidence indicate that coal mines are also one of the major contributors to environmental impacts, specifically water related impacts. The impacts of acid mine drainage will not be solved in the short to medium term, and is most likely to persist. The challenges associated with water will persist long after mining has ceased. It is, therefore, crucial to investigate water treatment or management actions before the life of mine is over.

Acid mine drainage from coal mines is normally associated with elevated metals, sulphate and acidity (Zagury *et al.*, 2007). Enslin (2010) states that acid mine drainage with associated heavy metals pose a significant aquatic ecological risk. Mine drainage can have detrimental effects on the receiving environment, which include water bodies, fauna and flora, and infrastructure.

Coal mining will continue in South Africa for many more years to come. Environmental impacts are, therefore, expected to persist long after mining operations have ceased, specifically water quality and quantity related impacts. It is critical to ensure sustainable water treatment and/or management methods are investigated still during the operational phase of a mine. This approach will reduce the water liability of the mine during post-closure phases.

Coal mining is normally associated with the following residual or latent water related impacts (Heath, *et al.*, 2004):

- *Impacts on surface & groundwater quality*, which is mainly caused by acid and sulphate that are generated from the oxidation of acid producing sulphide minerals, such as pyrite. Metals are liberated when the pH of water becomes acidic;
- *Impacts on surface and groundwater flow patterns*, which is caused by the disturbance of the aquifers during mining. This in turn impacts detrimentally on surface water (disturbance of natural drainage lines) and groundwater flow patterns (geohydrological properties, such as porosity and hydraulic conductivity of aquifers, are altered).

Potential sources of acid mine drainage on coal mines include, but are not limited to: waste rock dumps, discard facilities, open cast operations, discharge of poor quality water, seepage, and spillages (Akcil & Koldas, 2006). The Mineral and Petroleum Resources Development Act, Act 28 of 2002 states that all mining operations must be rehabilitated and closed upon cessation of activities, before a closure certificate can be obtained from the regulatory authorities (South Africa, 2002). Backfilled open cast coal mine pits have the potential to decant, which causes discharge of poor quality water into the natural catchment, if no remedial actions are implemented.

Mccarthy & Pretorius (2009) presents the following management practices for mine impacted water:

Evaporation dams*:* This method entails the construction of shallow dams that allow water to accumulate and evaporate. The cost of these systems is high due to strict lining requirements and high maintenance cost. The other downside to this method is that once the water is evaporated, sludge remains and needs to be removed, which also contributes to additional environmental risks and cost. In addition, these dams require large surface area for construction and operation. The use of evaporation dams is, therefore, limited by high construction and operating cost, as well as large footprint area requirements. The re-use of water is also not possible when using these systems. Evaporation dams might be suitable to manage mine impacted water on condition that these systems are lined to prevent leakages, which could impact detrimentally on soil and groundwater quality (U.S. EPA, 1975). Each site must be assessed to evaluate the suitability of evaporation dams for managing mine impacted water.

Irrigation: Research was conducted on using acid mine drainage water for irrigation purposes. The acidic conditions need to be neutralized before irrigated, but might still contain high sulphate and metal concentrations that could impact on soil quality and impede optimal vegetation growth. Sulphate in irrigated water can accumulate in the soil, and impact on agricultural productivity, or the sulphate could leach from the soil profile to the aquifers, contaminating groundwater systems. This is normally the case where the topsoil is of insufficient depth. Irrigating with mine impacted water is, therefore, a viable option, on condition that the irrigated water quality meets regulatory standards. Vermeulen & Usher (2009) state that irrigating with mine impacted water could result in salt built-up in the soil profile, specifically the upper 2 m. Vermeulen & Usher (2009) further state that impacts on groundwater quality caused by irrigation with mine impacted water depend mostly on the occurrence of clay lenses within the soil profile, and that impacts on groundwater quality were more significant in areas overlain by sandy soils than clayey soils.

***Limiting oxygen ingress into backfilled operations**:* Pyrite oxidation with subsequent acid generation is dependent on various factors, of which oxygen availability forms an integral part. Acid generation can be limited by preventing the ingress of oxygen into the backfilled pit, therefore limiting the contact of backfill material with oxygen. Encapsulating the backfilled pit to prevent oxygen ingress could limit acid, with associated metals and sulphate, to be generated and liberated. Encapsulation can be conducted by means of replacing the overburden and topsoil material that were removed during the initial phases of the mining process as soon as mining is completed. Some open cast pits are flooded after backfilling to prevent oxygen ingress. Detailed aquifer characterisation will be required to ensure water quality objectives are met. Villian (2014) supports the method of limiting oxygen ingress into backfilled open cast pits. Proper capping of the backfilled material in the open cast pit with topsoil will be required to ensure oxygen ingress is limited. Appropriate soil type, thickness and compaction of replaced soil will be instrumental in ensuring oxygen ingress into the backfilled sulphuric rich material is limited.

***Acid neutralization**:* Acid water seeping from decommissioned coal mines can be neutralized by the addition of neutralization agents such as sodium hydroxide or calcium carbonate (active treatment). The construction, operations, and potential expansion of existing active treatment systems can be costly (Taylor, *et al.*, 2005) compared to passive water treatment systems. The adaptability of water treatment systems on post-decommissioned mined sites is critical to ensure system effectiveness and production of the desired water quality. This is specifically the case where treatment of decant water is considered. The volume and quality of decant water can vary considerably depending on the recharge characteristics of the open cast pit, as well as the geochemical signature of the backfill material. The volume of decant can increase significantly during high rainfall periods, and decrease considerably during dry periods. The neutralization of acid water can be effective, but high sulphate concentration can still persist in the water. The addition of chemicals to acid water for neutralization purposes is an ongoing process and requires careful monitoring and maintenance. Active treatment of acid mine water is, therefore, only viable for the short to medium term, but should not be considered as a permanent solution for post-decommissioning scenarios (Younger, 2000).

***Water purification**:* Conventional water treatment facilities can be constructed to improve water quality. These treatment facilities, such as reverse osmosis, operate at a cost in excess of R 10.00/m^3 to treat impacted water to potable water quality standards. Capital expenditure (CAPEX) in excess of R 300 million for a 20 mega litre (Ml) treatment facility can be expected. The use of conventional water treatment methods, such as engineered treatment facilities (reverse osmosis), are accompanied by theft or vandalism, and require high maintenance and monitoring to ensure sufficient treatment. Reverse osmosis plants produce a waste product (brine) from the water treatment processes (Hutton *et al*., 2009), which requires further management. Depending on the feed water quality, the sludge is normally hauled to a designated landfill site, such as Holfontein (South African waste management facility) for further disposal.

Controlled release*:* This method entails the release of poor quality water into a natural system during high rainfall events. Water of poor quality is released into the natural catchment during flood periods in such a manner that the concentration of contaminants is reduced by means of a dilution effect (Mey & Van Niekerk, 2009). The impacted water is contained within the boundaries of the mine and released during times when storm water run-off is high. This approach is, however, not accepted by regulatory authorities. The management of discharge by means of controlled release can only be effective when the mine is in operation. This approach will not be feasible on decommissioned or closed mines, especially where service-infrastructure is limited.

The environmental impacts of coal mining in South Africa have not reached its peak yet. Various operational coal mines are nearing the end of their life of mine, and have initiated the rehabilitation of their operational footprints. Many mines neglected the approach of concurrent rehabilitation, and abandoned mined sites without proper rehabilitation or management of poor quality water (Munnik, *et al.,* 2010). Other mines followed the approach of concurrent rehabilitation, whereby the environmental impacts were better managed and mitigated. Concurrent rehabilitation of disturbed sites enables mines to restore ecosystems still during the operational period of the mine. A lack of concurrent rehabilitation could result in an overall higher environmental risk during the post-mining phases (Van Zyl, *et al.*, 2012).

Backfilled open cast pits tend to fill with water until a state of equilibrium is reached with the surrounding groundwater levels (Ardejani *et al*., 2007). The flooding of open cast pits immediately after backfilling could prevent oxygen ingress, and reduce the potential of acid generation (Vermeulen *et al.,* 2014). Some open-cast pits, however, are not flooded immediately, which provide an opportunity for oxygen to enter the open cast mine workings, and promote acid generation. The potential for open cast pits to generate acid and impact detrimentally on the receiving environment is, therefore, high, and the best fit mitigation or management methodology is still a pending matter.

Excess sulphate in water bodies can be considered an indicator for impacts from mining activities (Bosman, 2009). It is evident that mining activities in the Mpumalanga coalfields are currently impacting detrimentally on catchment water quality. Research, as conducted by Mccarthy & Pretorius (2009), has shown that water from current mining operations in the Witbank coalfields entering the Witbank and Middelburg dams add up to 30 Mm^3/a, which will increase to 44 Mm^3/a

in 2030. Improving the quality of this water will cost approximately R 440 million per annum in 2030. The longevity of acid generation depends on the pyrite content of the backfilled material, the oxygen concentration, and water content within the pit voids. Acid generation will, therefore, eventually decline as pyrite oxidation nears completion, but proactive measures must still be taken to ensure that any potential impacts are sufficiently mitigated. Water in the Middelburg dam deteriorated to such a standard that it is no longer fit for human consumption. It is expected that water in the Witbank dam might represent a similar quality as in Middelburg dam, if no mitigation is implemented.

Water quality data was obtained from the Department of Water & Sanitation (DWS) for the Middelburg dam and Witbank dam. *Figure 1* and *Figure 2* present water quality data collected from 2000 to 2017 in the Middelburg dam and Witbank dam. It is evident that sulphate concentration in the Middelburg dam exceeds the set standard (250 mg/L) (DWS, 2017). The water quality in the Witbank dam is of better quality, but sulphate concentration is bordering the legal limit of 250 mg/L. Attempts have been made to improve water quality in the Witbank area by means of installing a treatment plant in the Brugspruit area, but there are doubts about the sufficiency of the plant to sustainably improve water quality in the long term (Limpitlaw *et al*., 2005). The eMalahleni Water Reclamation Plant was also commissioned, which demonstrated that it is possible to treat mine impacted water to drinking water quality standards. The cost associated with this treatment is, however, extremely high. The plant has a capacity of 20Ml per day (Hutton *et al*., 2009), with a construction cost of approximately R 300 million (Naidoo, 2007). The water quality of the Olifants River will, therefore, continue to deteriorate if no alternative water treatment method is proposed. There are many different technologies that have been developed to improve the quality of impacted water originating from the local mining areas. Some of these treatment technologies have demonstrated to be successful in improving water quality, while others have failed.

Many mines in South Africa are approaching the end of their life of mine and impacts on water quality will, therefore, persist if no alternative treatment methods are developed, especially for post-decommissioning sites. The use of biological passive treatment systems seem to be the most feasible and practical solution for decommissioned sites, but further research is still required to properly design these systems to be sustainable in improving water quality (Mccarthy & Pretorius, 2009).

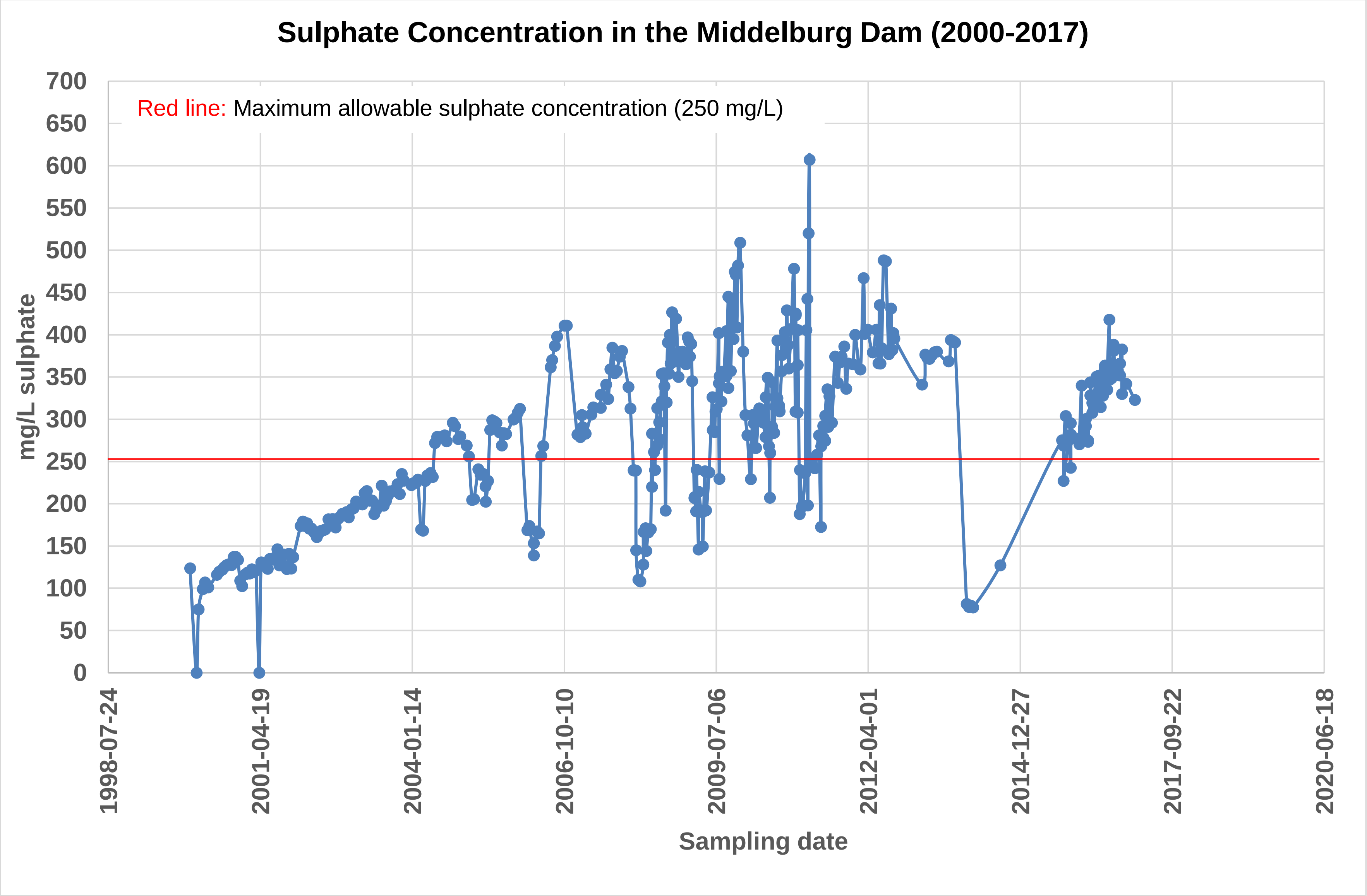


Figure 1: *Change in sulphate concentration in the Middelburg Dam (DWS, 2017).*

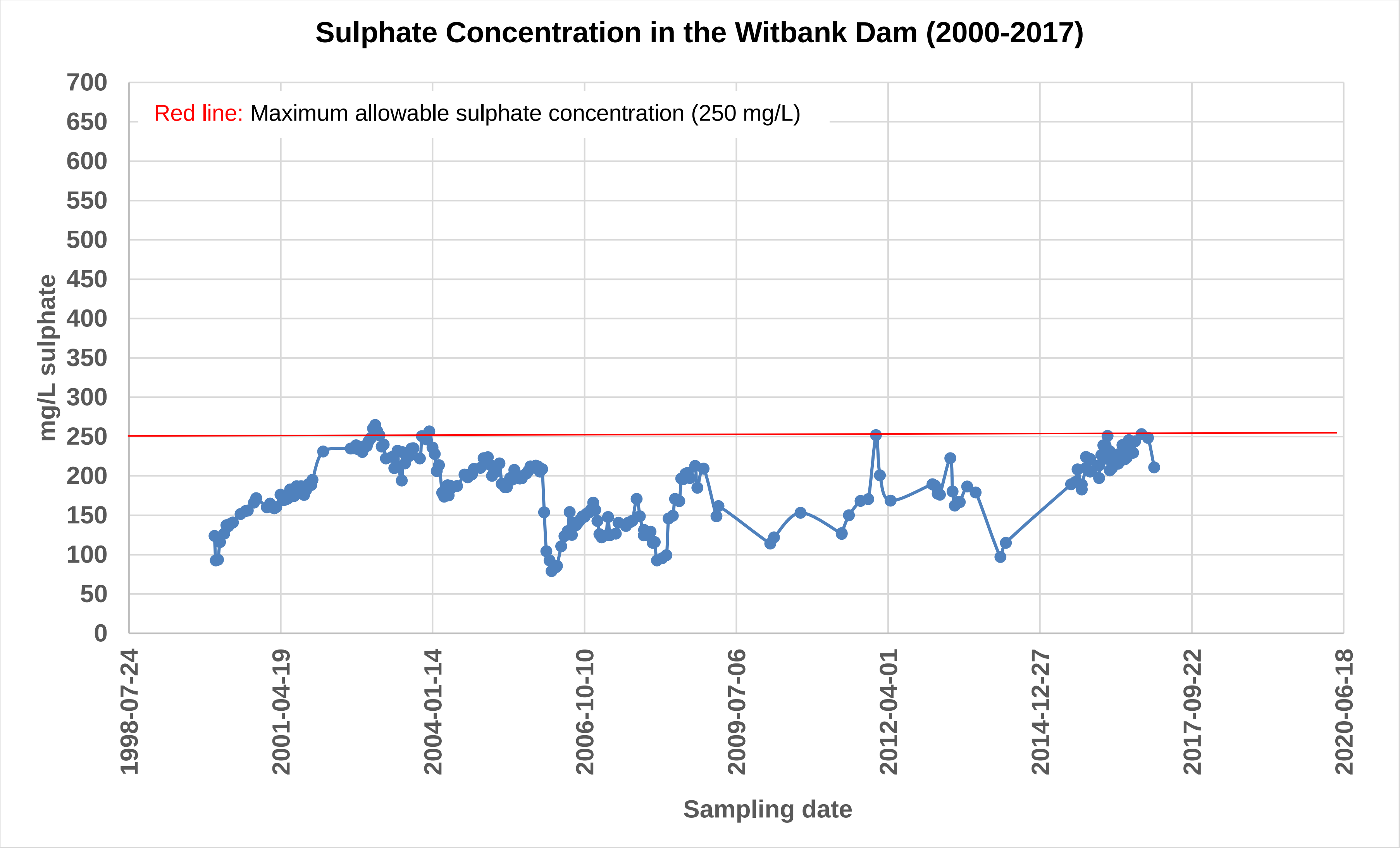


Figure 2: *Change in sulphate concentration in the Witbank Dam (DWS, 2017).*

2.2. BACKGROUND ON THE USE OF BIOLOGICAL PASSIVE TREATMENT SYSTEMS

Subsurface flow biological passive treatment systems (or anaerobic wetlands) can be used to improve the quality of impacted water, specifically water containing elevated sulphate concentration. Tilley *et al.,* (2014), provides an illustration of a typical biological passive treatment system *(Figure 3).*

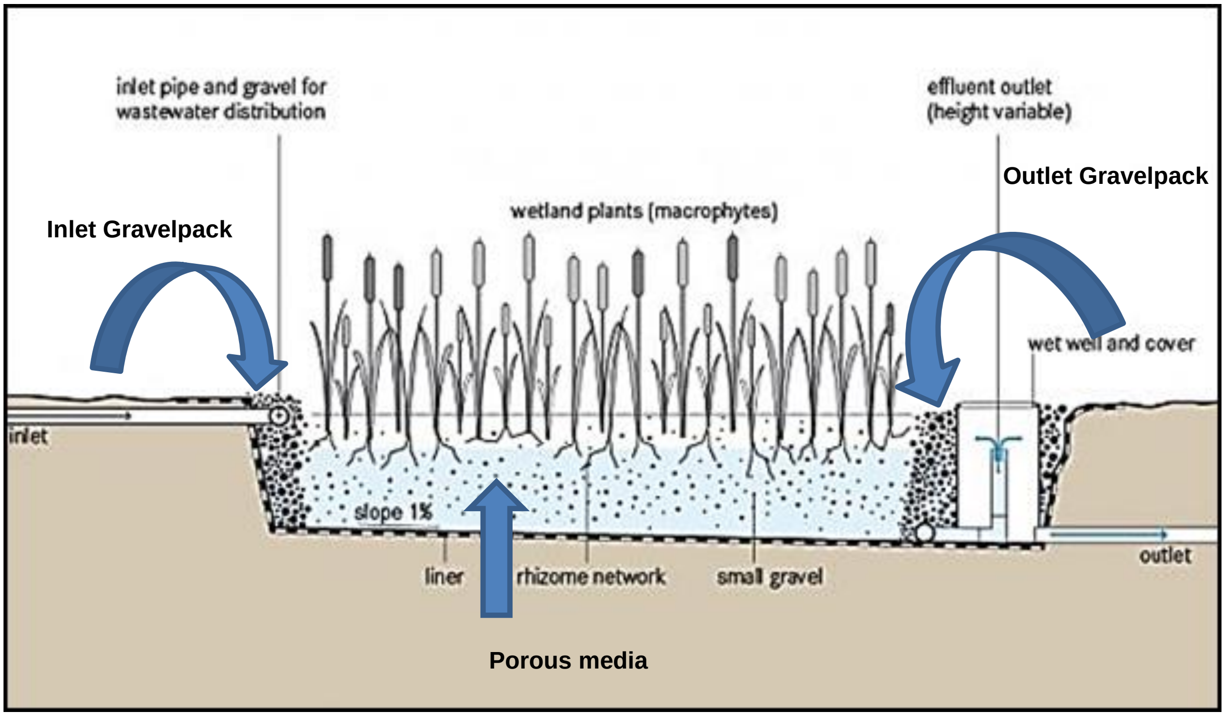


Figure 3: *Illustration of a subsurface flow wetland (modified from Tilley et al., 2014).*

These systems are mostly characterised by the following components:

Inlet: Impacted water requiring treatment will enter the wetland by means of a manifold distributer pipe to ensure water is distributed across the entire bed or substrate area of the wetland.

Inlet Gravelpack: A gravelpack is placed immediately after the manifold distributer pipe to prevent clogging of inlet pipes if backwashing occurs, as well as reduce direct impact of inflowing water on the wetland substrate. The gravelpack also acts as a first phase treatment (physical filtration unit).

Porous media (substrate): The substrate of the wetland can consist of a multitude of material types, depending mostly on the quantity and quality of water requiring treatment, and the desired product water quality. The substrate component of the wetland system provides a habitat for microbes to establish, which play a pivotal role in biological passive treatment. The hydraulic conductivity (K) together with the porosity (n) of the substrate also determines the HRT of the system, which are instrumental to effectively improve water quality. The substrate can be planted with wetland vegetation to aid in water contaminant uptake. Aerobic (promotes oxidation) treatment systems are more prone to be planted with wetland plants than anaerobic (promotes reduction) treatment systems.

Outlet Gravelpack: This gravelpack component of the treatment system prevents biomass washout from the substrate, and prevents clogging of the collection manifold.

Outlet: The treated water is then collected at a central point for discharge or re-use, depending on the quality of effluent (product) water.

Conventional active water treatment technologies, such as chemical treatment, can be effective for achieving water quality objectives. These systems, however, can be associated with high construction and maintenance costs (Johnson & Hallberg, 2005). Development of effective passive techniques to replace or complement active technologies can reduce water management liability associated with mining activities, especially for post-decommissioned sites.

Various studies have focused on the impact acid mine drainage has on the receiving environment. Some case studies indicate that water originating from mine residue deposits might have near neutral pH conditions (Johnson & Hallberg 2005), but still have a detrimental impact due to elevated sulphate concentrations (Madzivire *et al.*, 2009).

Biological passive water treatment is accomplished by means of physical, biological, and chemical mechanisms. Improving water quality in biological passive treatment systems is mostly accomplished by means of the following mechanisms:

- Settlement of suspended solids (Mayes *et al.*, 2009);
- Physical filtration (Burke & Banwart, 2002);
- Direct uptake of contaminants by plant roots (Batty & Younger, 2003);
- Ion exchange and organic complexation (Mayes *et al.,* 2009);
- Sulphate reduction and metal sulphide precipitation (Mayes *et al*., 2009).

Biological passive treatment systems (such as wetlands) have been recognised as a method to improve water quality (Gusek, 2008). Local environmental conditions, redox state of ions to be removed, and water chemistry determines whether these reactions will occur under oxidising (aerobic) or reducing (anaerobic) conditions (Gusek, 2008).

The management of water quality is the primary environmental challenge faced by the mining industry. The replacement of active treatment systems with passive treatment systems might reduce environmental and financial liabilities post-mine closure. The effectiveness and sustainability of biological passive treatment systems are, however, still to be proven.

Researchers at the Colorado School of Mines considered sulphate reducing bacteria in an anaerobic habitat as an appropriate treatment method for improving the quality of acid mine drainage water (Gusek, 2008). The primary focus was, however, on metal removal from mine impacted water and less research has been conducted on sulphate removal.

2.3. DIFFERENT TYPES OF BIOLOGICAL PASSIVE WATER TREATMENT SYSTEMS IMPLEMENTED FOR TREATING ACID MINE DRAINAGE WATER

The design of biological passive water treatment systems is mostly determined by the chemistry of the water to be treated. The Environmental Protection Agency (U.S. EPA, 2014) provides a list of passive and active treatments systems that can be used for the treatment of mine impacted water. The following section presents three types of biological passive treatment systems that can potentially be used for metal and sulphate removal from mine impacted water:

2.3.1. Constructed wetlands

2.3.1.1. Technology description: These systems utilise soil, organic material, rock, and wetland plants as substrate to host and facilitate microbial processes for water treatment. These systems can either be aerobic or anaerobic, depending on the contaminant removal mechanisms required (Costello, 2003). These systems are constructed to treat water passively over a long period. The main mechanisms of contaminant removal are plant uptake, volatilization, biological reduction or oxidation, precipitation, and settlement (Vymazal, 1998). Microbes are able to immobilise metals in acid mine drainage water (Johnson and Hallberg, 2005). The primary advantage of these systems is relatively low capital cost required for construction, and low maintenance and operational cost compared to conventional treatment methods, such as reverse osmosis.

2.3.1.2. Water constituents treated: The constituents that can be treated include a wide spectrum of constituents, such as vanadium, chromium, manganese, iron, cobalt, nickel, copper, zinc, arsenic, selenium, aluminium, phosphorus, sulphur, molybdenum, gold, cadmium, antimony, barium, mercury, titanium, silver, radium, uranium and lead (Gusek, 2009). Wetlands can treat mine impacted water within a wide range of pH conditions (acidic, neutral, or alkaline mine drainage water). The pH of influent water forms a critical part of the effectiveness of microbial processes to remove contaminants from impacted water (Rodriguez-Sanchez, *et al.*, 2014).

2.3.1.3. Operations: Wetlands are either anaerobic (subsurface flow, thus promoting reducing conditions) or aerobic (water flows on surface, thus promoting oxidising conditions). In anaerobic wetlands**,** water flows through a substrate that consists of a porous media, such as gravel, sand, or compost (or any other type of organic material) (Halverson, 2004). The substrate surface can be planted with aquatic plants. Plants can either be planted on the surface of the substrate, or be planted in a separate aerobic unit. Comments have been made in recent studies that the roots of the plants might promote oxygen ingress into the substrate, and inhibit optimal microbial functionality, specifically inhibiting the growth of sulphate reducing bacteria (Vymazal, 2011). A designed aerobic wetland is similar to a natural wetland, with water flowing over the substrate surface. The design of constructed wetlands for the treatment of mine impacted water varies from site to site. The most important factors that must be considered include: pH of influent water, substrate type, redox conditions, solid support, hydraulic residence time (HRT), sulphate concentration of influent water, metal concentration of influent water, temperature, and chemical oxygen demand (COD) to sulphate ratio (Gomez, 2013).

If mine impacted water has an alkaline pH, aerobic wetlands can be used to promote metal oxidation (Budeit, 2007). An anaerobic wetland consisting of organic material can be used for sulphate reduction (Morrison, 2005). A great benefit of constructed wetlands is the adoptability of the system. Aerobic or anaerobic units can be added as required.

2.3.1.4. Maintenance & monitoring: Monitoring is required on a periodic basis to ensure that treatment efficiency is maintained. Optimal functionality of microbes is highly dependent on organic carbon as a source of nutrients. One of the limiting factors of these systems is the lack of readily available organic carbon within the substrate (Mirjafari & Baldwin, 2016), which might be an ongoing maintenance requirement (Kousi *et al.*, 2015). Another factor that determines whether the system will be effective in removing required contaminants is continuous flow through the substrate. Challenges were also encountered with substrate clogging mainly caused by metal sulphide precipitates (Figueroa *et al.*, 2007). The replacement of the entire substrate might be required after a period of operation (mostly 2 to 3 years), depending on the degree of clogging, availability of organic carbon, and ability to manage HRT.

2.3.1.5. System limitations: Constructed wetlands require a large area of land and sufficient supply of water to function optimally. Influent mine water might require pre-treatment, if the pH is too low (Jong & Parry (2005) or metal concentrations are too high (Cabrera *et al.*, 2006) for sulphate reduction by sulphate reducing bacteria. The release of immobilised contaminants might occur during high flow periods. The development of preferential flow paths within the substrate could reduce HRT, which reduces the contact time between mine impacted water and microbes. These systems might also not be appropriate if stringent product water qualities (potable standards) are required.

2.3.1.6. Effectiveness: The effectiveness of a constructed wetland to improve water quality in Tennessee U.S.A. is illustrated in *Table 1*.

Table 1: *Water constituents treated by a constructed wetland (ITRC, 2010).*

Constituent	Influent (mg/L)	Effluent (mg/L)
pH (-log(H^+))	4,28	7,16
Aluminium	1,423	0,055
Iron	0,211	0,133
Manganese	1,148	0,294
Copper	0,197	0,017
Zinc	0,640	0,197
Sulphate	110	104
Hardness	97	142
Acidity	37	<1
Alkalinity	<1	45

The effluent water quality obtained from a constructed anaerobic wetland in Tennessee indicated that the wetland successfully reduced acidity and concentration of most metals. Only 10% to 30% of the sulphate was reduced. *Table 2* provides a general rule of practice that can be expected regarding the treatment efficiency of constructed wetlands (ITRC, 2010).

Table 2: *General treatment efficiency of constructed wetlands.*

Constituents	Removal rate (%)
Acidity	75-90%
Sulphate	10-30%
Iron	>80-90%
Aluminium	>90%
Copper	>80-90%
Zinc	>75-90%
Cadmium	>75-90%
Lead	>80-90%

2.3.2. Biochemical reactors

2.3.2.1. Technology description: These systems operate very similar to anaerobic constructed wetlands, except that vegetation normally does not form part of the treatment methodology. These systems use microbes to transform contaminants and increase pH in the impacted water (Zagury & Neculita *et al.*, 2007). These systems are normally operated within a closed environment and are operated anaerobically. These systems are also referred to as sulphate reducing bioreactors or anaerobic bio-substrates.

2.3.2.2. Constituent treated: These systems can effectively increase pH and remove sulphate and metals such as iron, manganese, copper, aluminium, zinc, cadmium, cobalt, nickel, and mercury. Sulphate and nitrate can also be removed successfully (Hammack *et al.,* 2006).

2.3.2.3. Operations: These systems can be active and/or passive. Active treatment refers to the addition of chemicals to accelerate the chemical reactions. An organic carbon substrate is used as a source of nutrients for bacteria (Johnson *et al*., 2016). A neutralization agent might be required if influent water has high acidity. The optimal functioning of microbes plays an important role in the effectiveness of these systems (Zagury,*et al*., 2007). The organic substrate can be a mixture of carbon sources such as liquid ethanol, manure, wood chips, spent mushroom, fish bones, chitin, and/or sewage wastes (Gomez, 2013). HRT can vary from 1 to 5 days, depending on microbial kinetics and treatment intensity required. A benefit that bioreactors have over constructed wetlands is that the environment in which the microbes function can be manipulated or engineered to optimal condition as required, whereas in constructed wetland changes to the environmental or microbial habitat conditions are not always possible.

2.3.2.4. Maintenance & monitoring: The maintenance of these systems is very similar to that of constructed wetlands. Several components must be maintained to ensure effective functionality of the system, which include: influent water pH, influent water metal concentrations, influent water volume per unit of time to be treated, availability of organic carbon as nutrients for microbial processes, sufficient HRT, and removal of any precipitates formed. The organic substrates will also have to be replaced after a period of operation to ensure effective treatment is sustained. Regular monitoring is required to track system success.

2.3.2.5. System limitation: The design of the system is based on specific parameters and characteristics of influent water, such as pH, flow rate, temperature, and the type and concentration of ions in water to be treated. The availability of sufficient surface area is a challenge when considering these systems. Cold temperatures can inhibit optimal functioning of microbes. The treatment system can, however, be designed to fit most environments. Controlling the influent flow rate is a critical factor in system success. Other limitations also include: depletion of organic carbon, low pH conditions, very high metal concentration of influent water inhibiting optimal microbial functionality, and sulphide toxicity to sulphate reducing bacteria. Substrate clogging might also be another challenge that decreases the effectiveness of the system. Inert gravel can be added to the substrate to prevent clogging by means of enlarging the pore spaces and improving the porosity of the substrate. Maintenance and monitoring of system infrastructure will be required to ensure optimal functionality. Another limitation to the system is the production of waste that is produced from metal sulphide precipitates.

2.3.2.6. Effectiveness: A bioreactor was constructed and tested in South Africa, near Vryheid in Kwazulu-Natal province in 2015. The treatment system was designed to neutralise acidity, remove metals, and reduce sulphate concentration from acid mine drainage water. The results obtained from the Vryheid Coronation Colliery bioreactor are indicated in *Table 3* (Pulles *et al*., 2016).

Table 3: *Effluent results from biological passive treatment at Vryheid Coronation Colliery.*

Constituent	Influent (mg/L)	Effluent (mg/L)
pH (-log(H^+))	3,03	6,94
Sulphate	1594	621
Alkalinity	0	487
Aluminium	33,64	0,45
Iron	25,45	0,31
Manganese	14,38	7,12
Ammonia	0,3	17,48
Phosphate	0,86	10,7

It’s evident from *Table 3* that the treatment system at Vryheid Coronation Colliery was successful in increasing the pH, and removing most contaminants from the water. Sulphate was removed by more than 50%. Additional treatment would be required to further improve the water quality.

An integrated bioreactor was installed at an abandoned metal (gold, zinc, silver, and lead) mine in Montana, U.S.A. The results obtained from the bioreactor are indicated in *Table 4* (Bless et al., 2006; Mine Waste Technology, 2004; NOAA, 2002).

Table 4: *Treatment efficiency for Surething Mine.*

Constituent	Influent (mg/L)	Effluent (mg/L)
pH	2.58	7.31
Aluminium	29.5	<0.04
Arsenic	0.127	<0.01
Cadmium	0.208	<0.00009
Copper	2.35	<0.003
Iron	15	<0.014
Lead	0.151	0.004
Manganese	26.7	0.037
Zinc	22.7	<0.007
Ammonium	0.11	0.37
Sulphate	591	239

It is evident from *Table 4* that sulphate was only reduced with approximately 60%. Some challenges encountered with the bioreactor was prolonged exposure to acid mine water with high metal concentrations. It also became evident that manganese require separate treatment with aerobic treatment components.

2.3.3. Permeable reactive barriers

2.3.3.1. Technology description: These systems are an *in situ* permeable treatment zone designed to improve water quality from a contaminated groundwater plume or seepage zone. These systems can be constructed as a *funnel-and gate* or *continuous* permeable reactive barrier (Courcelles, 2014). Both these methods require excavation to place the substrate and intercept the groundwater plume. The rate of groundwater movement determines the size of the permeable reactive barrier required to achieve desired water quality objectives (U.S. EPA, 1997).

2.3.3.2. Constituent treated: These can include, but are not limited to: radionuclides, trace metals, and anion contaminants. Trace metals treated include hexavalent chromium, nickel, lead, uranium, technetium, iron, manganese, selenium, copper, cobalt, cadmium and zinc. Anion contaminants include sulphate, phosphate, and arsenic (U.S. EPA, 2014).

2.3.3.3. Operations: The most commonly used permeable reactive barrier configuration is a continuous trench in which the treatment material is backfilled to intercept the groundwater plume (ITRC, 2005). The *funnel-and-gate* barrier can guide the groundwater plume to a designated treatment zone. The continuous barrier design transects the plume, and contaminated groundwater flow through the reactive media. The permeable reactive barrier is normally installed as close as possible to the bedrock to ensure that the water requiring treatment does not flow underneath the treatment zone. Another critical factor is to ensure that the hydraulic conductivity of the reactive media is greater that the hydraulic conductivity of the aquifer. This will prevent the groundwater plume from flowing around the reactive barrier (U.S. EPA, 1998). The reactive material degrades or immobilises contaminants by means of microbial processes. Several factors must be addressed when considering permeable reactive barriers for contaminant remediation: the geometric configuration of the plume, groundwater flow direction, hydraulic conductivity of aquifer material, and contaminant concentrations must be known to achieve the required treatment performance. The microbial processes within the reactive material must reduce the contaminant concentrations to within acceptable standards. The precipitation of sulphide metals could cause plugging (clogging) of the substrate.

2.3.3.4. System limitation: The system can take several years to reach optimal functionality, mainly due to the time it takes for the microbes to stabilise and reach maturity. Controls must be installed to monitor system performance. Plugging caused by metal sulphide precipitation can reduce treatment efficiency of the reactive barrier if not managed properly. The reactive material will have to be replaced if it becomes saturated and clogged with precipitates.

2.3.3.5. Effectiveness: A permeable reactive barrier was constructed at a uranium mine site in Durango, Colorado, with influent water containing 359 µg/L selenium. Effluent (treated) water only contained 8 µg/L selenium after treatment (U.S. EPA, 2014). Another permeable reactive barrier at the Monticello Mill Tailings site in Utah was constructed to remove uranium, vanadium, arsenic, selenium, molybdenum, and nitrate from mine impacted water. The hydraulic conductivity of the substrate decreased due to sulphide precipitation and caused groundwater to flow over the permeable reactive barrier. Desired water qualities were, therefore, not achieved.

In 2005, a pilot-scale permeable reactive barrier was installed at a metal smelting facility, located near Helena, Montana, U.S.A. to treat arsenic in groundwater. Monitoring results indicated that mine impacted water with arsenic concentrations > 25 mg/L were treated to concentrations < 2 mg/L in effluent water. Sulphate concentrations of effluent water were, however, not measured (U.S. EPA, 2005 and 2008).

A permeable reactive barrier was also installed at a Nickel Mine in Ontario, Canada. The water quality results are indicated in (Benner, et al., 1999; Benner et al., 2002; Johnson et al., 2000; Mayer et al., 2006; RTDF, 2000).

Table 5 (Benner, et al., 1999; Benner et al., 2002; Johnson et al., 2000; Mayer et al., 2006; RTDF, 2000).

Table 5: *Treatment efficiency of permeable reactive barrier at Nickel Mine.*

Constituents	Influent (mg/L)	Effluent (mg/L)
pH	2.8-5.9	6.7
Aluminium	130	<1.0
Copper	3.0	<0.01
Iron	250-1350	80
Nickel	0.12-30	<0.1
Zinc	1.0	<0.015
Sulphate	2500-5200	840
Alkalinity	<1-60	2300

(Benner, et al., 1999; Benner et al., 2002; Johnson et al., 2000; Mayer et al., 2006; RTDF, 2000).

Table 5 is indicative that sulphate was reduced significantly. Challenges were encountered with regard to maintaining a constant hydraulic conductivity throughout the barrier, which reduces the treatment efficiency over time.

2.4. SULPHUR, PYRITE OXIDATION, AND MICROBES

2.4.1. Sulphur and microbes

Micro-organisms play an essential role in sulphur uptake from the environment. Sulphate is consumed as a nutrient and reduced to sulphide. Sulphate reducing bacteria are anaerobic micro-organisms that occur in anoxic habitats, where they use sulphate as a terminal electron acceptor (reduction of sulphate) for the degradation (oxidation) of organic compounds Organic compounds (amino acids, sugars and fatty acids) are fermented by fermentative bacteria. The fermentation of organic compounds by sulphate reducing bacteria is optimal in an anaerobic environment where sufficient sulphate and readily available organic carbon is available (Muyzer & Stams, 2008). The products from the fermentation process include acetate, propionate, butyrate, lactate and hydrogen, which can be utilised by sulphate reducing bacteria (Muyzer & Stams, 2008). The oxidation of sulphide bearing minerals can cause detrimental impacts to the receiving environment by means of acid and sulphate that are produced, if exposed to oxygen and water. A very common sulphide bearing mineral is pyrite (Muyzer & Stams, 2008).

2.4.2. Pyrite oxidation

Coal deposits in South Africa are associated with shales, mudstones, siltstones, and sandstones (Karoo Supergroup) host rock. These deposits host coal seams, which contain minerals such as quartz, carbonates, sulphides, and pyrite (Aphane & Vermeulen, 2015).

Acid mine drainage is generated when reactive minerals, such as pyrite, are exposed to oxygen and water (Tsukamoto *et al.*, 2004). The oxidation of pyrite can be illustrated by the following reactions (Akcil & Koldas, 2006):

$$FeS_2 + 7/2O_2 + H_2O \rightarrow Fe^{2+} + 2SO^{2-}{}_4 + 2H^+$$

(Reaction 1)

The above-mentioned reaction represents the oxidation of sulphide mineral that produces dissolved iron, sulphate, and hydrogen. The dissolved iron, sulphate, and hydrogen contribute the total dissolved solids (TDS) and acidity in the receiving water course. The following reaction presents the oxidation of ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}):

$$Fe^{2+} + 1/4O_2 + H^+ \rightarrow Fe^{3+} + 1/2H_2O \qquad \text{(Reaction 2)}$$

Ferric iron will precipitate at pH 2.3 and pH 3.5 as $Fe(OH)_3$ (Iron(III) oxide-hydroxide) and jarosite ($KFe^{3+}{}_3(OH)_6(SO_4)_2$).

$$Fe^{3+} + 3H_2O \rightarrow Fe(OH)_{3\ solid} + 3H^+$$

(Reaction 3)

Pyrite can be oxidised by ferric iron that does not precipitate immediately:

$$FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2SO^{2-}{}_4 + 16H^+$$

(Reaction 4)

A combination of the above-mentioned reactions where $Fe(OH)_3$ precipitates, can be presented as:

$$FeS_2 + 15/4O_2 + 17/2H_2O \rightarrow Fe(OH)_3 + 2SO_4^{2-} + 4H^+$$

(Reaction 5)

The oxidation of pyrite by ferric iron can also be illustrated as:

$$FeS_2 + 15/8O_2 + 13/2Fe^{3+} + 17/4H_2O \rightarrow 15/2Fe^{2+} + 2SO^{2-}{}_4 + 17/2H^+$$

(Reaction 6)

Dissolved metals liberated by acidic conditions pose a danger to ecosystem health, if acidic water enters natural water courses (Maree & Du Plessis, 1994). The consequences of acid mine drainage differ from site to site, depending mostly on the pH, and metal and sulphate concentrations of the acid mine drainage water. The primary factors that determine the rate of acid mine drainage generation are (U.S. EPA, 1994):

- Mineral composition of sulphide bearing mineral;
- Type and quantity of neutralization minerals present;
- Reaction surface area;
- Oxygen and water content;
- Presence and type of bacteria.

When acid products are flushed from the sulphide rocks, it might encounter an acid consuming mineral that has the capacity to neutralise the acidity (neutralization capacity of minerals). The acid produced can be neutralized by minerals such as calcium carbonate. If the neutralization capacity of the surrounding rocks is exceeded, then excess acid remains (Eary & Williamson, 2006). This excess acid can then be mobilised to the receiving environment. The neutralization capacity of the surrounding rocks can, however, not remove any sulphate or metals produced from the pyrite oxidation process. Any metals and sulphate produced from pyrite oxidation can be flushed to the surrounding groundwater systems or be transported to surface water systems. Many coal (and gold) mines encounter the challenge of excess sulphate concentration in decanting or seepage water.

The potential of coal discard material to generate sulphate and acidity, and potentially impact determinately on the receiving environment, can be determined by means of the following analytical methods (Lawrence & Wang, 1997; Price *et al*., 1997; Sober *et al*., 1978; Soregaroli & Lawrence, 1998; and Usher *et al.,* 2003):

- **Acid base accounting (ABA):** ABA is a general term used to describe the acid potential (AP), neutralization potential (NP), and the net neutralization potential (NNP) of the material. The NNP is calculated by means of subtracting the AP from the NP, which provides an indication on whether any excess acidity will remain, or whether the material will be able to neutralise the produced acid. A NNP < 0 entails that the host material does not have the capacity to completely neutralise the produced acidity, while a NNP > 0 entails that the host material has the capacity to neutralise the produced acid. The neutralization potential ratio (NPR) of the discard material can also be used to determine the potential for acid generation. A NPR of < 1:1 is indicative that the discard material has the potential to generate acid, while a NPR of > 4:1 shows no indication for acid generation.
- **Sulphur content:** The sulphide-sulphur content of the material provides an indication on whether the material has the potential to yield acidity. A sulphide-sulphur content of at least 0.3% is required for sustainable long term acid-generation.

2.5. ENVIRONMENTAL, HUMAN, AND ANIMAL HEALTH IMPACTS CAUSED BY ELEVATED SULPHATE CONCENTRATION IN WATER

The Department of Water Affairs (DWS) provides two water quality guidelines that can be used as a baseline to assess the impacts of excess sulphate concentration on the receiving environment. These two documents include: *Water Quality for Livestock Watering (Volume 5, 1996)* and *Water Quality for Aquatic Ecosystems (Volume 7, 1996).* These two guidelines are very applicable to the South African mining context in that most coal mines aim at rehabilitating disturbed areas back to grazing end land use. The impacts associated with decanting or seepage water from decommissioned coal mines in South Africa are normally related to livestock and aquatic ecosystems health. These two guidelines describe the characteristics of the sulphate anion as follow:

Sulphate can form salts with various cations such as potassium, sodium, calcium, magnesium, barium, lead, ammonium, and other metals. Sulphur has physiological roles in that it forms a component of amino acids, hormones, enzymes, carbohydrates, and metabolic processes. Excess sulphate can cause diarrhoea and poor productivity in livestock not adapted to higher sulphate concentration in water. The adverse effects of excess sulphate intake are normally associated with sodium and magnesium sulphate. Calcium sulphate showed negligible effects as compared to other sulphate salts. Sulphate concentrations in excess of 1000 mg/L in water can cause the following symptoms in livestock (DWA, 1996):

- Diarrhoea;
- Adverse palatability effects;
- Poor productivity;
- Poor water intake.

The effects of high sulphate concentration on livestock health depend on the age, type, and weight of the livestock. Cadmium and zinc sulphate can have an adverse effect on fish. Copper and manganese sulphate is normally soluble in water. Toxicity of zinc has been associated with salts, such as zinc sulphate (DWA, 1996).

The World Health Organization (WHO) (2004) provides the following synopsis of sulphate detectability and the effects on humans:

Sulphate can be tasted in drinking water at concentrations ranging from 250-1000 mg/L: 350 mg/L for sodium sulphate, 250-1000 mg/L for calcium sulphate, and 400-600 mg/L for magnesium sulphate (NAS, 1977). Sulphate concentration at which drinking water has an offensive taste is approximately 1000 mg/L and 850 mg/L for calcium and magnesium sulphate respectively (Zoeteman, 1980).

Cocchetto & Levy, (1981) state that the ingestion of 8.0 g of sodium sulphate could cause some degree of diarrhoea. A laxative effect is caused when water with a sulphate concentration in excess of 1000 mg/L is ingested (U.S. EPA, 1999b). Dehydration has also been reported as a common side effect from the ingestion of high concentrations of magnesium or sodium sulphate (Fingl, 1980). The dehydration is normally caused from diarrhoea (U.S. EPA, 1999a,b). The development of cataracts has been reported to develop as a result of ingesting water containing sulphate concentration in excess of 600 mg/L (U.S. DHEW, 1962 & Chien *et al.*, 1968), although humans can adapt to higher concentrations over time (U.S. EPA, 1985). A decrease in gastrointestinal retention of food can be experienced with ingestion of high concentration sulphate (Heizer, *et al*., 1997). It can be concluded that the ingestion of high concentration of sulphate can have possible adverse effects on human, livestock, and ecosystem health. The above-mentioned studies thus prove that mitigation measures are required to reduce elevated concentration of sulphate in impacted waters before discharged into the natural catchment.

2.6. SULPHUR TRANSFORMATION

The reduction of sulphate in mine impacted water by means of sulphate reducing bacteria requires an environment that enables and promotes sulphate reduction to occur. The pathway for biological sulphur transformation is illustrated in *Figure 4*. Sanchez Andrea *et al*., (2014) provide a synopsis of the chemical and biological transformations of sulphur:

- *Chemical sulphur transformation* entails atmospheric and terrestrial redox processes.
- *Biological sulphur transformation* entails the use of micro-organisms that catalyse the oxidation and reduction reactions of sulphur compounds. These reactions include:
    - *Dissimilatory sulphur reduction*, where sulphate is reduced to sulphide, which is coupled to energy preservation and biomass growth;
    - *Assimilatory sulphate reduction*, where the reduced sulphide is assimilated in biomass, proteins, amino-acids, fungi, and micro-organisms;
    - *Mineralization* of organic compounds with hydrogen sulphide release;
    - *Sulphide oxidation* by O_2, NO^-_3, Fe^{3+}, or Mn^{4+} as electron acceptors by lithotrophic and phototrophic bacteria, producing sulphur and sulphate;
    - *Disproportionation*, where oxidation and reduction of sulphur compounds (thiosulfate, sulphide, and sulphur) to sulphate and sulphide occurs.

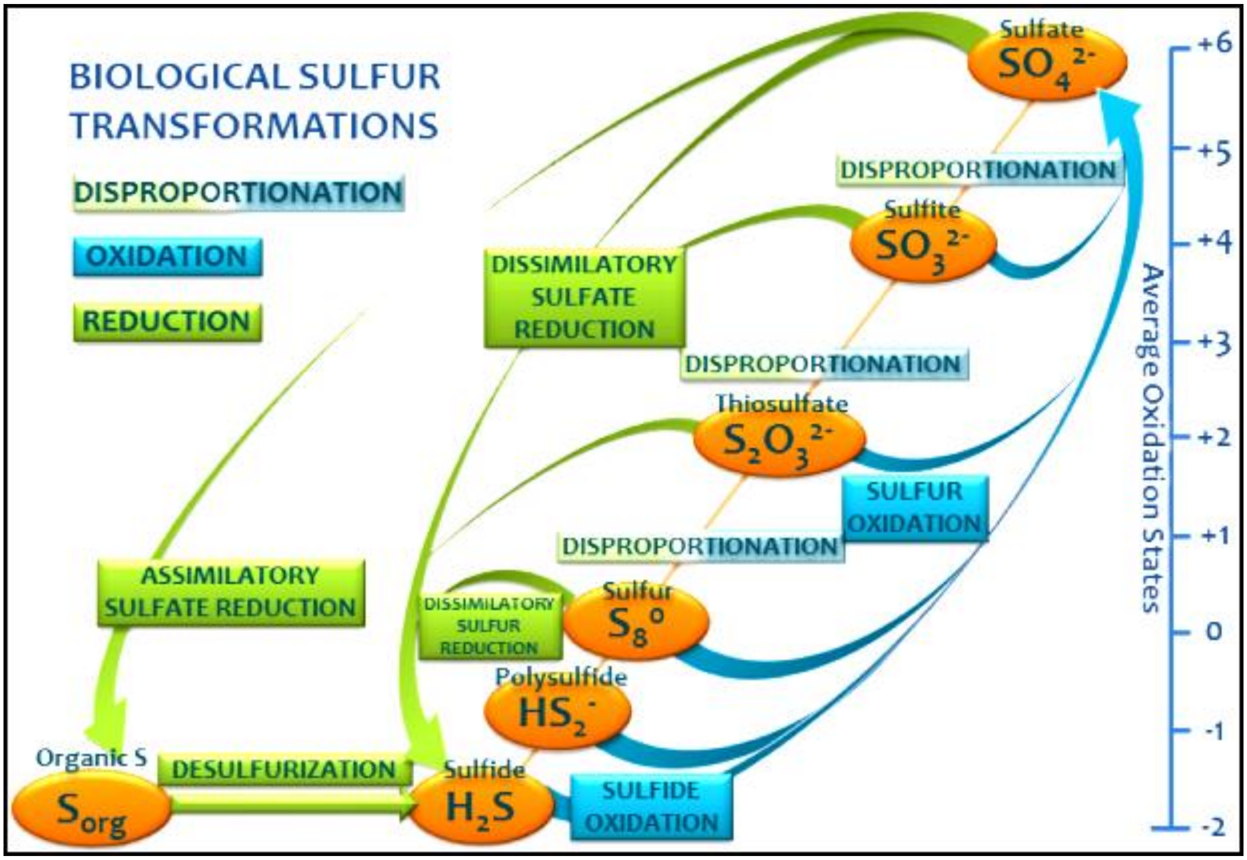


Figure 4: *The process of biological sulphur transformations (Sanchez-Andrea et al., 2014).*

2.7. REDOX SEQUENCE FOR SULPHATE REDUCTION

The redox environment is instrumental in achieving optimal bacteria functioning (Alesia, 2014). Understanding the sequence in which chemical species are reduced or oxidised are fundamental to the success of any biological passive treatment system. The *reduction* of chemical species occurs in the following sequence (Appelo & Postma, 2013): oxygen reduction, denitrification, Mn^{4+} oxide → Mn^{2+}, Fe^{3+} oxide → Fe^{2+}, SO_4^{2-} reduction, and CH_4 fermentation. The *oxidation* reactions occur in the following sequence: oxidation of organic matter, sulphide → SO_4^{2-}, oxidation of Fe^{2+}, NH_4^+ → NO_3^-, and the oxidation of Mn^{2+}. These sequences in which oxidation-reduction reactions occur are presented in the *Figure 5*.

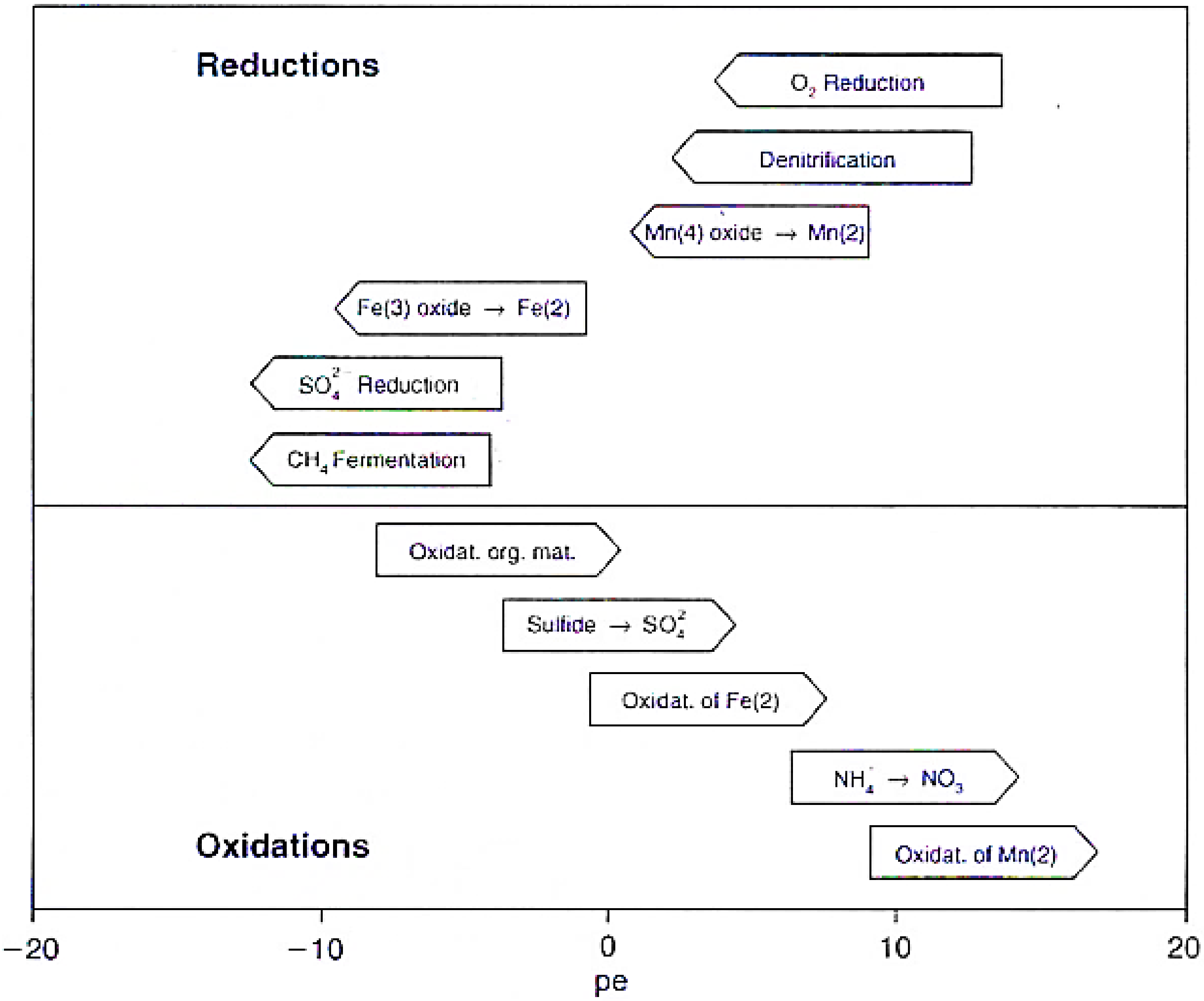


Figure 5: *Sequence of redox reactions at pH 7 in natural waters. (Appelo & Postma, 2013).*

Redox processes can play a fundamental role in the success of biological passive treatment systems. The redox conditions within a biological passive treatment system are of critical importance for optimal sulphate reduction. A reduction reaction will commence with an oxidation reaction that is located at a lower pe (redox potential) (origin of arrow in *Figure 5*). The reduction of sulphate can be combined with the oxidation of organic matter, but not with the oxidation of ferrous iron, for example (Appelo & Postma, 2013). The redox conditions in biological passive treatment systems have implications for the sequence in which contaminants are removed from the mine impacted water. A critical controlling factor is the pH at which treatment occurs. For example, biological oxidation of manganese does not occur rapidly in the presence of ferrous iron (Liu, *et al.*, 2014). Metal sulphide precipitation in anaerobic bio-substrates is not recommended for manganese removal. It was found that pH 9.5 was optimum for Mn^{2+}-oxidation. The presence of sulphate causes a deceleration of Mn-oxidation (Balcioglu & Demirkol, 2013). The removal of manganese is, therefore, recommended prior to sulphate reduction.

Iron can either be removed in reducing or oxidising systems. In reducing systems, sulphate is reduced to sulphide. Iron could couple with sulphide, and produce a metal sulphide precipitate (FeS_2, FeS). Work undertaken by Batty & Younger, (2003) indicates that aerobic wetlands can be used as a polishing stage to remove residual iron from the water. Oxidising systems remove iron through oxidation of ferrous iron, once the pH has been raised sufficiently. The product water will have a low pH once ferrous iron is oxidised to ferric iron (precipitate) (Akcil & Koldas, 2006). The oxidation of ferrous iron depends on oxygen concentration and pH of water. The oxidation of ferrous iron to ferric iron requires water to have high oxygen content, with a pH of ideally 8.5. Cascading is a method by which aeration can be promoted, which is normally conducted during the first stages of treatment.

2.8. BIOLOGICAL TREATMENT OF SULPHATE

2.8.1. Background on biological sulphate reduction and metal removal

Sheoran *et al*., (2010) state that sulphate reducing bacteria can occur in various anoxic environments, such as wetland sediments, cattle rumens and geothermal vents. Sulphate reducing bacteria are prokaryotes that function in reducing anaerobic environments that contain sufficient sulphate concentration (Sheoran *et al*., 2010). Sulphate reducing bacteria is either heterotrophic (using organic compounds) (Logan *et al.*, 2005), or autotrophic (using hydrogen as an electron donor and CO_2 as carbon source) (Kilborn Inc., 1996).

According to Lorax Environmental (2003), anaerobic wetlands are suitable for biological sulphate removal from mine impacted water. This is accomplished by means of utilising sulphate reducing bacteria. Maree *et al.,* (2000) also states that sulphate, metals and acidity can be removed from mine impacted water by means of biological sulphate reduction.

Biological treatment systems utilise sulphate reducing bacteria to reduce sulphate to sulphide, which also lead to the removal of metals and acidity (Zagury *et al.*, 2007). Sulphide is a product formed when sulphate undergoes reduction. The produced sulphide can react with metal oxides to form metal sulphide precipitate. The produced metal sulphides normally have a low solubility. An additional carbon source must be introduced to increase the rate of sulphate reduction (Brahmacharimayum & Kumar, 2012), if the initial pool of organic carbon is depleted. Sulphate reducing bacteria can generate hydrogen sulphide and carbonate alkalinity, promoting metal sulphide precipitation and increasing the pH of the water being treated. An increase in alkalinity of treated water is mostly experienced in sulphate reducing bioreactors, which is caused by the oxidation of organic matter (Maree *et al.,* 2000). Various applications of these biological passive treatment systems have been considered to treat acid mine water. Some of these applications include, but are not limited to: anaerobic bioreactors, constructed anaerobic wetlands and permeable reactive barriers (Kilborn, 1999).

Simplified reactions that represent biological oxidation of acetic acid, sulphate reduction, and metal sulphide precipitation, are illustrated in Reaction 7 and Reaction 8 (Middleton & Lawrence, 1977, and Doshi, 2006):

$$CH_3COOH + SO_4^{2-} \rightarrow H_2S + 2HCO_3^{-1}$$

(Reaction 7)

$$H_2S + M^{2+} \rightarrow MS + 2H^+$$

(Reaction 8)

CH_3COOH represents acetic acid produced from the oxidation of organic matter. Sulphate reducing bacteria uses simple organic compounds, such as acetic acid, as electron donors to reduce sulphate and produce sulphide (Logan *et al.*, 2005). The produced H_2S reacts with a metal (M^{2+}) to form a metal sulphide precipitate (MS) (Doshi, 2006). The presence of other electron acceptors such as ferric iron and nitrate can prevent sulphate reduction or oxidise sulphides produced by sulphate reduction (Lovley & Phillips, 1987).

Sulphate reducing bacteria can survive in a wide range of environmental conditions (Muyzer & Stams, 2008). The availability of organic carbon controls the rate of sulphate reduction (Eger & Wagner, 2003). Insufficient organic carbon can be regarded as a potential limiting factor for optimal sulphate reduction in biological passive treatment systems (Roychoudhury & Cappellen, 2003). A study conducted by Chen, *et al.*, (2014) indicates that readily available organic carbon could improve the rate of sulphate reduction significantly. Sources of organic carbon can include: sawdust, cattle manure, wood chips, cow manure, horse manure, poultry manure, sheep manure, sewage sludge, peat, pulp mill, molasses, and compost (Sheoran *et al.*, 2010).

Data on the longevity of biological passive treatment systems are limited. Decreases in the rate of sulphate reduction in biological passive treatment systems have been reported (Watzlaf *et al.*, 2000 and Benner *et al.*, 2002). Biological treatment systems are normally associated with vegetation planted on the surface of the substrate. Vymazal (2011) indicates that sulphate reduction is higher in unplanted anaerobic biological treatment systems, which is indicative that plant roots might inhibit microbial sulphate reduction, due to the development of preferential pathways that promote oxygen ingress into the anaerobic substrate via root canals formed. The use of vegetation in anaerobic bio-substrates is thus not recommended.

There is still some concern whether biological passive treatment systems are effective in reducing sulphate concentrations in mine impacted water to within acceptable or desired concentrations (Kuchler *et al.*, 2006). Optimising the design of these systems could improve the effectiveness of treatment.

2.8.2. Biological treatment system design factors

There are several factors to consider when designing a biological substrate for sulphate reducing bacteria to establish. These include: pH of influent water, substrate type, redox potential, availability of solid substrate, hydraulic residence time, sulphate concentration of influent water, metal concentrations of influent water, temperature, and chemical oxygen demand (COD) to sulphate ratio. Each of these factors is briefly described below.

2.8.2.1. pH: Alkalinity is produced in bioreactors by processes associated with sulphate reducing bacteria, such as the oxidation of organic matter to available carbon. These systems, therefore, have the potential to neutralise acid rich water (Johnson & Hallberg, 2005). According to Jong & Parry (2005), sulphate reducing bacteria can function within pH ranges from 4 to 6, but is inhibited when the pH is < 3.5. Metal precipitation will require a higher pH, preferably > 7.5. Sulphide and certain organic acids are known to be toxic to anaerobic bacteria, which become available at low pH (Rzeczycka & Blaszczyk, 2005).

2.8.2.2. Substrate/energy sources: The substrate mainly has two purposes: (1) provide carbon source, and (2) manage HRT through the system. The availability of an appropriate electron donor is critical for successful biological sulphate reduction (Logan *et al*., 2005). Organic matter is broken down by a variety of microbes. Studies indicate that slow decomposition of organic matter into useable available products for sulphate reducing bacteria could be a rate limiting factor in the sulphate reduction process (Logan *et al.*, 2005). Drury (2006) states that a significant challenge with biological passive treatment systems is determining the biodegradability of the organic carbon source, which plays a pivotal role in the rate of sulphate reduction. Drury (2006) further states that the biodegradability of the organic carbon source will decrease over time, thus also reducing the rate of sulphate reduction. Cellulose is normally the main component of the substrate after long periods of treatment. Sulphate reducing bacteria requires simple organic carbon sources for optimal sulphate reduction. Simple organic compounds are those that are immediately available to sulphate reducing bacteria, while complex organic compounds first need to undergo degradation by other microbes before they become available to sulphate reduction bacteria.

Simple organic carbon sources can include: alcohols (methanol and ethanol), organic acids (acetate, lactate, formate, and pyruvate) and sugars (sucrose). Tsukamoto *et al.*, (2004) concurs that sulphate reducing bacteria uses easily degradable organic matter (methanol, ethanol, and lactate), polylactic acid (Edenborn, 2004), and simple carbohydrate monomers (e.g., glucose or sucrose) (Mizuno *et al.*, 1998). Logan *et al.,* (2005) states that a variety of substrates can be used in biological treatment systems, which may include, but are not limited to: spent mushroom compost, leaves, sawdust, leaf mulch, wood chips, molasses, hay, straw, cow manure, horse manure, dried poultry waste, and sewage sludge. The most abundant source of organic matter is plant material, consisting primarily of lignin and polysaccharides (Logan *et al.,* 2005). Drury (2006) modelled sulphate reduction within a biological reactor using sawdust and cow manure as organic carbon source. Well degraded (high initial age) organic material performed better than organic material that was less degraded (low initial age).

Logan *et al.,* (2005) provides a synopsis of the decomposition process of organic matter. The *first step* in the decomposition of organic matter is that polysaccharides are degraded into alcohols, fatty acids, and H_2 by enzymes excreted by fermenting bacteria. *Step two* involves the degradation of alcohols and fatty acids to acetic acid, formic acid, hydrogen and carbon dioxide. The *final step* entails the oxidation of fermentative intermediates (acetate, formate, and hydrogen) *(Figure 6).*

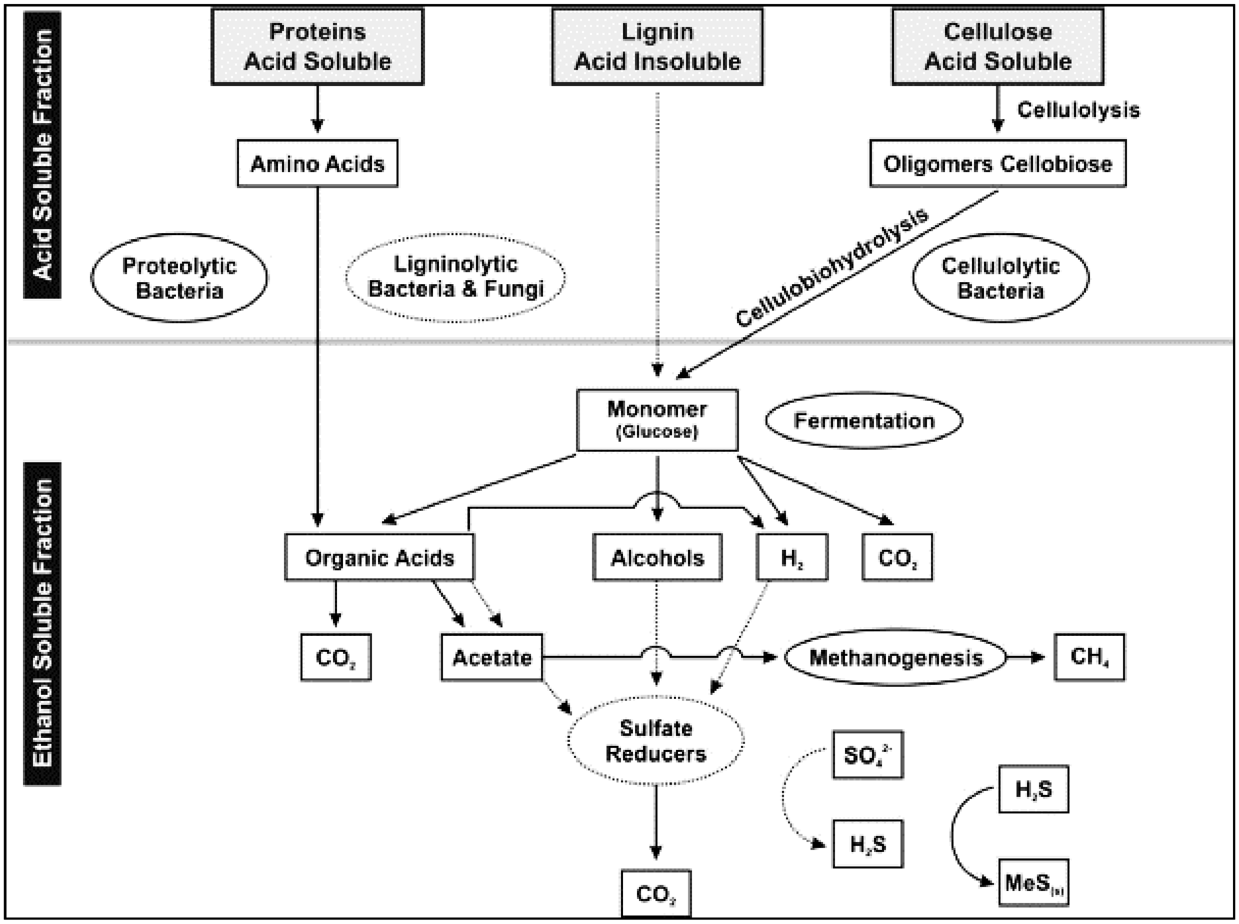


Figure 6: *Decomposition of organic matter to useable carbon (Logan et al., 2005).*

The chemical signature of the organic carbon source within the substrate plays a pivotal role in the success of any biological passive treatment system. A first stage evaluation on the adequacy of the compost to be used within a biological system includes (Cornell Waste Management Institute, 1996):

- **Carbon-nitrogen ratio:** Carbon and nitrogen are both essential nutrients for optimal microbial functionality. A general good practice standard for C/N ratio is 30:1 (30 parts carbon for 1 part nitrogen by weight). Lower ratios imply a low carbon and excess nitrogen content. Lower ratios will lead to nitrogen being lost as ammonia gas. Higher ratios will cause nitrogen content to be insufficient, which in turn might cause microbial death. The C/N ratio also provides an indication of the degree of degradation. High ratios (> 30:1) is an indication that the material still has a low initial age (not degraded significantly yet), while lower ratios (< 10:1) entail that the material has degraded significantly.
- **Trace metals and salts:** The compost material must have low concentrations of trace metals and salts. Guidelines for metal and salt concentrations in compost material are, however, limited. Compost material can vary greatly with regard to its chemical signature.

The concentration of these constituents should not impact detrimentally on effluent (treated) water quality during the treatment period. This is especially the case during the early stages of treatment when some salts and metals are liberated from the compost material and ends up in the receiving water course. It's indicated that these constituents normally occur in ample concentrations in compost.

- **pH:** An ideal pH for compost to host microbes ranges from 4 to 6. The degradation of organic matter is commonly associated with the release of organic acids that lower the pH. Low pH conditions (pH < 3.5) could lead to a reduction in some microbial activity.
- **Electrical conductivity (EC):** This parameter is used to determine the overall salt content of the compost. High EC (> 2000 mS/m) could cause negative impacts on the receiving water course. Most salts are, however, flushed from the substrate during the early stages of treatment.

2.8.2.3. Redox potential: For optimal performance, sulphate reducing bacteria needs an anoxic and reduced microenvironment with a redox potential (Eh) < -250 millivolt (mV) (Martins *et al.*, 2008). Batch and column laboratory bioreactors successfully treated mine impacted water at Eh values of –100 mV to –200 mV, at HRTs of 23 days and 150 days respectively (Cocos *et al*., 2002 and Gibert *et al*., 2004). The redox potential of each chemical species that requires treatment must be considered and taken into consideration during the design phase of the treatment system.

2.8.2.4. Availability of solid substrate: Sulphate reducing bacteria requires a solid substrate (sand and/or gravel) onto which they can attach (Lyew & Sheppard, 1999). Higher sulphate reduction rates are achieved if sulphate reducing bacteria have access to a solid support, compared to suspended bacteria (Glombitza, 2001). A substrate type with large pore spaces is preferred, because it minimizes the plugging of the bioreactor and treatment is more efficient (Sheoran *et al*., 2010). The substrate is also used to control the flow, specifically the HRT within the system. The addition of sand to organic material should change the hydraulic properties of the substrate, which impact on the HRT, and eventually the treatment efficiency of the system.

2.8.2.5. Hydraulic residence time (HRT): The hydraulic properties, such as porosity and hydraulic conductivity of a substrate, influence the HRT required to achieve the desired water quality standards. HRTs are, therefore, specific to each bioreactor, depending mostly on the desired effluent (product) water quality (Sheoran *et al*., 2010). The general accepted HRT in anaerobic bioreactors for optimal sulphate reduction is 5 days (Kuyucak *et al.*, 2006). Different organic substrates were tested for use in biological passive treatment systems. One study indicated that sheep manure did not promote sulphidogenesis at a HRT of 0,73 days. Increasing the HRT to 2,4 and 9 days improved sulphate reduction rates to 18% and 27% respectively (Gibert *et al.*, 2004). A shorter HRT might not allow adequate time for sulphate reducing bacteria to neutralise acidity, precipitate metals, or reduce sulphate sufficiently. Shorter HRTs might also result in the washing out of biomass from the reactor substrate. Yarwood *et al.,* (2006) stated that bacterial growth and accumulation within a porous substrate significantly impacted the hydraulic properties of a biological treatment system. Small variation in hydraulic conductivity of the substrate could entail significant changes in HRTs, and might result in decreased sulphate reduction (Benner *et al.*, 2002). Recently, sawdust has been increasingly used as a substrate in biological treatment systems, mainly due to a significantly higher hydraulic conductivity. When sawdust is used, there is, however, an increased potential for substrate compaction. Pre-wetting the substrate before treatment would aid to provide a more consistent hydraulic conductivity and a more constant flow rate (Bolis *et al.,* 1992). The HRT to achieve the required sulphate reduction and metal sulphide precipitation will differ between reactor and substrate types. Different substrate mixtures should, therefore, be investigated to obtain the desired HRT and treatment efficiency.

2.8.2.6. Sulphate concentration: The effect sulphate concentration of the influent water has on the performance of sulphate reducing bacteria varies between different biological passive treatment systems. A trend is noticed where the efficiency of sulphate reduction, specifically sulphate reduction rate, is higher at influent water containing sulphate concentration > 2000 mg/L, compared to influent water containing < 400 mg/L sulphate concentration (Tsukamoto *et al*., 2004 and Jong & Parry, 2004). Sheoran *et al.*, (2010) supports this statement by stating that an increase in sulphate concentration from 1.0 g/L to 5.0 g/L improved the volumetric sulphate reduction rate from 0.007 to 0.075 g/L/h respectively. Dev & Bhattacharya, (2014), however, states that sulphate reduction rates and overall functionality of sulphate reducing bacteria decrease with sulphate feed concentrations in excess of 1500 mg/L.

2.8.2.7. Effect of sulphide on sulphate reduction: It is shown that sulphate reducing bacteria have two sulphide threshold inhibition levels: undissociated sulphide and total sulphide. These threshold levels mostly depend on the environmental pH. At a pH < 7.2, undissociated sulphide (H_2S) is dominant (O’Flaherty & Colleran, 1998). At a pH > 7.2, the total sulphide is responsible for the inhibitory effect (O’Flaherty & Colleran, 1998) and occurs mostly in the form HS^- and S^{2-} (Al-Zuhair *et al*., 2008). The sulphate reducing bacteria is less sensitive for total sulphide when the pH ranges from 6.8 to 8.0 and more sensitive for undissociated sulphide concentration. Sulphate reduction rates can be inhibited by undissociated sulphide by as much as 50% when the pH is > 8.0 (O’Flaherty & Colleran, 1998).

2.8.2.8. Effect of metals: Sulphate reducing bacteria is capable of consuming high quantities of metals (Elsilk *et al.*, 2014). However, batch studies showed that high metal concentrations could inhibit bacterial growth, decrease sulphate reducing capacity, and perhaps be fatal to microbes (Cabrera *et al.*, 2006). Mine impacted water normally contains heavy metals (iron, zinc, copper, manganese and lead), which might become toxic or have an inhibitory effect on the activity of sulphate reducing bacteria, if threshold concentrations are exceeded. A study conducted by Elsilk *et al.,* (2014) indicates that a certain strain of bacteria (PS2010) can tolerate heavy metals such as cadmium, copper, cobalt, zinc and lead at minimum inhibitory concentrations of 0.6, 2.0, 0.8, 4.0, and 3 mM (millimol) respectively. Some strains of sulphate reducing bacteria have been found to function in water containing 100 ppm (parts per million) copper and 30 ppm iron (Garcia *et al*., 2001). It was noted that undissociated and dissociated metals can be inhibitory to sulphate reducing bacteria (Cabrera *et al.*, 2006).

2.8.2.9. Temperature: Doshi, (2006) states that low temperatures inhibit sulphate reducing bacteria. Tsukamoto *et al.,* (2004) states that sulphate reducing bacteria can still function optimally at 6°C. Sulphate reduction can occur within a wide range of temperatures, depending mostly on the type of bacteria. Optimum growth of *Desulfovibrio* bacteria has been found to be at temperatures ranging from 20°C to 35°C (Sheoran, *et al.,* 2010). Temperatures in excess of 40°C could inhibit the optimal functioning of sulphate reducing bacteria (Sheoran, *et al.,* 2010). Decreasing the temperature to below 10°C could reduce the sulphate reducing rate by 50% (Sheoran *et al.,* 2010). It was found that temperature changes between 20°C and 35°C had negligible effect on the functionality of sulphate reducing bacteria (Al-Zuhari *et al.*, 2008).

2.8.2.10. COD to sulphate ratio: This ratio is an important parameter affecting the competition between sulphate reducing bacteria and other anaerobic bacteria (Gomez, 2013). Moon *et al.*, (2015) state that the COD/sulphate ratio effects the decomposition of organic matter, specifically the organic carbon end products (propionate, lactate, formate, acetate, butyrate) that are produced from the oxidation or organic matter. Celis-Garcia *et al*., (2006) states that a COD to sulphate ratio of 0,67 was sufficient for degradation of organic matter for optimal sulphate reduction. Studies conducted by Dev & Bhattacharya (2014) indicate that optimal sulphate reduction, specific growth rate, and sulphide generation were observed at a COD/sulphate ratio of 8,94 in a medium containing marine waste extract. A general trend is observed where optimal sulphate reduction occurs at COD/sulphate ratios > 1,0.

2.8.2.11. Selecting treatment components: Gusek (2008) developed a flow chart that facilitates the process of selecting a passive water treatment system *(Figure 7)*. The starting point is characterising the influent water chemistry, and the flow rates. Metal concentrations of influent water will play a critical role in selecting the treatment components of the passive treatment system. Net alkaline water with nitrate, selenium, sulphate, cyanide, uranium, and other metals will entail that one of the treatment components should be a biochemical reactor for sulphate reduction and metal removal through metal sulphide precipitation. Net alkaline water without sulphate and metals will only require a settling pond, followed by an aerobic wetland. Net acidic water will normally entail pre-treatment such as limestone drains to increase the low pH and remove excess acidity.

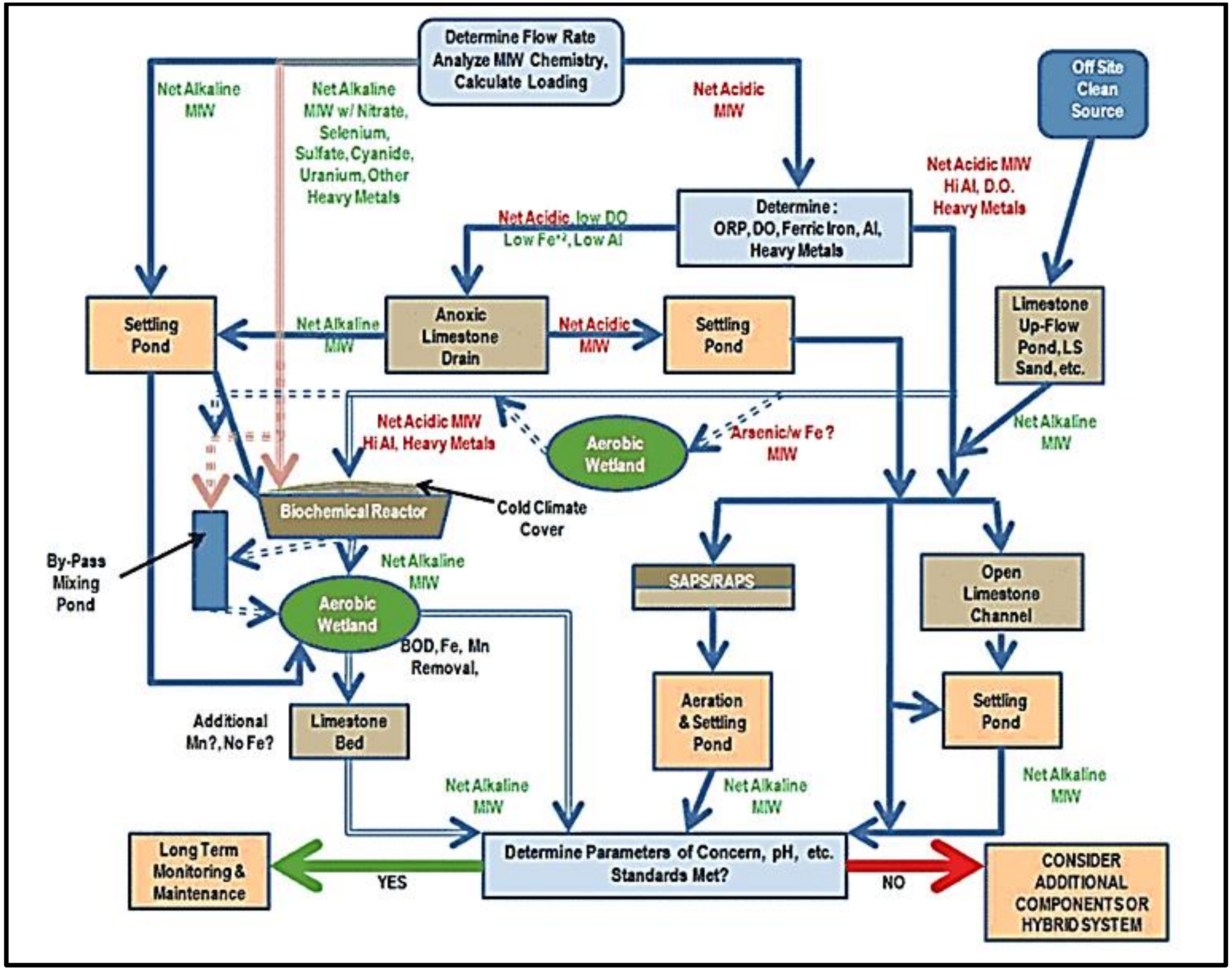


Figure 7: *Selecting treatment components of a passive treatment system (Gusek, 2008).*

2.9. HYDRAULIC RESIDENCE TIMES WITHIN SUBSTRATES

2.9.1. Flow rate

The flow rate within substrates is primarily governed by the following factors (Heath, 1983):

- **Hydraulic conductivity**: Hydraulic conductivity (K) is defined as the volume of water flowing through a cross-sectional area under hydraulic gradient (i).
- **Bed slope:** Steeper slopes will allow water to flow more rapidly through the substrate as compared to a flatter slope.
- **Inflow rate:** If inflow rate is higher than what the system allows, a spill will occur, unless the treatment system is a sealed unit in which pressure can build up.
- **Intactness of substrate material and interconnectivity of voids:** The development of voids within the substrate (preferential flow paths) could cause differentiated flow rates to occur. A continuous interconnected network of voids, pore spaces, and fissures within the substrate will lead to an increased flow rate through the substrate. The effective porosity of the substrate will be a governing factor to determine the HRT. It is expected that the effective porosity will be similar to the total porosity in unconsolidated material, whereas consolidated material will present a lower effective porosity and higher total porosity. This is mostly due to the occurrence of “dead-end-pockets” within consolidated material, which is absent in unconsolidated material.
- **Hydraulic gradient:** The hydraulic gradient is a function of the pressure head. The higher the pressure head, the higher the hydraulic gradient. The head at which water flows into the treatment system also plays a pivotal role in the flow rate and HRT within a substrate. The higher the head, the higher the flow rate will be, and the shorter the HRT. Inflow is, therefore, related to the head, which governs hydraulic gradient.

A critical success factor of anaerobic bio-substrates is determining the hydraulic conductivity (K) of the selected substrate. Hydraulic conductivity can be calculated by means of the following Darcy equation:

$$K = \frac{Q}{iA}$$

(Equation 1)

Where,

Q = Volumetric flow rate (m^3/unit time),

i = Hydraulic gradient (∆H/L) (dimensionless),

A = Cross-sectional area of flow (m^2),

K = Hydraulic conductivity (m/unit time).

Figure 8 illustrates the relationship between the parameters in Equation 1.

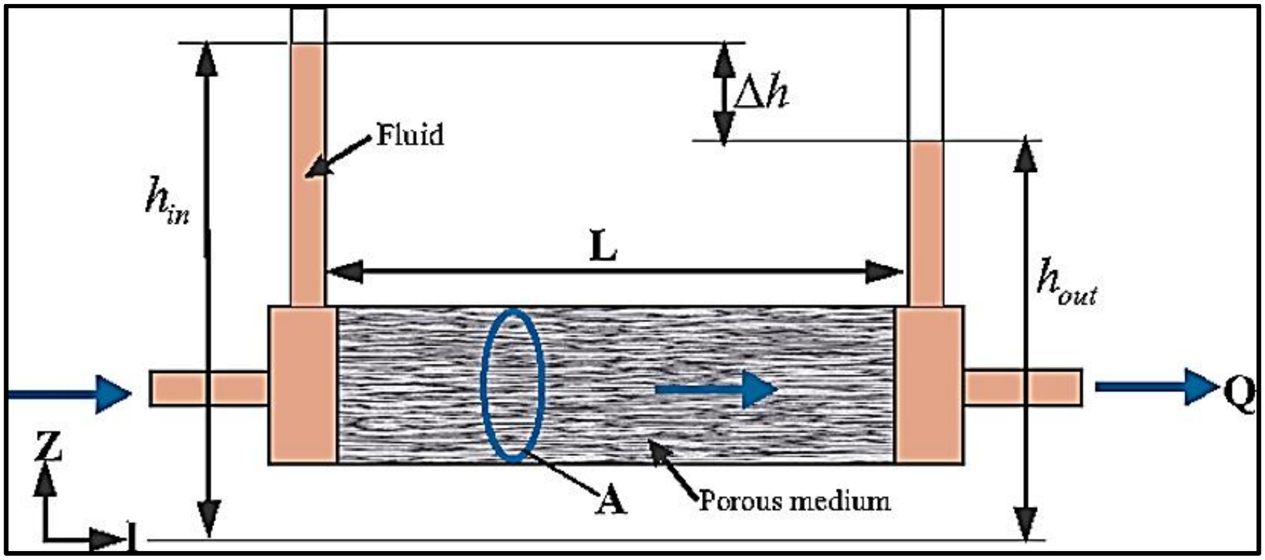


Figure 8: Darcy Apparatus (Aumann & Ford, 2002; Kresic, 2007).

Two other parameters that need to be calculated in order to calculate HRT within a substrate, is Darcy velocity (V_d) and Seepage velocity (V_s). Darcy velocity assumes the entire substrate contributes to flow (complete interconnectivity of pores), whereas Seepage velocity considers the effective porosity of the substrate material. Seepage velocity is regarded as being more representative of actual groundwater velocity, because it considers that not all pores within the substrate are connected and contribute to discharge. Only the interconnected pores contribute to flow and discharge within a porous medium. Darcy velocity can be calculated as:

$$V_d = \frac{Q}{A}$$

(Equation 2)

Where,

Q = Volumetric flow rate (m^3*/unit time),*

A = Cross-sectional area of flow (m^2*),*

V_d *= Darcy velocity (m/unit time).*

Darcy velocity can also be calculated as a function of hydraulic conductivity (K) and hydraulic gradient (i):

$$V_d = Ki$$

(Equation 3)

Where,

K = Hydraulic conductivity (m/unit time);

i = Hydraulic gradient (i);

V_d *= Darcy velocity (m/unit time).*

The interconnectivity of pores within organic material and pure washed river sand is regarded as being significant. It is expected that effective porosity will be equal to total porosity due to the unconsolidated nature of such materials (Nnaemeka, 2010), as well as the absence of “dead-end-pockets”. The total porosity (hereafter referred to as porosity) can be calculated as (ASTM, 1998):

$$n = \left(1 - \frac{P_b}{P_d} \right) \times 100$$

(Equation 4)

Where,

P_b *= Dry density (g/*cm^3*),*

P_d *= Relative density (g/*cm^3*),*

n = Porosity (%).

The Seepage velocity (V_s) is obtained from dividing the Darcy velocity by the porosity of the substrate material, which can be calculated as (Watson & Burnett, 1995):

$$V_s = \frac{V_d}{n}$$

(Equation 5)

Where,

V_d = Darcy velocity (m/unit time),

n = Porosity (index),

V_s = Seepage velocity (m/unit time).

2.9.2. Tracers and Reactors

Groundwater tracers are chemical or biological substances injected into groundwater systems to provide an indication of aquifer characteristics (Eliot, 2014), by means of measuring changes in concentration of the particular tracer over time and space. Tracers can be divided into environmental or artificial tracers. Environmental tracers (chemical constituents, isotopes etc.) are mostly used to obtain a regional understanding of the hydrogeology of an area, while artificial tracer (radioactive tracer, chemical tracers, tracers that can be activated, labelling material etc.) are used for site specific analysis (Centre for Water Sciences and Management, 2016). Tracers are normally used in groundwater studies to determine HRT, flow paths, or degree of mixing within an aquifer (Bruckner, 2016). Tracers used should at least hold the following characteristics: low sorptivity, high water solubility, and not present as an environmental risk (Winter, 2006). Tracers can also be used in reactors to determine the mixing properties within the substrate.

Tracers can be injected at a certain concentration into the substrate at the inlet of a reactor, and the change in tracer concentration measured at the outlet over time. Davis & Davis (2003) describe three types of flow within reactors. These are illustrated in *Figure 9* to *Figure 11.* When considering a plug flow reactor (PFR), it is assumed that every molecule spends an equal time within the reactor. No mixing is expected within these systems (no axial mixing) *(Figure 9).* Tracer is injected at a certain concentration at time t_0, and a sudden pulse is observed at the outlet at time t_1. The influent and effluent tracer concentration is very similar.

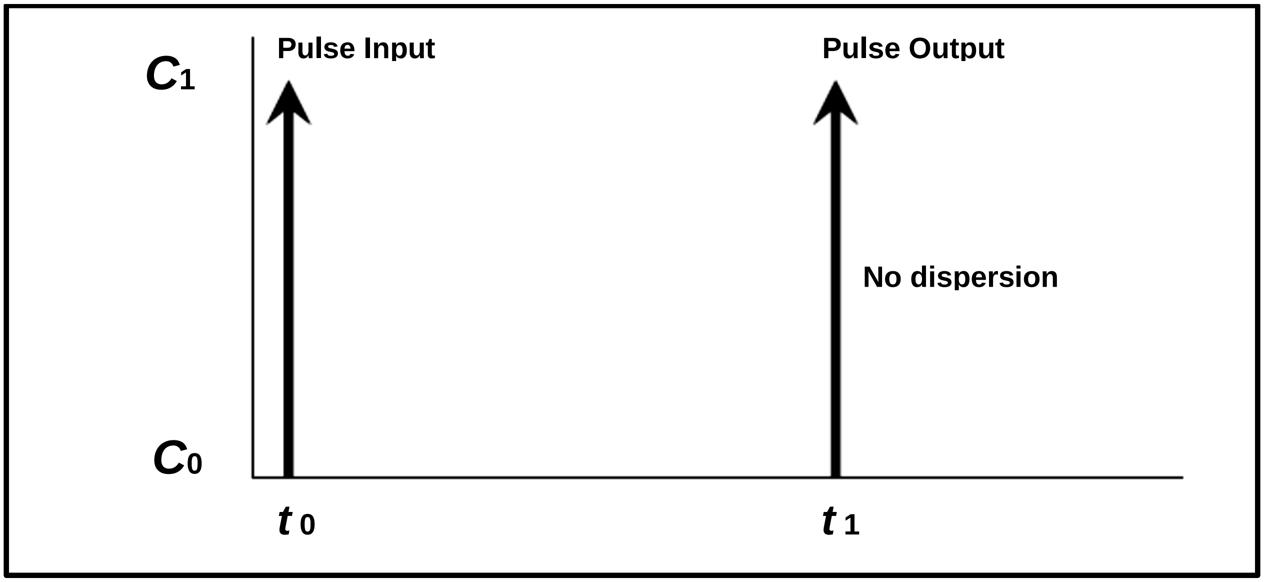


Figure 9: *Dispersion within a plug flow reactor (Colorado Education, 2014).*

In a continuous stirred reactor (CSTR), the substrate mixture within the reactor is identical in concentration and temperature to the effluent (products) *(Figure 10).* These systems are, therefore, considered to be completely mixed. Tracer is injected at a certain concentration $t_{0,}$ and the tracer concentration of the effluent decreases gradually over time (t_1).

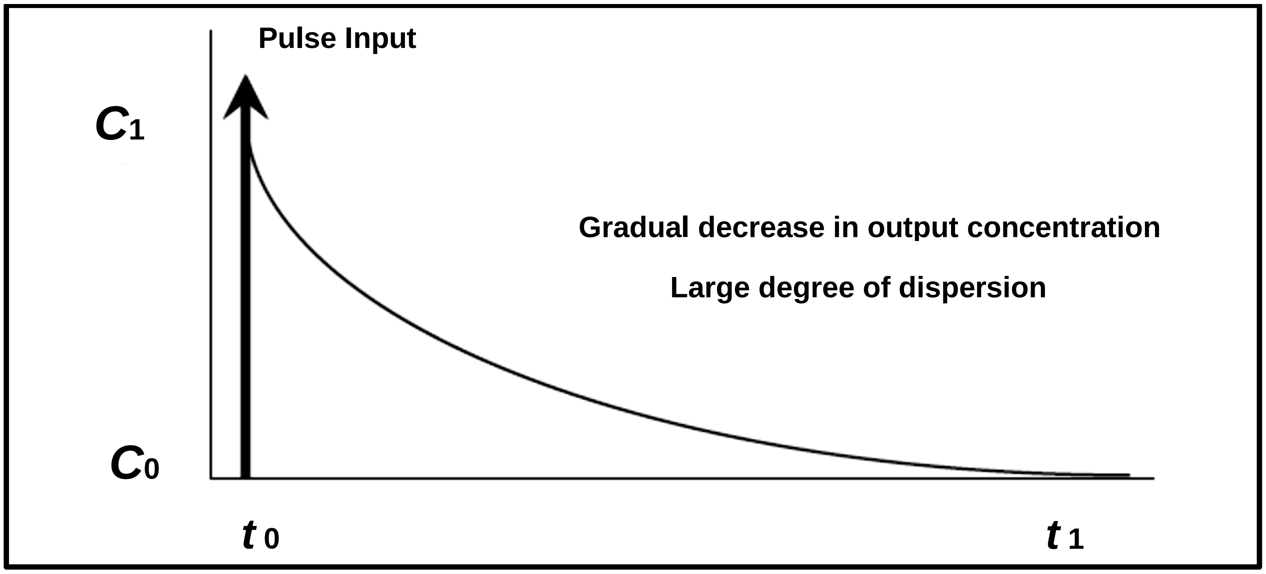


Figure 10: *Dispersion within a continuous stirred reactor (Colorado Education, 2014).*

Non-ideal flow reactors are characterised by dispersion that has an output concentration response between a PFR and a CSTR. The tracer concentration of effluent at the outlet will increase gradually (t_0), but then decrease more rapidly over time (t_1) *(Figure 11).* It must be noted that the change in outlet concentration can also increase rapidly, and decrease gradually, depending on the degree of dispersion.

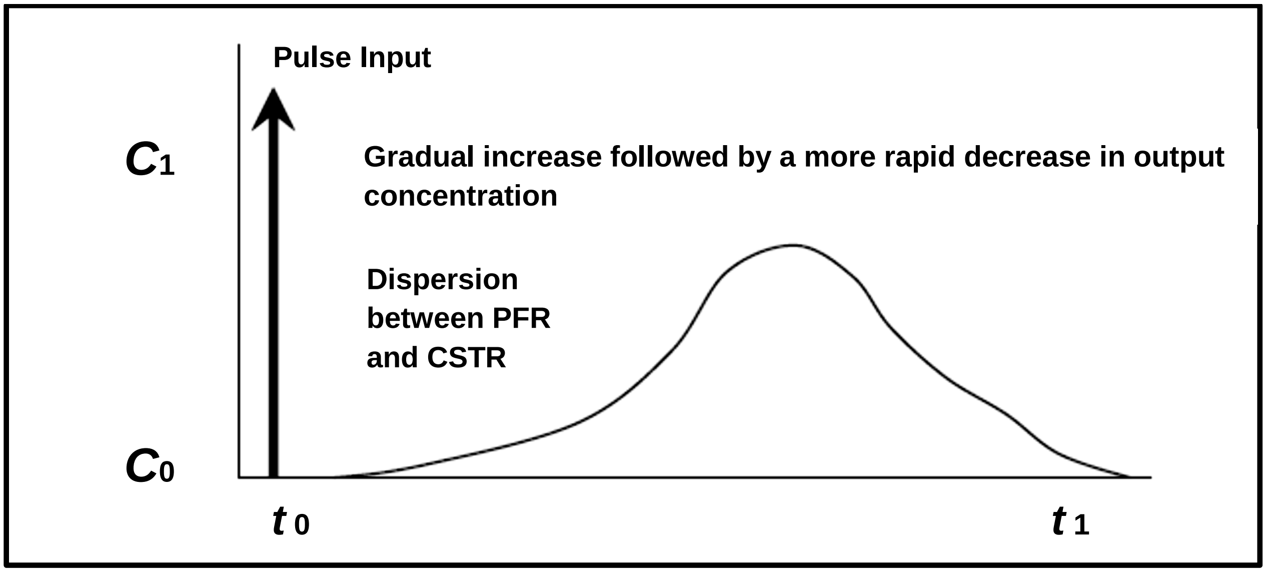


Figure 11: *Concentration of tracer* species – *non-ideal flow (Colorado Education, 2014).*

The HRT of a substrate is a function of the change in outlet tracer concentration over time. If a tracer is injected into a PFR, an impulse of the tracer will appear at the outlet, because there is no fluid mixing occurring within the reactor substrate. If a tracer is injected into a CSTR, an exponential decay in tracer concentration will be observed, which is caused by complete tracer mixing within the reactor substrate. For non-ideal flow reactors, the outflow concentrations will be between PFR and CSTR (Levenspiel, 1999; Davis & Davis, 2003)).

It is expected that anaerobic reactors consisting of organic substrates will be representative of non-ideal flow characteristics. Non-ideal flow conditions are characterised by preferential flow paths, channelling, recycling of the fluid, or dead zones in the substrate (Levenspiel, 1999).

The radial convergent test (RCT) can be used to interpret breakthrough curves obtained from tracer analysis and model results obtained from tracer testing. RCT can be used to conduct 1-dimensional transport modelling within an aquifer (Xu, *et al.,* 1998). Radial convergent can be calculated as:

$$c(r, t) = \frac{\Delta M}{2Q\sqrt{\pi \propto_L V_s t^3}} \exp\left[- \frac{(r-V_s t)^2}{4D_L t}\right]$$

(Equation 6)

Where,

ΔM = Injected mass of tracer per unit section (g/m^2),

Q = Volumetric flow rate (m^3/unit time),

α_L = Longitudinal dispersivity (m),

V_s = Seepage velocity (m/unit time),

t = Time,

r = Radial distance of flow (m),

D_L = Longitudinal dispersion coefficient (m^2/unit time),

$C(r,t)$ = Concentration at distance with time.

Equation 7 can be used to estimate porosity as a function of Darcy velocity (V_d) and fitted Seepage velocity ($V_{s\ Fitted}$), as obtained from the RCT:

$$\text{Porosity} = \frac{Ki}{V_{s\ \text{Fitted}}}$$

(Equation 7)

Where,

K = Hydraulic Conductivity (m/unit time),

i = Hydraulic gradient (dimensionless),

$V_{s\ Fitted}$ = Seepage velocity fitted during RCT.

The output concentrations obtained from tracer testing can also be used to calculate HRTs within substrates. Levenspiel (1999) calculates the mean HRT as follows:

$$\bar{t} = \frac{\sum_{i=0}^{n} t_i \, c_i \, \Delta t_i}{\sum_{i=0}^{n} c_i \, \Delta t_i}$$

(Equation 8)

Where,

t_i =*Time of measurement* C_i,

C_i = *Concentration at time* t_i;

Δt_i = *Time difference between consecutive measurements,*

$\bar{t}$ = *Mean hydraulic residence time.*

CHAPTER 3: METHODOLOGY

Biological passive treatment systems utilise a multitude of chemical, biological, and physical processes to aid in improving the quality of impacted water. It is, therefore, not feasible to address all gaps in a single study. The following gaps were identified for further investigation:

1. Chemical characterisation of selected organic carbon source: A wide spectrum of organic carbon sources have been investigated to be used in biological passive treatment systems in various studies. The selection of an organic carbon source depends on a multitude of factors, such as:

- Chemical signature of the organic carbon source, specifically the C:N ratio, metal concentrations, and electrical conductivity.
- Degree of organic material breakdown that occurred. Simple organics are preferred by sulphate reducing bacteria oppose to complex organics.
- Local availability of material.
- Cost.

2. Investigation on whether dissolved organic carbon (DOC) will be readily available: The capacity of the organic matter to release sufficient quantities of organic carbon for optimal functioning of sulphate reducing bacteria.

3. Investigation of hydraulic properties within different substrate mixtures: Another factor to consider is the hydraulic properties of the selected substrate mixtures. The hydraulic conductivity (K) need to be calculated from volumetric flow rate (Q), hydraulic gradient (i), and cross-sectional area (A) in order to design an anaerobic bio-substrate that would be suitable to host sulphate reducing bacteria for sulphate reduction. The Seepage velocity (V_s) needs to be calculated to determine HRT.

Tracers can also be used to calculate HRT within each substrate mixture. The results obtained from the tracer experiment can be validated by means of the radial convergent test (RCT), during which the mass of tracer injected (ΔM), longitudinal dispersivity (α_L) and seepage velocity are used as fitting parameters.

4. Kinetic modelling of sulphate reduction rates with acetate as organic carbon source: The kinetic model provides an indication of the expected sulphate reduction rates at an experimentally measured available organic carbon source, as well as the impact that dissolved organic carbon has on sulphate reduction rates. The kinetic model provides the HRT that is required to reduce feed sulphate concentration to desired product sulphate concentration. A generalised mathematical function was developed from the kinetic model results that represent sulphate reduction as a function of available acetate concentration and HRT.

5. Case study: Applying the outcomes from the conducted investigations to design a site specific biological passive treatment system for a decommissioned mine site: The outputs from all the experiments conducted, as well as the kinetic model, were used to design a site specific biological passive treatment system, with emphasis on the anaerobic bio-substrate. The objective was to reduce sulphate concentration in mine impacted water to within acceptable concentration.

The above-mentioned gaps were addressed according to the following methodology:

3.1. CHEMICAL CHARACTERISATIONAND DISSOLVED ORGANIC CARBON (DOC) OF SELECTED ORGANIC CARBON SOURCE

The chemical characterisation of the selected organic carbon source provides a point of departure to evaluate the suitability of the organic carbon source to act as a substrate to host sulphate reducing bacteria. The following experiments were conducted to determine the suitability of the organic matter to act as an organic carbon source:

Experiment 1: Chemical signature of organic carbon source

Compost was selected as the preferred organic carbon source to utilise in the anaerobic bio-substrate of the biological passive treatment system. The organic carbon substrate mixtures should be selected based on the chemical signature of the organic carbon source, potential of the organic carbon source to provide sufficient dissolved organic carbon for sulphate reducing bacteria to function optimally, as well as on the local availability of material relative to the location of the site. The substrate mixtures consisted of compost as the organic carbon source, and washed river sand as the solid support. The compost consists mostly of animal manure (mixtures of chicken and horse manure), decomposed bark (mixtures of *Pinus* and *Eucalyptus* bark), and woodchips. The compost used should preferably have as low as possible electrical conductivity (EC), sulphur, nitrogen, and metal concentrations. The compost was obtained from horses that graze primarily on pasture crops, mainly for the expected low nitrogen content in the manure. Manure is required to activate the microbial processes within the substrates. The decomposed bark and woodchips provide the organic carbon source for microbes. Washed river sand was used together with compost to better control the HRT within each substrate mixtures, prevent clogging caused by biofouling or plugging (metal sulphide precipitation), and act as a solid support for microbes. The compost was analysed to determine the concentrations of the following: nitrogen (N), sodium (Na), sulphur (S), phosphorus (P), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), boron (B), arsenic (As), carbon (C), C:N (carbon nitrogen ratio), and electrical conductivity (EC). The results obtained from the analysis would be indicative of the suitability of the compost to host sulphate reducing bacteria, and eventually aid in improving the quality of mine impacted water.

Experiment 2: Dissolved Organic Carbon (DOC)

. The readily availability of dissolved organic carbon provide an indication of the potential of the organic substrate to host sulphate reducing bacteria and successfully aid in improving water quality. An experiment was conducted with pure compost and pure washed river sand to evaluate the change in dissolved organic carbon concentration over time. A critical success factor was to prevent oxygen ingress into the substrates in order to simulate field conditions as best possible. The experiment consisted of two separate large columns that host the pure compost and pure sand, each with a smaller column fitted to the top of the larger column. The smaller columns were also filled with pure compost and pure washed river sand, similar to that of the larger columns. The purpose of the smaller columns was to act as reservoirs to fill the larger columns with water after water was extracted for measurements. The water from the smaller columns was of similar quality, specifically with respect to dissolved organic carbon concentration. A non-return valve was fitted to each of the columns to ensure that only gasses produced within the columns could escape from the system. The experiment was conducted over a period of 44 days during which dissolved organic carbon measurements were taken. It was assumed that these two substrates would present end-members (maximum and minimum) of expected dissolved organic carbon concentration.

The dissolved organic carbon was measured by means of a *Sievers 900 TOC analyser*. The calibration entails a multi-point calibration (absolute 0, 0.5 mg/L, and 50 mg/L).

The instrument used to conduct the measurements, as well as the method of analyses and results obtained, are approved by the South African National Accreditation System (SANAS). Theinstrument undergoes regular inspection and calibration to ensure accurate results are obtained.

3.2. HYDRAULIC PROPERTIES OF DIFFERENT SUBSTRATE MIXTURES.

Experiment 3: Constant head permeability test

A constant head permeability test was conducted on each of the substrate mixtures. The constant head permeability test was specifically selected to simulate conditions in the field, where a relatively constant pressure head drives flow through the substrate. Hydraulic conductivity (K) can then be calculated, as a function of volumetric flow rate (Q), hydraulic gradient (i), and cross-sectional area (A). Different compost and sand substrate mixtures were developed and tested under specific experimental conditions.

The substrate mixtures that were used during this experiment include the following (*Table 6*):

Table 6: *Substrate compositions.*

	Compost	**Sand**
Substrate 1	100%	0%
Substrate 2	70%	30%
Substrate 3	50%	50%
Substrate 4	30%	70%
Substrate 5	0%	100%

The design of an anaerobic bio-substrate is based on the groundwater flow principles of Darcy. Each of the above-mentioned substrate mixtures were placed under specific experimental conditions to obtain an understanding of the flow that occurs within each of the substrate mixtures under constant head flow conditions. The calculated hydraulic conductivity (K) was used as an input parameter to the upscale model. The Darcy velocity (V_d) was calculated as a function of volumetric flow rate (Q), and cross-sectional area (A).

The substrate mixtures were placed in a column, after which flow was initiated for at least 12 hours to allow the material to settle. Flow was initiated at a constant hydraulic head. Volumetric flow rate (Q) and hydraulic gradient (i) were measured in order to calculate hydraulic conductivity (K) as a function of the cross-sectional area (A).

Experiment 4: Dry density, Relative density, and Porosity

The substrate mixtures were oven dried at 105^{o}C for 12 hours, after which the samples were weighed. The dry density was calculated by means of dividing the dry sample weight (post-oven drying) by the total substrate volume (pre-oven drying). The relative density of the material was determined by means of a *Micromeritics AccuPyc II 1340 Gas Pycnometer*. The pycnometer provides the relative density of a sample relative to a reference density. This method of density measurement is prescribed by the American Society for Testing and Materials (ASTM D5550). Calibration of the Pycnometer is conducted by means of reference volume spheres. The Pycnometer at the Civil Engineering Department of the University of Pretoria was used for calculating the relative density of each substrate mixture. It was confirmed that the Pycnometer was calibrated to produce accurate results. Porosity for each substrate mixture was calculated as a function of dry density and relative density (ASTM, 1998).

Experiment 5: Tracer testing and Radial Convergent Test (RCT)

The results obtained from the tracer test can be used to estimate seepage velocity, from which HRT can be calculated. Tracer testing was conducted under similar hydraulic conditions as presented in Experiment 3. A pulse injection tracer test was conducted by means of injecting the tracer with a syringe at the inlet of each column. A tracer solution was developed from potassium chloride (KCl), with a concentration of 0.005 M that was injected into each of the substrate mixtures during active flow. 40 ml of tracer was injected into each of the substrates. The electrical conductivity (EC) of the effluent (outflow) water was measured by means of an *Extech 400 EC* meter. This unit has a self-calibration setting to allow for accurate measurements. EC measurements were taken in random intervals from when the tracer was injected until the output tracer concentration stabilised to pre-injection (baseline) levels. Dispersivity and Seepage velocity were used as fitting parameters during the radial convergent test (RCT), which was used to validate the results obtained from the tracer experiment. The radial convergent test (RCT) was also used to estimate porosity from the fitted Seepage velocity ($V_{s\ \mathrm{Fitted}}$).

3.3. KINETIC MODELLING

A kinetic model was developed by using PHREEQC model code. The model is based on available kinetic data as obtained from Middleton & Lawrence, (1977). The kinetic data, together with the dissolved organic carbon concentration, as obtained from Experiment 2, were used as inputs to the simulation. The modelling was conducted with different feed sulphate concentrations and based on the dissolved organic carbon concentration released from pure compost over a 44-day period (Experiment 2), as it is assumed that the pure compost (100%) would yield the maximum organic carbon concentration. A model was also conducted to determine the effect of available dissolved organic carbon on the rate of sulphate reduction. The outputs from the kinetic model were used to develop a generalised mathematical function that represents sulphate reduction as a function of available acetate concentration and HRT.

3.4 CRITICAL REVIEW OF OBTAINED DATA

An error or uncertainty in experimentally obtained data can be a result of the following:

Systematic errors: These types of errors could occur as a result of poorly calibrated measuring instruments or faulty readings caused by dysfunctional measuring devices. Systematic errors impact on the accuracy of the measurements.

Random errors: These errors impact on the precision of the measurements. Measuring devices are mostly limited by the precision of their measurements. Sources of random errors can be the smallest scale to which an instrument can measure.

Obtained data was critically evaluated by means of conducting an error analysis on experimentally measured data. The effect of the error on the design of the upscale model was then calculated. Statistical methods used to calculate the error on the data obtained are presented in Appendix A.

3.5. CASE STUDY (UPSCALE MODEL)

The outcomes from the experiments and modelling were used to design a full-scale biological passive treatment system, with emphasis on an anaerobic bio-substrate that is based on site specific conditions at a decommissioned coal mine in Mpumalanga province of South Africa. The lowest surface elevation of the pit has been decanting since 2012. A biological passive treatment system was identified as a potential water treatment method. The design of the biological passive treatment system consists of two stages: conceptual design and detailed design.

3.5.1. Conceptual design

The conceptual design was conducted by means of the following methodology:

- The first step in selecting a biological passive treatment system is to characterise the water chemistry of the mine impacted water that requires treatment. Water from coal mines are normally characterised by a low pH, with elevated iron, manganese, aluminium, sulphate, and other trace metal concentrations (Oelofse, 2008). The pH levels of the decant water is, however, near neutral.
- The removal of ferrous iron from solution will be the *first step* in the treatment process. Ferrous iron will only precipitate if the pH is near neutral and the water is well oxygenated (Florence, *et al.*, 2016).
- The methodology that was followed to achieve these ideal conditions for ferrous iron precipitation was to design a system that will increase the pH by using an anoxic limestone bed. It is crucial to prevent any Fe-oxide formation within the limestone bed, which will cause armouring of the limestone and cause the limestone to become ineffective (Watzlaf, 2000).
- After the pH has been raised sufficiently, the *second step* will involve oxygenating the water for ferrous iron precipitation as ferric iron by means of

cascading. The ferrous iron will start to precipitate as ferric iron once the pH is > 7 and the water is well oxygenated (Leavitt, 2016).

- The *third step* entails the settlement of ferric iron precipitate within a settling pond. The precipitation of ferrous iron as ferric iron is a rapid and aggressive process. Other metals such as aluminium will precipitate together with ferric iron, and most aluminium will be removed from solution in the first treatment process. Co-precipitation is, however, dependant on sufficient dissolved oxygen concentration in the water, and near alkaline pH levels (Bertsch, *et al*. 1989). The precipitation of ferric iron will lower the pH of the water.
- The *fourth step* entails the removal of manganese (Mn^{2+}) as Mn^{5+} precipitate (Hallberg, 2005). Manganese will not oxidise sufficiently if the pH is < 9 (Hallberg, 2005), ferrous iron is still present in high concentrations (Nairn & Hedin, 1993), or the water is insufficiently oxygenated (Florence, *et al*., 2016).
- The water will have a low pH after ferrous iron has precipitated as ferric iron, which entails that the pH must be raised for manganese precipitation. The pH is increased by using an anoxic limestone bed, and the water is oxygenated through cascading. The manganese precipitate will then settle within the next settlement pond.
- Most iron, manganese, and aluminium are now removed from the water, but the water still contains elevated sulphate concentration, which is removed from solution through biological reduction in an anaerobic bio-substrate.
- Sulphate reduction occurs optimally when the pH is near 5 (Jong & Parry, 2005), the dissolved oxygen concentration within the influent water is low (Muyzer & Stams, 2008), and excessive metal concentrations are removed from solution (Cabrera *et al.*, 2006).
- The water already has a relatively low pH due to the preceding manganese precipitation process. High dissolved oxygen concentration in the water will cause sulphate reducing bacteria to be less active near the inlet of the anaerobic bio-substrate and only become effective near the outlet.
- The *fifth step* involves the use of an anoxic sump to reduce the dissolved oxygen concentration before entering the anaerobic bio-substrate. Residual iron, aluminium and other trace metals such as, arsenic, copper, zinc, cadmium, selenium, nickel, and lead will also be removed within the anaerobic bio-substrate from solution through metal sulphide precipitation. The precipitate will not be significant, because most iron, aluminium, and manganese have been removed through oxidation and precipitation in the preceding treatment processes.

This methodology will cause less clogging of pores within the anaerobic bio-substrate by means of first removing most of the metals. In an effective composted system, the effluent (treated) water will have a low dissolved oxygen concentration and, therefore, it is regarded as good practice to follow an anaerobic treatment system with an aerobic wetland, which oxygenate water and remove residual iron, aluminium, and manganese by means of oxidising processes and plant uptake.

3.5.2. Detailed design

The focus of this study is primarily the reduction of sulphate in mine impacted water. The detailed design will, therefore, only focus on the anaerobic bio-substrate unit of the biological passive treatment system. The detailed design of the anaerobic bio-substrate is based on site specific conditions (available land area, feed water quality, volume of water to be treated per day, groundwater level in relation to surface elevation), as well as results obtained from the experimental work and modelling.

The dimensions of the anaerobic bio-substrate were calculated as follow:

- **Length** of treatment system, as a function of required HRT (from kinetic model), and Seepage velocity (V_s) of substrate material.
- **Cross sectional area (A)** (depth and width) of treatment system, as a function of:
  - Volume of water to be treated per day (Q);
  - Hydraulic gradient (i) representative of field conditions;
  - Hydraulic conductivity (K) as calculated from Q, i, and A from constant head permeability test.

The sulphate concentration in the feed water determines the volume of compost required. The volume of compost required is a function of:

- The fraction of compost that would become available to sulphate reducing bacteria (simple organic carbon);
- Molar mass of compost;
- Maximum expected operational life of the treatment system;
- Moles compost required to remove sufficient quantities of sulphate from impacted water;
- Sulphate/acetate utilisation rate;
- Dry density of compost.

CHAPTER 4: EXPERIMENTAL DESIGNS AND SETUP

This chapter provides a discussion on the design and setup of each experiment. The experiments conducted in this study include the following:

- Characterising the compost that is proposed to be used as an organic carbon source;
- Determining whether a suitable redox potential could be achieved within the compost substrate;
- Determine whether dissolved organic carbon would be readily available from the compost;
- Determine the hydraulic properties within each substrate mixture under a constant head flow;
- Conduct tracer testing to calculate HRT within each substrate mixture.

4.1. CHEMICAL CHARACTERISATION OF ORGANIC CARBON SOURCE

The objective of this experiment was to conduct a baseline characterisation of the compost material proposed to be used in the anaerobic bio-substrate component of the biological passive treatment system. The chemical signature of the compost was determined by means of the following methodology:

- **Method references for % N:** The Dumas (Gonick, 1945) combustion method was used to calculate percentage nitrogen as total %.
- **Reference for ash % (to calculate % C**): The material was ashed at 500°C.
- **Moisture 60°C:** Weight loss at 60°C.
- **Cations:** Cation concentrations were determined by means of nitric/perchloric acid digestion. Readings were obtained from *Inductively coupled plasma* mass spectrometry (ICP).
- **pH and EC:** A 1:10 water extract was used to measure the pH and EC. Obtaining the correct consistency is fundamental to ensure the extract is representative of the actual compost pH and EC.

4.2. DISSOLVED ORGANIC CARBON (DOC)

An additional experiment was conducted to determine whether the selected compost would be able to host sulphate reducing bacteria, specifically relating to dissolved organic carbon. The objective of this experiment was to assess how dissolved organic carbon concentration within pure compost changes over time in an anaerobic bio-substrate, with pure river sand as control. The conceptual experimental setup is presented in *Figure 12*.

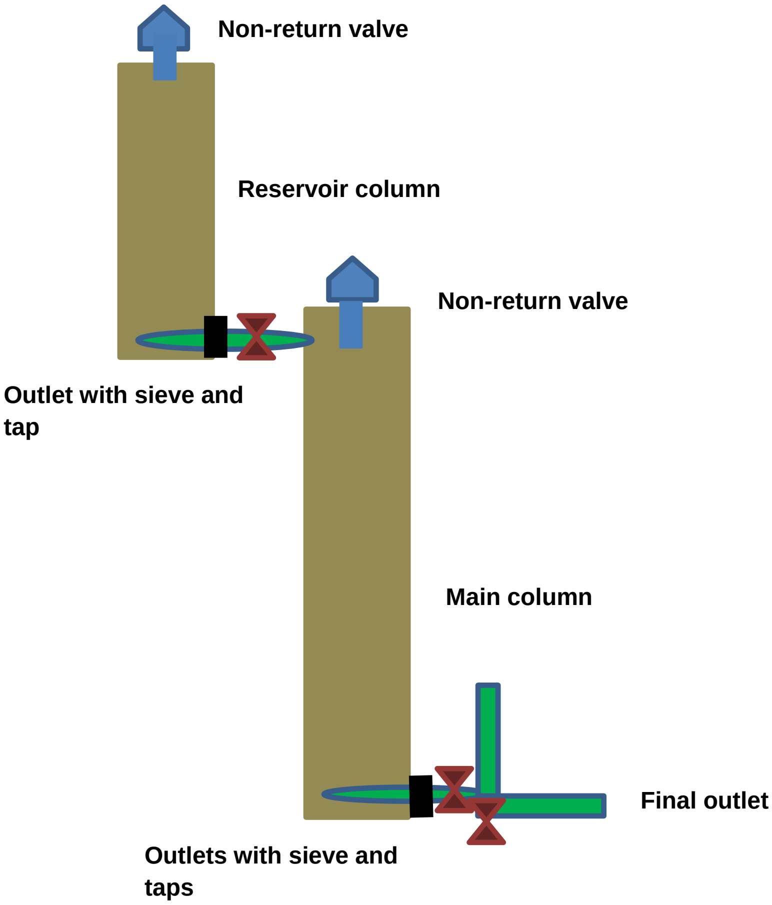


Figure 12: *Conceptual experimental design for dissolved organic carbon measurements.*

This experiment was conducted as follows:

- 100% compost and 100% sand were used as substrates (it is assumed that these would represent end-members (maximum and minimum) of potential changes in dissolved organic carbon (DOC) concentration over time).
- The compost and sand substrates were placed in two separate columns, which were sealed to prevent oxygen ingress (this was conducted to simulate conditions in an anaerobic reactor);
- The columns were wrapped with dark plastic bags to prevent light from penetrating the substrates;
- Each column was completely saturated with distilled water. This was accomplished by means of pre-wetting the substrate before placed in the columns. Water was also added sequentially as substrate material was placed within each column. This would

ensure that the substrates are wetted equally across the column length, and that differentiations in moisture condition across the substrate length are prevented. Distilled water was used to more accurately measure dissolved organic carbon released from the substrates.

- An additional shorter column was placed on top of the longer column for both the compost and sand substrates. The purpose of the shorter columns was to act as reservoirs for when water is tapped from the longer columns for DOC measurements. The tapped water from the longer columns would need to be replaced by water that is similar dissolved organic carbon concentration. The tapped water from the longer columns was thus replaced by water from the shorter columns after each measurement.
- Non-return valves were placed on the top of each column to prevent O_2 ingress, but allow gas (such as methane) outlet.
- Dissolved organic carbon concentration measurements were taken in intervals over a period of 44 days. The measurements were taken in random intervals.

Figure 13 presents the experimental setup to evaluate how dissolved organic carbon concentration change over time within pure compost (100%) and pure sand (100%).

Figure 13: *Experimental setup to track changes in dissolved organic carbon concentration over time.*

1: Reservoir columns *2: Taps to manage water flow from reservoir columns to main columns*
3.: Main columns *4. Final outlet taps* *5. Non-return valves to prevent O_2 ingress, but allow CH_4 outlet*

4.3. HYDRAULIC PROPERTIES OF SUBSTRATE MIXTURES

The objective of this experiment was to determine the hydraulic properties (volumetric flow rate (Q), and hydraulic gradient (i), and hydraulic conductivity (K)) within the five (5) different substrate mixtures under constant head flow. *Figure 14* provides a conceptual design of the experimental setup.

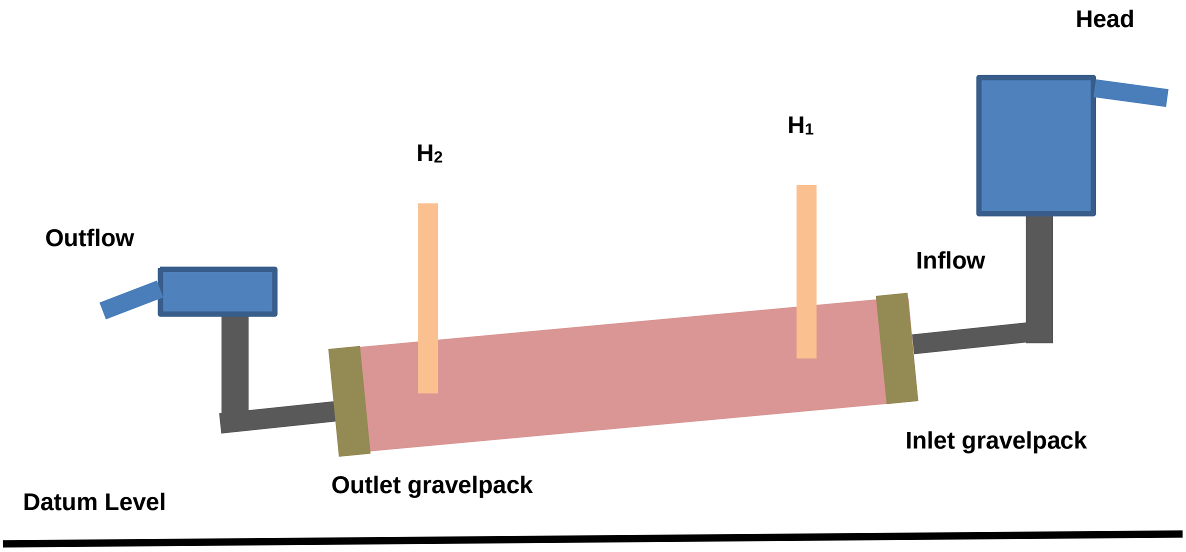


Figure 14: *Conceptual experimental design for constant head permeability test.*

The final site location of the anaerobic bio-substrate was not determined yet at the time that the experiment was conducted. The experiment was developed to represent flow under a constant head flow conditions. The following design specifications were applied:

- Constant head of 0.632 m (± 0.018 m);
- Bed slope of 1:43 (or 2.3 %);
- Flow length of 250 mm;
- Cross sectional area of flow 0.0093 m^2.

The constant head permeability test was conducted as follows:

- The substrate mixtures were carefully placed within each column with no physical compaction conducted.
- A gravelpack was placed at the inlet of each column to promote the distribution of flow across the entire substrate area, and prevent the development of preferential flow paths. The outlet gravelpack prevented substrate material to be washed out, and prevent clogging of outlet pipe.
- The inlet and outlet tubes were fitted to the centre of each column. Flow was initiated through the substrates in order for the substrate material to settle and compact under

gravimetrical flow conditions. Substrate material was then added to each column after settlement occurred to ensure each column is completely filled with substrate material. Flow was initiated again and left for a period of approximately 12 hours before any flow measurements were taken. It was crucial to ensure that the substrate mixtures were completely saturated, and that steady state flow was achieved before measurements were taken.

Figure 14 presents the experimental setup that was used to assess the change in hydraulic gradient (i) and volumetric flow rate (Q) within each of the mentioned substrate mixtures. Hydraulic conductivity (K) was then calculated as a function of volumetric flow rate (Q), hydraulic gradient (i), and cross-sectional area (A).

The hydraulic gradient (i) was measured for each of the substrate mixtures. The hydraulic gradient was calculated as follows:

$$i = \frac{H_1 - H_2}{L}$$

(Equation 9)

Where,

$(H_1 – H_2) = \Delta H$ (change in water level relative to datum level),

L = Length of column,

i = Hydraulic gradient (i).

Figure 15 provides an illustration of the experimental setup for the constant head permeability test.

Figure 15: *Experimental setup to assess hydraulic properties within each substrate mixture by means of a constant head permeability test.*

1: Bucket of water placed at 0.632m head   2: Columns containing substrate mixtures at 1: 43 bed slope
3. Outlet tubes fitted to the centre of each column   4: Gravelpack at inlet and outlet of each column
5. Hydraulic gradient test tubes   6. Outflow from outlets

4.4. DRY DENSITY, RELATIVE DENSITY, and POROSITY

A separate experiment was conducted to calculate the porosity (n) of each substrate mixture. It is expected that the effective porosity will equal the total porosity due to the unconsolidated nature of the substrate material.

The substrate mixtures were each placed in a graduated cylinder. The total volume of the cylinder was 500 ml (0.00050 m^3). The columns were filled with substrate material to align with the total volume of the cylinder (0.00050 m^3). The substrate mixtures were only placed and not compacted. The substrates were then oven dried for 12 hours at 105^{o}C to remove all retained water. The substrates were weighed post-drying to determine the dry substrate mass. The dry density was calculated as a function of post-oven dried weight and total volume of that substrate prior to drying. Dry density was, therefore, calculated as:

$$p_b = \frac{M_d}{M_t}$$

(Equation 10)

Where,

M_d *= Mass of substrate material after oven dried,*

M_t *= Total volume of substrate material prior to drying,*

P_b *= Dry density.*

The relative density was then determined for each of the substrate mixtures by means of a gas Pycnometer (*Figure 16*). The volume and density of the substrate material were calculated from the measured decrease in pressure when Helium gas of a known amount was injected into the chamber. The Helium gas filled all available pore spaces, after which the volume and density of the sample was calculated. The oven dried sample was weighed and placed in the chamber of the Pycnometer, and volume and relative density were calculated as a function of change in pressure. Average room temperature was 20,55ºC. Three cycles of calculations were conducted for each substrate mixture.

Figure 16: *Sample measuring and Pycnometer to calculate relative density.*

The porosity was then calculated according to Equation 4.

4.5. TRACER TESTING

Tracer tests were conducted on the experimental setup as presented in *Figure 15*, under the similar hydraulic conditions (volumetric flow rate (Q), hydraulic gradient (i), hydraulic conductivity (K), and bed slope) as during the constant head permeability test. The same experimental setup was used to conduct tracer tests on five different substrate mixtures (*Table 6*). The objective was to calculate the mean HRT within each of the substrates, as well as to estimate porosity by means of the radial convergent test (RCT). Potassium Chloride (KCl) was used as the tracer solution, mainly due to the inability of the substrate material to absorb the substance. Tracer was injected through pulse injection by means of a syringe. 40 ml of tracer solution with a concentration of 0,005 M was injected. An *Extech 400 EC* meter was used to measure change in electrical conductivity (EC) (µS/cm) over time at the outlet. EC measurements were taken until the outlet concentration of the tracer stabilised. *Figure 17* presents the experimental setup to conduct tracer testing on each substrate mixture, which is similar than the constant head permeability test. Three consecutive rounds of pulse injections were conducted on each substrate mixture.

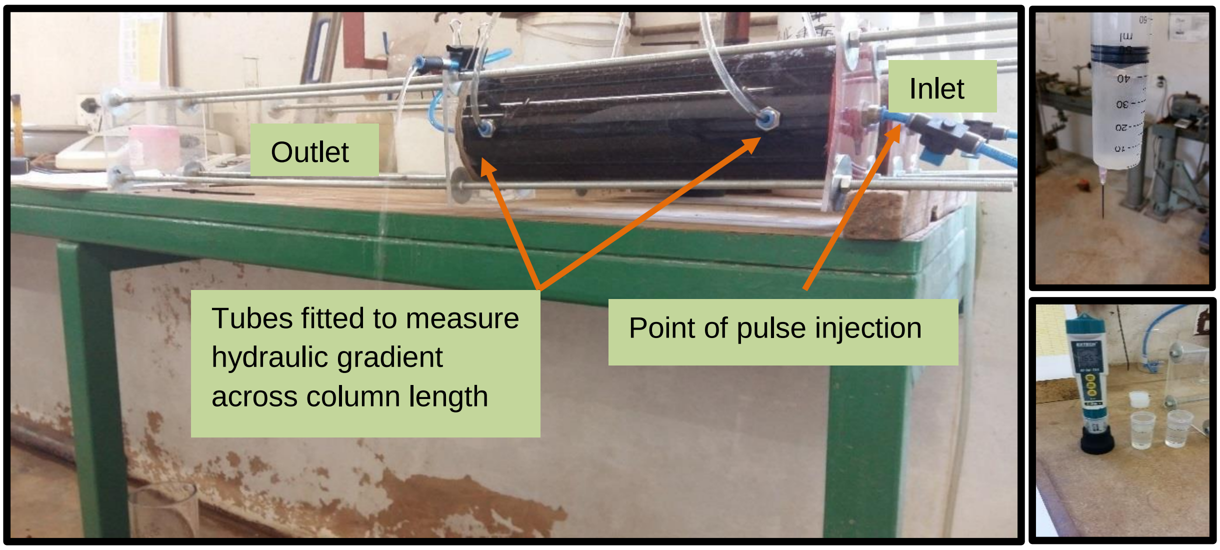


Figure 17: *Experimental setup for tracer testing.*

CHAPTER 5: NUMERICAL MODELLING

5.1. STOICHIOMETRY AND KINETIC MODEL SETUP

Kinetic modelling was conducted using the geochemical software PHREEQC (Parkhurst & Appelo, 1999), in order to obtain a first order estimation of the feasibility of sulphate reducing bacteria within an anaerobic bio-substrate to reduce sulphate.

The following assumptions were made during the development of the kinetic model and upscaling of model design:

Assumption 1: The availability of organic carbon might be a rate limiting factor for sulphate reduction, if high sulphate concentration occurs and organic carbon (acetate) is not readily available in sufficient quantities (as stipulate by Middleton & Lawrence (1977)).

Assumption 2: The dissolved organic carbon (DOC) concentration from pure compost (100%) is the maximum to be available to sulphate reducing microbes (868 mg/L). It is expected that other substrates that contain less compost will yield lower DOC concentration, or require longer HRT to produce similar DOC concentration. This also accounts for the achieved redox potential achieved in the pure compost. It is expected that substrate mixtures with less compost will require a longer standing time to reach a suitable redox potential.

Assumption 3: The oxidation of compost will result primarily in the release of acetate, which is readily available for sulphate reduction processes (as stipulated by Israel, 2015).

Assumption 4: The mol ratio of sulphate to acetate utilisation is 1:1 (as presented by reaction 9, $CH_3COOH + SO_4^{2-} \rightarrow H_2S + 2HCO_3^{-1}$ (Middleton & Lawrence (1977).

Assumption 5: The mol ratio of compost (woodchip based organic material) to acetate is 1.68 (as stipulated by Israel, 2015).

Assumption 6: Only 30% of organic matter (compost) by mass will be available for sulphate reduction (as presented by Neculita *et al.*, 2007b).

Assumption 7: Pure sand (100%) contains no compost. No organic matter will, therefore, be available for oxidation. No sulphate reduction will be able to occur within the pure sand substrate.

Assumption 8: The hydraulic residence times as used in the kinetic model are still below what are required for methane production. It is assumed that all sulphate will be removed from the mine impacted water, before methane is produced.

Assumption 9: It is expected that the mixing properties of the substrate from which the kinetic data was sourced will be similar to that of the anaerobic bio-substrates.

Assumption 10: The model developed in PHREEQC assumed that all of the dissolved organic carbon is available for the bacteria to use, and this will be an overestimation in field conditions.

Assumption 11: The model does not account for factors that might inhibit sulphate reducing bacteria.

5.1.1. PHREEQC

PHREEQC is the model code used to conduct aqueous geochemical modelling. The programme is based on an ion-association aqueous model with the ability to calculate speciation and saturation-indices, batch reactions with 1-dimensional transport calculations for reversible reactions and irreversible reactions.

5.1.2. Model Setup

PHREEQC model code was used to conduct predictive kinetic modelling for acetate utilisation and sulphate reduction, as a function of time. Sulphate reduction kinetic data was obtained from Middleton & Lawrence (1977). Dissolved organic carbon (DOC) was measured as part of Experiment 2. The results obtained from experiment 2 were used as input to the PHREEQC model. The outputs from the PHREEQC model firstly include sulphate reduction rates as a function of varying sulphate feed concentration, with constant acetate concentration. Secondly, sulphate reduction as a function of varying acetate concentration with constant sulphate feed concentration. Israel, (2015) provides a brief overview of the breakdown of wood. Most compost materials consist of a large portion of woodchips. The oxidation of a simple organic carbon source can be illustrated by means of the following reaction (Middleton & Lawrence, 1977):

$$CH_3COOH + SO_4^{2-} \rightarrow H_2S + 2HCO_3^{-}$$

(Reaction 9)

The degradation of woodchips is described by Browning (1963). Wood mostly consists of carbohydrates, phenolic substances, terpenes, aliphatic acids, alcohols, proteins, and inorganic substances. Fifty percent (50%) of the mature wood weight consists of carbohydrates, whereas polysaccharides and lignin make up the larger part of wood. The cellulose hydrolysis yields products of D-glucose, while the non-cellulose polysaccharides part yields acetyl groups, uronic acids & methoxy-uronic acids, pentoses, and hexoses. Microbes normally compete for nutrients from the oxidation of H_2, acetate, and formate during microbial redox reactions (Israel, 2015). The rate of microbial growth can thus be linked to the availability of the above mentioned organic carbon sources (Israel, 2015). During the breakdown of organic matter to simple useable organic compounds, acetate can be considered to be the first breakdown product (Israel, 2015). Acetic acid is regarded as being an immediate available carbon source for well degraded organic material. Organic matter that has sufficiently degraded should be used in the anaerobic bio-substrate. *Figure 18* provides an illustration of the breakdown products of wood.

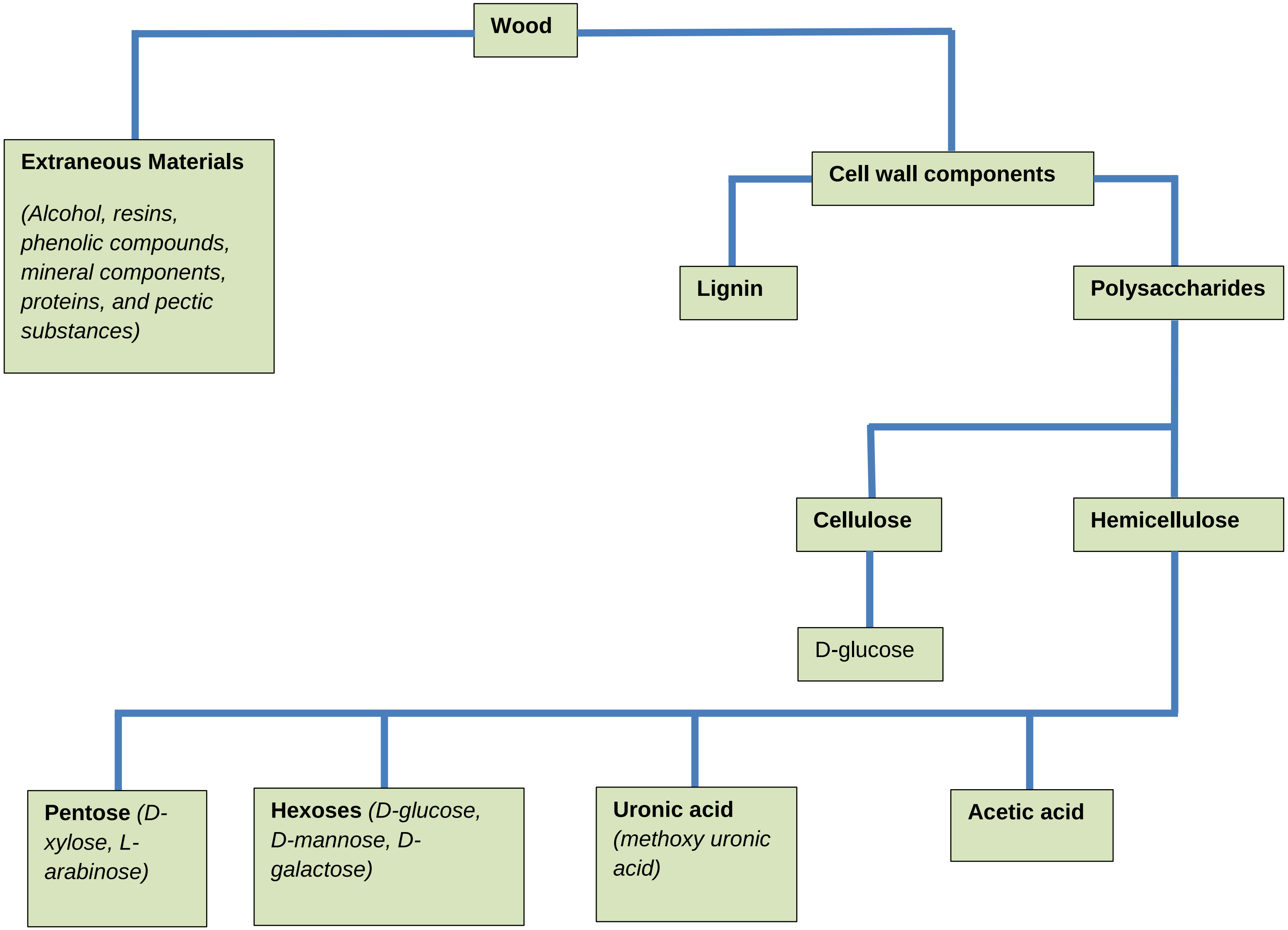


Figure 18*: Breakdown of woodchips (Browning, 1963).*

Middleton & Lawrence, (1977) provide a kinetic model to simulate sulphate reduction with acetate as organic carbon source. The Monod function can be used to best illustrate acetate utilisation by sulphate reducing bacteria*:*

$$r_s = - \frac{k\,S\,x}{k_s + S}$$

(Equation 11)

Where,

k = Maximum specific rate of organic carbon utilisation (T^{-1})*;*

S = Dissolved organic carbon concentration (M/L^3)*;*

x = Bacteria concentration (M/L^3)*;*

k_s *= Half-velocity coefficient* (M/L^3)*;*

r_s *=Volumetric reaction rate of organic carbon* ($M/L^3\,T$)*.*

The relationship between bacterial growth and organic carbon utilisation can be illustrated as:

$$r_x = Yr_s - bX$$

(Equation 12)

Where,

Y = Yield coefficient (M/M);

b = Decay coefficient (T^{-1})*;*

r_x *= Volumetric rate of bacterial cell growth* (M/L^3) *T.*

The following steady state equations can be used to describe bacterial growth within a completely mixed flow reactor. These include:

$$\theta = \frac{1}{\mu} = \frac{X}{r_x}$$

(Equation 13)

$$\theta = \theta_c$$

(Equation 14)

$$S_1 = \frac{K_e\,(1+b\theta_c)}{\theta_c(Yk-b)-1}$$

(Equation 15)

$$X = \frac{Y\,(S_o - S_1\,)}{1+\,b\theta_c}$$

(Equation 16)

$$-r_s = \frac{S_0 - S_1}{\theta}$$

(Equation 17)

$$C_1 = C_0 - \alpha\,(S_0 - S_1 - \beta\,X)$$

(Equation 18)

Where,

Θ_c *= Solids retention time (T),*

μ *= Net specific growth rate of bacteria* (T^{-1}),

θ *= Hydraulic residence time (T),*

S_1 *= Dissolved organic carbon concentration of effluent* (M/L^3),

S_o *= Organic carbon concentration of influent* (M/L^3),

X *= Bacteria concentration* (M/L^3),

R_s *= Volumetric reaction rate of organic carbon* (M/L^3),

K *= Maximum specific rate of organic carbon utilisation* (T^{-1}),

Y *= Yield coefficient (M/M),*

K_e *= Half-velocity coefficient (M/M),*

b *= Decay coefficient* (T^{-1}),

C_o *= Electron acceptor concentration of influent* (M/L^3),

C_1 *= Electron acceptor concentration of effluent* (M/L^3),

α *= Mass of electron acceptor consumed to oxidise one unit mass of organic carbon, (M/M),* *β = Mass of organic carbon required to synthesize one unit mass of bacterial cells (M/M).*

Equation 19 can be used for estimating bacterial mass, which is based on electron acceptor utilisation:

$$X = \frac{1}{\beta}\left[(S_o - S_1) - \frac{1}{\propto}(C_0 - C_1)\right]$$

(Equation 19)

Equation 20 to Equation 22 describe the growth of bacteria, oxidation of acetate, and the reduction of sulphate.

$$\frac{d_S}{d_t} = -\frac{kSX}{k_s + S}$$

(Equation 20)

$$\frac{d_x}{d_t} = -Y\frac{d_S}{d_t} - bX$$

(Equation 21)

$$\frac{d_c}{d_t} = \alpha\left[\frac{d_S}{d_t} + \beta\left(\frac{d_x}{d_t}\right)\right]$$

(Equation 22)

Where

S = Organic carbon concentration (M/L^3),

X = Biomass concentration (M/L^3),

C = Electron acceptor concentration (M/L^3).

It was required that acetic acid be defined in PHREEQC, as it is not a standard component in the PHREEQC database. The data blocks SOLUTION_MASTER_SPECIES and SOLUTION_SPECIES were used to define the thermodynamic properties of acetic acid *(Appendix B).*

Middleton & Lawrence (1977) present kinetics of sulphate reducing bacteria during sulphate reduction through acetate utilisation in a batch reactor. The model setup was conducted in PHREEQC model code, during which acetic acid and sulphate utilisation by sulphate reducing bacteria were modelled as a base case model. The outputs of the base case model, as compiled by Middleton & Lawrence (1977), are presented in *Figure 19* and *Figure 20*. The numerical outputs from the model can be viewed in Appendix C.

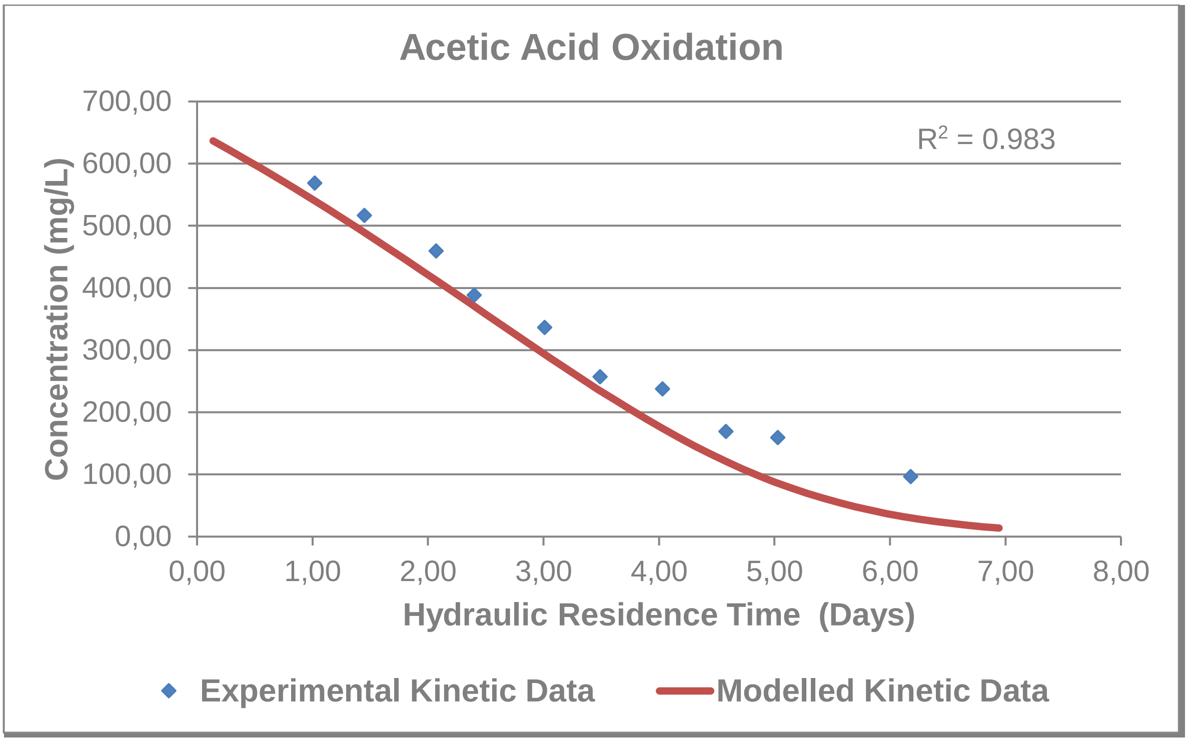


Figure 19: *Base case model-acetic acid oxidation (Middleton & Lawrence, 1977).*

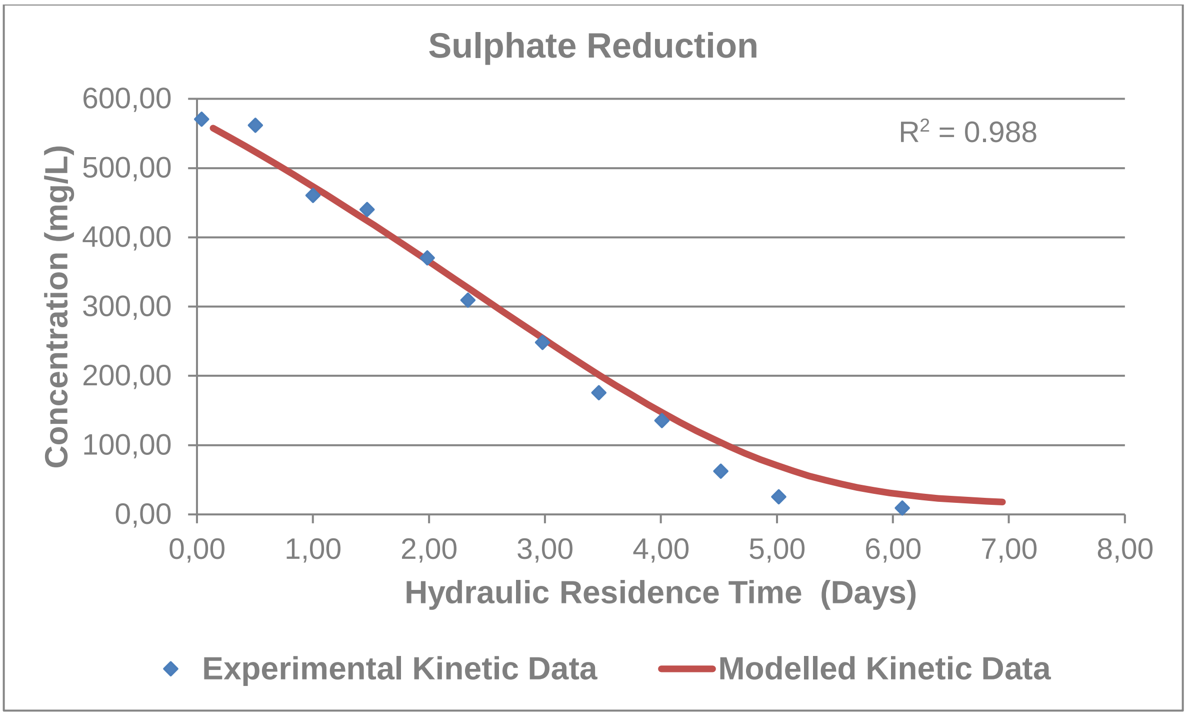


Figure 20: *Base case model—sulphate reduction (Middleton & Lawrence, 1977).*

The kinetic model provided the HRT required to reduce sulphate concentration from feed concentration to desired concentration, which was used as input to the upscale model. The outputs from the upscale model include geometry of proposed anaerobic bio-substrate, as well as volume of compost required to reduce a specific feed sulphate concentration to desired concentration. *Figure 21* presents a schematic on the developed model components.

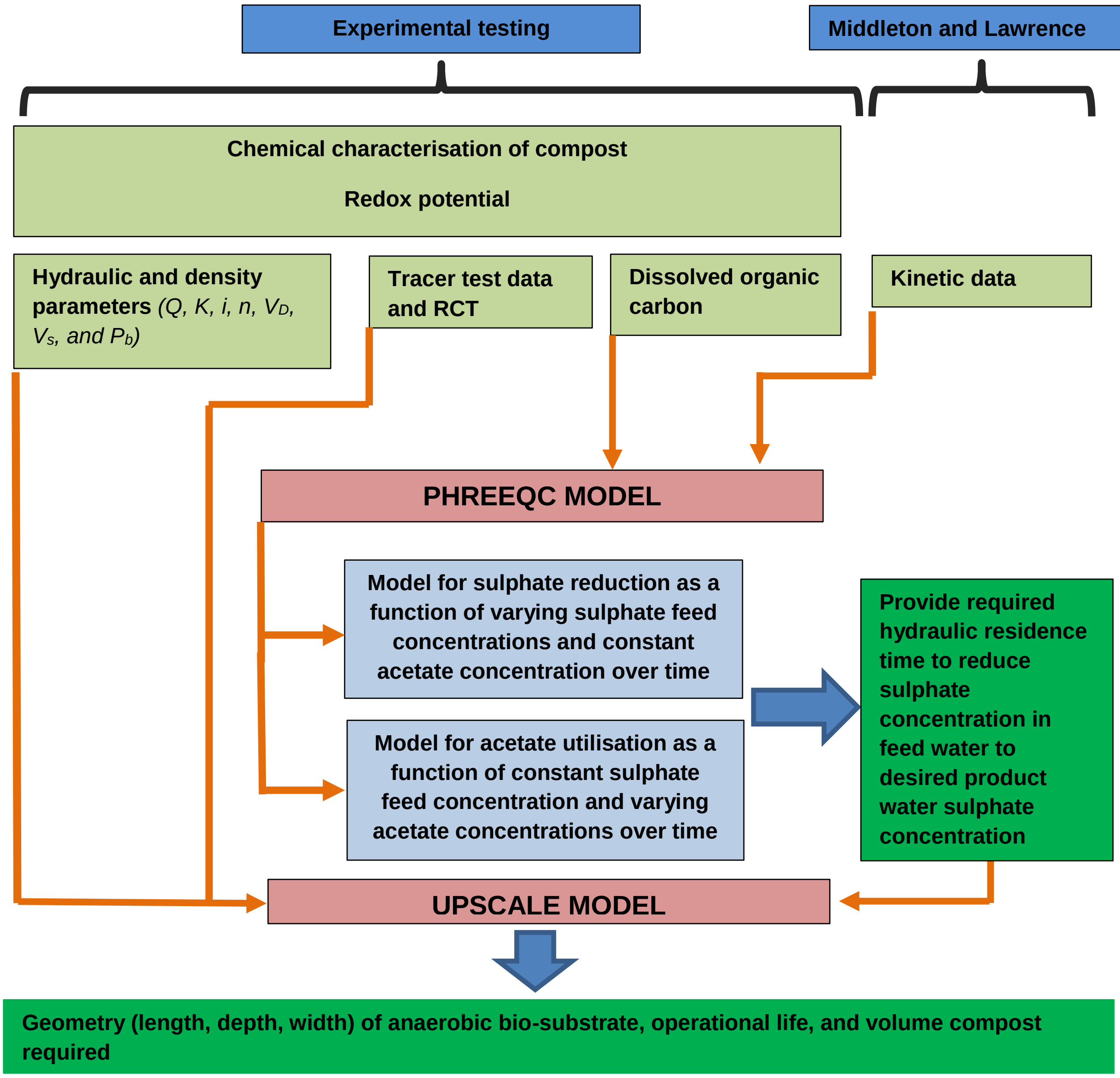


Figure 21: *Schematic on model inputs and outputs.*

CHAPTER 6: RESULTS AND DISCUSSION

This chapter provides the results obtained from the physical experimental work conducted, as well as the kinetic model.

6.1. PHYSICO-BIOCHEMICAL EXPERIMENTS

The results for the experiments conducted in Chapter 4 are presented in this chapter. The results are presented in the following sequence:

A. Chemical signature of the organic carbon source (compost)

B. Changes in dissolved organic carbon concentration within the 100% compost and 100% sand substrates over time

C. Hydraulic properties of different substrate mixtures under constant head flow conditions

D. Dry density, relative density, and porosity

E. Tracer testing and RCT

6.1.1. Chemical signature of organic carbon source

Chemical analysis was conducted on pure compost material to determine the chemical signature of the organic carbon source. The results obtained from the tests conducted are presented in *Table 7.*

***Table 7**: Chemical properties of compost used.*

Constituent	Unit	Value
N	**%**	0,77
Na	**mg/kg**	960
S	**%**	0,12
P	**%**	0,14
Fe	**mg/kg**	21720
Mn	**mg/kg**	338
Cu	**mg/kg**	23
Zn	**mg/kg**	73
B	**mg/kg**	9
As	**%**	47,35
pH		6,8
C	**%**	30,61
C:N	**Ratio**	39,75:1
EC	**mS/m**	54,2

N: Nitrogen; Na: Sodium; S: Sulphur; P: Phosphorus; Fe: Iron; Mn: Manganese; Cu: Copper; Zn: Zinc; B: Boron; As: Arsenic; C: carbon; C:N: Carbon Nitrogen Ratio; EC: Electrical Conductivity

The objective of the chemical analysis on the compost material was to obtain a baseline understanding of the chemical signature of the compost. Compost can consist of a wide variety of degraded material, and each compost type is, therefore, unique in terms of its chemical signature. Product water quality will resemble the chemistry of the compost during the early stages of treatment. Salts and nutrients will be flushed from the compost to the receiving water courses during the early stages of operation. The quality of the compost impact directly and indirectly on the effectiveness of the proposed anaerobic bio-substrate. Direct impacts entail the release of salts, metals, or acidity to water flowing through the substrate. Indirect impacts relate more to biodegradability of the organic material. Direct impacts to water quality are normally noted during the early stages of treatment when some of the metals and salts are flushed out of the substrate. Indirect impacts occur as a result of the biodegradability of the compost, which is evident in the long term.

The results of the chemical analysis conducted on the compost *(Table 7)* present as follow:

Total nitrogen and carbon ratio (C/N): The nitrogen and carbon ratio of the compost is 39.7: 1. This entails that the compost material has sufficient concentrations of nitrogen and carbon for optimal microbial functionality. The ratio also implies that the compost has a low initial age, meaning that the organic matter has not undergone extensive degradation yet. This entails that the organic matter will release large quantities of electrons during the early stages of degradation, but will reach a steady state during which the degradation is slower and more consistent. The C/N ratio is thus ideal for optimal biological sulphate removal.

Trace metals and salts: Literature provides an indication that trace metals and salts can occur in ample concentrations in compost. The concentrations of trace metals and salts can vary significantly between organic materials. Standard universal concentration thresholds are, therefore, not available for organic material. It is, however, noted that the iron concentration is > 20 000 mg/kg. The assumption can thus be made that the effluent (product) water will contain high iron concentrations, which is flushed from the organic material. It is expected that this phenomenon will only be for the initial treatment stage and reach a steady state as the system operates.

pH: The pH of the compost is slightly acidic (6.8). This can be an indication that the compost has started to degrade, during which organic acids are released. Reducing environments might further lower the pH, causing excess acidity to become available, and inhibit optimal microbial activity.

Electrical conductivity: The EC of the compost is 54,2 mS/m. Compost with an EC of > 2000 mS/m is regarded as being salty (Geolab, 2013). The available salt load within the compost is, therefore, very low. The compost is ideal for biological passive water treatment from an EC perspective.

The chemical signature of the compost proves to be suitable for usage in an anaerobic bio-substrate.

6.1.2. Dissolved organic carbon

An experiment was conducted to track the change in dissolved organic carbon concentration within pure compost and pure washed river sand over time. The experiment was conducted over a period of 44 days. The results obtained from this experiment are indicated in *Figure 22*. Tabulated results are presented in Appendix D.

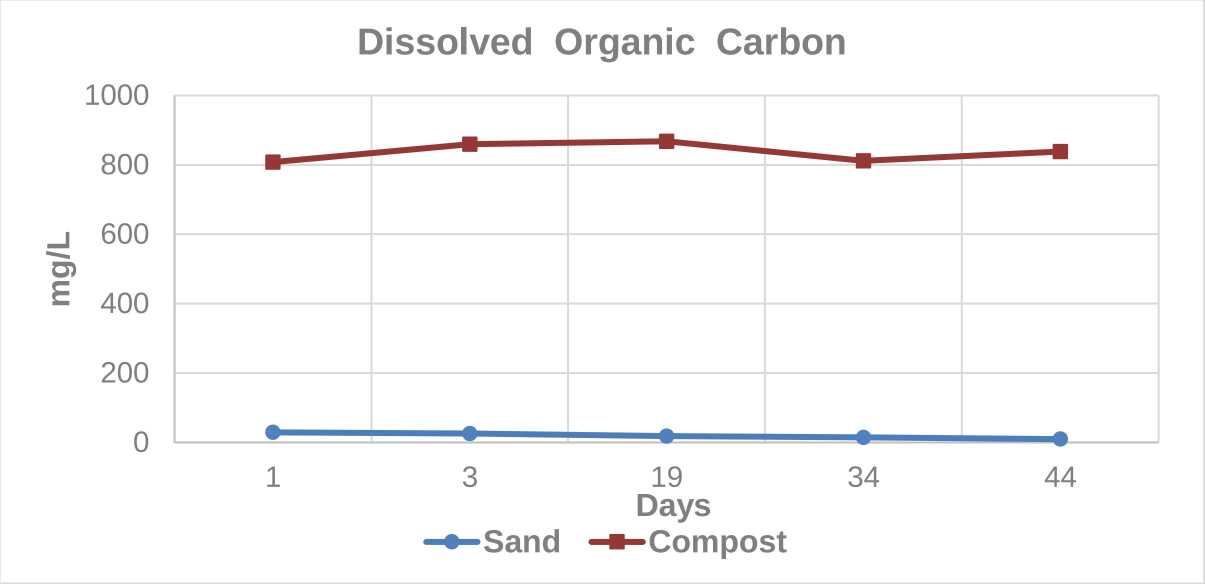


Figure 22: *Change in dissolved organic carbon concertation within 100% compost and 100% sand substrates over time*

The objective of the experiment was to evaluate whether dissolved organic carbon will remain readily available over time. Dissolved organic carbon was only measured sporadically to obtain a baseline understanding of changes that could occur over time. The dissolved organic carbon concentration within the compost column remained high, whereas the dissolved organic carbon concentration in the sand column remained low. No organic compounds were, however, available for oxidation in the sand column. The dissolved organic carbon content within the compost column reached a peak at day 19 (868 mg/L), and decreased towards day 34. This might be attributed to most of the dissolved organic carbon being readily available during the first day of the experiment. As testing continued, most of this readily available organic carbon was flushed from the substrate. The dissolved organic carbon then increased slightly towards day 44, which implies that the organic compounds underwent further oxidation, and released dissolved organic carbon to the pore water.

6.1.3. Hydraulic properties of substrate mixtures

Constant head permeability tests were conducted for five (5) different substrate mixtures (*Table 6*). Volumetric flow rate (Q) and hydraulic gradient (i) were measured to calculate hydraulic conductivity (K). *Table 8* to *Table 10* present the results obtained from the experiment conducted. See Appendix E for detailed experimental results.

Table 8: *Volumetric flow (Q) rate results for different substrate mixtures.*

Volumetric flow rate (Q) (m^3/d)						Error Analysis		
	Round 1	**Round 2**	**Round 3**	**Round 4**	**Round 5**	**Average**	**Standard Deviation**	**Uncertainty**
100%C	0,283	0,274	0,322	0,343	0,342	0,313	0,033	0,313 ± 0,033
70%C30%S	0,162	0,321	0,351	0,365	0,351	0,310	0,084	0,310 ± 0,084
50%C50%S	0,343	0,283	0,302	0,384	0,339	0,330	0,039	0,330 ± 0,039
30%C70%S	0,254	0,217	0,204	0,286	0,256	0,243	0,033	0,243 ± 0,033
100%S	0,178	0,562	0,565	0,640	0,635	0,516	0,193	0,516 ± 0,193
Average	0,243	0,332	0,349	0,404	0,384			

Table 9: *Hydraulic gradient (i) for different substrate mixtures.*

Hydraulic Gradient (i) (Index)						Error Analysis		
	Round 1	**Round 2**	**Round 3**	**Round 4**	**Round 5**	**Average**	**Standard Deviation**	**Uncertainty**
100%C	0,896	1,016	0,968	0,932	0,880	0,938	0,055	0,938 ± 0,055
70%C30%S	1,396	1,200	1,136	1,000	0,916	1,130	0,186	1,130 ± 0,186
50%C50%S	1,050	1,228	1,088	1,092	1,216	1,135	0,081	1,135 ± 0,081
30%C70%S	1,368	1,440	1,444	1,332	1,360	1,389	0,050	1,389 ± 0,050
100%S	0,760	0,788	0,780	0,680	0,680	0,738	0,054	0,738 ± 0,054
Average	1,094	1,134	1,083	1,007	1,101			

Table 10: *Hydraulic conductivity (K) for different substrates.*

Hydraulic conductivity (K) (m/d)						**Error Analysis**		
	Round 1	**Round 2**	**Round 3**	**Round 4**	**Round 5**	**Average**	**Standard Deviation**	**Uncertainty**
100%C	33,762	28,791	35,623	39,546	41,687	35,882	5,049	35,882 ± 5,049
70%C30%S	12,440	28,718	32,992	39,186	41,060	30,879	11,421	30,879 ± 11,421
50%C50%S	35,026	24,744	29,716	37,666	29,864	31,403	5,048	31,403 ± 5,048
30%C70%S	19,909	16,147	15,140	23,021	20,162	18,876	3,213	18,876 ± 3,213
100%S	25,110	76,532	77,674	100,949	100,131	76,079	30,812	76,079 ± 30,812
Average	25,249	34,986	38,229	48,073	46,581			

Table 8 to *Table 10* present data obtained from constant head permeability tests. The objective of conducting constant head permeability testing was to determine the hydraulic properties within each of the substrate mixtures under constant head flow. The data obtained from the testing is input data to the upscale model. The primary outcome from the test work is to calculate the hydraulic conductivity (K). Five rounds of consecutive measurements were taken on each of the substrate mixtures. The objective of conducting five rounds of consecutive measurements was to allow time for the substrate material to settle and prevent differential movement within the column when subjected to constant head flow.

It is expected that the volumetric flow rate (Q) and hydraulic conductivity (K) should both decrease with decreasing compost content, and that the hydraulic gradient (i) should increase with decreasing compost content. The results obtained from the 100% sand substrate do not present this trend. The results from the 100% sand substrate could potentially not be representative of actual flow conditions within this substrate. This could be due to a number of reasons, such as:

- Experimental errors, such as changes in inflow rate, measurement or reading errors, backwashing etc.
- Differential movement of material within column;
- Development of preferential flow paths within the substrate;
- Clogging of inlet and outlet tubes.

The results from the 100% sand substrate were, therefore, not used in further hydraulic calculations. Only the results obtained from the 100% compost, 70% compost 30% sand, 50% compost 50% sand, and 30% compost 70% sand substrates have been considered for calculation purposes.

Volumetric flow (Q) (m^3/d): The 30% compost 70% sand substrate presented the lowest average volumetric flow rate (0,243 m^3/d), while the 50% compost 50% sand substrate presented with the highest average volumetric flow rate (0,330 m^3/d). The 100% compost presented with an average volumetric flow rate of 0,313 m^3/d, while the 70% compost 30% sand substrate presented an average volumetric flow rate of 0,310 m^3/d. Variance is observed between the results obtained from the different rounds of measurement for each substrate. Standard deviation of the mean was used to calculate the uncertainty of the results obtained. The results obtained from the standard deviation of the mean would be indicative whether the average of the different rounds of measurement can be used as a design parameter. The results from the standard deviation of mean are indicative that the deviation is significant. The greatest uncertainty is observed with the 70% compost 30% sand substrate (0,310 ± 0,084), while the 100% compost substrate presented with the lowest uncertainty (0,313 ± 0,033). The use of an average would, therefore, not be an accurate representation of volumetric flow within the selected substrate. A correlation (R^2) was calculated between change in percentage sand versus change in volumetric flow rate (*Figure 23*). A weak negative correlation is observed between change in percentage sand and change in volumetric flow rate. A decrease in volumetric flow rate is observed with increasing sand percentage.

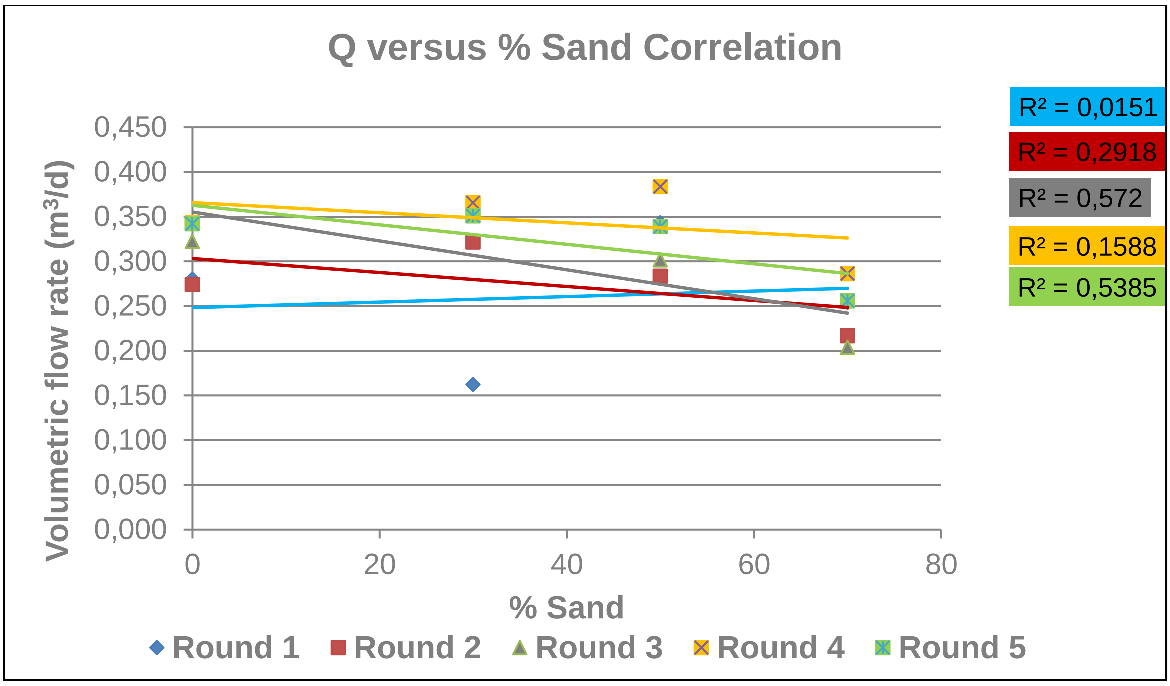


Figure 23: *Volumetric flow (Q) and percentage sand correlation (R^2).*

Hydraulic gradient (i): The 30% compost 70% sand substrate presented with the highest average hydraulic gradient (1,389), while the 100% compost substrate presented with the lowest average hydraulic gradient (0,938). The 70% compost 30% sand, and 50% compost 50% sand substrates presented with hydraulic gradients of 1,130 and 1,135 respectively. Standard deviation of the mean was calculated to determine whether the average between the different measuring rounds of a substrate mixture would be representative of the hydraulic gradient within each of the substrate mixtures. The greatest uncertainty is observed in the 70% compost 30% sand substrate (1,130 ± 0,186), while the 30% compost 70% sand substrate presented with the lowest uncertainty (1,389 ± 0,050). The uncertainty observed from using the average is significant. The use of the average would, therefore, not be representative of the hydraulic gradient within the selected substrate. A correlation (R^2) was calculated between the change in percentage sand in a substrate versus change in hydraulic gradient. A moderately strong positive correlation was observed. The hydraulic gradient (i) increases with increasing sand percentage (*Figure 24*).

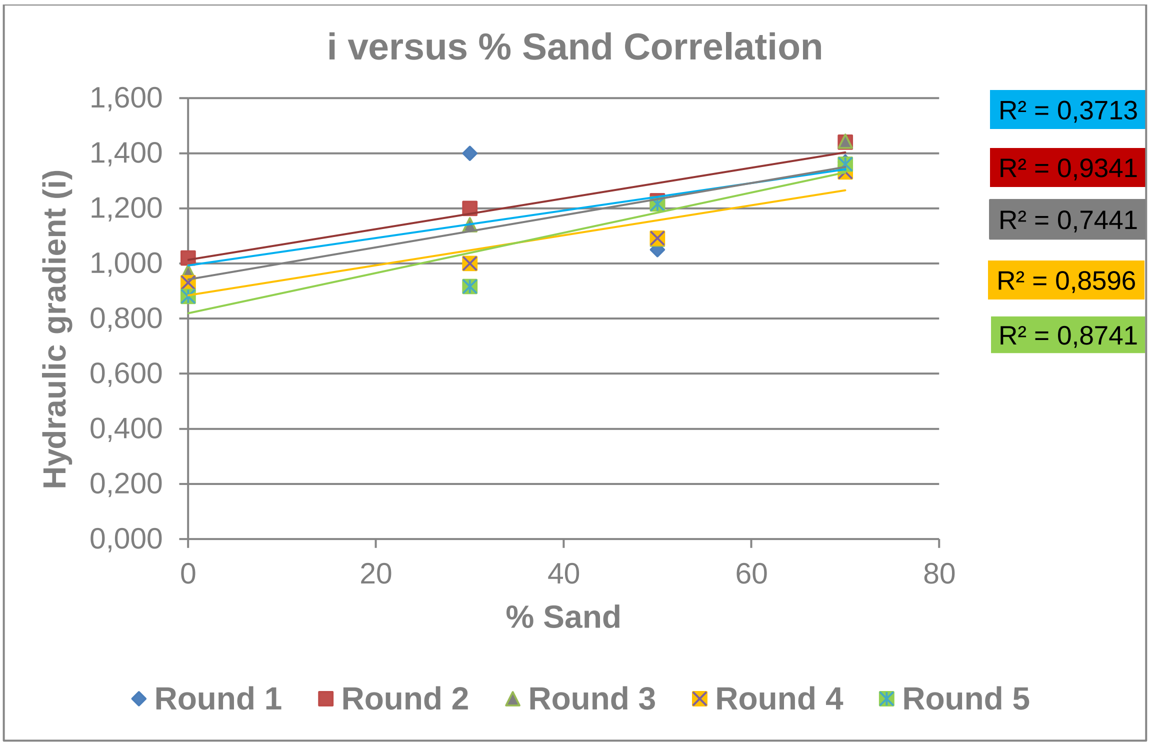


Figure 24: *Hydraulic gradient (i) and percentage sand correlation.*

Hydraulic conductivity (K): The hydraulic conductivity was calculated from the measured volumetric rate (Q), hydraulic gradient (i), and cross-section area (A) (Equation 1). The highest average hydraulic conductivity was obtained from the 100% compost substrate (35,882 m/d), while the lowest hydraulic conductivity was observed in the 30% compost and 70% sand substrate (18,876 m/d). The 70% compost 30% sand, and 50% compost 50% sand substrates presented very similar hydraulic conductivities, 30,879 m/d, and 31,403 m/d respectively. The most significant error was evident in the 70% compost 30% sand substrate (30,879 ± 11,421), while the 100% compost presented with the lowest error (35,882 ± 5,049). The standard deviation of the mean is significant, and the average hydraulic conductivity for each of the substrates is not representative of the hydraulic conductivity within each substrate mixture. A correlation was calculated between change in substrate sand percentage and hydraulic conductivity (K). A moderately strong negative correlation is observed between change in percentage sand and change in hydraulic conductivity. The hydraulic conductivity tends to decrease as the percentage sand increases.

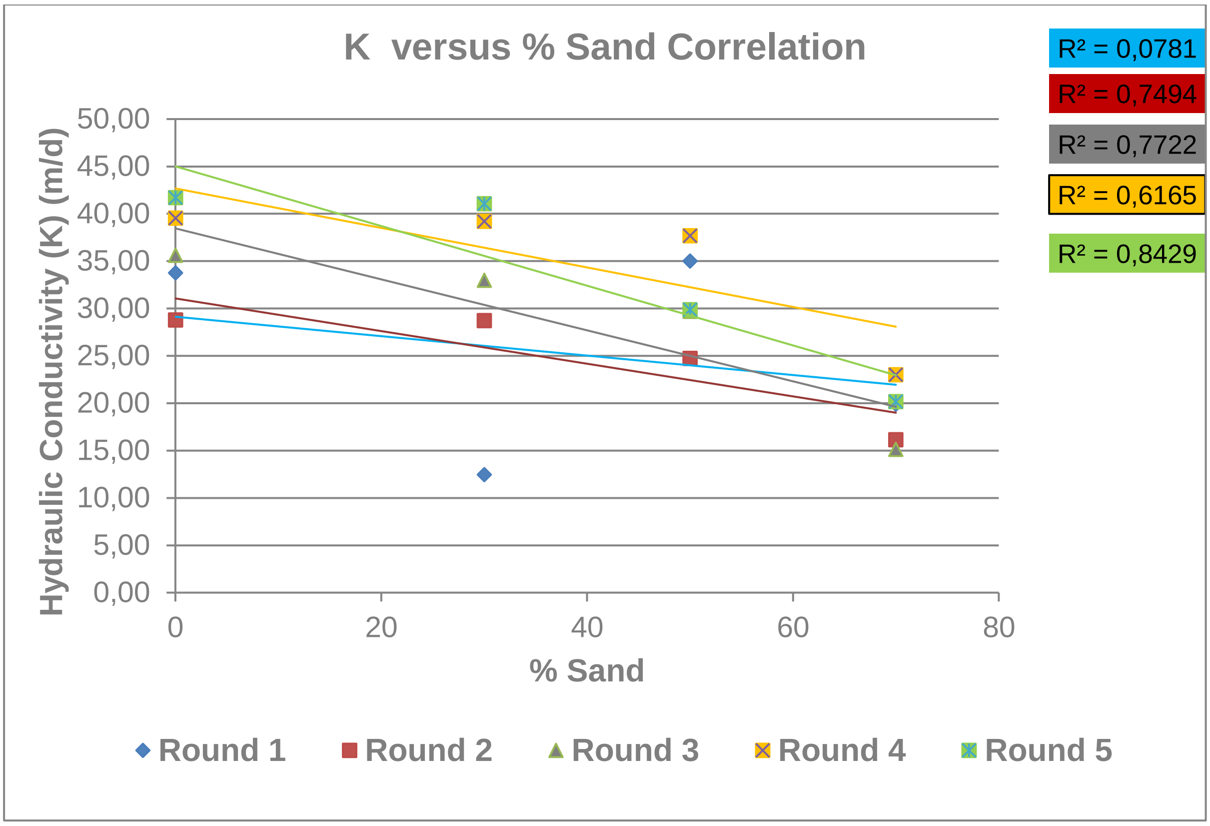


Figure 25: *Hydraulic conductivity (K) and percentage sand correlation.*

The standard deviation between the five rounds of measurement for each substrate mixture is significant. The variation in data can possibly be explained by the following experimental errors.

Systematic errors:

- Stopwatch: The stopwatch was used to determine the time it takes to fill a 2000 ml cylinder for measuring the volumetric flow rate (Q). No error was observed on the functioning of the stopwatch, and calibration is not applicable.
- Graduated cylinder: A cylinder was used to collect 2000 ml water from the outflow of the substrate to measure volumetric flow rate (Q). No systematic error was applicable.
- Ruler: A ruler was used to measure the height of the water column in pressure tubes. No systematic error was applicable.

Random errors:

- Stopwatch: The smallest measuring unit taken is 1 second. The error value on the stopwatch, therefore, is ± 0.5 seconds. The error needs to be converted to potential error on volumetric flow rate.
- Graduated cylinder: Outflow was measured from the substrate until the cylinder was filled to the 2000 ml mark. No random errors noted during the measurement.
- Ruler: The ruler used to measure height of the water column in the pressure tubes measures up to 1 mm. The ruler, therefore, has an error of ± 0.5 mm.

The calculated standard deviations of the mean indicate that the average value for volumetric flow rate and hydraulic gradient are not an accurate representation of flow within the substrate. Calculating hydraulic conductivity from the average volumetric flow rate and hydraulic gradient will yield results with significant error. Five rounds of measurements were taken to eliminate potential significant differential movement of substrate material within the column. Correlation (R^2) was calculated between the change in substrate sand percentage versus change in volumetric flow rate (Q), hydraulic gradient (i), and hydraulic conductivity (K). Round 5 presented the best correlation for calculated hydraulic conductivity.

The random (measurement) errors for volumetric flow rate, hydraulic gradient, and hydraulic conductivity were calculated by using the “product” rule. The results from the error calculation for round 5 results are presented in *Table 11*. The maximum error on hydraulic conductivity is < 1%, which is indicative that the precision of the measurements is high. The variance in the experimental results can possibly be ascribed to the development of preferential flow paths, differential movement of material, or clogging of the outlets.

Table 11: *Experimental error calculation for hydraulic conductivity.*

	100%C	**70%C30%S**	**50%C50%S**	**70%S30%C**
Q (m^3/d)				
Actual reading	0,342	0,351	0,339	0,256
Error	0,0007	0,0008	0,0007	0,0004
Error %	0,1980	0,2298	0,1962	0,1483
i				
Actual reading	0,880	0,916	1,216	1,360
Error	0,0040	0,0040	0,0040	0,0040
Error %	0,4545	0,4367	0,3289	0,2941
A (m^2)	0,0093	0,0093	0,0093	0,0093
k (m/d)				
Actual reading	41,687	41,060	29,864	20,162
Error %	0,6526	0,6665	0,5251	0,4424
Error	0,2720	0,2737	0,1568	0,0892

6.1.4. Dry density, Relative Density and Porosity

Each of the substrate mixtures were dried in an oven at 105°C for 12 hours. The dry density for each of the substrate mixtures were calculated by means of Equation 10. The following results were obtained for each of the substrate mixtures (*Table 12*):

Table 12: *Dry density for each of the substrate mixtures.*

	100%C	**70%C30%S**	**50%C50%S**	**70%S30%C**	**100%S**
Dry density (kg/m^3)	469,90 ± 10	838,58 ± 10	1033,00 ± 10	1212,44 ± 10	1464,28 ± 10

The results obtained indicate that the 100% compost substrate has the lowest dry density (469,90 ± 10), while the 100% sand substrate has the highest dry density (1464,28 ± 10). The other substrates have dry densities that fall between the 100% compost and 100% sand substrates. An error analysis was conducted on the results obtained. The scale used to weigh the substrate material has a precision error of 0,005 g. The calculated error value on the dry density for each of the substrate mixtures is ± 10 kg/m^3.

A Pycnometer was used calculate relative density. The results obtained from the Pycnometer are presented in *Table 13*.

Table 13: *Relative density for different substrate mixtures as obtained from gas Pycnometer.*

Substrates	Cycle 1 (kg/m^3)	Cycle 2 (kg/m^3)	Cycle 3 (kg/m^3)	Average (kg/m^3)
100%C	1608,50 ± 0,05	1602,60 ± 0,05	1596,09 ± 0,05	1602,70 ± 0,05
70%C30%S	2190,00 ± 0,05	2185,20 ± 0,05	2178,10 ± 0,05	2184,50 ± 0,05
50%C50%S	2469,30 ± 0,05	2464,10 ± 0,05	2461,90 ± 0,05	2465,10 ± 0,05
70%S30%C	2574,80 ± 0,05	2569,20 ± 0,05	2566,70 ± 0,05	2570,20 ± 0,05
100%S	2648,90 ± 0,05	2645,90 ± 0,05	2643,50 ± 0,05	2645,33,10 ± 0,05

Refer to Appendix F for results obtained from each cycle.

The pycnometer has a precision error of 0,05 kg/m^3 as indicated in *Table 13*.

Porosity (n) was calculated for each of the substrate mixtures as a function of dry density and relative density (Equation 4). The results obtained are presented in *Table 14*.

Table 14: *Porosity results.*

	100%C	70%C30%S	50%C50%S	70%S30%C	100%S
Porosity (n)	0,7068 ± 0,0084	0,6161 ± 0,0076	0,5809 ± 0,0073	0,5282 ± 0,0069	0,4377 ± 0,0052

The 100% compost substrate presented with the highest porosity (0,7068 ± 0,0084), while the 100% sand substrate presented with the lowest porosity (0,4377 ± 0,0052). The other substrate mixtures presented with porosities that fall between the 100% sand and 100% compost substrate. An error analysis was also conducted for the results obtained from the Pycnometer (*Table 14*). The results present a strong negative correlation (R^2) between the change in percentage sand (per volume) versus the change in experimentally determined porosity (*Figure 26*). It is, therefore, evident that an increase in a substrate's sand content (by volume) decreases the porosity, compared to compost that increases the porosity.

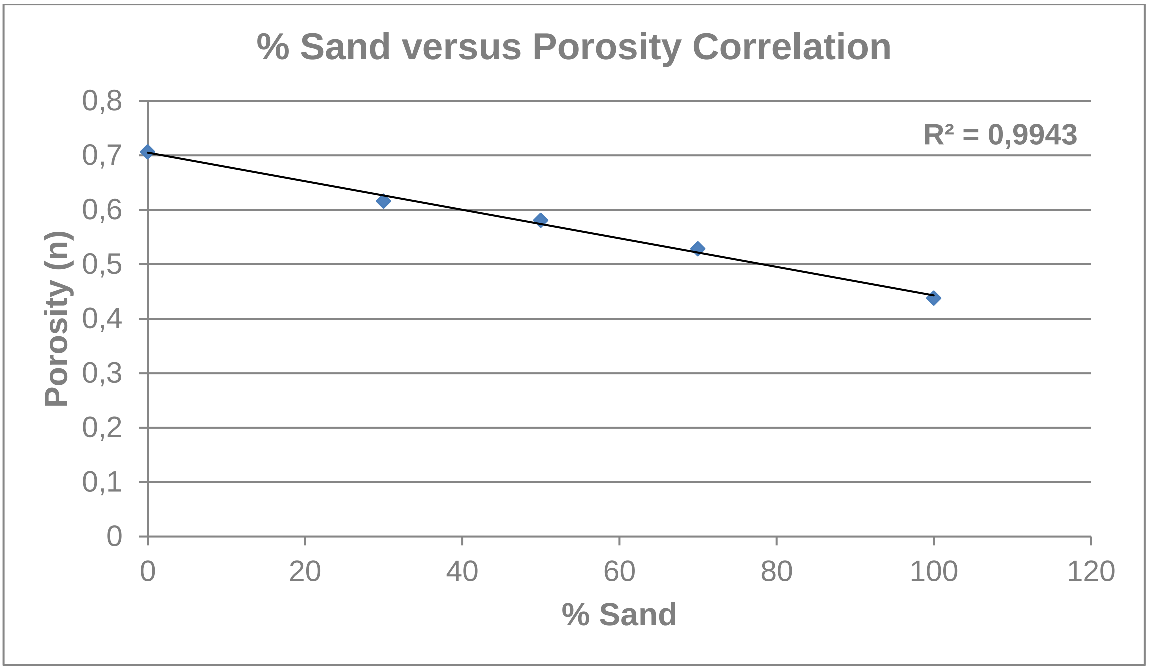


Figure 26*: Correlation between change in percentage sand versus change in porosity.*

6.1.5. Tracer testing

Tracer testing was conducted to determine the HRT within each substrate mixture (Appendix G). The tracer tests were conducted under similar experimental and hydraulic conditions as the constant head permeability test, which include volumetric flow (Q), hydraulic gradient (i), head, bed slope, and hydraulic conductivity (K).

Dispersion can be approximated by making use of the radial convergence test (RCT). The longitudinal dispersivity (α_L) provides an indication of the dispersion within the substrate. The mass of tracer injected (ΔM), longitudinal dispersivity (α_L), and Seepage velocity (V_s) were used as fitting parameters. The longitudinal dispersion coefficient (D_L) was calculated as a product of Seepage velocity (v_s) and longitudinal dispersivity (α_L). The radial flow (r) was set as the length of the column. *Table 15* present the results obtained from the RCT analysis.

It is evident from the results presented in *Table 15* that the 100% sand substrate has the greatest degree of longitudinal dispersion (α_L) (0,065 m). This substrate, however, contains no organic matter, which entail that this substrate will not be able to supply sufficient nutrients to sulphate reducing bacteria for sulphate reduction. The results obtained from the constant head permeability test also indicate that an error might have occurred during the experimental run in the 100% sand substrate. The 100% sand substrate will, therefore, not be considered for calculations. The 30% compost 70% sand substrate present with a longitudinal dispersion of 0,035 m, which is higher than the longitudinal dispersion for the remaining substrate mixtures.

Table 15: *Results from RCT analysis.*

		ΔM	Q	α_L	D_L	v_s
100%C	Round 1	0,010	0,00028	0,018	0,0006	0,0319
	Round 2	0,012	0,00028	0,018	0,0006	0,0310
	Round 3	0,012	0,00028	0,018	0,0006	0,0319
70%C30%S	Round 1	0,017	0,00019	0,025	0,0004	0,017
	Round 2	0,016	0,00019	0,025	0,0005	0,018
	Round 3	0,016	0,00019	0,020	0,0004	0,018
50%C50%S	Round 1	0,010	0,00029	0,020	0,0008	0,038
	Round 2	0,013	0,00029	0,022	0,0008	0,038
	Round 3	0,012	0,00030	0,025	0,0010	0,042
30%C70%S	Round 1	0,010	0,00021	0,035	0,0013	0,038
	Round 2	0,010	0,00021	0,020	0,0007	0,035
	Round 3	0,010	0,00021	0,030	0,0010	0,035
100%S	Round 1	0,028	0,00035	0,060	0,0016	0,026
	Round 2	0,032	0,00035	0,065	0,0170	0,026
	Round 3	0,032	0,00035	0,050	0,0012	0,024

ΔM: injected mass of tracer injected per unit section (g/m^2)   *Q: volumetric flow rate (m^3/minute)*   *α_L: Longitudinal dispersion (m)*

D_L: Longitudinal dispersion coefficient (m^2/minute)   *V_s: Seepage velocity (m/minute)*

Porosity can be estimated from the RCT. The estimated porosity is calculated as a function of Darcy velocity and fitted Seepage velocity (Equation 6). The estimated porosity for each substrate mixture, together with the experimentally calculated porosity for each substrate mixture is presented in *Table 16*.

Table 16: *Porosity results (experimental and RCT fit).*

	100%C	**70%C30%S**	**50%C50%S**	**30%C70%S**	**100%S**
	Experimentally calculated porosity				
	0,7068	0,6161	0,5809	0,5282	0,4377
	Estimated porosity (RCT)				
Round 1	0,8152	1,5044	0,6725	0,4986	1,7918
Round 2	0,7975	1,4466	0,6725	0,5484	1,7918
Round 3	0,7975	1,4749	0,6052	0,5484	1,7918
Average	0,8034	*1,4753*	0,6501	0,5318	*1,7918*

During the constant head permeability test it was noted that the 100% sand substrate might contain errors. The 100% sand substrate can, therefore, be regarded as unrepresentative of flow conditions that can be expected under constant head flow conditions. The estimated porosity values for the 100% sand substrate (*1,7918)* are, therefore unrepresentative of substrate characteristics. The 70% compost 30% sand substrate also present porosity values (*1,4753)* that are unrepresentative of substrate characteristics. All hydraulic parameters (Q, i, K, and A) were kept constant during the constant head permeability test, as well as the tracer experiment. The error in the results obtained for these two substrates can possibly be ascribed to differential movement of material, development of preferential flow paths, or clogging of outlet pipe that caused the skewed tracer results.

The expected HRT for each substrate under specific hydraulic conditions was calculated as a function of column length by means of the following methods:

- Calculated Seepage velocity from constant head permeability test (Equation 5).
- Fitted Seepage velocity during radial convergent test (RCT) (Equation 6).
- Change in observed tracer output concentration from pulse injection (Levenspiel method) (Equation 8).

Table 17 presents the obtained HRTs.

Table 17: *Hydraulic residence times (HRTs).*

	Calculated Seepage velocity from CHPT					
	Round 1	**Round 2**	**Round 3**	**Round 4**	**Round 5**	**Average**
100%C	7,90	8,07	6,86	6,44	6,46	7,61
70%C 30%S	11,23	5,68	5,20	4,99	5,20	7,37
50%C 50%S	5,08	6,15	5,78	4,54	5,14	5,67
30%C 70%S	6,43	7,53	8,01	5,71	6,39	7,32
100%S	8,26	2,61	2,60	2,30	2,31	4,49
	Tracer output concentrations (Levenspiel method)					
	Round 1	**Round 2**		**Round 3**		**Average**
100%C	9,81	10,15		9,34		9,77
70%C 30%S	14,15	12,94		16,34		14,48
50%C 50%S	11,70	13,10		12,90		12,57
30%C 70%S	8,19	10,20		13,34		10,58
100%S	8,83	8,12		10,00		8,98
	Fitted Seepage velocity (RCT)					
	Round 1	**Round 2**		**Round 3**		**Average**
100%C	7,80	8,00		7,80		7,87
70%C 30%S	14,40	13,80		14,10		14,10
50%C 50%S	6,70	6,70		6,00		6,47
30%C 70%S	6,50	7,20		7,20		6,97
100%S	9,50	9,50		10,30		9,77

RCT: Radial convergent test *HRT presented in minutes* *C: Compost* *S: Sand*
CHPT: Constant head permeability test

The possible error in the constant head permeability test for the 100% sand substrate and the tracer test for the 70% compost 30% sand substrate is evident in the HRTs obtained for these specific substrates. The 50% compost 50% sand substrate also present higher HRTs as calculated from the tracer data, however, the porosity results are similar than the experimental porosity calculated. The HRT for the 50% compost 50% sand as calculated from the RCT is similar than the experimentally calculated HRT.

Pearson Correlation (r) and Root Mean Square Error (RMSE) were used firstly to test the goodness of fit between observed tracer results and RCT, and secondly to assess the error from the mean between model runs. Refer to Appendix H for RCT fits.

6.2. KINETIC MODEL

Two kinetic models were developed to simulate sulphate reduction and acetate utilisation as a function of HRT. The first model was developed to simulate the utilisation of sulphate and acetate at varying feed sulphate concentrations with a single feed acetate concentration. The feed sulphate concentrations include: 500 mg/L, 700 mg/L, 900 mg/L, and 1200 mg/L. The dissolved organic carbon concentration of 100% compost was assumed to be the maximum to be released (868 mg/L). The results obtained from the first model are indicative that the available acetate concentration was insufficient to reduce feed sulphate concentration of 1200 mg/L to ≤ 250 mg/L. The available acetate concentration was, however, sufficient to reduce feed concentrations of 500 mg/L, 700 mg/L, and 900 mg/L to ≤ 250 mg/L sulphate at HRTs of 2,2 days, 3,5 days, and 5,3 days respectively. It is evident from the model outputs that optimal sulphate reduction rates are dependent on sufficient acetate concentration. The results from the first model are presented in *Figure 27* (numerical model data outputs presented in Appendix I).

The best fit line was plotted against the modelled data for varying feed sulphate concentrations (first model). The following generalised mathematical function was used to find the best fit line for each feed sulphate concentration:

$$y = \frac{A_1 - A_2}{1 + \left(\frac{x}{x_0}\right)^p} + A_2$$

(Equation 23)

Where,

x = Desired HRT (time unit),

y = final sulphate output concentration (mg/L).

Other function parameters are described by Equation 24 to *Equation 27*

A best fit line was plotted for each of the modelled sulphate concentrations. Each of the parameters mentioned in Equation 23 were used to create the best fit line. The value for each specific parameter is presented in Appendix I. The best fit line for modelled sulphate concentration with varying feed sulphate concentrations provided the following functions for each of the parameters:

$$A_1 = 1{,}0168\ x - 38{,}879$$

(Equation 24)

$$A_2 = 0{,}6299\,x - 404{,}670$$

(Equation 25)

$$p = -\,0{,}964\ln x + 9{,}424$$

(Equation 26)

$$x_0 = 0{,}2799\,x^{0.397}$$

(Equation 27)

Where,

x = Sulphate feed concentration (mg/L)

A second model was developed to simulate the utilisation of sulphate and acetate at varying feed acetate concentrations with a single feed sulphate concentration. The aim of this model was to optimise the concentration of acetate required for sulphate reduction. This model was conducted for the following acetate concentrations: 200 mg/L, 500 mg/L, 700 mg/L, and 800 mg/L. Sulphate was introduced at a concentration of 500 mg/L for each of the acetate feed concentrations. The results obtained from this model are indicative that sulphate reduction is dependent on sufficient supply of acetate. A feed acetate concentration of 200 mg/L was insufficient to reduce feed sulphate concentration from 500 mg/L to ≤ 250 mg/L. Acetate feed concentrations of 500 mg/L, 700 mg/L, and 800 mg/L could reduce feed sulphate concentration of 500 mg/L to ≤ 250 mg/L at 3,0 days, 2,40 days, and 2,0 days respectively. The results obtained from this model are presented in *Figure 28* (numerical model data outputs are presented in Appendix I).

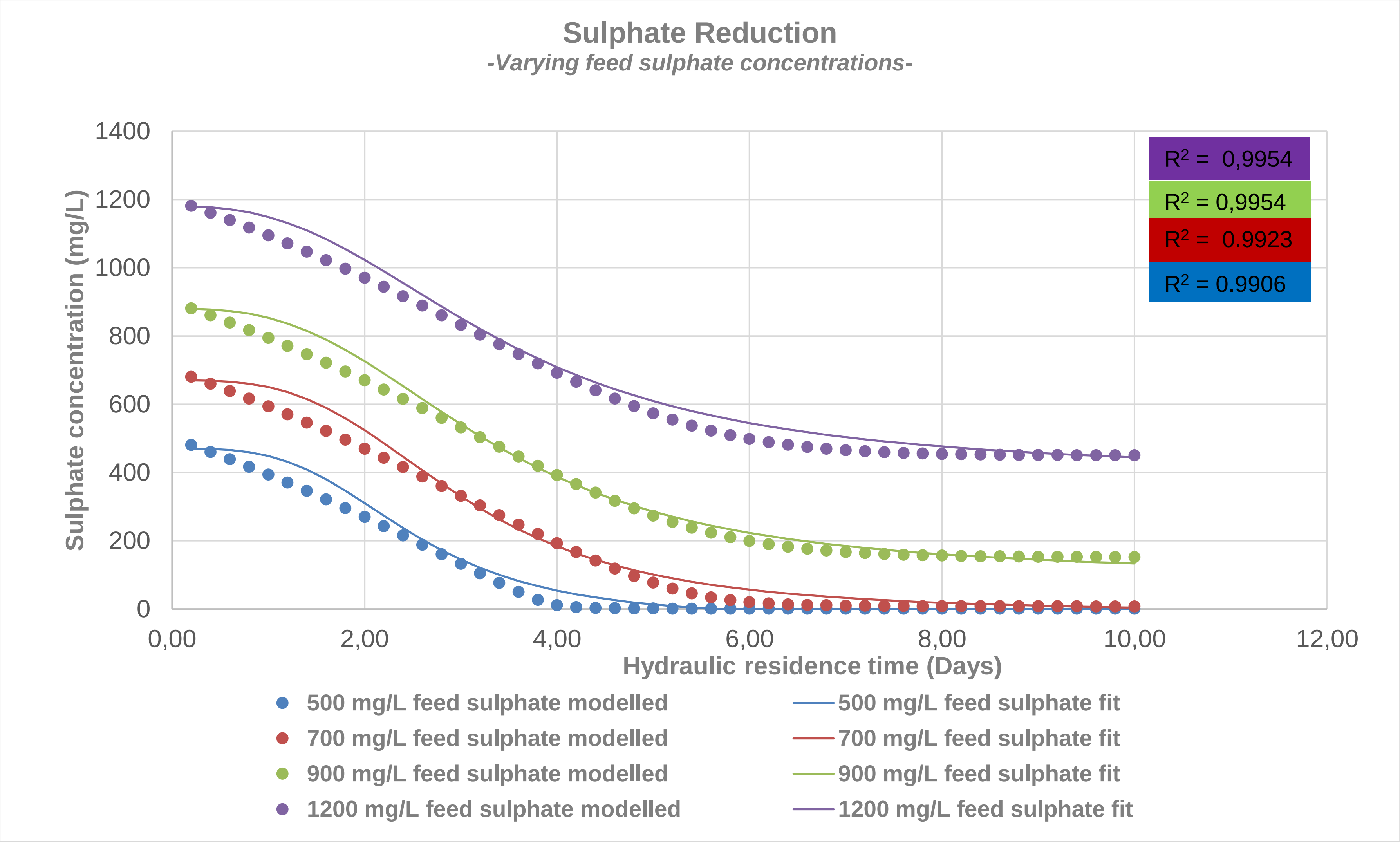


Figure 27: *PHREEQC model results for different feed sulphate concentrations with a single feed acetate concentration.*

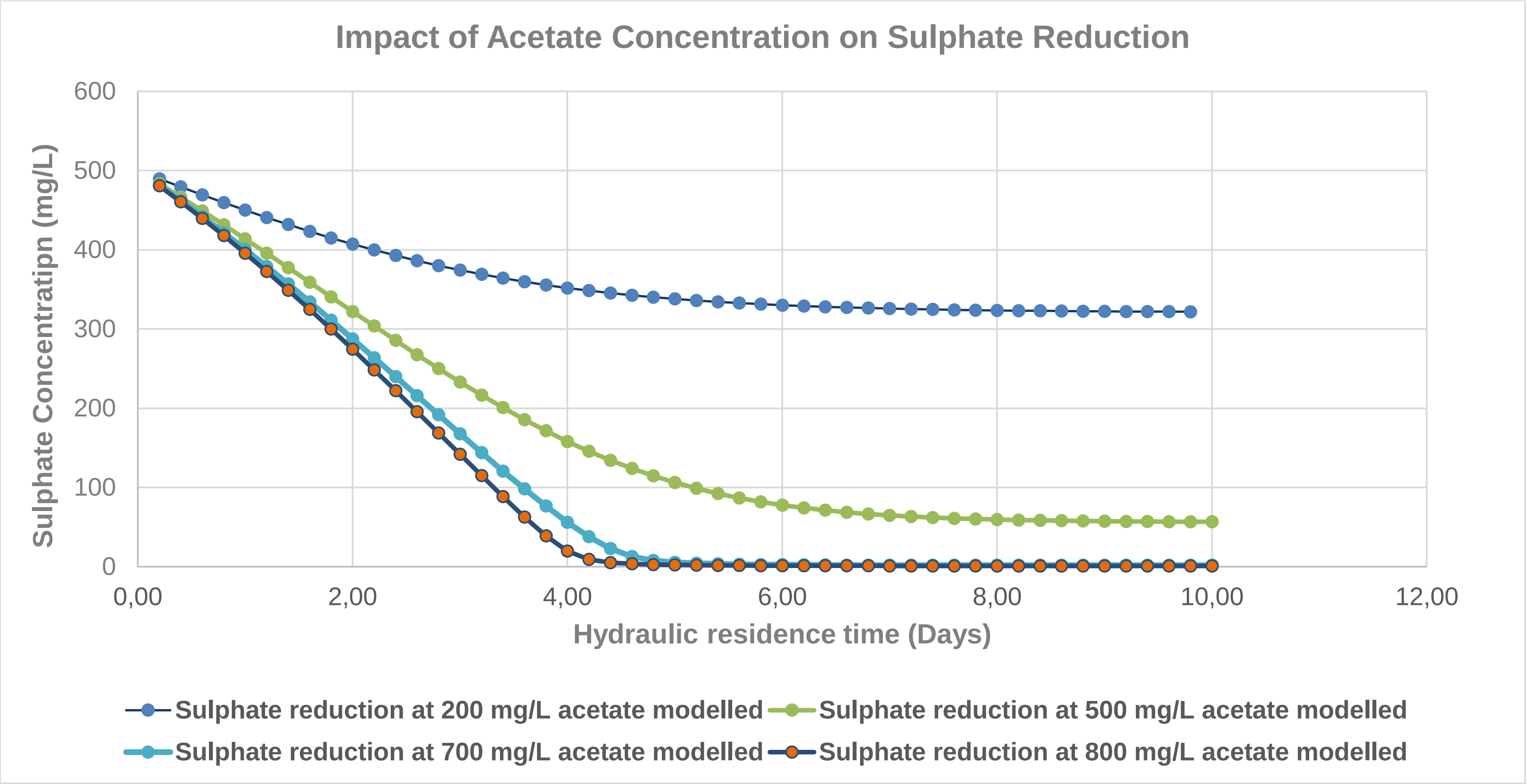


Figure 28: *PHREEQC model results for impact of acetate concentration on sulphate reduction rates.*

CHAPTER 7: CASE STUDY

7.1. PROJECT BACKGROUND

An area was mined for coal resources by means of open cast methods between 1998 and 1999. The mined site is located approximately 10 km south west of Belfast town, in Mpumalanga province, South Africa. The estimated extent of the open cast surface area comprised 56 Ha. The maximum pit depth was estimated to be 25 m at the high-wall. The open cast pit was backfilled after mining ceased in 2000, and the surface rehabilitated. An official closure certificate, as required by the Mineral and Petroleum Resources Development Act, Act 28 of 2000, was never obtained from the regulatory authority (Department of Mineral Resources). The mining company, therefore, remains liable for the site until a closure certificate is granted. After decommissioning, the site was only used for grazing purposes by a local farmer. Environmental impacts, such as surface subsidence, sporadic soil surface acidification, and decanting started to become evident on site. The responsible mining company was, therefore, obligated to further rehabilitate the effected environment to as near as possible to the pre-mining state. Emphasis was, however, placed on treating or better managing the decant water, which currently is discharging into the natural catchment. A pre-directive was issued by the applicable catchment management agency to rectify the decanting situation as soon as possible. The end land use objective is to rehabilitate the site to a standard where the rehabilitated open cast pit has little to no detrimental environmental impacts on the receiving environment, and for the land to be used for grazing purposes by local farmers.

Biological passive water treatment was selected as the preferred method for managing the decant situation, due to the following reasons:

- The decant water quality is of such nature that more intense treatment by advanced treatment technologies is not required;
- The proposed water treatment system is less labour intensive as compared other more advanced treatment technologies;
- Requires less maintenance and monitoring as compared to more advanced technologies, such as reverse osmosis. The maintenance and management of the system can be conducted by the end land user;
- Requires less service-infrastructure (electricity, pumps, pipes, filters etc.) as compared to more advanced technologies;
- The aesthetic value of a biological passive treatment system is greater than that of other technologies that require steel structures.

7.2. POST-DECOMMISSIONING ENVIRONMENTAL CONDITIONS

7.2.1. Climate

The study area is located in the Eastern Transvaal Highlands, Mpumalanga province of South Africa. The site obtains most of its rain during the summer months (October to April). The average annual rainfall is 650 mm. No rain is expected to occur during the winter months. The mean daily temperature in the summer varies from 14,5ºC to 18,9ºC, while the mean daily temperature in the winter varies from 9,1ºC to 12,3ºC (EMPr, 1996).

7.2.2. Topography and drainage

The current site has an undulating topographical character. The site falls within two Quaternary catchments (X11C and X11D). The current decant point is situated at an elevation of approximately 1770 mamsl. North-flowing runoff and seepage enter the Klein Komati catchment, while south-flowing runoff and seepage enter the Blesbokspruit catchment. General hydrological characteristics of the catchments are provided in *Table 18*.

Table 18: *Catchment Characteristics (Imperata 2012).*

Catchment	Mean annual precipitation	Mean annual run off	Mean annual evaporation
X11C (Blesbokspruit)	715 mm	10,3 million m^3	1435 mm
X11D (Klein Komati)	744 mm	40,7 million m^3	1414 mm

Figure 29 illustrates the flow path of decant water discharging into the natural catchment. The decant water only flows into the quaternary catchment X11C. Quaternary catchment X11D, therefore, remains unaffected by run-off from the decant point. The topography and drainage characteristics of the site and surroundings are indicated in *Figure 30*.

Figure 29: *Surface run-off from the site and possible receptors (Harck, 2014).*

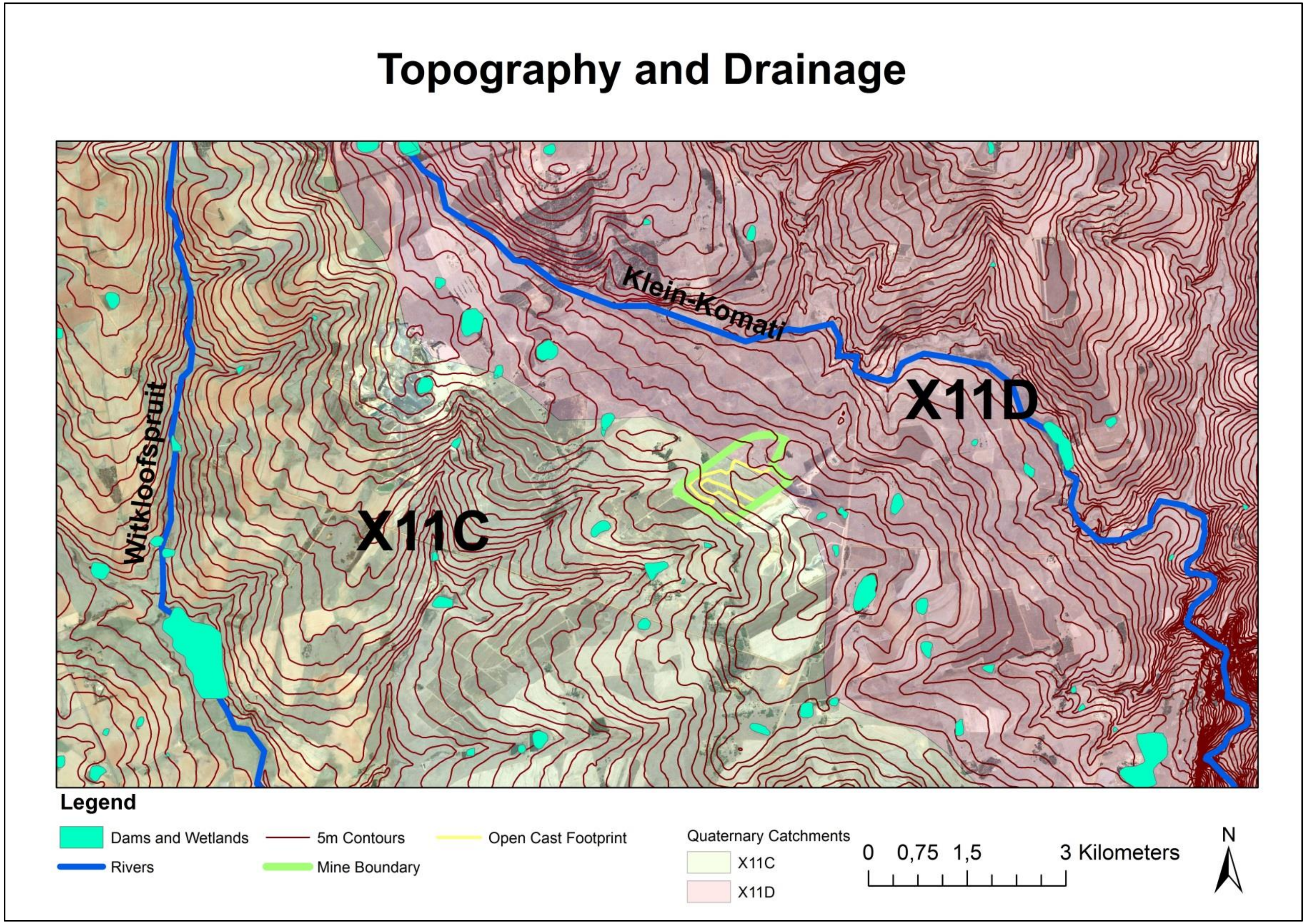


Figure 30: *Topography and drainage of study area.*

7.2.3. Geology and soils

The area is underlain by rocks of the Karoo Supergroup on older Mokolian intrusives and extrusives. The Karoo Supergroup consists mostly of sandstones, shales, and coal. The Vryheid formation contains the coal resources, which forms part of the Middle ECCA Group. The sedimentary succession overlies the Dwyka Formation, comprising of diamictites and tillites at the base of the Karoo sequence. Igneous intrusives (dolerite dykes) of late Karoo age characterise the Mpumalanga coal fields, which were also mapped in the mined pit (Steenekamp, 2011).

The number 2 coal seam was mined at this specific site. The upper seams (4 and 5 seam) that occur above the number 2 seam have been eroded away. The mined coal seam varied in thickness. This is clearly evident in the final geometry of the pit where a portion was not mined due to the absence or too thin availability of the coal seam. The unsaturated soil zone is composed of sandy loam soils (reddish-yellowish brown). According to the Soil Classification Working Group (2006), the pre-mining soil forms vary from sandy loam soil forms (Hutton/Clovelly) in the upper slope regions to soils with some wetness deeper in the profile (Avalon) lower down the slopes. Katspruit soil form is also evident in lower lying areas. A wetland soil form was identified at the decant point in the south-western corner of the site. *Figure 31* provides a representative borehole log of the pre-mining environment.

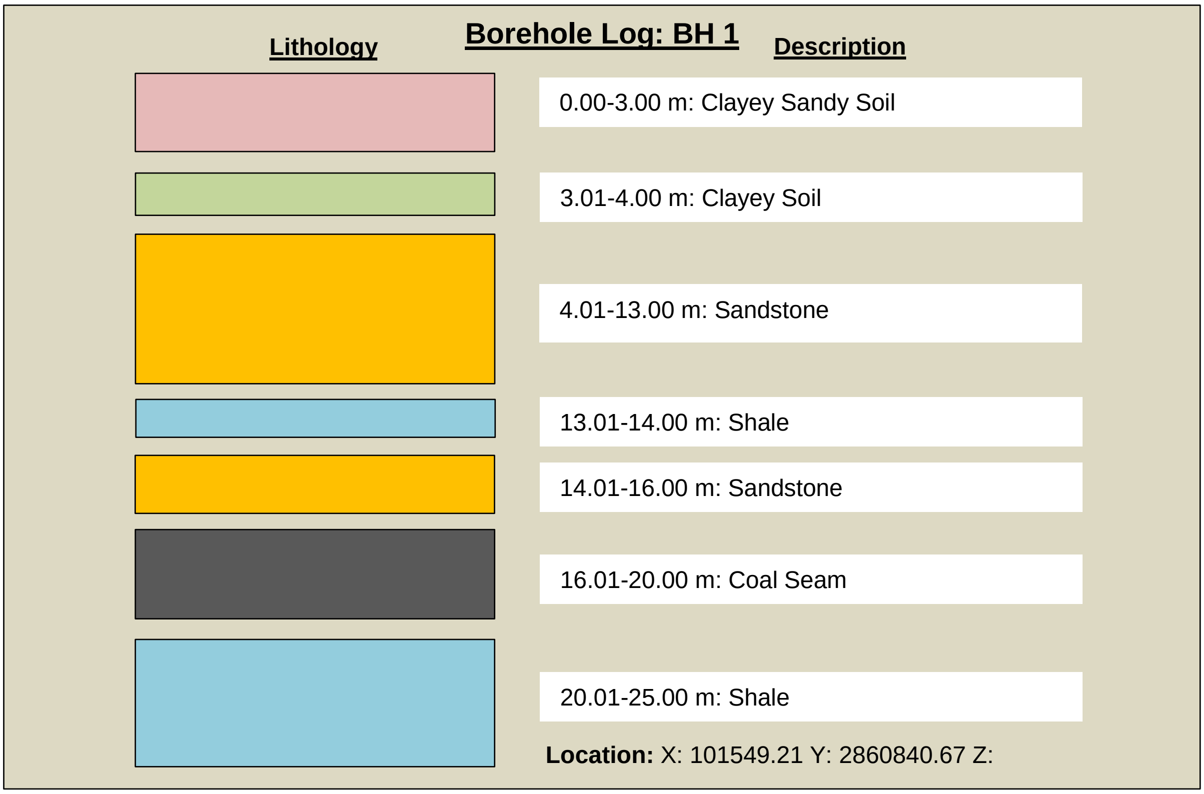


Figure 31: *Typical borehole log drilled during exploration phase.*

7.2.4. Site specific groundwater recharge

The open cast pit was backfilled with overburden material, which primarily consists of waste rock and coal discard. The surface was then covered with subsoil and topsoil. A soil depth survey was conducted to evaluate whether the thickness of the replaced soil adhere to commitments made in the EMPr. The results from the soil survey indicated that soil depth varies between 141 mm to >1000 mm across the rehabilitated open cast pit area. The hydraulic conductivity and thickness of the replaced soil layer determine the infiltration rate, amount of run-off, and the effective recharge to the underlying aquifer (Steenekamp, 2011).

A hydrogeological study was conducted by Steenekamp, (2011) to obtain a baseline understanding of groundwater flow characteristics. The following results were obtained:

- The decant point has an elevation of approximately 1770 mamsl;
- A total void volume of 3 600 000 m^3 was estimated for the open cast pit;
- A porosity of 25% (0.25) was applied during the modelling;
- The surface area of the pit was estimated to be 56 Ha;
- Decanting started (2010) approximately 10 years after decommissioning (2000);
- This calculates to an effective recharge rate of 22% (0.22) of MAP (mean annual precipitation) in backfilled pit;
- An effective recharge rate of 2% of MAP has been estimated for the natural (undisturbed) regional area.

7.2.5. Groundwater level

SRTM 90 elevation data was used as the digital elevation model (DEM). Groundwater level data was obtained from the National Groundwater Archive (NGA), as well as from field measurements. The correlation between topography and groundwater level was calculated. *Figure 32* illustrates that a high correlation occurs between the groundwater level elevations and the topography. Bayesian interpolation was then used to generate the groundwater level map.

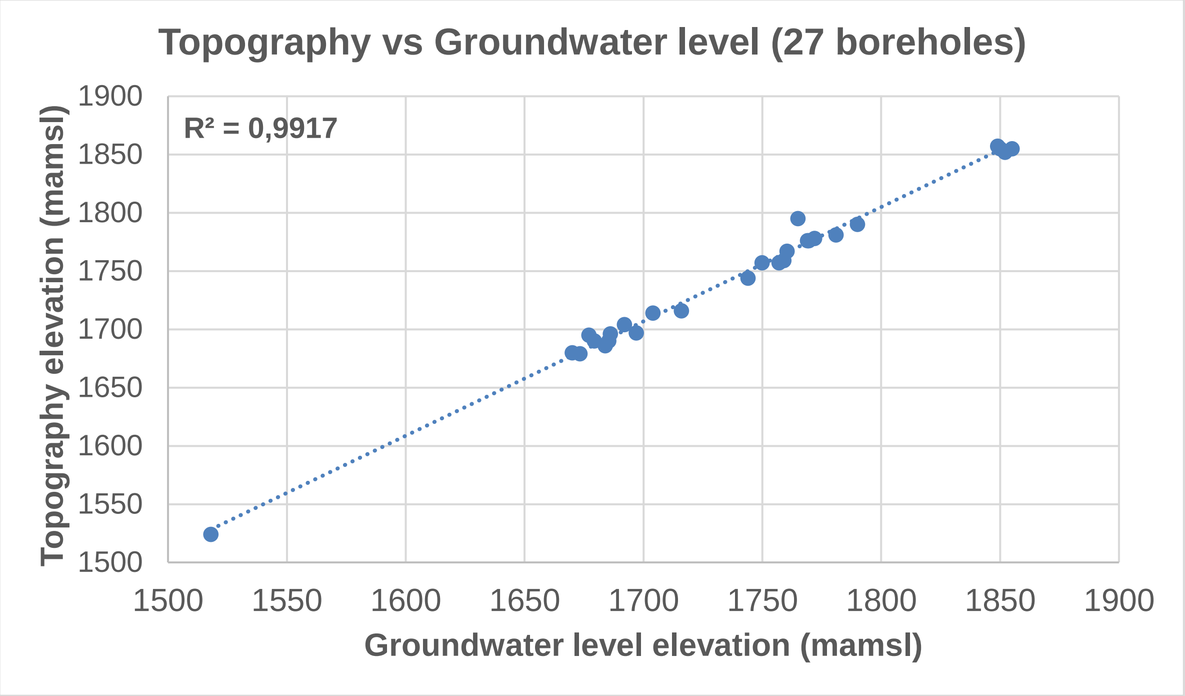


Figure 32: *Correlation between surface topography and groundwater level.*

Figure 33 presents the spatial distribution of the boreholes used in generating the groundwater contour map (See Appendix J for borehole information). *Figure 34* presents a groundwater contour map generated for the site.

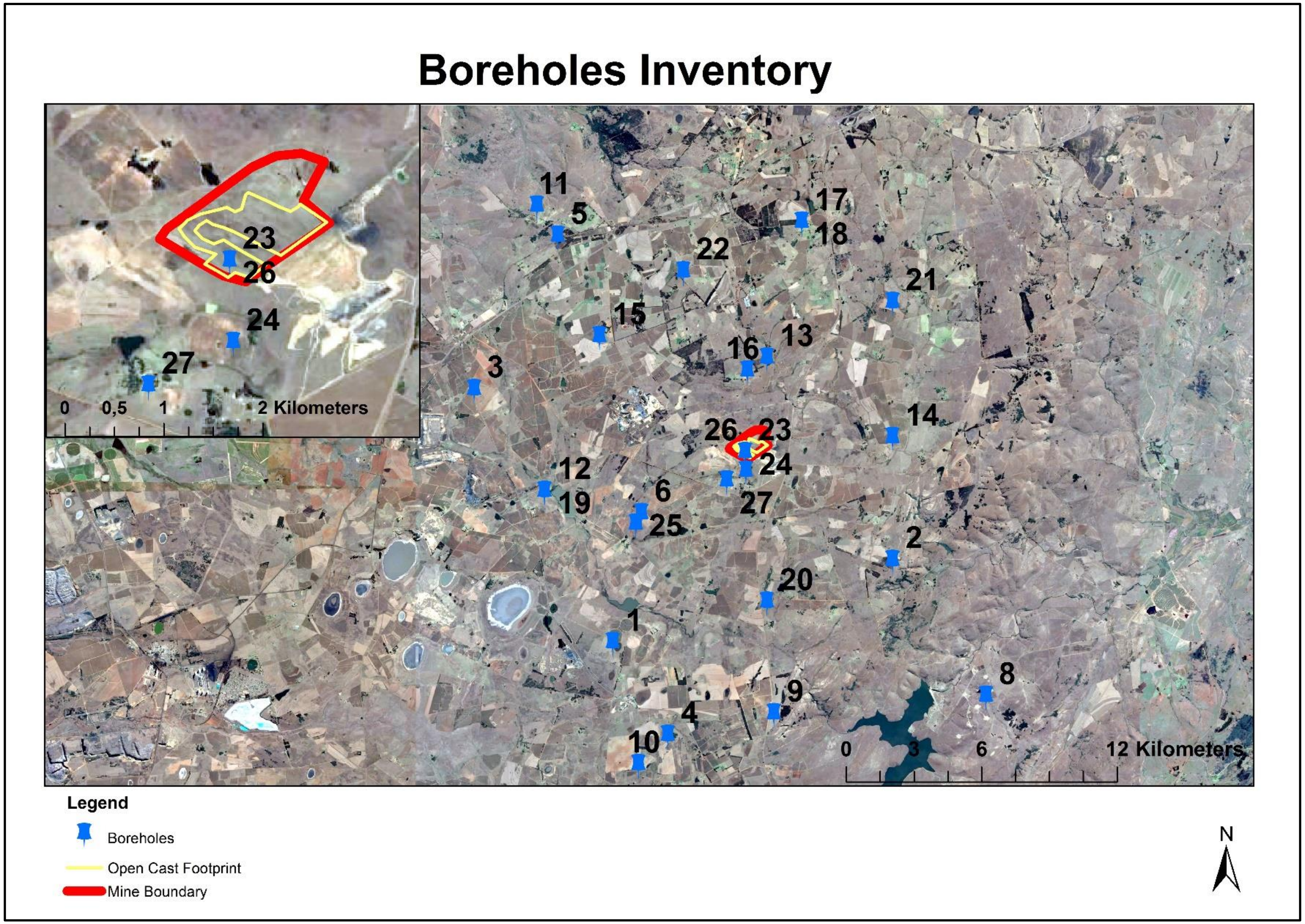


Figure 33: *Location of boreholes used to develop groundwater contour map relative to the mine site boundary*.

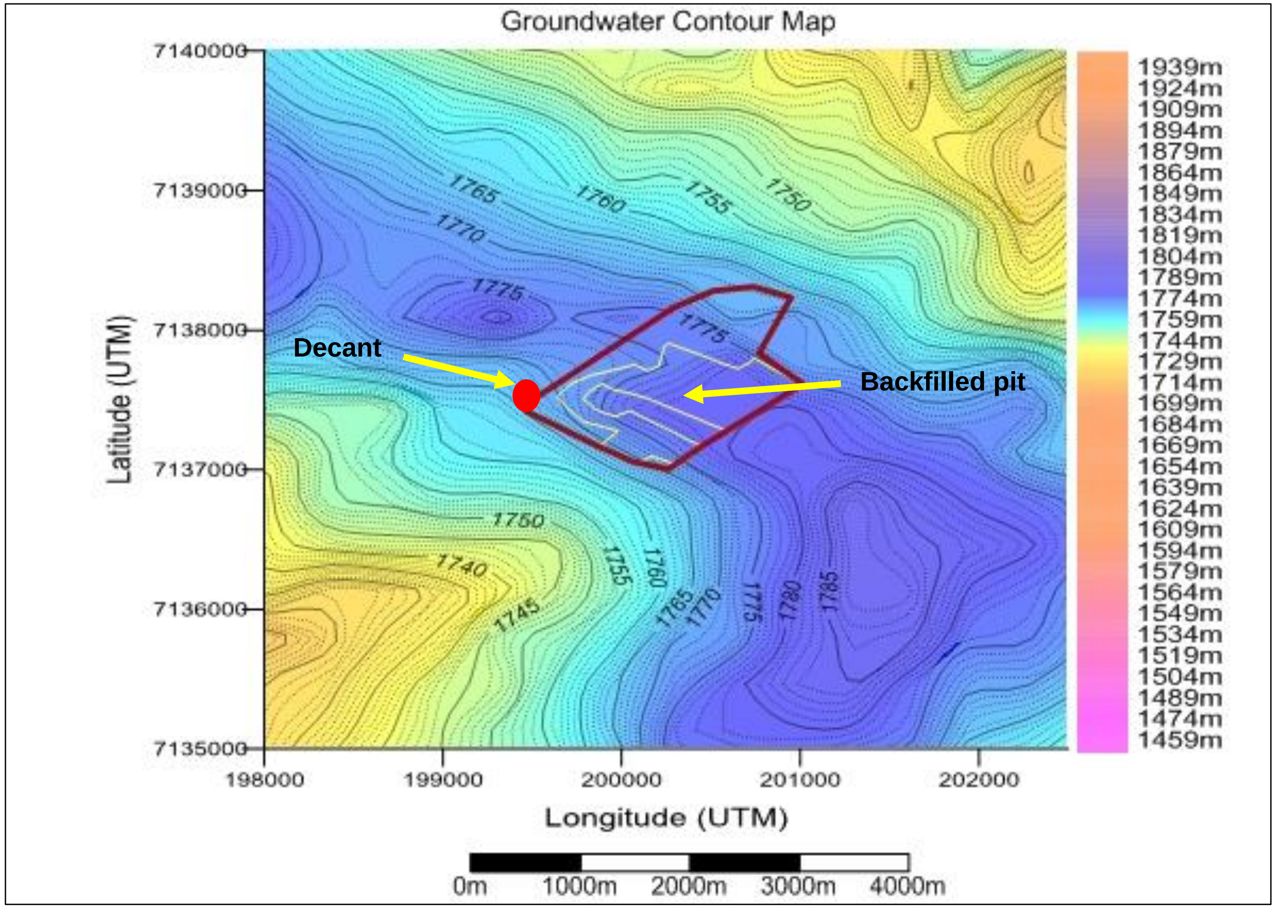


Figure 34: *Groundwater contour map for study area.*

7.3. DECANT WATER QUALITY AND EXPECTED IMPACT

A monitoring borehole was drilled in the deepest part of the backfilled open cast pit to obtain a sample of backfilled material. Chemical analyses (static acid base accounting) were conducted on the waste rock material, primarily to determine the sulphur content and acid generating potential. The results from the analyses are presented in *Table 19*:

Table 19: *Results from acid base accounting analysis.*

Parameter	Value
Total Sulphur (%)	0,40
Sulphate Sulphur as S (%)	0,21
Sulphide Sulphur (%)	0,19
Paste pH	5,9
Acid Potential (kg/t)	6,25
Neutralization Potential (NP)	0,993
Nett Neutralization Potential (NNP)	-5,3
Neutralization Potential Ratio (NPR) (NP: AP)	0,16
Interpretation	Potentially acid generating

The results presented in *Table 19* entail the following:

- **Paste pH**: The paste pH of the sample is slightly acidic (5,9), which entails that acidity can be generated and mobilised in the short-term. The origin of the acidity could be from oxidation and weathering of sulphide minerals.
- **Total sulphur**: The total sulphur is 0,4%, of which 0,19% is sulphide sulphur, and 0,21% is sulphate sulphur. The sulphate sulphur could be the source of the acidity that is evident in the paste pH. The potential of the discard to generate acidity is indicated by the sulphide sulphur content. Sulphide sulphur < 0,3 % is considered as containing insufficient sulphide to generated acidity over the long term. Short term acid can, however, be generated by the oxidation of the discard material.
- **Acid generating potential (AP):** The acid generating potential is calculated to be 6,25 kg $CaCO_3$/t, which is significantly higher than the neutralization potential (NP) of 0,993 kg $CaCO_3$/t. This is indicative that the acid neutralization potential of the discard material is limited.
- **Nett neutralization potential (NNP) (NP-AP):** The nett neutralization potential is negative for potential acid generating material. The NNP for the discard presented as -5,3, which is indicative of being potentially acid forming.

- **Nett neutralization potential ratio (NNR) (NP/AP):** The nett neutralization potential ratio for the discard material is 0,16 which is indicative of material that is potentially acid generating.

The results from the ABA analysis are indicative that the discard material within the backfill pit still has the potential to generate acid, which could develop into acid drainage. A portion of the sulphide sulphur in the discard material already oxidised to sulphate, which is evident in the water quality at the decant point. The pH of the decant water is, however, near neutral, which is indicative that the material still has sufficient neutralization potential to inhibit acid to be liberated from the oxidation zone, or that the results obtained from the single discard sample are not representative of all discard in the backfilled pit (Harck, 2014).

Hydrogeochemical modelling was conducted to determine the impact on downstream water quality, specifically relating to impacts caused by elevated sulphate concentration in the decant water. The results from the modelling indicated that the elevated sulphate concentration (approximately 700 mg/L) in the decant water could potentially impact detrimentally on the receiving environment, if no mitigation measures are implemented (Harck, 2014). *Figure 35* illustrates the location of the decant point and discharge flow direction. Downstream of the decant point is an existing attenuation dam that is used for drinking purposes by cattle.

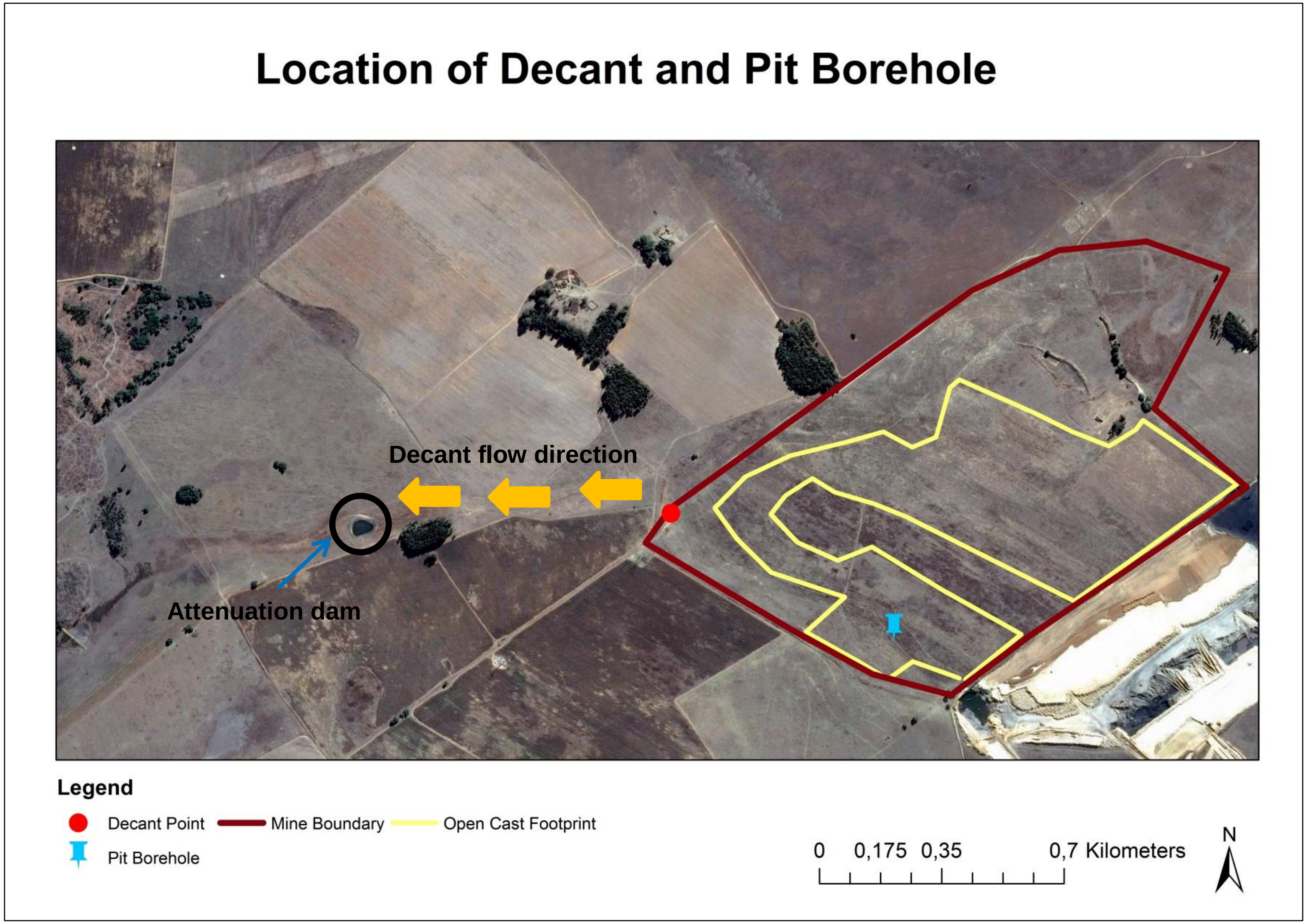


Figure 35: Location of decant and pit borehole.

7.4. PROPOSED CONCEPTUAL WATER TREATMENT SYSTEM

A conceptual biological passive treatment system was designed to fit site specific conditions. The following factors were considered during the design phase of the treatment system:

- **Available land area**: The area of land available for implementation of the water treatment system can be a limiting factor. Land availability is fortunately not a limiting factor at the study area. The decant point is situated directly upstream of a non-perennial drainage line. An area of approximately 8.1 Ha is available for construction of the proposed water treatment system.
- **Topography**: The site area selected for construction of the proposed treatment system presents with a change in surface elevation of 5 m over a 500 m distance. This calculates to a gradient of 0,01. The bed slope of the biological passive water treatment system will be mended to fit desired flow velocity within the substrate. The desired gradient of the bed slope is approximately 0,05 for each of the different substrate mixtures.
- **Volume of water to be treated per unit time:** The volume of water that decant is mostly dependant on the amount of rainfall and effective recharge. The decant volume averages at approximately 100 m^3/day. A safety factor of 10% will be applied to allow sufficient treatment capacity. The treatment system will, therefore, be designed to treat a maximum of 110 m^3/day.
- **Water quality constituents to be removed:** The primary water quality constituent to be removed is sulphate. Sporadic elevations of aluminium, manganese, and iron also require removal in order to meet discharge standards. The proposed treatment system will be designed to remove a minimum of 450 mg/L sulphate (feed concentration of 700 mg/L sulphate, to a target water quality of 250 mg/L sulphate). Successful removal of sulphate by means of an anaerobic bio-substrate is dependent on a number of factors. These factors include the following:
    - **pH of inflow water**: The pH of inflow water is near neutral (pH 7). Sulphate reducing bacteria requires a slightly acidic pH to function optimally.
    - **Substrate type:** Anaerobic bio-substrates utilise microbes that reduce or oxidise the constituents of concern. In the case of this study, the microbes will oxidise the organic carbon source in order to reduce the sulphate anions in the decant water. The microbes require easily degradable carbon sources, such as acetate, which is a breakdown product of organic matter, such as compost. The laboratory experiment conducted on the pure compost present the maximum concentration of dissolved organic carbon released. It is expected that substrates containing less compost might produce lower concentrations of

dissolved organic carbon, or require further degradation to produce the desired organic carbon concentration. This will, however, lead to a more rapid depletion of the total organic pool in substrates that contain less compost and more sand. It is expected that the compost contains natural spores of sulphate reducing bacteria that will be activated once a suitable redox state is achieved, and sufficient nutrients become available. Inoculation may be required if bacterial growth is insufficient.

- **Redox potential:** The redox potential within the substrate is a critical factor to consider when designing an anaerobic bio-substrate. The feed water should contain low dissolved oxygen concentration. The ingress of oxygen into the substrate must be prevented in order to ensure sulphate reducing bacteria functions optimally. Excess oxygen could inhibit optimal growth, or even cause microbial death. A redox potential of at least -200mV must be obtained within the anaerobic bio-substrate.. A long lag time might be required before a suitable redox potential for sulphate reducing bacteria is achieved in substrates that contain less compost.
- **Solid support:** The selected organic matter should be accompanied by an appropriate solid support, such as sand. The purpose of the sand is to prevent microbes from being flushed from the system. The microbes attach to the sand, retaining the microbes within the anaerobic bio-substrate. Different substrate mixtures presented with differentiating hydraulic characteristics. A detailed discussion on the selection of an appropriate substrate will follow.
- **HRT:** The HRT of substrates is a function of the hydraulic properties of a particular substrate, specifically the Seepage velocity (V_s) and distance of flow. The proposed anaerobic bio-substrate needs to be designed to ensure sufficient sulphate reduction occurs. The results from the kinetic model, specifically the generalised mathematical model, were used to approximate the required HRT to remove sufficient sulphate from feed water. A detailed discussion on HRT and how that applies to the design of the anaerobic bio-substrate will follow.
- **Feed sulphate concentration:** The proposed anaerobic bio-substrate is designed for a feed sulphate concentration of 700 mg/L.
- **Metal concentrations:** The removal of metals prior to sulphate reduction could enhance the success of sulphate removal from mine impacted water. The pre-treatment processes include:
    - Further increasing the pH of feed water by means of an anoxic limestone drain to promote precipitation of iron and aluminium after the

    pH was raised sufficiently. The anoxic conditions within the limestone drain will prevent immediate oxidation and precipitation of iron and associated metals, therefore, preventing armouring of the limestone. The anoxic limestone drain will be followed by cascading to oxygenate the water for iron and some aluminium precipitation.

    - The cascading will be followed by a settling pond for the settling of precipitated iron and aluminium hydroxides. The precipitation process causes the pH of the product water to become more acidic.
    - A second anoxic limestone drain and cascade are planned for the precipitation of manganese, which requires a high pH and sufficiently oxygenated water.
    - A second settling pond is planned for the settling of precipitated manganese. The pH of the water after manganese precipitation will be slightly acidic, which is optimal for sulphate reduction.
    - The removal of metals prior to sulphate reduction will prevent metal toxicity to microbes, as well as prevent excessive clogging of the anaerobic substrate.
    - An anoxic sump and drain are planned to follow the manganese settling pond. The feed water must have a low dissolved oxygen concentration for sulphate reducing bacteria to function optimally.
    - Six anaerobic bio-substrate units are planned to follow the sump. The selected substrate mixtures will be placed within a void, and capped with topsoil to prevent oxygen to come in contact with sulphate reducing bacteria. The surfaces of the anaerobic bio-substrates will not be planted with vegetation.
    - The anaerobic bio-substrate will be followed by an aerobic wetland, planted with wetland vegetation. The aerobic wetland will act as a polishing agent for excess nitrates and residual metals.
- **Temperature:** Sulphate reduction can be inhibited by extremely low or high temperatures. The study area is located in an area where low temperatures can occur during winter months. Capping of the anaerobic bio-substrate with topsoil could prevent the inhibiting effect of low temperatures on sulphate reducing bacteria.
- **COD/sulphate ratio:** Controlling the dissolved oxygen concentration in feed water is a critical system success factor for optimal sulphate reduction.

- **Operational maintenance and monitoring requirements:** The mining company is currently the lawful land owners. They propose to sell the land to an adjacent farmer, who plans to utilise the land for agricultural purposes, mainly for grazing. The selected treatment system must fit the final end land use of the site. The maintenance and monitoring capacity of the final land owner is low, hence the need to select a water treatment system that requires low operational maintenance and monitoring, as compared to that required by conventional treatment technologies.
- **CAPEX and OPEX**: The construction and operation of water treatment systems can be costly. A water treatment system must be selected with reasonably low construction and operational expenditure in relation to conventional treatment systems, such as reverse osmosis.
- **Treatment longevity**: It is expected that the backfilled open cast pit will continue to decant for an indefinite period of time, mostly depending on fluctuating groundwater levels. The quality of the decant water will depend on the sulphide sulphur content of the discard material, which is expected to deplete over time. Optimal functioning of the selected water treatment system will be instrumental in ensuring the integrity of the downstream environment.

Figure 36 illustrates a conceptual passive treatment system proposed for the study area.

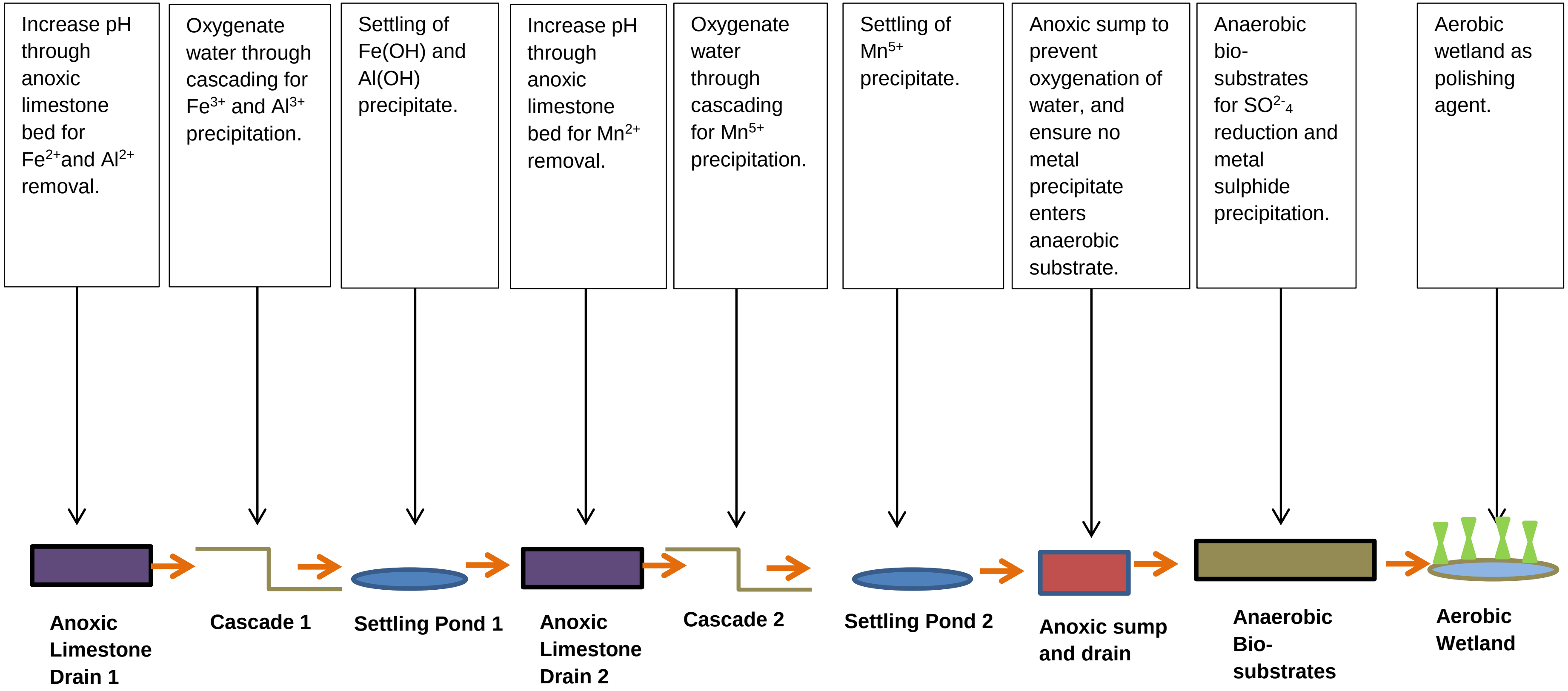


Figure 36: *Conceptual layout of a passive water treatment system for the study area.*

7.5. DETAILED DESIGN

Emphasis will be placed on the detail design of the anaerobic bio-substrate for sulphate reduction. The preceding treatment components will remain a conceptual discussion for the purposes of this study. The detail design of the anaerobic bio-substrate consists of two design components: (1) geometric design (length, width, and depth), and (2) maximum operational life of the system, which is a function of the quantity of organic carbon required to reduce sulphate to within acceptable concentration. The detail design of the biological treatment system entails the following:

Step 1, **Feed water quantity and quality that requires treatment:** The sulphate concentration in the decant water is approximately 700 mg/L. The volume of water that decants from the backfilled open cast void is approximately 100 m^3/day. The anaerobic bio-substrate component was designed to reduce sulphate concentration from 700 mg/L to ≤ 250 mg/L. The system was designed to treat an average of 100 m^3/day. A 10% safety factor was applied to allow for periods when excess decant occurs, such as during high rainfall events. The maximum capacity of the system is therefore 110 m^3/day. The anaerobic bio-substrate has been divided into six independent treatment units. Each of the treatment units has been designed to treat 18,33 m^3/day. This approach will enable better management of the system. Cumulatively, the six units will be able to treat a maximum volume of 110 m^3/day.

Step 2, Required HRT: The developed generalised mathematical model was used to calculate the required HRT to reduce feed sulphate concentration from 700 mg/L to <250 mg/L. The kinetic model indicates that a HRT of at least 3,5 days is required to reduce feed sulphate concentration to < 250 mg/L (249,59 mg/L), as required by the applicable catchment management agency. A safety factor was applied in the design of the anaerobic bio-substrate. A HRT of 4,0 days was applied to ensure sufficient contact time between feed water and sulphate reducing bacteria in the substrate, which reduced sulphate concentration to 195,20 mg/L.

Step 3, Hydraulic conductivity (K): The hydraulic conductivity (K) for each substrate mixture as calculated from the constant head permeability test for round 5 was applied. It is expected that round 5 of the constant head permeability test would be most representative of flow within each of the substrates.

Step 4, Porosity (n): The porosity (n) as experimentally calculated for each substrate mixture was applied.

Step 5, Hydraulic gradient (i): A cross-section of the topography versus groundwater level was developed for the proposed location of the anaerobic bio-substrate. The cross-section is indicative of whether the groundwater level will be breached during excavation, and aid in setting the site specific hydraulic gradient (i) (refer to Appendix J for cross-section).

Step 6, Cross-sectional area (A): The cross-sectional area (A) was subsequently used as a design parameter to align the calculated hydraulic gradient (i) with the site specific hydraulic gradient (i).

Step 7, Seepage velocity (V_s): The Seepage velocity (V_s) was calculated as a function of the experimentally obtained hydraulic conductivity (K), calculated hydraulic gradient (i), as well as experimentally obtained porosity (n) for each specific substrate mixture.

$$V_s = \frac{Ki}{n}$$

Step 8, Length: The length of the anaerobic bio-substrate was calculated as a function of Seepage velocity (V_s) and HRT.

$$\text{Length} = V_s \times \text{HRT}$$

Ste 9, Depth: The excavation depth of the anaerobic bio-substrate was set at 1,2 m. The topography versus groundwater level cross-section was used to confirm that excavation depth does not breach the groundwater level when a specific hydraulic gradient (i) is applied.

Step 10, Width: The width of the anaerobic bio-substrate was calculated as a function of cross-sectional area (A) and depth.

$$\text{Width} = \frac{A}{\text{Depth}}$$

Step 11, Total void volume: The total void volume (m^3) was calculated as a function of length, depth, and width.

$$\text{Total void volume} = \text{Length} \times \text{Depth} \times \text{Width}$$

Step 12, Calculate sulphate removed: The sulphate removal rate (mg/d) was calculated by multiplying the volumetric flow (Q) (L/d) by the quantity sulphate that is expected to be removed (difference between feed and product water sulphate concentration).

The molar mass (M) of sulphate is 96,06 g/mol (or 96062,60 mg/mol) *(Table 20*). The moles of sulphate removed per day (mol/d) were calculated by multiplying the sulphate removal rate (mg/d) by the molar mass of sulphate.

Table 20: *Upscale model inputs.*

Molar mass sulphate	96,06	g/mol
	96062,60	mg/mol
Molar mass compost	170,00	g/mol
Ratio (Compost/Acetate)	1,68	
Easily degradable compost	30 % of mass	
Compost dry density	469,90	kg/m^3

(Reaction **7** indicates that the mol ratio between dissolved organic carbon (acetate) and sulphate utilisation is 1:1. It can, therefore, be assumed that the mol sulphate removed (mol/d) from the water equal the mol dissolved organic carbon (mol/d) utilised by sulphate reducing bacteria. Israel, (2015) calculated the mol compost to mol acetate ratio to be 1.68. The mol compost required per day can, therefore, be calculated to be the mol compost to mol acetate ratio (1.68), multiplied by the dissolved organic carbon removal rate (mol/d) *(Table 20).* It is assumed that only 30 % of the compost (by mass) will be easily degradable and available to microbes for sulphate reduction (Neculita, *et al.*, 2007b). The dry density of the compost (496,90 kg/m^3) was calculate experimentally and used to calculate the volume of compost required to effectively reduce sulphate to within acceptable concentrations *(Table 20)* over the maximum operational life of the treatment system*.* The operational life of the treatment system was used as a design parameter to align the required compost to reduce sulphate to desired concentration with the volume of compost as calculated during the hydraulic design.

Step 13, Identify suitable substrate mixture. The following criteria were used to evaluate each of the substrate mixtures for suitability to be used in the anaerobic bio-substrate.

- **Operational life**: Available compost within the substrate mixture to release sufficient acetate for sulphate reduction. The easily degradable portion of compost will determine the operational life of the treatment system. An additional organic carbon source may have to be added to ensure treatment persists, once the initial pool of organic carbon is depleted.
- **Solid support**: Substrate must consist of solid support, such as sand, to prevent microbes being flushed from the substrate.
- **Confidence in experimental and model results**: The results obtained from experimental and model work would be instrumental in selecting a suitable substrate.

- **Appropriate hydraulic characteristics:** The substrate mixture must be able to manage the required water volumes at a specific HRT to adequately improve water quality by means of hosting sulphate reducing bacteria.

Figure 37 illustrates the proposed footprint area of the biological passive treatment system, together with associated treatment components.

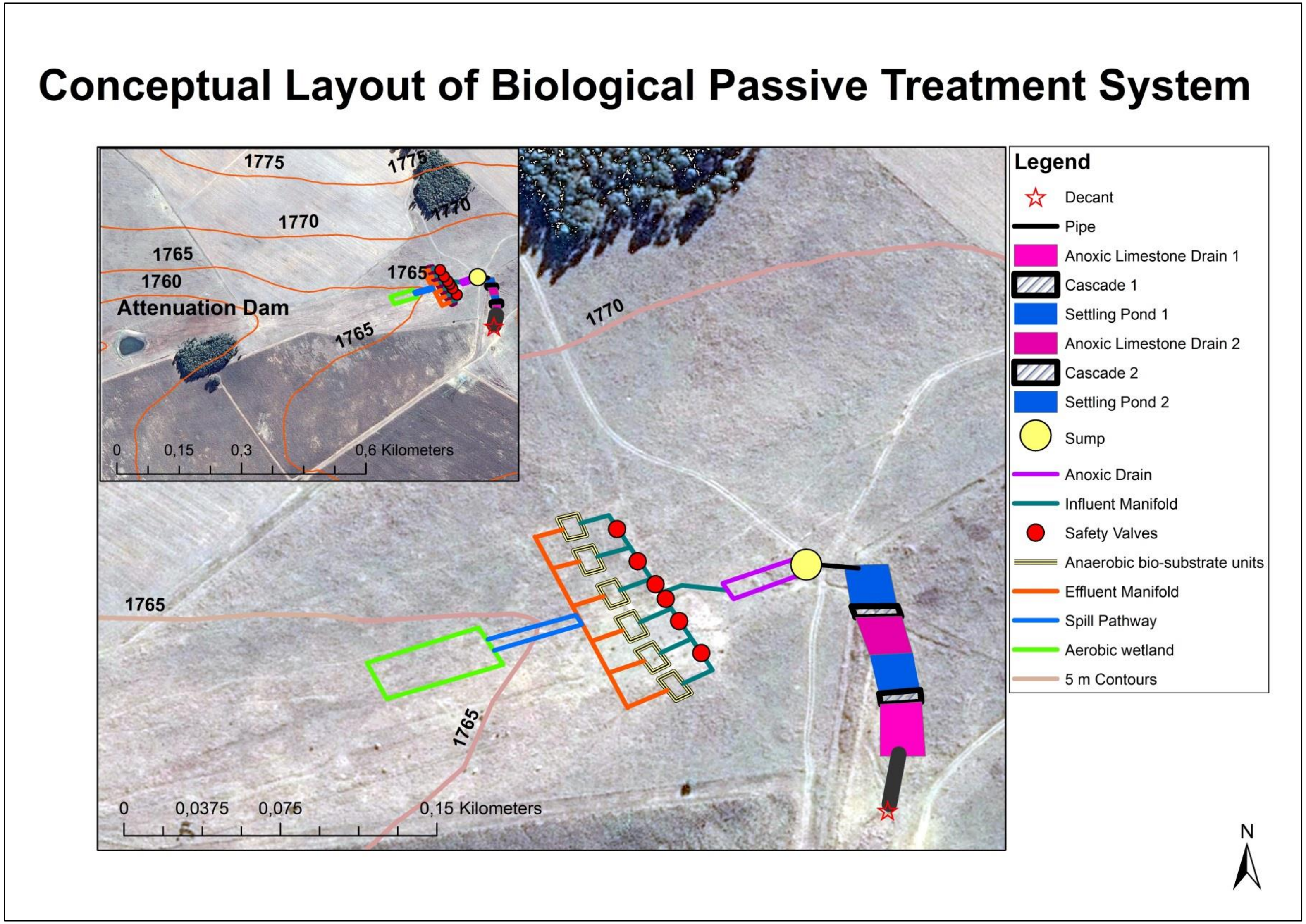


Figure 37: *Conceptual layout of biological passive treatment system.*

The 50% compost 50% sand substrate was selected as the preferred substrate to be used. The substrate contains equal amounts of compost and sand, which enable the substrate to adequately support sulphate reducing bacteria by means of supplying sufficient amounts of organic carbon, as well as solid support to prevent biomass from being flushed from the system. The 30% compost 70% sand substrate will require more frequent maintenance due to the lower compost content of the substrate. Experimental data obtained indicated some degree of error that occurred during tracer testing on the 70% compost 30% sand substrate. The use of this substrate was, therefore, excluded. The 100% sand substrate contains no compost material. This substrate will, therefore, be inadequate to host sulphate reducing bacteria. The 100% compost will have the longest operational life, but contains no solid support. This substrate can, therefore, also not be used as a substrate in the anaerobic bio-substrate. The 50% compost 50% sand substrate is, therefore, the most appropriate substrate to use to in the design of the anaerobic bio-substrate.

Table 21 presents the outputs from the detail design of the anaerobic bio-substrate with the 50% compost 50% sand substrate. It must be noted that the design presented in *Table 21* is for only one anaerobic bio-substrate unit. Six of these units will be required to meet decant volumes that require treatment.

Table 21: *Selected substrate mixture – 50%C 50%S.*

Design Parameters	**Value**	**Unit**	**Source Term**	**Error value**	**Error %**
Hydraulic design					
Volumetric flow (Q) rate	18,33	m^3/day	Decant volume, plus 10% safety factor	-	-
	18333,33	L/day		-	-
Hydraulic Conductivity (K)	29,864	m/d	Experimentally calculated during CHPT	0,2720	0,9109
Porosity (n)	0,5809		Experimentally calculated	0,0069	1,195
HRT	4,0	Days	General Mathematical Model	-	-
Hydraulic gradient (i)	0,051		Calculated (site specific)	-	-
Seepage velocity	2,61	m/d	Calculated	0,055	2,106
Length	10,43	m	Calculated	0.220	2,106
Depth	1,20	m	Design parameter	-	-
Cross-sectional area (A)	12,10	m^2	Design parameter	-	-
Width	10,08	m	Calculated	-	-
Void Volume	126,24	m^3	Calculated	2,658	2,106

Design Parameters	**Value**	**Unit**	**Source Term**	**Error value**	**Error %**
Volume compost required (50% of substrate volume)	63,12	m^3	Calculated	1,329	2,106
Volume sand required (50% of substrate volume)	63,12	m^3	Calculated	1,329	2,106
Dry density compost	469,90	Kg/m^3	Experimentally calculated	10,00	2,128
Mass compost required	29660,30	Kg	Calculated	1255,769	4,234
Physicochemical design factors					
	Value	**Unit**	**Source Term**	**Error value**	**Error %**
Molar mass sulphate	96,06	g/mol	Literature	-	-
	96062,60	mg/mol		-	-
Molar mass compost	170,00	g/mol	Literature	-	-
Ratio (compost/acetate)	1,68		Literature	-	-
Useable compost by mass	0,30		Literature	-	-
Dry density of compost	469,90	Kg/m^3	Experimentally calculated	10,00	2,128
Bio-physical design *(calculate compost required to reduce sulphate from 700 mg/L to < 250 mg/L)*					
	Value	**Unit**	**Source Term**	**Error value**	**Error %**
Influent sulphate concentration	700,00	mg/L	Decant water quality	-	-
Target sulphate concentration	250,00	mg/L	DWS (IUCMA) requirement	-	-
Effluent (product) sulphate concentration	195,20	mg/L	General Mathematical Model with 4 day HRT	-	-
Sulphate removed	504,80	mg/L		-	-
	9254666,67	mg/day		-	-
Moles sulphate removal rate	96,34	mol/day	Calculated	-	-
Dissolved organic carbon removal rate	96,34	mol/day	Calculated	-	-
Moles compost required that is available to microbes	161,75	mol/day	Calculated	-	-
Total microbial available compost required over operational life	52246,80	moles	Calculated	-	-
Mass available compost required	8881955,32	gram	Calculated	-	-
Useable and unusable mass compost (simple and complex organics)	29606517,72	gram	Calculated	-	-
Useable and unusable mass compost (simple and complex organics)	29606,52	Kg	Calculated	-	-

Design Parameters	Value	Unit	Source Term	Error value	Error %
Total volume compost required	63,01	m^3	Calculated	-	-
Maximum operation time	323.00	days	Design parameter	-	-

Refer to Appendix K for detail design on other substrate mixtures.

Each anaerobic bio-substrate unit will consist of the following components:

Liner: The entire anaerobic bio-substrate will have to be lined in order to prevent seepage to groundwater. The type of liner will depend on the quality of influent water.

Inlet pipe: The inlet pipe will collect water from the influent manifold, which will transport water to the inlet manifold located at the head of the anaerobic bio-substrate.

Inlet manifold: The inlet manifold will distribute water equally across the anaerobic bio-substrate via the inlet gravelpack.

Inlet gravelpack: The inlet gravelpack will reduce direct impact of water flowing from the manifold to the substrate. The gravelpack will also promote the distribution of water across the entire cross-sectional area of the substrate. Another function of the gravelpack is that it will prevent clogging of the manifold by the organic matter if backwashing occurs.

Anaerobic bio-substrate: This component acts as the primary treatment component, specifically to host sulphate reducing bacteria and manage HRT.

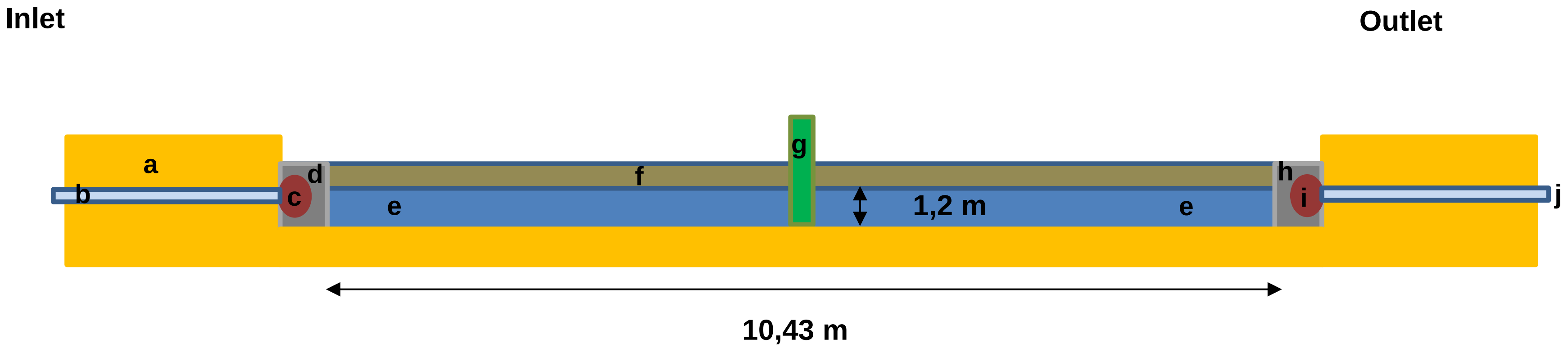
Topsoil cap: Conventional wetlands were planted with vegetation on the surface to extract metals from the waste water. Literature indicates that the roots of vegetation could cause preferential flow paths to develop, which enables oxygen to penetrate the anaerobic zone. This reduces optimal functionality of sulphate reducing bacteria. The topsoil cap will prevent very low temperatures to impact on microbial functionality, as well as protect the anaerobic bio-substrate from atmospheric impacts caused by high rainfall events, hail, wind, and also against agricultural impacts, such as grazing.

Piezometers: *In-situ* monitoring of processes within the anaerobic bio-substrates will have to be conducted in order to track changes in redox potential, dissolved organic carbon concentration, COD/sulphate ratios, and obtain an understanding of chemical and microbial processes involved. The piezometers could also act as dozing stations for the addition of organic carbon, once the maximum operational life of the substrate is reached. One piezometer will be installed within each of the anaerobic bio-substrate units.

Outlet gravelpack: The outlet gravelpack fulfils a similar function as the inlet gravelpack. The outlet gravelpack will reduce the potential for clogging of the effluent manifold.

Outlet pipe: The outlet pipe will transport water to the effluent manifold, which flows into the spill pathway and to the aerobic wetland.

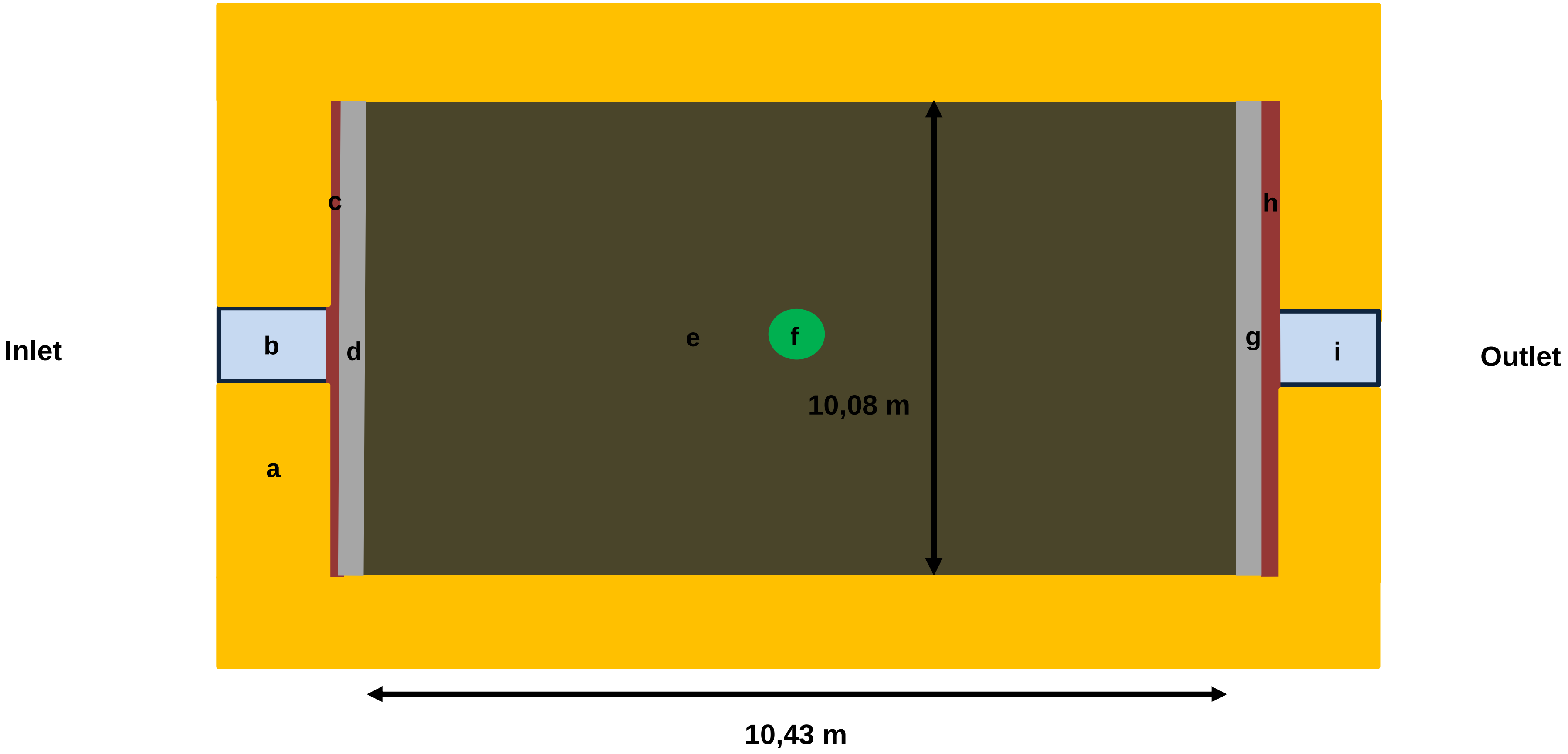
Figure 38 and *Figure 39* provide an illustration of a cross-section along the length of the anaerobic bio-substrate and top view of an anaerobic bio-substrate unit.



a: *Liner* ***b:*** *Inlet pipe* ***c:*** *Inlet manifold* ***d:*** *Inlet gravepack* ***e:*** *Anaerobic bio-substrate* ***f:*** *Topsoil cap* ***g:*** *Piezometer* ***h:*** *Outlet gravelpack*

i: *Outlet manifold* ***j:*** *Outlet pipe*

Figure 38: *Longitudinal cross-section of anaerobic bio-substrates.*



a: *Liner* ***b:*** *Inlet pipe* ***c:*** *Inlet manifold* ***d:*** *Inlet gravelpack* ***e:*** *Topsoil cap* ***f:*** *Piezometer*
g: *Outlet gravelpack* ***h:*** *Outlet manifold* ***i:*** *Outlet pipe*

Figure 39: *Top view of anaerobic bio-substrate.*

7.6. ENVIRONMENTAL AUTHORISATION REQUIREMENTS

The construction of the anaerobic bio-substrate will trigger activities as mentioned in the National Environmental Management: Waste Act, Act 59 of 2008 that relate to liner requirements for waste water management facilities. Specific reference is made to regulations No. 636 and 636 that specify the assessment methodology for the management of facilities containing waste water, as well the liner requirements for such a facility, respectively.

The anaerobic bio-substrate will be constructed within a non-perennial drainage line. A stream diversion will, therefore, be required to separate clean and dirty water run-off, and to reinstate natural hydrological function. The hydrological characteristics, as well as the riparian zone of the non-perennial stream will be altered. The construction of the anaerobic bio-substrate will, therefore, require a water use licence, specifically relating to section 21 of the National Water Act, Act 36 of 1998:

- 21 (c): “Impeding or diverting the flow of a watercourse.”
- 21 (f): “Discharging waste or water containing waste into a water resource through a pipe, canal, sewer, or any other conduit.”
- 21 (i): “Altering the bed, bank, course, or characteristics of a watercourse.”

Regulations of the National Environmental Air Quality Act, Act 39 of 2004 should also be considered for possible methane gas release from the anaerobic bio-substrate.

Authorisation will, therefore, be required from both the Department of Water and Sanitation (DWS) and Department of Environmental Affairs (DEA).

7.7. SAFETY, MAINTENANCE AND MONITORING REQUIREMENTS

The longevity and success of the biological passive treatment system will require careful periodic maintenance and monitoring. The following safety, maintenance and monitoring frameworks should be considered:

7.7.1. Safety Requirements

- The entire treatment system must be fenced-off to prevent cattle grazing and trampling from impacting on functionality of treatment components. This specifically relates to the anaerobic bio-substrates and aerobic wetland.
- The sump must be sealed to prevent humans, animals, or any other objects from falling into the sump. The lit of the sump must, however, be able to open. The sump will act as one of the monitoring locations.

- All other safety requirements pertaining to waste water treatment facilities will be applicable to this system as well.

7.7.2. Maintenance Requirements

Regular maintenance work must be conducted on the functionality of each treatment component. The treatment components must be inspected for the following:

- Ensure armouring of anoxic limestone drains are prevented by means of preventing oxygen ingress. The limestone will have to be replaced after a period, either due to armouring of limestone, or limestone disassociation over time.
- Ensure cascades are not clogged by metal precipitates.
- The metal precipitates within each of the settling ponds will have to be removed to ensure sufficient capacity remains within each of the ponds. The removed metal precipitates will have to be disposed of at a designated hazardous landfill site, such as Holfontein.
- All pipes will have to be inspected internally to ensure clogging is prevented.
- The sump is the primary flow-through from the pre-treatment components to the anaerobic bio-substrates. The sump will have to be inspected to ensure clogging is prevented.
- The anaerobic bio-substrates will have to be replaced, either when the organic carbon is depleted, or when the substrate is clogged by sulphide metal precipitates. The benefit of the multi-component anaerobic bio-substrates is that treatment can still continue in the other five units, while maintenance work is conducted on one unit.
- The topsoil cap of the anaerobic bio-substrate will have to be cleared of vegetation to prevent the roots of vegetation from penetrating the substrate. An alien and invasive management programme will also have to be developed.
- Peak flows could cause portions of the substrate to be flushed out of the system. These portions will need to be replaced.

7.7.3. Monitoring Requirements

The functioning of this treatment system will require intense monitoring to ensure sufficiency is maintained. Monitoring of the following will be required:

- Continuous monitoring devices will be ideal for monitoring inflow volumes. Other methods such as flow meters on pipes can be installed to provide an indication of flow as well. Influent flow volumes must specifically be monitored during high rainfall events, as well as during extreme dry periods. The treatment success and longevity of the system greatly depends on constant flow. The monitoring of outflow volumes should also be conducted to track whether any water losses occur throughout the system. The expected water losses will be by means of evaporation, evapotranspiration (only aerobic unit), leakages, or seepage to groundwater.
- Monitoring of water qualities throughout the system will be instrumental in proving treatment success. Water quality monitoring is recommended at the following locations:
    - In pit groundwater;
    - Decant location;
    - Within each settling pond;
    - Sump;
    - Piezometers situated within anaerobic bio-substrates;
    - After anaerobic bio-substrates, prior to aerobic wetland;
    - Final effluent from aerobic wetland.
- The following suite of water quality constituents should be analysed for:
    - pH;
    - EC or TDS;
    - Metals and trace metals (aluminium, manganese, iron, zinc, lead, chromium, cobalt, copper, selenium, antimony, boron, barium, nickel, cadmium);
    - Anions (nitrate, nitrite, sulphate, ortho-phosphate);
    - Odour testing for methane gas from anaerobic bio-substrate;
    - Alkalinity (calcium and magnesium carbonates);
    - Chemical and biological oxygen demand;
    - Dissolved organic carbon (only in anaerobic bio-substrates);
    - Redox potential (only *in-situ* measurement);
    - Temperature (only *in-situ* measurement within anaerobic bio-substrates);
    - Bacterial plate count within anaerobic bio-substrates and aerobic wetland.

- Monitoring should also be conducted within the existing dam downstream of the treatment system. This dam can act as a final water quality monitoring point before discharged into the natural catchment.

The frequency of monitoring will be stipulated in the water use licence as dictated by the Department of Water and Sanitation, and Department Environmental Affairs.

CHAPTER 8: CONCLUSION AND FURTHER RESEARCH

8.1. CONCLUSION

Coal mining contributed greatly to economic development in South Africa. Coal mining, however, is associated with numerous environmental challenges, specifically water related impacts. The coal mining process mostly entails the disturbance of surface and groundwater systems. Open cast mining contributes significantly to the physical and chemical alteration of both surface and groundwater systems. Many coal mines in South Africa already reached their life of mine. These mined out sites were decommissioned and most abandoned without further investigation into residual or latent impacts that might arise. Water related impacts are part of the suite of environmental challenges that remain post-mining. The coal seems are mostly surrounded by host rock that contains sulphide bearing minerals, such as pyrite. The activities associated with open cast mining can promote or activate the oxidation of sulphide bearing minerals, which in turn generate acid. Minerals such as pyrite get oxidised when in contact with sufficient water and oxygen. The products from the oxidation process are acid that could cause metals to become liberated and readily available to the surrounding environment, and excess sulphate. Most open cast pits are backfilled once mined out. These backfilled pits have the tendency to decant once the groundwater level intercepts the topography.

Extensive research has been conducted on the use of active and passive treatment systems to improve water quality of mine impacted water. More coal mines will reach their life of mine within the next 10 years. Water related impacts will, however, persist long after decommissioning. The management of contaminated water during the operational phase is not a significant challenge for mines, mostly due to the success of active treatment systems. Concerns are mostly related to the management of this water during the post-decommissioning phases. Decommissioned sites are mostly characterised by a lack of service-infrastructure, and very little to no monitoring and maintenance capacity. Water treatment systems that can function with as little as possible intervention would be an ideal solution. The successful design, implementation, and operation of such a system are, however, still a pending matter. Most biological passive water treatment systems were developed for the removal of metals from mine impacted water. Less attention has been given to the use of biological passive treatment systems to remove sulphate from mine impacted water.

The use of biological passive treatment systems to remove sulphate from mine impacted water was, therefore, further investigated. This project was inspired by a decommissioned mine site where decant water is flowing into the natural catchment, with sulphate concentration exceeding the regulated standard.

A detailed literature study was conducted on the use of different biological treatment systems to reduce sulphate concentration in mine impacted water. Constructed wetlands, bioreactors, and permeable reactive barriers were identified as being the primary treatment methodologies. Literature also indicates that certain design factors must be taken into account in order to successfully design, implement, and operate a biological passive treatment system. The literature study was indicative that gaps do exist, specifically relating to better understanding the hydraulic (flow) properties within different substrate mixtures, as well as the capacity of sulphate reducing bacteria within an organic carbon substrate to successfully reduce sulphate concentration to within acceptable standards.

Anaerobic bio-substrates can possibly be used to host sulphate reducing bacteria to aid in water treatment. Sulphate reducing bacteria utilises simple organic carbon as a source of nutrients. Compost and sand substrate mixtures were selected for further investigation, specifically relating to better understanding the hydraulic properties of each substrate mixture under constant head flow.

Compost that consists of *Eucalyptus* bark, *Pinus* bark*,* woodchips*,* and horse manure, was chemically analysed to obtain a baseline understanding of the chemical signature of the compost. Various methods were used to analyse and determine the concentration and content of each constituent. The results obtained indicate that the selected compost is suitable to use within an anaerobic bio-substrate. The C/N ratio indicates that sufficient carbon and nitrogen are available to supply microbes with electrons for biological sulphate reduction. Other compost quality constituents such as EC, pH and ion concentrations are also within acceptable concentrations to provide a suitable habitat to host sulphate reducing bacteria and not impact detrimentally on effluent water quality.

The objective of the experiment was to measure change in dissolved organic carbon (DOC) concentration over time. This experiment was conducted for a period of 44 days.. A DOC concentration of 868 mg/L was measured in the compost substrate, while the sand substrate presented with a DOC concentration of 29,10 mg/L. DOC was readily available from the first measuring round. The DOC results achieved from the compost was used as input to the PHREEQC model to simulate sulphate reduction and acetate utilisation. It is, however, expected that the other substrate mixtures that contain less compost would require longer standing time before a similar redox potential is reached. This will also be the case for the DOC concentration. Substrates with lower compost volumes might contain lower concentrations of DOC, or require more time to oxidise and produce similar DOC.

The selected compost was mixed with washed river sand. Five compost sand mixtures were developed for further testing. The compost sand mixtures include: 100% compost, 70%

compost 30% sand, 50% compost 50% sand, 30% compost 70% sand, and 100% sand. The substrate mixtures were placed within cylindrical columns for constant head permeability testing. The outcomes from this experiment include the following:

- Volumetric flow rate (Q) was measured. Five consecutive measurements were conducted for each substrate in order to allow the material within the columns to settle, and eliminate potential measuring errors caused by differential movement of substrate material.
- Hydraulic gradient (i) was measured.
- Hydraulic conductivity (K) was calculated as a function of cross-sectional area (A), hydraulic gradient (i), and volumetric flow rate (Q).

Standard deviation was calculated to evaluate whether an average of the five measurements would be representative of flow within each of the substrate mixtures. The standard deviations were indicative that significant variance occurs between the obtained results for each of the substrates, which entail that an average of the five measuring rounds would not be a good representative of flow within the substrate. Correlation was calculated between change in percentage sand versus change in volumetric flow rate, hydraulic gradient, and hydraulic conductivity.

A weak negative correlation was observed between change in percentage sand versus change in volumetric flow rate. A decrease in volumetric flow rate is observed with increasing sand percentage.

A moderately strong positive correlation was observed between hydraulic gradient (i) versus increase in sand percentage. The hydraulic gradient (i) increased with increasing sand percentage

A moderately strong negative correlation was observed between change in percentage sand versus change in hydraulic conductivity (K). The hydraulic conductivity (K) tends to decrease as the percentage sand increases.

The hydraulic conductivity (K) calculated from the constant head permeability test would act as input data to the upscale design model. An average value of the five measuring rounds would not be represented of flow, therefore the use of a single measuring round. Measurement round 5 presented with the best correlation between change in percentage sand versus change in hydraulic conductivity. Round 5 of the constant head permeability test was, therefore, selected as being the most representative of flow within each of the substrates, and to be used in the upscale design model.

A kinetic model was developed in PHREEQC model code to conduct predictive modelling on sulphate reduction. Sulphate reduction kinetic data was obtained from literature. Two model runs were conducted: (1) sulphate and acetate utilisation with varying feed sulphate concentrations, but similar input acetate concentrations, and (2) sulphate and acetate utilisation with constant sulphate feed concentration, but varying available acetate concentrations. A generalised mathematical model was developed to calculate the sulphate concentration in the product water (post-treatment). The generalised mathematical model is a function of feed sulphate concentration, HRT, and available acetate concentration. The results obtained from the first model run indicated that a minimum HRT of 3,5 days will be required to reduce feed sulphate concentration from 700 mg/L to 249,59 mg/L. A maximum sulphate concentration of 250 mg/L is allowed within the IUCMA. The results also indicated that sulphate reduction will cease once acetate is depleted. The second model indicated that sulphate reduction rate is greatly dependant on the availability of readily available organic carbon, in this case, acetate. The rate of sulphate reduction decreases as acetate concentration decreases. The results were indicative that sulphate reduction is greatly dependant on the availability of organic carbon (acetate).

Dry density was calculated for each of the substrate mixtures. The dry density of the substrate mixtures varied greatly from 1464,28 kg/m^3 (100% sand substrate) to 469,90 kg/m^3 (100% compost substrate). The dry density of the compost was particularity important as input to the upscale design model. The relative density of each substrate mixture was also calculated by means of a gas Pycnometer. The porosity of each substrate mixture was then calculated as a function of dry density and relative density. The 100% compost has a porosity of 70,68%, while the 100% sand has a porosity of 43,77%.

Tracer tests were conducted to calculate HRT, Seepage velocity, and estimate porosity. A pulse injection tracer test was conducted by means of using a KCl solution as tracer. The change in tracer output concentration was measured over time. Radial convergent test (RCT) was conducted to validate the results obtained from the tracer test. The obtained estimated porosity values and HRTs from the tracer and RCT differ significantly for the 100% sand, and 70% compost and 30% substrate as compared to the constant head permeability test, even though hydraulic properties during the tracer tests were similar than that of the constant head permeability test. Possible reasons for this could have been differential movement of substrate material during testing, clogging of outlet pipe, or the development of preferential flow paths.

A biological passive treatment system was developed for a decommissioned coal mine site. The site is characterised by decant water with elevated sulphate concentrations impacting on downstream water courses. A study conducted indicated that the decant water will impact detrimentally on receiving water courses if no remediation is attempted. A proposed passive treatment system was developed with pre-treatment components to remove most of the metals, such as aluminium, iron, and manganese from the mine impacted water prior to sulphate reduction in the anaerobic bio-substrates. Anoxic limestone drains were proposed to increase the pH for metal oxidation. Cascades were proposed to promote the oxygenation of water. Settling ponds were then proposed following the cascades for settling of metal precipitate.

A detail design was conducted for the anaerobic bio-substrate treatment component. The total volume of water to decant is 100 m^3/day. A 10% safety factor has been allowed for to ensure sufficient treatment capacity exists during times of higher decant volume. The anaerobic bio-substrate has, therefore, been designed to treat a maximum of 110 m^3/day. The anaerobic bio-substrate consists of six independent units. Each will be able to treat 18,33 m^3/day.. The generalised mathematical model was used to calculate the sulphate concentration in the product water post- treatment. Although a HRT of only 3,5 days are required, a safety factor has also been applied. A HRT of 4,0 days was applied, after which the sulphate concentration was reduced from 700 mg/L to 195,20 mg/L. The 50% compost 50% sand substrate was selected as the preferred substrate to host sulphate reducing bacteria within the anaerobic bio-substrate. This substrate contains sufficient compost to act as electron donors to sulphate reducing bacteria, as well as sufficient sand for solid support. Hydraulic conductivity (K) and porosity (n) for this specific substrate was applied. The cross-sectional area (A) was used as a design parameter, specifically to design according to the site specific hydraulic gradient (i). The Seepage velocity (V_s), geometric configuration, void volume, and quantity compost and sand required were calculated. The amount of compost that would be available to microbes was calculated. The maximum operational life of the 50% compost 50% sand substrate was calculated to be 323 days, after which the initial pool of organic carbon will then be depleted. An additional organic carbon source will then have to be supplied to ensure continuous treatment.

The following conclusions can be derived from the work conducted:

- The literature study conducted provided great insight into the bio-chemical processes and hydraulic behaviour within different material types under constant head flow conditions. The literature study laid the foundation for further work to be conducted.
- The results obtained for each of the hydraulic parameters from the constant head permeability test are indicative that flow within compost sand mixtures can vary greatly, even within a single substrate.
- A moderately strong correlation was obtained between change in percentage sand within the substrate versus change in hydraulic conductivity (K).
- The predictive modelling indicated that readily available organic carbon is instrumental for successful microbiological sulphate reduction.

- It became evident that the dissolved organic carbon was readily available to sulphate reducing bacteria.
- A generalised mathematical model was developed to determine HRT for feed sulphate to be reduced to within acceptable concentration.
- The 50% compost 50% sand substrate was selected as the preferred substrate.
- Continuous monitoring and maintenance will be required to ensure sufficient and efficient treatment. This specifically refers to ensuring both the hydraulic integrity of the system, as well as the pool of sulphate reducing bacteria are maintained.

The modelling results are indicative that a biological passive treatment system that utilises an organic carbon source and sulphate reducing bacteria can be effective in improving water quality by means of reducing feed sulphate concentration from 700 mg/L to < 250 mg/L. The modelling results will, however, need to be validated by means of experiments that measure the acetate concentration and sulphate reduction.f

8.2. FURTHER RESEARCH

This study focused on the fundamental hydraulic and micro-biological processes involved in biological sulphate reduction. Further research would include:

8.2.1. Biochemical processes

- Experimental testing on sulphate reduction rates within the different sand compost mixtures.
- Experimental testing on the effect influent sulphate concentration has on sulphate reducing bacteria.
- Experimental testing on the effect of sulphide on nutrient uptake of sulphate reducing bacteria.
- Impact of different metal types and concentrations on sulphate reducing bacteria.
- Experimental testing on the rate of organic carbon oxidation within each of the different sand compost mixtures.
- Changes in oxidation-reduction gradients over time within each of the different sand compost mixtures.
- Evaluating different compost types to act as a carbon source.
- Evaluating different organic material types to act as a carbon source.
- Evaluating the occurrence of different types of microbes during different stages of treatment.

8.2.2. Hydrological processes

- Flow rates at which biomass gets washed out from the substrate.
- Conduct modelling on the hydrological flow patterns and mixing properties within each of the different sand compost substrate mixtures.
- Conduct further test work on the flow properties within different organic materials (sawdust, grass, woodchips, pulp, and plant based compost vs manure based compost).

The upscaling of laboratory experiments to pilot or bench scale would aid in obtaining more realistic data that can be used for design and further modelling work. The development of research projects from laboratory concept to full scale implementation would add significantly to the knowledge base on the feasibility of biological passive treatment systems in post-mining environments. The validation of all modelling work is crucial to the credibility of this research project.

REFERENCES

AKCIL, A. AND KOLDAS, S. 2006. Acid Mine Drainage (AMD): Causes, Treatment and Case Studies. Elsevier, Sciencedirect, Journal of Cleaner Production, 14(2006), 1139-1145.

ALESIA, F.D. 2014. Optimisation of elemental sulphur recovery during acid mine water treatment. Faculty of Engineering Built Environment and Information Technology, University of Pretoria.

AL-ZUHAIR, S., EL-NAAS, M.H. AND AL-HASSANI, H. 2008. Sulfate inhibition effect on sulfate reducing bacteria. J. Biochem. Tech, (2008), 1(2): 39-44.

APHANE, V. AND VERMEULEN, P.D. 2015. . Acid mine drainage and its potential impacts on the water resource in the Waterberg Coalfield. Institute of Groundwater Studies, University of the Free State, Bloemfontein, South Africa.

APPELO, C.A.J. AND POSTMA, D. 2013. Geochemistry, Groundwater and Pollution. 2nd ed. Leiden: A.A. Balkema Publishers. 439 p.

ARDEJANI, F.D., SINGH, R., BAAFI, E., SHAFAEI, S.Z., AND ARYAFAR, A. 2007. Prediction of post-mining groundwater recovery pattern in an unconfined aquifer to predict opencast mine backfill settlement. IMWA Symposium 2007: Water in Mining Environments, R.Cidu & F. Frau (Eds).

ASTM INTERNATIONAL.1998. Standard test method for saturated hydraulic conductivity, water retention, porosity, particle density, and bulk density of putting green and sports turf root zone. Designation: F-1815-97.

AUMANN, C.A. AND FORD, E.D. 2002. Modelling tree water flow as an unsaturated flow through a porous medium. Journal of Theoretical Biology, 219 (4): 415-429.

BALCIOGLU, E.B. AND DEMIRKOL, G.T. 2013. The effects of sulphate, nitrate, and humic substances on Mn(II) oxidation with atmospheric oxygen in drinking water. Pol. J. Environ. Stud. Vol 22, No. 6 , 1601-1607.

BATTY, L.C. AND YOUNGER P.L. 2003. Effects of external ion concentration upon seedling growth and uptake of Fe and phosphate by the common reed, *Phragmites australis* (Cav.) Trin ex. Steudel. Annals of Botany, 92(2003): 801-806.

BENNER, S.G., BLOWES, W.D., GOULD, R.B., HERBERT, JR., AND PTACEK, C.J., 1999. Geochemistry of a permeable reactive barrier for metals and acid mine drainage. Environmental Science and Technology. 33:2793-2799.

BENNER, S.G., BLOWES, D.W., PTACEK, C.J. AND MAYER, K.U. 2002. Rates of sulfate reduction and metal sulphide precipitation in a permeable reactive barrier. Elsevier, Applied Geochemistry, 17 (2002), 301-320.

BERTSCH, P.M., MILLER, W.P., ANDERSON, M.A. AND ZELAZNY, L.W. 1989. Co-precipitation of iron and aluminium during titration of mixed Al^{3+}, Fe^{3+}, and Fe^{2+} solutions. Clays and Clay Minerals, Vol. 39 No. 1, 12-18.

BLESS, D. JOYCE, H., AND PARK, B. 2006. MWTP demonstrates integrated passive biological system for treating acid rock drainage. Technology News and Trends.

BOLIS, J. L., WILDEMAN, T.R. AND DAWSON, H.E. 1992. Hydraulic Conductivity of Substrates used for Passive Acid Mine Drainage Treatment. Proceedings America Society of Mining and Reclamation, pp 79-89, DOI: 10.21000/JASMR92010079.

BOSMAN, C. 2009. The hidden dragon: nitrate pollution from open-pit mines-a case study from the Limpopo Province, South Africa. International Mine Water Conference. Hyperlink:{http://www.miningweekly.com/article/environmental-impact-weighs-heavy-on-opencast-mining-operations-2012-05-11/rep_id:3650}. Accessed 1 April 2017.

BRAHMACHARIMAYUM, B. AND KUMAR, G.P. 2012. A study of efficiency of five different carbon sources on sulphate reduction. Journal of Environmental Research and Development, Vol. 7, No. 1.

BROWNING, B.L. 1963. The chemistry of wood, John Wiley and Sons Ltd., Glen House, London.

BRUCKNER, M.Z. 2016. Using hydrological tracers to characterise a watershed. Microbial Life, Educational Resource. Hyperlink: http://serc.carleton.edu/microbelife/research_methods/environ_sampling/hydrotrace.html. Accessed: 5 April 2017.

BUDEIT, D. 2007. Role of accelerated oxidation for removal of metals from mine drainage. National Meeting of the American Society of Mining and Reclamation.

BURKE, S.P. AND BANWART, S.A. 2002. A geochemical model for removal of iron$(II)_{(aq)}$ from mine water discharges. Elsevier, Applied Geochemistry 17 (2002): 431-443.

CABRERA, G.[A], PEREZ, R.[B], GOMEZ, J.M.[A], ABALOS, A.[B] & CANTERO, D.[A] 2006. Toxic effects of dissolved heavy metals on *Desulfovibrio vulgaris* and *Desulfovibrio* sp. Strains. Department of Chemical Engineering, Food Technology and Environmental Technologies, Faculty of Sciences, University of Cadiz (UCA), 11510 Puerto Real, Cadiz, Spain. [b] Biotechnology Centre for Industrial Studies, Faculty of Natural Sciences, University of Oriente (UO), 10500 Santiago de Cuba, Cuba. 0304-3894/$ Elsevier.

CELIS-CARCIA, L.B., RAZO-FLORES, E. AND MONROY, O. 2006. Performance of a down-flow fluidized-bed reactor under sulphate reducing conditions using volatile fatty acids as electron donors. Biotechnology and Bioengineering, Vol 97, No. 4:771-779.

CENTRE FOR WATER SCIENCES AND MANAGEMENT. 2016. Tracer tests. North West University.

CHEN, Y., WEN, Y. ZHOU, J., TANG, Z. LI, L., ZHOU, Q., and VYMAZAL, J. 2014 Effects of cattail biomass on sulphate removal and carbon sources competition in subsurface-flow constructed wetlands treating secondary effluent. doi: 10.1016/j.watres.2014.03.077

CHIEN L *ET AL*. 1968. Infantile gastroenteritis due to water with high sulphate content. Canadian Medical Association Journal, 99:102–104.

COCCHETTO DM, LEVY G. 1981. Absorption of orally administered sodium sulfate in humans. *Journal of Pharmaceutical Sciences*, 70:331–333.

COCOS, I.A., ZAGURY, G.J., CLEMENT, B. AND SAMSON, R. 2002. Multiple factor design for reactive mixture selection for use in reactive walls in mine drainage treatment. Elsevier, Water Research, 32(2002) 167-177.

COLORADO EDUCATION. 2014. Cascade simulation of non-ideal reactors. Hyperlink:ceae.colorado.edu/.../Nonideal%20reactor%20analysis%20CSTR%20cascadeAccessed: 5 April 2017.

CORNELL WASTE MANAGEMENT INSTITUTE. 1996. Cornell composting, science & engineering. Hyperlink: http://compost.css.cornell.edu/chemistry.html. Date Accessed: 28 November 2016.

COSTELLO, C. 2003. Acid mine drainage innovative treatment technologies: national network of environmental management studies fellow.

COURCELLES, B. 2014. Guidelines for preliminary design of funnel-and-gate Reactive Barriers. Proceedings of the 4th International Conference on Environmental Pollution and Remediation, Prague, Paper No. 81.

DAVIS, M.E. AND DAVIS, R.J. 2003. Fundamentals of chemical reaction engineering. McGraw-Hill Companies, New York. pp. 263 and 275.

DEPARTMENT OF WATER AFFAIRS AND FORESTRY (DWA). 1996. South African water quality guideline (second edition). Volume 5: Agricultural Use: Livestock Watering.

DEPARTMENT OF WATER AFFAIRS AND FORESTRY (DWA). 1996. South African water quality guidelines. Volume 7: Aquatic Ecosystem.

DEPARTMENT WATER AND SANITATION (DWS). 2017. Resource quality information.

DEV, S. AND BHATTACHARYA, J. 2014. Sulfate reduction and growth kinetics of sulfate reducing bacteria while using marine waste extract as nitrogen source. An Interdisciplinary Response to Mine Water Challenges-Sui Sun & Wang (eds), China University of Mining and Technology.

DOSHI, S.M. 2006. Bioremediation of acid mine drainage using sulfate-reducing bacteria. national network of environmental management studies, university of indiana. pp. 1-72.

DRURY, W.J. 2006. Modelling of sulphate reduction in anaerobic solid substrate bioreactors for mine drainage treatment. Mine Water and the Environment, International Mine Water Association.

EARY, L.E. and Williamson, M.A. 2006. Simulations of the neutralising capacity of silicate rock in acid mine drainage environments. 7th International Conference on Acid Rock Drainage.

EDENBORN, H.M. 2004. Use of poly (lactic acid) amendments to promote the bacterial fixation of metals in zinc smelter tailings. Elsevier, Bioresource Technology, 92(2004): 111-119.

EGER, P. AND WAGNER, J. 2003. Wetland treatment systems-How long will they really last?

ELIOT, T. 2014. Environmental tracers. Water, 6(2014): 3264-3269.

ELSILK, S.E., EL-RAHEEM, ABD., EL-SHANSHOURY, R. AND ATEYA, P.S. 2014. Accumulation of some Heavy Metals by Metal Resistant Virulent *Bacillus anthracis* PS2010 isolated from Egypt. African Journal of Microbiology Research, 8(12): 1266-1276.

ENSLIN, F., VAN DER MEY, L. AND WAANDERS, F. 2010. Acid leaching of heavy metals from bentonite clay, used in the cleaning of acid mine drainage. The Journal of the Southern African Institute of Mining and Metallurgy, vol 110: 187-191.

ESKOM HOLDINGS SOC LTD. 2016. Coal Power. Hyperlink: http://www.eskom.co.za/AboutElectricity/ElectricityTechnologies/Pages/Coal_Power.aspx. Accessed: 5 November 2016.

FIGUEROA, L., MILLER, A., ZALUSKI, M. AND BLESS, D. 2007. Evaluation of a two-stage passive treatment approach for mining influenced waters. National Meeting of the American Society of Mining and Reclamation.

FINGL E. 1980. Laxatives and cathartics. In: Gilman AG et al., eds. *Pharmacological basis of therapeutics*. New York, NY, MacMillan Publishing.

FLORENCE, K., SAPSFORD, D., AND WOLKERSDORFER, C. 2016 Mechanisms of iron removal during passive treatment of AMD in a vertical flow reactors. ICARD/IMWA Conferences.

GARCIA, C., MORENO, D.A., BALLESTER, A., BLAZQUEZ, M.L. AND GONZALEZ, F. 2001. Bioremediation of an industrial acid mine water by metal tolerant sulphate-reducing Bacteria. Elsevier, Minerals Engineering, 14(9): 997-1008.

GEOLAB. 2013. Personal communication.

GIBERT, O., DE PABLO, J., CORTINA, J.L. AND AYORA, C. 2004. Chemical characterisation of natural organic substrates for biological mitigation of acid mine drainage. Elsevier, Water Research, 38(2004): 4186-4196.

GLOMBITZA, F. 2001. Treatment of acid lignite mine flooding water by means of microbial sulphate reduction. Elsevier, Waste Management, 21(2001) 197-203.

GOMEZ, D.K.V. 2013. Simultaneous sulfate reduction and metal precipitation in an inverse fluidized bed reactor. Wageninger University, Netherlands.

GONICK, H., TUNNICLIFF, D.D., PETERS, E.D., LYKKEN, L. AND ZAHN, V. 1945. Determination of nitrogen by combustion improved dumas apparatus and recycle procedure. Ind. Eng. Chem, Anal. Ed., 17 (11), pp 677-682.

GUSEK, J.J. 2008. Passive Treatment 101: An overview of the technologies. EPA/National Groundwater Association’s Remediation of Abandoned Mine Lands Conference, Denver.

GUSEK, J.J. 2009. A Periodic Table of Passive Treatment for Mining Influenced Water. National Meeting of the American Society of Mining and Reclamation.

HALLBERG, K.B. and Johnson, D.B. 2005. Biological manganese removal from acid mine drainage in constructed wetlands and prototype bioreactors. Science of the total environment 338 (2005) 115-124.

HALVERSON, N.V. 2004. Review of constructed subsurface flow vs. surface flow wetlands. WSRC-TR-2004-00509. Revision 0.

HAMMACK, R.W., DE VEGT, A.L., AND SCHOEMAN, A.L. 2006. The removal of sulphate and metals from mine waters using bacterial sulphate reduction: pilot plant results. Mine Water and the Environment, International Mine Water Association.

HARCK, T. 2014. Eerstelingfontein backfill and pit water assessment. PMM13-094-D5.

HEATH, R.C. 1983. Basic groundwater hydrology U.S. geological survey water supply, paper 2220.

HEATH, R., MOFFETT, M., BANISTER, S. 2004. Water related impacts of small scale mining. WRC Report No 1150/1/04. ISBN No 1-77005-119-8.

HEIZER WD et al. 1997. Intestinal effects of sulfate on drinking water in normal human subjects. Digestive Diseases and Sciences, 42(5):1055–1061.

HUTTON, B., KAHAN, I., NAIDU, T. AND GUNTHER, P. 2009. Operating and maintenance Experience at the eMalahleni Water Reclamation Plant. International Mine Water Conference.

INTERSTATE TECHNOLGY REGULATION COUNCIL (ITRC). 2005. Technical regulatory guideline: permeable reactive barriers: lessons learned/new directions.

INTERSTATE TECHNOLOGY REGULATION COUNCIL (ITRC). 2010. Technology overview as part of a web-based technical and regulatory guidance constructed treatment wetland. Hyperlink: {http://www.itrcweb.org/miningwaste-guidance/to_const_treat.htm} Date accessed: 2 April 2017.

ISRAEL, S. 2015. *In Situ* denitrification of nitrate rich groundwater in South Africa. Dissertation presented for the degree of PhD Soil Science in the Faculty of Agriculture, Stellenbosch University.

JONG, T. AND PARRY, D.L. 2004. Removal of sulphate and heavy metals by sulphate reducing bacteria in short-term bench scale upflow anaerobic packed bed reactor runs. Elsevier, Water Research 37 (2003) 3379-3389.

JONG, T. AND PARRY, D.L. 2005. Evaluation of the stability of arsenic immobilized by microbial sulphate reduction using tclp extractions and long-term leaching techniques. Elsevier, Chemosphere, 60(2005) 254-265.

JOHNSON, B., SANTOS, A.L., FALAGAN, C., JONES, R.M., GRAIL, B.M., HOLANDA, R. AND HEDRICH, S. 2016. Developing sustainable biotechnologies for mine water treatment in the 21st century. Proceedings of IMWA 2016.

JOHNSON, D.B. AND HALLBERG, K.B. 2005. Acid mine drainage remediation options: a review. Elsevier, ScienceDirect, 338(2005), 3-14.

JOHNSON, R.H., BLOWES, D.W., ROBERTSON, W.D., AND JAMBOR, J.L. 2000. The hydrogeochemistry of the Nickel Rim Mine Tailings impoundment, Sudbury, Ontario. Journal of Contaminant Hydrology 41:49-80

KILBORN INCORPORATED. 1996. Review of passive systems for treatment of acid mine drainage, phase 2. Lakeshore, Toronto. 49 p.

KIRBY, C.S. AND BRADY, J.A.E. 1998. Field determination of Fe^{2+} oxidation rates in acid mine drainage using a continuously-stirred tank reactor. Elsevier, Applied Geochemistry, 13(4) 909-520.

KOUSI, P., REMOUNDAKI, E. AND TSEZOS, M. 2015. Sulphate-reducing bioreactors: current practices and perspectives.

KRESIC, N. 2007. Hydrogeology and Groundwater Modelling. CRC Press.

KUCHLER, A., GUNTER, K. AND CHRISTIAN, K. 2006. Passive biological treatment systems of mine waters at wismut sites. 9th International Mine Water Congress. WISUTEC Wismut Environmental Technologies Gmhb, Chemnitz, Germany

KUYUCAK, N., CHABOT, F. AND MARTSCHUK, J. 2006. Successful implementation and operation of a passive treatment system in an extremely cold climate, northern quebec, Canada. 7th International Conference on Acid Rock Drainage (ICARD), March 26-30, 2006, St. Louis MO. R.I. Barnhisel (ed.) Published by the American Society of Mining and Reclamation (ASMR), 3134 Montavesta Road, Lexington, KY 40502.

LEAVITT, B.R. 2016. Aeration of mine water using a TROMPE. Patent Pending, Consulting Hydrogeologist 2776 S-Bridge Rd Washington, Pa. 15301.

LEVENSPIEL, O. 1999. Chemical reaction engineering. 3rd edition. John Wiley & Sons, New York. p. 257-267, 295.

LIMPITLAW, D., AKEN, M., LODEWIJKS, H. AND VILJOEN, J. 2005. Post-mining rehabilitation, land use and pollution. Sustainable Development in the life of coal mining in South Africa: The South African Institute of Mining and Metallurgy.

LIU, J., ZHANG, H., YOU, K. AND YUAN, Y. 2014. Different filter material on the northeast biological iron manganese removal effect of groundwater in the countryside. Applied Mechanics and Materials, Vols. 522-524 (2014): 465-468.

LYEW, D. AND SHEPPERD, J. 1999. Sizing considerations for gravel beds treating acid mine drainage by sulfate reduction. Environmental Quality 28(3): 6 and 1025.

LOGAN, M.V., REARDON, K.F., FIGUEROA, L.A., MCLAIN, J.E.T. AND AHMANN, D.M. 2005. Microbial community activities during establishment, performance, and decline of bench-scale passive treatment systems for mine drainage. Elsevier, Water Research, 39(2005): 4537-4551.

LORAX ENVIRONMENTAL. 2003. Treatment of sulphate in mine effluents. International Network for Acid Prevention.

LOVLEY, D.R. AND PHILLIPS, E.J.P. 1987. Competitive mechanisms for inhibition of sulfate reduction and methane production in the zone of ferric iron reduction in sediments. Applied and Environmental Microbiology, Vol. 53, No. 11: 2636-2641.

MADZIVIRE, G., PETRIK, L.F., GITARI, W.M., BALFOUR, G., VADAPALLI, V.R.K. AND OJUMU, T.V. 2009. Role of pH on sulphate removal from circumneutral mine water using coal fly ash. Abstracts of the International Mine Water Conference. pp 462-471.

MAREE, J.P., GREBEN, H. AND MNQANQENI. 2000. Simultaneous removal of sulphate and sulphide with the biosure process from coal acid mine drainage. Department of Water, Environmental and Forestry Technology, CSIR, Pretoria. WISA Biennial Conference, Sun City, South Africa.

MAREE, J.P. AND DU PLESSIS, P. 1994. Neutralization of acid mine water calcium carbonate. Pergamon, Wat. Sci Tech., 29(9):258-296.

MARTINS, M.S.F.[A], SANTOS, E.S.[A], BARROS, R.J.J.[B] AND COSTA. M.C.S.S.[A] 2008. Treatment of acid mine drainage with sulphate-reducing bacteria using a two-stage bioremediation process. [a]Centro de Ciências do Mar, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal. [b] Centro de Investigação de Química e Catálise, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro.

MAYER, K.U., Benner, S.G., Blowes, D.W. 2006. Process-based reactive transport modelling of a permeable reactive barrier for the treatment of mine drainage. Journal of Contaminant Hydrology 85:195-211.

MAYES, W.M., BATTY, L.C., YOUNGER, P.L., JARVIS, A.P., KOIF, M., VOHLA, C. AND MANDER, U. 2009. Wetland treatment at extremes of pH: A review. Elsevier, Sciencedirect, 407 (2009): 3944-3957.

MCCARTHY, T.S. & PRETORIUS, K. 2009. Coal mining on the Highveld and its implications for future water quality in the Vaal River system. Abstracts of the International Mine Water Conference, Proceedings USBN Number: 978-0-9802623-5-3. Document Transformation Technologies cc. Pretoria, South Africa. pp. 56, 61-64.

MEY, W.S. AND VAN NIEKERK, A.M. 2009. Evolution of mine water management in the Highveld coalfields. International Water Conference, 978-0-9802623-5-3.MIDDLETON, A.C. AND LAWRENCE, A.WM. 1977. Kinetics of microbial sulfate reduction. Water Pollution Control Federation, 49 (7:1699-1670).

MINE WASTE TECHNOLOGY PROGRAM. 2004. Annual Report: Integrated Passive Biological Treatment Process Demonstration, Mine Waste Technology Program.

MIRJAFARI, P. AND BALDWIN, S.A. 2016. Decline in performance of biochemical reactors for sulphate removal from min-influenced water is accompanied by changes in organic matter characteristics and microbial population composition. Water (2016) (8): 124.

MIZUNO, O., LI, Y.Y. AND NOIKE, T. 1998. The behaviour of sulphate-reducing bacteria on acidogenic phase of anaerobic digestion. Elsevier, Water Research, 32(5):1626-1634.

MOON, C., SINGH, R., VEERAVALLI, S.S., SHANMUGAM, S.R., CHAGANTI, S.R., LALMAN, J.A., AND HEATH, D.D. 2015. Effect of COD:SO_4^{2-} ratio, HRT and linoleic acid concentration on mesophillic sulfate reduction: reactor performance and microbial population dynamics. Water, 2015(7):2275-2292.

MORRISON, M. 2005. Biochemical interactions in a compost wetland for spoil heap leachate remediation at Quaking Houses, Country Durham, UK.

MUNNIK, V., HOCHMANN, G., HLABANE, M., AND LAW, S. 2010. The social and environmental consequences of coal mining in South Africa: a case study. A joint initiative of Environemntal Monitoring Group, Cape Town, South Africa and Both ENDs, Amsterdam, The Netherlands.

MUYZER, G. AND STAMS, A.J.M. 2008. The ecology and biotechnology of sulphate-reducing bacteria. Natuer Publishing Group, Vol 6: 441-454.

NAIDOO, B. 2007. Mining Weekly: “Reclamation Project moves ahead”.

NAIRN, B. AND HEDIN, R.S. 1993. Contaminant removal capabilities for wetlands constructed to treat coal mine drainage. Boca Raton (1993), p. 187-95.

NAS. 1977. Drinking water and health. Washington, DC, National Research Council, National Academy of Sciences.

NECULITA, C.M., ZAGURY, G.J. AND KULNIEKS, V. 2007a. Short-term and long-term bioreactors for acid mine drainage treatment, proceedings of the annual international conference on sols, sediment, water and energy. Scholar works umass, 12(2).

NECULITA, C., ZAGURY, G.J., AND BUSSIERE, B. 2007b. Efficiency of three reactive mixtures of organic wastes for the treatment of highly contaminated acid mine drainage. Ottawa 2007.

NNAEMEKA, E. 2010. Petroleum Reservoir Engineering Practice. Prentice Hall.

NOAA, 2002. Divisional Normals and Standard Deviations of Temperature, Precipitation and Heating and Cooling Degree Days, 1971-2000. Climatography of the United States No. 85. National Climatic Data Center, Aseville, NC.

O' FLAHERTY, V. AND COLLERAN, E. 1998. Effect of sulphate addition on volatile fatty acid and ethanol degradation in an anaerobic hybrid reactor. Elsevier, Bioresource Technology, 68(1999):101-107.

OELOFSE, S. 2008. Mine water pollution-acid mine decant, effluent and treatment: a consideration of key emerging issues that may impact the state of the environment. CSIR, Natural Resources and the Environment.

PARKHURST, D.L. AND APPELO, C.A.J. 1999. User's guide to PHREEQC (version 2)-A computer program for speciation, batch-reaction, one-dimensional transport, and inverse geochemical calculations. Water-Resource Investigations Report 99-4259.

PRICE, W.A., MORIN, K. & HUTT, N. 1997. Guidelines for the prediction of acid rock drainage and metal leaching for mines in British Columbia: Part 11. Recommended procedures for static and kinetic testing. In: Proceedings of the Fourth International Conference on Acid Rock Drainage. Vol 1. May 31 – June 6. Vancouver, BC., pp. 15 – 30.

PULLES, W., LODEWIJKS, H.M., TOERIE, A., MUHLBAUER, R., VAN NIEKERK, J.A AND RICHART, A. 2016. Passive treatment of acid mine drainage at Vryheid coronation colliery, South Africa. Mine Closure 2016, Perth, ISBN 978-0-9924810-4-9.

REMEDIATION TECHNOLOGY DEVELOPMENT FORUM (RTDF). 2000. Nickel rim Mine Site. Accessed July 21, 2006.

RODRIGUEZ-SANCHEZ, A., GONZALEZ-MARTINEZ, A., MARTINEZ-TOLEDO M.V., GARCIA-RUIZ, M.J., OSORIO F. AND GONZALEZ-LOPEZ, J. 2014. The effects of influent characteristics and operational conditions over the performance and microbial community structure of partial nitrification reactors. Water (2014) (6), 1905-1924.

ROYCHOUDHURY, A.N. AND CAPPELLEN, P. 2003. Kinetics of microbial mediated reactions: dissimilatory sulphate reduction in saltmarch sediments (Sapelo Island, Georgie, USA). Estuarine, Coastal and Shelf Science 56 (2003) 1001-1010.

RZECZYCKA, M. AND BLASZCZYK, M. 2005. Growth and activity of sulphate-reducing bacteria in media containing phosphogypsum and different sources of carbon. Polish Journal of Environmental Studies Vol.14, No. 6 (2005), 891-895.

SANCHEZ-ANDREA, I., SANZ, J.L., BIJMANS, M.F.M. AND STAMS, A.J.M. 2014. Sulphate reduction at low ph to remediate acid mine drainage. Elsevier, Journal of Hazardous Material, Hazmat-15625.

SHEORAN, A.S., SHEORAN, V. AND CHOUDHARY, R.P. 2010. Bioremediation of acid rock drainage by sulphate reducing prokaryotes: a review. Elsevier, Sciencedirect, Minerals Engineering, 23(2010), 1073-1100.

SOIL CLASSIFICATION WORKING GROUP. 2006. A taxonomic system for South Africa Department of Agricultural Development, Pretoria.

SOREGAROLI, B.A. & LAWRENCE, R.W. 1998. Update on waste characterisation studies. proc. mine design, Operations and Closure Conference. Polson, Montana.

SOUTH AFRICA. 1998. National Environmental Water Act (NWA), Act 36 of 1998, section 21.

SOUTH AFRICA. 2002. Mineral and Petroleum Resources Development Act 28 of 2002 (MPRDA), section 43.

SOUTH AFRICA. 2004. National Air Quality Act (NACA), Act 39 of 2004.

SOUTH AFRICA. 2008. National Environmental Management: Waste Act (NEMWA), Act, 59 of 2002, regulation No. 633 and 636.\

STEENEKAMP, 2011. Department Water Affairs report. Eerstelingsfontein Geohydrology Study and Sub-Surface Flow Model.

ENVIRONMENTAL MANAGEMENT PROGRAMME (EMPr). 1996. Sumo Coal Colliery.

TAYLOR, J., PAPE, S. AND MURPHY, N. 2005. A summary of passive and active treatment technologies for acid and metalliferous drainage (AMD). Earth System (Pty) Ltd.

TILLEY, E., ULRICH, L., LUETHI, C., REYMOND, P. AND ZURBRUEGG, C. 2014. Compendium of sanitation systems and technologies. 2nd Edition. Duebendorf, Switzerland: Swiss Federal Institute of Aquatic Science and Technology (Eawag).

TIWARY, R.K. AND DHAR, B.B. 1994. Environmental pollution from coal mining activities in Damodar River Basin, India. Mine water and the environment, 13 (1994): 1-10.

TSUKAMOTO, T.K., KILLION, H.A. AND MILLER, G.C. 2004. Column Experiments for Microbiological Treatment of Acid Mine Drainage: low-temperature, low-pH and matrix investigations. Elsevier, Water Research, 38 (2004): 1405-1418.

U.S. DHEW. 1962. Drinking water standards. 1962. Washington, DC, US Department of Health, Education and Welfare, Public Health Service; US Government Printing Office (Publication No. 956).

U.S. EPA. 1975. Inactive and abandoned underground mines: Water pollution, prevention and control. EPA-440/9-75-007.

U.S. EPA. 1985. National primary drinking water regulations; synthetic organic chemicals, inorganic chemicals and microorganisms; proposed rule. US Environmental Protection Agency. *Federal Register*, 50(219):46936.

U.S. EPA. 1994. Technical document: acid mine drainage prediction. Office of solid waste, Special waste branch, Washington. 9 p.

U.S. EPA. 1997. Permeable reactive subsurface barriers for the interception and remediation of chlorinated hydrocarbon and chromium (VI) plumes in ground water. EPA/600/F-97/008.

U.S. EPA. 1998. Permeable reactive barrier technologies for contaminant remediation. EPA/600/R-98/125.

U.S. EPA. 1999a. Health effects from exposure to high levels of sulfate in drinking water study. Washington, DC, US Environmental Protection Agency, Office of Water (EPA 815-R-99-001).

U.S. EPA. 1999b. Health effects from exposure to high levels of sulfate in drinking water Workshop. Washington, DC, US Environmental Protection Agency, Office of Water (EPA 815-R-99-002).

U.S. EPA. 2005. Prevention of acid mine drainage generation from open-pit highwalls. EPA/600/R-05/060.

U.S. EPA 2008. Field application of a permeable reactive barrier for treatment of arsenic in ground water. Office of Research and Development, EPA 600/R-08/093.

U.S. EPA. 2014. Reference guide to treatment technologies for mining-influenced water. EPA 542-R-14-001. 2pp. 3-30 and 34-37.

USHER, B.H., CRUYWAGEN, L-M., DE NECKER, E. & HODGSON, F.D.I. 2003. Acid-base: accounting, techniques and evaluation (abate): recommended methods for conducting and interpreting analytical geochemical assessments at opencast collieries in South Africa. Water Research Commission Report No 1055/2/03. Pretoria.

VAN ZYL, H., BOND-SMITH, M., MINTER, T., BOTHA, M. AND LEIMAN, A. 2012. Financial provisions for rehabilitation and closure in South African Mining: Discussion document on challenges and recommended improvements. Independent Economic Research ,University of Cape Town.

VERMEULEN, P.D., DEYSEL, L-M, MACDONALD, N. AND APHANE, V. 2014. Spoils handling from coal mines in the Waterberg coalfield area, South Africa. an interdisciplinary response to mine water challenges. Institute for Groundwater Studies, University of the Free State.

VERMEULEN, D. AND USHER, B. 2009. Operation and monitoring guidelines and the development of a screening tool for irrigating with coal mine water in Mpumalanga Province, South Africa. Vol 35.

VILLIAN, L. 2014. Reclamation of acid-generating waste rock by in-pit backfilling and sealing: an evaluation of the Kimheden Mine Site, Northern Sweden. Lulea University of Technology, Department of Civil, Environmental and Natural Resources Engineering. Division of Geosciences and Environmental Engineering.

VYMAZAL, J. 1998. Removal mechanisms and types of constructed wetlands. Constructed Wetlands for Wastewater Treatment in Europe.

VYMAZAL, J. 2011. Plants used in constructed wetlands with horizontal subsurface flow: a review. Hydrobiologia (2011) 674: 133-156.

WATSON, I. AND BURNETT, A.D. 1995. Hydrology: An Environmental Approach. Lewis Publishers.

WATZLAF, G.R., SCHROEDER, K.T. AND KAIRIES, C. 2000. Long-term performance of alkalinity producing passive systems for the treatment of mine drainage. National Meeting of the American Society for Surface Mining and Reclamation, Tampa, Florida.

WINTER, F. 2006. Using tracer techniques to investigate groundwater recharge in the Mount Carmel aquifer, Israel.

WORLD HEALTH ORGANIZATION (WHO). 2004. Sulphate in drinking water. Background document for development of WHO Guidelines for Drinking-water Quality. WHO/SDE/WSH/03.04/114.

XU, Y., VAN TONDER, G.J., VAN WYK, B., AND VAN WYK, E. 1998. Recharge-incorporated approximate solution to tracer experiments under pumping conditions. Water SA, Vol. 24 No. 1.

YARWOOD, R.R., ROCKHOLD, M.L., NIEMET, M.R., SELKER, J.S. AND BOTTOMLEY, P.J. 2006. Impact of Microbial growth on Water Flow and Solute Transport in Unsaturated Porous Media. Water Resource Research, 42(W10405):1-11.

YOUNGER, L. 2000. The adoption and adaptation of passive treatment technologies for mine waters in The United Kingdom. Mine Water and the Environment (2000), 19: 84-97.

ZAGURY, G.J.[A], NECULITA, C.[A] AND BUSSIERE[B]. 2007. Passive Treatment of Acid Mine Drainage nn Bioreactors: Short Review, Applications, And Research Needs. Department of Civil, Geological, and Mining Engineering - École Polytechnique de Montréal, Montreal, QC, Canada. Department of Applied Sciences - Université du Québec en Abitibi-Témiscamingue, Rouyn-Noranda, QC, Canada & NSERC Polytechnique/UQAT Industrial Chair in Environment and Mine Waste Management and Canada Research Chair on Abandoned Mine Site Rehabilitation.

ZOETEMAN, B.C.J. 1980. Sensory assessment of water quality. New York, NY, Pergamon Press.

APPENDIX A: Statistical Methods

Standard deviation of the mean (σ_m)

Multiple rounds of measurements were taken of volumetric flow rate (Q) and hydraulic gradient (i) to calculate hydraulic conductivity (K). The objective of conducting standard deviation of the mean was to assign an uncertainty to the average, and whether it would be correct to use the average of the measurements in the upscale design of the treatment system. The standard deviation of the mean was calculated:

$$\sigma_m = \frac{\sigma}{\sqrt{n}}$$

Where,

σ = *Standard deviation*

n = *Number of measurements*

σ_m = *Standard deviation of the mean*

Experimental Reading Errors

The objective of this calculation was to determine the uncertainty in the data caused by the scale or precision of the measuring instrument. The reading errors were calculated by means of applying the “product” and “sum” rules:

$$\delta(x+y) = \delta x + \delta$$

$$\delta(x-y) = \delta x + \delta y$$

OR

$$\frac{\delta\,(xy)}{xy} = \frac{\delta\,(x)}{x} + \frac{\delta\,(y)}{y}$$

$$\frac{\delta\,\left(\frac{x}{y}\right)}{\frac{x}{y}} = \frac{\delta x}{x} + \frac{\delta x}{y}$$

Where,

δ = Error or uncertainty

x and y = Measured values

The “sum” rule entails: if two values, each with associated errors, are added or subtracted, the error values are added. The “product” rule entails: if two values, each with associated errors, are multiplied or divided, the error percentages are added.

Root mean square error (RMSE)

The root mean squared error can be used to calculate the difference in results obtained between observed and modelled data. The RMSE can be calculated as:

$$\text{RMSE}=\sqrt{\frac{\sum_{i=1}^{n}(X_{obs,\,i}-X_{mod,\,i})^2}{n}}$$

Where,

$X_{obs,\,I}$ = Observed values at time i

$X_{mod,\,i}$ = Modelled values at time i

n = Number of measurement

RMSE = Root mean squared error

The RMSE was calculated for the tracer experiment outputs to identify the model that best fit the observed data.

Regression (R^2- Coefficient)

This coefficient was calculated as a goodness of fit between modelled and observed data by means of:

$$R^2 = \frac{SS_T - SS_E}{SS_T}$$

Where,

SS_T *= Total sum of squares*

SS_E *= Residual sum of squares of errors*

R^2 *= Regression coefficient*

Pearson Correlation (r)

The correlation coefficient between two sets of data can be expressed as:

$$r = \frac{\sum (x-\bar{x})(y-\bar{y})}{\sqrt{\sum (x-\bar{x})^2 \sum (y-\bar{y})^2}}$$

Where,

x *= Sample array 1*

y *= Sample array 2*

$\bar{x}$ *and* $\bar{y}$*= Sample array means*

r *= Pearson correlation*

APPENDIX B: PHREEQC Solutions

```
SOLUTION_MASTER_SPECIES; Acetic Acetic 0 60.5 60.5        # CH3COOH
SOLUTION_SPECIES; Acetic = Acetic; -log_k 0
```

RATES

```
 S_degradation
# dS/dt = -((k*S*X)/(K_8+S))                                          (mol Acitic Acid/L/T)
# k is the maximum specific rate of organic carbon utilization        (M/L3)>>>>(1/T)
# S is the organic carbon concentration                               (mol Acitic Acid/L/T)
# K_8 is half-velocity coefficient                              (M/L3)
 -start
  1 mu_max = parm(1); 2 k_half = parm(2); 3 Y = parm(3); #4 R = 1 + parm(4)
 10 S = tot("Acetic")
 20 if S < 1e-9 then goto 60
 30 B = kin("Biomass")                  # kin(".i.") gives moles of "Biomass"
 40 rate = -mu_max * ((S * B)/(k_half + S))
# 40 rate = -mu_max * (B / (Y * 8)) * (S /(k_half + S + S^2/8.65e-4)) / R
 50 dS = rate * time
 60 save dS                       # d mol(C8H10)
 70 put(rate, 1)                  # Store dS/dt for use in Biomass rate
 -end
```

Biomass

```
## dX/dt = -YdS/dt - bX
# Y is the yield coefficient                                          (M/M)
# b is the bacterial mass decay coeffient                       (1/T)

 -start
  1 Y = parm(1);  3 k_Bd = parm(2)
 10 rate_S = get(1)                     # Get degradation rate, multiply by Retardation
 20 B = m
 30 rate = -Y * rate_S - k_Bd * B
 40 dB = rate * time
 50 save -dB                            # dB is positive, counts negative to solution
 60 put(rate, 2)
 -end

 V4SO4_red
-start
 # 1 moles = 0
```

```
 # 2 rate = 0 # (mol /(L·second))
 3 rate_S = get(1)
 4 rate_dB = get(2)
 10 alfa = parm(1)
 20 beta = parm(2)

 100 rate = alfa*(rate_S+(beta*rate_dB))
 110 moles = rate * TIME
 210 SAVE moles
-end

SOLUTION 1; -units mg/L; Acetic 650; S(6)  570
KINETICS 1

 S_degradation;-formula Acetic 1; -m0 0
  -parms 5.4e-5 5.13e-3 0.065                         # mu_max, k_half, Y
 Biomass;-formula C 0;-m0 2.3e-4
  -parms 0.065 0

V4SO4_red
-formula  Acetic 1 SO4 1 H2S -1 HCO3 -2
-parms 1.3 0.1
-m0  0                                                      # initial moles of the kinetic reactant
-tol 1e-12
-steps 0.6e6 in 50
```

INCREMENTAL_REACTIONS

```
USER_GRAPH
 -headings time c_acetic Biomass
 -axis_scale y_axis 0 600 2 1; -axis_scale x-axis 0 6 1
 -axis_titles "Time / days" "mg / L"
 -start
 -plot_tsv_file figure5.phr
 -plot_tsv_file figure6.phr
 10 graph_x total_time / 86400
 20 graph_y tot("Acetic")*60500, tot("S(6)")* 96000
 -end
END
```

APPENDIX C: PHREEQC Model Validation (Base Case Model)

HRT	C_Acetate	HRT	C_Sulphate
1,02	568,49	0,04	570,58
1,45	516,86	0,50	561,94
2,07	459,45	1,00	460,67
2,40	388,28	1,46	440,04
3,01	336,65	1,98	370,38
3,49	256,99	2,33	309,41
4,03	237,46	2,98	248,49
4,58	169,01	3,46	175,56
5,03	159,33	4,01	135,33
6,18	96,49	4,52	62,40
		5,01	25,43
		6,08	9,28

HRT: Days *Concentration: mg/L*

HRT	C_Acetate	HRT	C_Sulphate
0,14	636,65	0,14	557,67
0,28	622,21	0,28	544,75
0,42	607,47	0,42	531,58
0,56	592,44	0,56	518,14
0,69	577,13	0,69	504,45
0,83	561,54	0,83	490,51
0,97	545,67	0,97	476,32
1,11	529,55	1,11	461,91
1,25	513,18	1,25	447,28
1,39	496,58	1,39	432,45
1,53	479,76	1,53	417,42
1,67	462,75	1,67	402,22
1,81	445,55	1,81	386,86
1,94	428,21	1,94	371,36
2,08	410,73	2,08	355,76
2,22	393,16	2,22	340,07
2,36	375,52	2,36	324,33
2,50	357,85	2,50	308,56
2,64	340,19	2,64	292,81
2,78	322,57	2,78	277,11
2,92	305,05	2,92	261,49

HRT	C_Acetate	HRT	C_Sulphate
3,06	287,67	3,06	246,01
3,19	270,47	3,19	230,71
3,33	253,52	3,33	215,64
3,47	236,87	3,47	200,84
3,61	220,56	3,61	186,38
3,75	204,66	3,75	172,29
3,89	189,22	3,89	158,64
4,03	174,30	4,03	145,47
4,17	159,94	4,17	132,84
4,31	146,20	4,31	120,78
4,44	133,11	4,44	109,36
4,58	120,72	4,58	98,59
4,72	109,05	4,72	88,53
4,86	98,12	4,86	79,19
5,00	87,94	5,00	70,59
5,14	78,52	5,14	62,75
5,28	69,86	5,28	55,67
5,42	61,93	5,42	49,34
5,56	54,73	5,56	43,74
5,69	48,20	5,69	38,85
5,83	42,33	5,83	34,63
5,97	37,08	5,97	31,03
6,11	32,39	6,11	27,99
6,25	28,23	6,25	25,44
6,39	24,56	6,39	23,32
6,53	21,32	6,53	21,56
6,67	18,48	6,67	20,11
6,81	15,99	6,81	18,91
6,94	13,82	6,94	17,92

HRT: Days *Concentration: mg/L*

APPENDIX D: Dissolved Organic Carbon

		Control (Sand)	Compost
Day No.	**Date**	**DOC (mg/l)**	**DOC (mg/l)**
1	2016/04/12	29,10	808,00
3	2016/04/14	26,10	860,00
9	2016/04/20	---	---
14	2016/04/25	---	---
19	2016/04/30	18,00	868,00
24	2016/05/05	---	---
29	2016/05/10	---	---
34	2016/05/15	14,90	812,00
39	2016/05/20	---	---
44	2016/05/25	9,70	838,00

DOC: Dissolved Organic Carbon *mg/L: Milligram per Litre*

APPENDIX E: Constant Head Permeability Test Data Sheets

Constant head permeability test-100% compost.

	Start Time	End Time	Time (hr:min:sec)	Time (min.)	Volume Q (ml)	Flow Rate Q (m^3/s)	h_1 (mm)	h_2 (mm)	L (mm)	Δh (mm)	Hydraulic Gradient í = Δh/L	k = Q/(í *A)
Run 1	16:10	16:20	0:10:09	10,2	2000	0.00000328	481	257	250	224	0,896	0,000393
Run 2	21:00	21:11	0:11:55	11,9	2270	0,00000317	500	246	250	254	1,016	0,000335
Run 3	13:00	13:08	0:8:56	8,9	2000	0,00000373	506	264	250	242	0,968	0,000413
Run 4	17:20	17:28	0:8:24	8,4	2000	0,00000397	503	270	250	233	0,932	0,000456
Run 5	19:15	19:23	0:8:25	8,4	2000	0,00000396	490	270	250	220	0,880	0,000482

Constant head permeability test-70% compost 30% sand.

	Start Time	End Time	Time (hr:min:sec)	Time (min.)	Volume Q (ml)	Flow Rate Q (m^3/s)	h_1 (mm)	h_2 (mm)	L (mm)	Δh (mm)	Hydraulic Gradient i = Δh/L	k = Q/(í *A)
Run 1	8:43	9:01	0:17:42	17,7	2000	0,00000188	520	171	250	349	1,396	0,000145
Run 2	8:28	8:37	0:8:58	9,0	2000	0,00000372	530	230	250	300	1,200	0,000332
Run 3	10:30	10:38	0:8:13	8,2	2000	0,00000406	525	241	250	284	1,136	0,000383
Run 4	12:12	12:20	0:7:53	7,9	2000	0,00000426	506	256	250	250	1,000	0,000453
Run 5	14:31	14:39	0:7:43	8,2	2000	0,00000406	505	276	250	229	0,916	0,000475

Constant head permeability test-50% sand 50% compost.

	Start Time	End Time	Time (hr:min:sec)	Time (min.)	Volume Q (ml)	Flow Rate Q (m^3/s)	h_1 (mm)	h_2 (mm)	L (mm)	Δh (mm)	Hydraulic Gradient i = Δh/L	k = Q/(í *A)
Run 1	17:00	17:08	0:8:24	8,4	2000	0,00000397	498	235	250	263	1,052	0,000404
Run 2	20:07	20:16	0:9:49	9,8	2063	0,00000350	517	220	250	297	1,188	0,000316
Run 3	21:45	21:55	0:10:09	10,2	2000	0,00000328	524	217	250	307	1,228	0,000287
Run 4	9:25	9:33	0:9:33	9,6	2000	0,00000349	513	241	250	272	1,088	0,000344
Run 5	14:10	14:17	0:7:30	7,5	2000	0,00000444	523	250	250	273	1,092	0,000436
Run 6	19:52	20:00	0:8:30	8,5	2000	0,00000392	537	233	250	304	1,216	0,000346

Constant head permeability test-70% sand 30% compost.

	Start Time	End Time	Time (hr:min:sec)	Time (min.)	Volume Q (ml)	Flow Rate Q (m^3/s)	h_1 (mm)	h_2 (mm)	L (mm)	Δh (mm)	Hydraulic Gradient i = Δh/L	k = Q/(í *A)
Run 1	17:10	17:21	0:11:21	11,4	2000	0,00000294	565	223	250	342	1,368	0,000230
Run 2	20:20	20:33	0:13:17	13,3	2000	0,00000251	572	212	250	360	1,440	0,000187
Run 3	21:56	22:10	0:14:08	14,1	2000	0,00000236	571	210	250	361	1,444	0,000175
Run 4	9:37	9:47	0:10:04	10,1	2000	0,00000331	562	229	250	333	1,332	0,000266
Run 5	19:40	19:51	0:11:15	11,3	2000	0,00000296	567	227	250	340	1,360	0,000233

Constant head permeability test-100% sand.

	Start Time	**End Time**	**Time (hr:min:sec)**	**Time (min.)**	**Volume Q (ml)**	**Flow Rate Q (m^3/s)**	**h_1 (mm)**	**h_2 (mm)**	**L (mm)**	**Δh (mm)**	**Hydraulic Gradient i = Δh/L**	**k = Q/(í *A)**
Run 1	19:10	19:16	0:16:09	16,2	2000	0,00000206	505	315	250	190	0,760	0.000291
Run 2	19:50	19:55	0:05:07	5,1	2000	0,00000651	497	300	250	197	0,788	0,000886
Run 3	20:29	20:03	0:05:06	5,1	2000	0,00000654	500	305	250	195	0,780	8,000898
Run 4	12:43	12:47	0:04:30	4,5	2000	0,00000741	493	323	250	170	0,680	0,000117
Run 5	14:47	14:51	0:04:32	4,5	2000	0,00000735	495	325	250	170	0,680	0,000116

APPENDIX F: Pycnometer Cycle Results

100%S						
Cycle #	**Volume (cm³)**	**Volume Deviation (cm³)**	**Relative Density (g/cm³)**	**Density Deviation (g/cm³)**	**Elapsed Time (mm:ss)**	**Temperature (°C)**
1	34,2184	0,0359	2,6489	0,0028	4:33	20,33
2	34,2565	0,0022	2,6459	0,0002	6:23	20,37
3	34,2881	0,0338	2,6435	0,0026	8:10	20,40

100%S			
Analysis Gas:	Helium	**Analysis Start:**	28/07/2017 11:18:19PM
Reported:	28/07/2017 11:35:27PM	**Analysis End:**	28/07/2017 11:27:25PM
Sample Mass:	90,6400 g	**Equilib. Rate:**	0,050 psig/min
Temperature:	20,37 °C	**Expansion Volume:**	74,6390 cm³
Number of Purges:	3	**Cell Volume:**	108,4400 cm³

Psig (pound-force per square inch gauge)

100C						
Cycle #	**Volume (cm^3)**	**Volume Deviation (cm^3)**	**Relative Density (g/cm^3)**	**Density Deviation (g/cm^3)**	**Elapsed Time (mm:ss)**	**Temperature (°C)**
1	10,3513	0,0378	1,6085	0,0058	5:31	20,54
2	10,3895	0,0004	1,6026	0,0001	7:49	20,54
3	10,4264	0,0374	1,5969	0,0058	10:05	20,58

100C			
Analysis Gas:	Helium	**Analysis Start:**	28/07/2017 9:36:04PM
Reported:	28/07/2017 9:47:56PM	**Analysis End:**	28/07/2017 9:47:21PM
Sample Mass:	16,6500 g	**Equilib. Rate:**	0,050 psig/min
Temperature:	20,55 °C	**Expansion Volume:**	74,6390 cm^3
Number of Purges:	3	**Cell Volume:**	108,4400 cm^3

Psig (pound-force per square inch gauge

30%C70%S						
Cycle #	**Volume (cm³)**	**Volume Deviation (cm³)**	**Density (g/cm³)**	**Density Deviation (g/cm³)**	**Elapsed Time (mm:ss)**	**Temperature (°C)**
1	26,4793	0,0475	2,5748	0,0046	4:09	20,44
2	26,5374	0,0106	2,5692	0,0010	5:44	20,44
3	26,5636	0,0368	2,5667	0,0036	7:20	20,45

30%C70%S			
Analysis Gas:	Helium	**Analysis Start:**	28/07/2017 12:10:29PM
Reported:	28/07/2017 12:18:44PM	**Analysis End:**	28/07/2017 12:18:20PM
Sample Mass:	68,1800 g	**Equilib. Rate:**	0.050 psig/min
Temperature:	20,44 °C	**Expansion Volume:**	74,6390 cm³
Number of Purges:	3	**Cell Volume:**	108,4400 cm³

Psig (pound-force per square inch gauge

50%C50%S						
Cycle #	**Volume (cm³)**	**Volume Deviation (cm³)**	**Density (g/cm³)**	**Density Deviation (g/cm³)**	**Elapsed Time (mm:ss)**	**Temperature (°C)**
1	23,3066	0,0393	2,4693	0,0042	4:59	20,40
2	23,3550	0,0091	2,4641	0,0010	6:57	20,44
3	23,3761	0,0303	2,4619	0,0032	8:54	20,45

50%C50%S			
Analysis Gas:	Helium	**Analysis Start:**	28/07/2017 11:56:11PM
Reported:	28/07/2017 12:07:38PM	**Analysis End:**	28/07/2017 12:06:06PM
Sample Mass:	57,5500 g	**Equilib. Rate:**	0,050 psig/min
Temperature:	20,43 °C	**Expansion Volume:**	74,6390 cm³
Number of Purges:	3	**Cell Volume:**	108,4400 cm³

Psig (pound-force per square inch gauge

70%C30%S						
Cycle #	**Volume (cm³)**	**Volume Deviation (cm³)**	**Density (g/cm³)**	**Density Deviation (g/cm³)**	**Elapsed Time (mm:ss)**	**Temperature (°C)**
1	16,7121	0,0427	2,1900	0,0056	4:52	20,44
2	16,7490	0,0058	2,1852	0,0007	6:27	20,41
3	16,8033	0,0485	2,1781	0,0063	8:28	20,45

70%C30%S			
Analysis Gas:	Helium	**Analysis Start:**	28/07/2017 11:42:33PM
Reported:	28/07/2017 11:53:20PM	**Analysis End:**	28/07/2017 11:52:12PM
Sample Mass:	36,6000 g	**Equilib. Rate:**	0.050 psig/min
Temperature:	20.43 °C	**Expansion Volume:**	74,6390 cm³
Number of Purges:	3	**Cell Volume:**	108,4400 cm³

Psig (pound-force per square inch gauge

APPENDIX G: Tracer Data

Tracer results for 100% compost

Round 1		Round 2		Round 3	
Time (minutes)	**EC (µS/cm)**	**Time (minutes)**	**EC (µS/cm)**	**Time (minutes)**	**EC (µS/cm)**
0,62	160,30	0,70	156,20	0,60	163,30
1,02	157,00	1,63	154,30	2,17	162,00
1,50	153,60	3,20	156,80	2,80	160,80
2,17	155,20	4,50	168,10	3,12	161,30
3,78	167,20	5,68	178,80	4,23	174,40
4,33	173,50	6,63	175,90	5,15	185,30
5,28	181,70	7,90	162,00	5,93	187,90
6,02	184,10	10,42	166,80	6,65	186,70
6,60	184,80	12,62	158,30	7,95	181,10
7,70	179,70	14,35	157,70	9,13	177,80
9,15	173,70	15,65	157,40	10,12	173,60
9,97	168,00	16,23	156,30	11,35	170,60
10,60	168,50	17,65	156,10	12,82	165,30
11,60	161,10	19,32	156,50	13,08	163,20
12,90	161,50	20,50	156,90	16,00	163,80
13,62	158,70	23,60	157,30	17,55	163,30
14,53	159,40	24,60	155,60	19,20	161,90
15,32	155,80			20,07	161,80
16,32	158,60			22,03	162,40
17,37	160,00			23,88	162,10
18,97	159,30			26,78	160,60
20,48	157,30			29,08	163,10
22,13	159,80				
24,70	159,60				

Tracer results for 70% compost 30% sand

Round 1		Round 2		Round 3	
Time (Minutes)	EC (µS/cm)	Time (Minutes)	EC (µS/cm)	Time (Minutes)	EC (µS/cm)
0,22	162,80	0,30	161,00	0,32	161,30
0,50	161,50	0,63	161,60	0,68	161,40
1,00	161,00	1,05	161,40	1,12	160,20
2,12	157,80	2,40	164,70	1,70	158,80
2,58	159,90	3,28	162,00	2,17	148,80
3,27	159,30	3,82	161,10	2,90	158,50
3,83	161,70	4,33	163,30	3,57	160,50
4,32	161,40	4,80	166,10	4,38	165,10
5,00	164,00	5,23	196,10	4,95	164,50
5,72	167,00	5,67	163,30	5,62	167,60
6,22	170,90	6,32	176,60	6,45	174,70
6,68	176,10	6,75	177,50	6,82	178,90
7,10	175,00	7,17	181,70	7,68	182,10
8,05	178,20	7,63	182,70	8,35	184,60
8,65	182,00	8,25	185,50	8,93	179,80
9,48	181,30	8,90	186,90	9,95	187,30
10,25	178,40	9,30	184,80	10,27	184,10
11,45	184,00	9,68	185,70	10,90	185,90
12,10	187,90	10,12	187,80	11,37	187,80
12,67	189,30	10,53	187,30	11,85	190,00
13,45	182,50	11,03	189,40	12,25	193,40
14,50	180,00	11,45	187,60	12,67	188,20
14,90	181,60	11,97	189,10	13,18	188,30
15,90	176,30	12,47	193,70	13,83	187,00
16,30	173,80	12,95	189,10	14,45	187,00
16,82	174,70	13,33	187,80	14,98	184,40
17,43	174,30	13,78	187,50	15,58	181,50
17,95	170,40	14,58	187,50	16,32	181,20
18,83	169,50	15,23	183,60	17,25	178,00
19,28	168,60	15,67	180,50	18,48	174,50
19,78	169,30	16,03	182,10	18,82	171,90
20,20	168,70	16,55	179,60	20,47	170,20
20,67	165,80	17,33	174,10	20,83	169,40
21,07	167,00	17,85	172,00	21,33	169,80
21,50	166,80	18,78	171,30	21,72	168,50
21,90	165,60	23,47	165,70	22,05	166,70
22,33	167,50	23,80	169,10	22,82	168,20
22,83	166,00	25,72	163,40	23,22	166,60
23,33	166,70	26,22	165,60	23,62	166,00
23,87	164,00	26,97	164,50	24,18	164,20
24,47	164,60	27,98	164,50	24,55	168,10
26,73	163,00	28,75	163,70	24,97	164,80

Round 1		Round 2		Round 3	
27,55	163,70			25,45	165,60
28,00	162,50			26,43	165,30
28,43	162,00			26,47	165,00
29,02	164,60			26,98	165,80
				27,60	164,90
				28,13	164,30
				28,62	168,70
				29,10	165,40
				29,63	165,30
				30,50	168,80
				31,03	165,60
				31,45	168,30
				31,85	167,00
				32,27	163,50
				32,78	165,30
				33,20	164,30
				33,58	165,10

Tracer results for 50% compost 50% sand

Round 1		**Round 2**		**Round 3**	
Time (minutes)	**EC (µS/cm)**	**Time (minutes)**	**EC (µS/cm)**	**Time (minutes)**	**EC (µS/cm)**
0,55	171,40	0,75	162,00	0,83	165,50
1,02	161,40	1,02	166,60	1,58	167,90
1,25	163,50	1,32	166,00	1,85	172,20
1,88	169,20	2,30	172,50	2,38	176,00
2,57	172,80	2,88	179,90	2,92	178,90
3,30	175,20	3,72	188,80	3,95	189,30
4,25	184,00	4,42	194,40	5,32	197,30
4,95	188,80	5,17	197,30	6,23	191,70
5,77	187,00	5,93	193,70	6,82	189,10
6,75	186,30	6,60	189,60	8,45	184,30
7,83	178,00	7,23	186,10	8,58	179,30
8,62	176,80	7,85	181,10	9,33	174,40
9,70	169,00	8,70	176,60	10,23	172,80
10,53	169,10	9,43	174,30	11,38	171,40
11,38	169,30	10,70	167,30	12,00	170,60
12,38	165,50	12,12	168,50	12,55	169,40
13,22	165,00	13,82	169,40	13,42	169,60
14,20	165,80	16,03	168,70	14,32	169,40
15,22	166,60	17,88	167,30	15,43	168,80
15,25	167,10	19,35	167,90	17,30	170,30
17,27	164,70	21,30	164,80	18,18	169,10
18,55	167,50	23,50	166,60	19,08	168,40
19,78	167,50	25,57	167,00	22,43	170,50
20,65	166,60	27,97	168,00	23,05	170,90
22,05	167,80	30,02	167,90	24,32	169,30
23,50	167,20	32,48	165,00	25,98	171,10
25,20	166,80	33,57	167,00	27,32	171,00
26,62	165,50	35,67	168,50	28,32	169,20
30,27	165,90			29,60	169,80
				31,32	170,10
				34,63	169,70

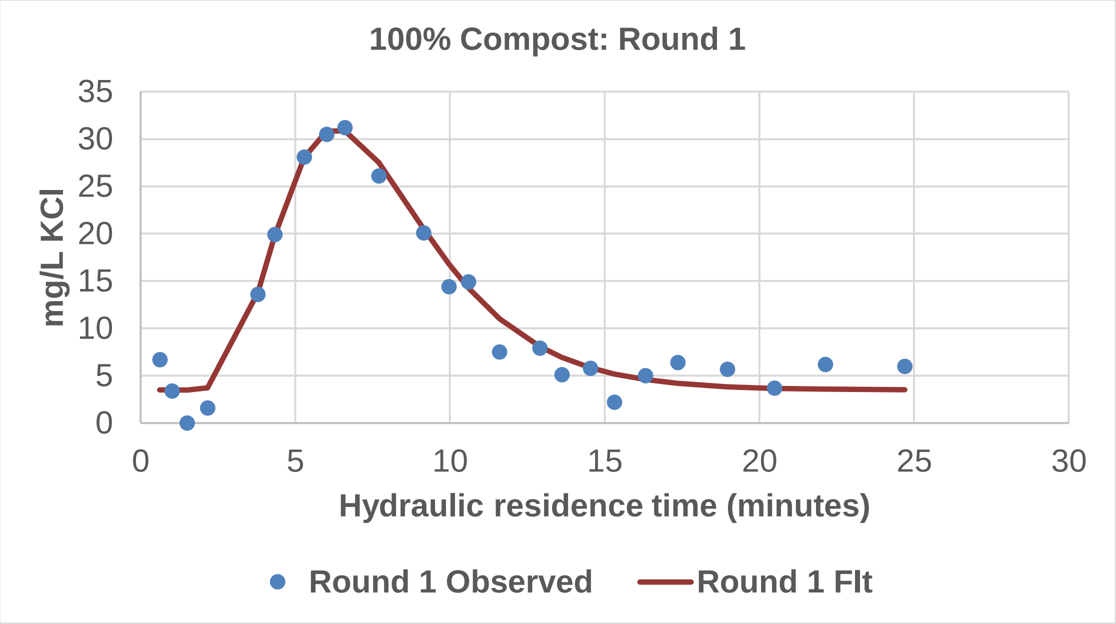
Tracer results for 30% compost 70% sand

Round 1		**Round 2**		**Round 3**	
Time (minutes)	**EC (µS/cm)**	**Time (minutes)**	**EC (µS/cm)**	**Time (minutes)**	**EC (µS/cm)**
0,53	171,90	0,72	173,10	1,00	165,80
1,03	171,30	0,93	169,30	1,30	168,60
1,25	169,00	2,95	171,60	1,52	166,40
1,97	177,50	4,15	191,90	2,23	178,90
2,90	183,50	4,95	199,80	3,58	193,80
3,67	192,20	5,78	200,00	4,63	197,90
4,35	196,40	6,42	197,60	5,37	200,00
5,20	198,10	7,23	194,70	6,20	196,50
5,80	192,80	8,28	190,20	6,93	192,80
6,82	186,60	9,78	185,00	7,53	188,70
7,50	183,20	10,63	178,50	8,75	180,60
9,07	177,30	12,47	175,40	9,87	180,30
9,73	176,20	13,25	172,70	10,80	178,00
10,52	172,80	14,88	172,10	11,37	175,00
11,32	175,40	15,87	169,60	13,28	174,40
12,02	169,80	18,40	167,70	13,72	174,70
13,25	172,70	19,15	167,70	14,85	172,70
14,57	171,30	19,95	170,50	16,23	170,90
15,55	167,90	21,07	170,90	18,30	170,90
16,37	165,30	22,28	165,90	19,28	171,50
17,10	171,20	23,12	169,80	20,32	172,90
18,68	171,00	25,73	172,00	21,05	171,50
19,68	171,40	27,08	170,80	22,60	170,70
21,37	171,00	28,43	171,00	23,57	170,70
				24,58	171,20
				25,45	171,10
				26,90	169,80
				29,57	169,50
				30,78	168,70
				33,72	169,40
				35,08	173,00
				36,47	170,90
				37,40	172,20
				39,35	167,20

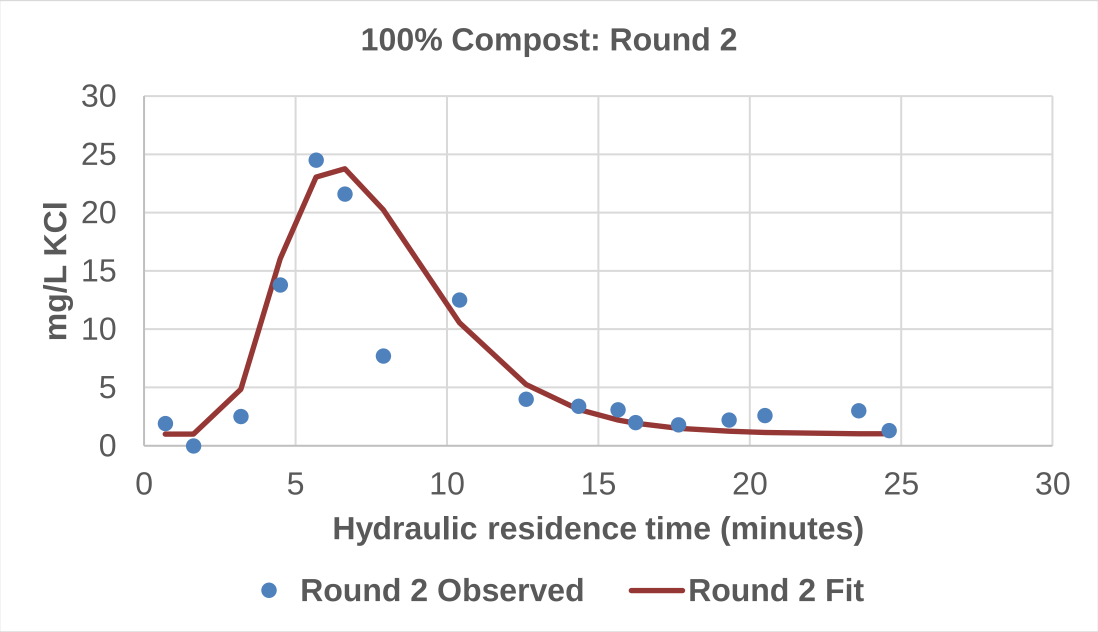
Tracer results for 100% sand

Round 1		Round 2		Round 3	
Time (minutes)	**EC (µS/cm)**	**Time (minutes)**	**EC (µS/cm)**	**Time (minutes)**	**EC (µS/cm)**
0,07	171,00	0,65	174,50	0,52	176,60
0,98	173,70	1,12	174,00	1,07	175,00
2,43	183,10	2,48	181,80	1,75	173,20
2,95	191,30	3,22	199,60	2,38	181,00
3,83	200,00	4,02	208,00	3,13	193,00
4,45	200,00	4,55	208,00	3,88	206,00
4,90	206,00	5,27	215,00	5,73	205,00
5,65	206,00	5,98	209,00	6,32	207,00
6,12	200,00	6,52	206,00	6,92	200,00
7,28	198,30	7,28	204,00	7,55	200,00
7,68	194,00	8,48	197,90	8,17	198,10
9,12	192,30	9,13	194,60	8,88	198,10
9,75	188,50	9,82	189,80	9,78	195,50
10,75	188,40	10,08	189,30	10,35	195,30
11,73	186,40	11,35	185,30	11,10	187,90
12,18	186,70	12,18	184,70	11,80	192,70
13,18	184,10	13,25	182,10	12,80	187,20
14,13	180,00	14,07	183,00	13,55	185,80
14,75	181,00	14,78	181,00	14,42	178,80
15,50	182,40	15,48	179,10	15,27	184,00
16,32	176,70	16,27	180,00	16,27	183,60
16,83	179,00	16,73	175,70	17,00	184,30
17,57	179,10	17,27	179,00	17,98	183,20
18,32	176,80	18,12	178,80	19,78	176,90
18,83	177,00	18,65	176,00	20,65	179,10
21,52	174,60	19,50	174,90	21,78	178,10
23,12	170,80	20,37	174,40	23,10	178,00
		20,90	172,00	24,53	170,40

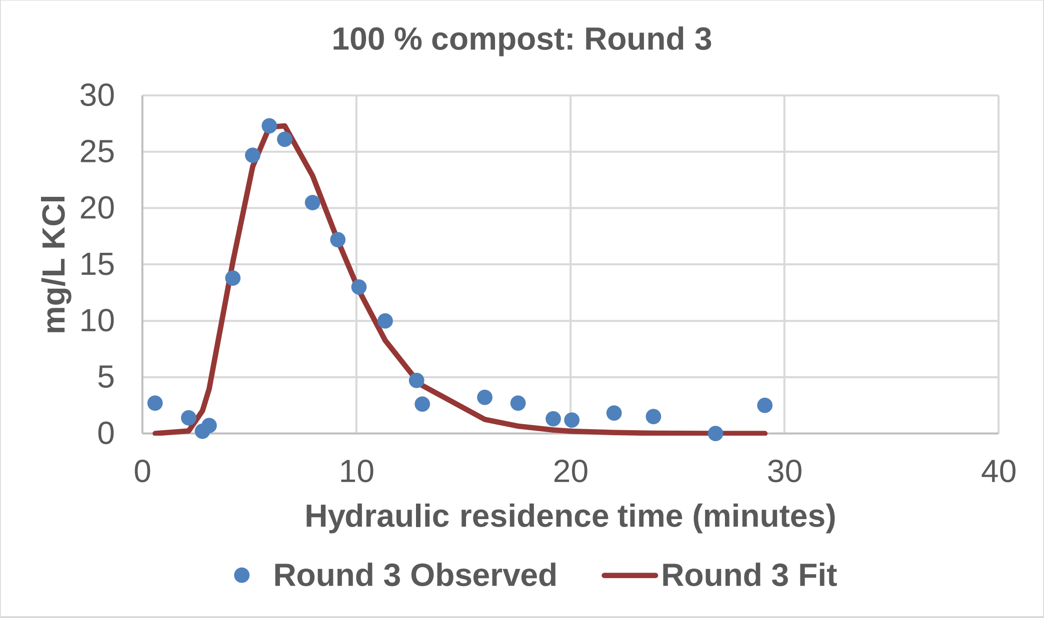
APPENDIX H: Radial Convergent Test (RCT)



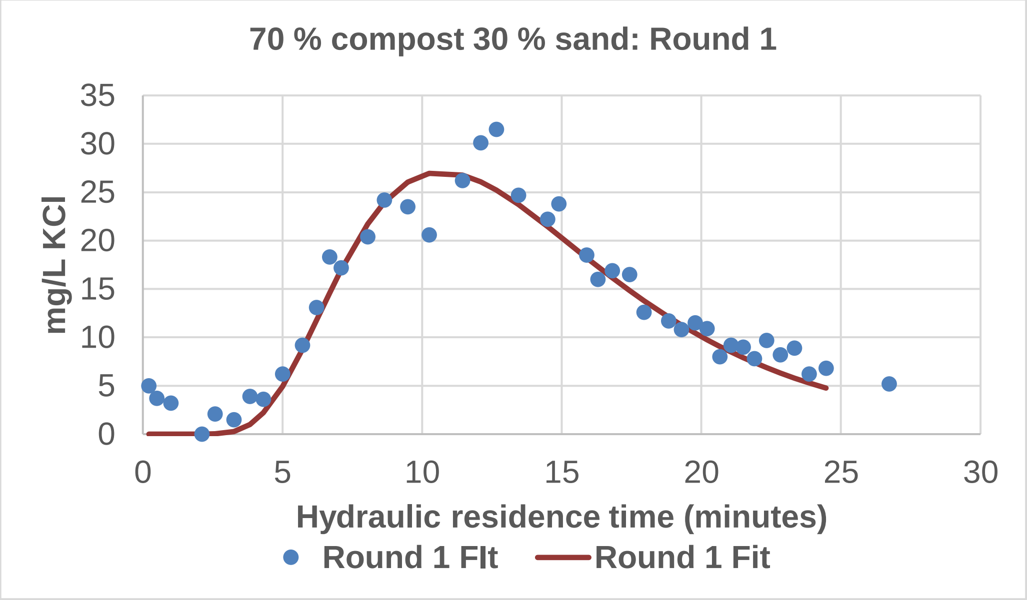
Results from tracer testing on 100 % compost-round 1 (Correlation (r): 98%; RMSE: 1.85 mg/L).



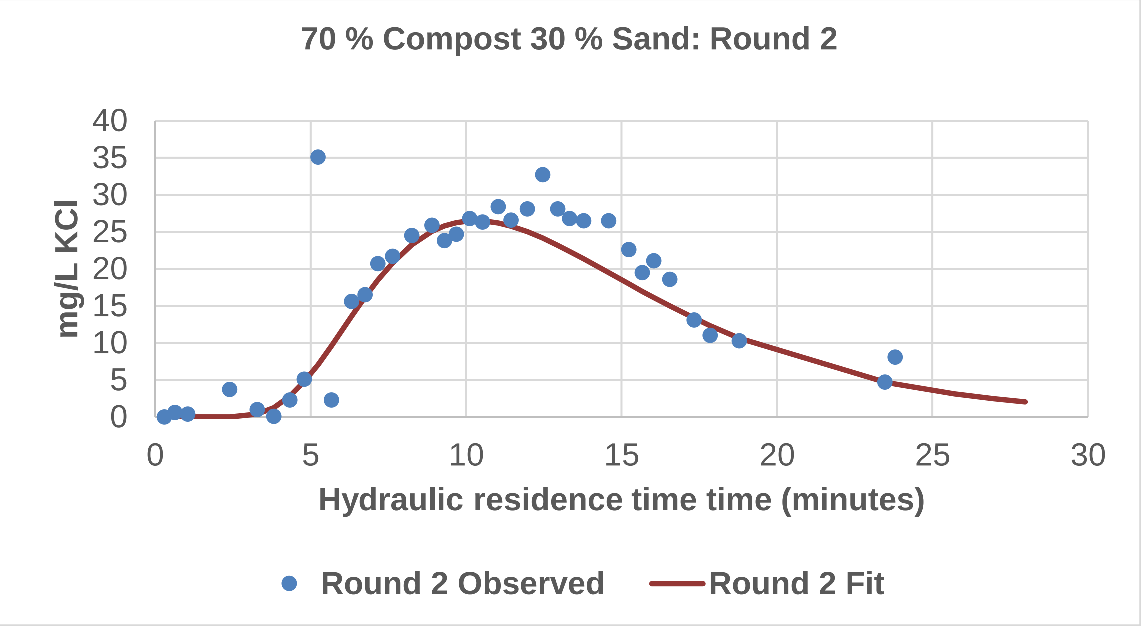
Results from tracer testing on 100 % compost-round 2 (Correlation (r): 92%; RMSE: 3.34 mg/L).



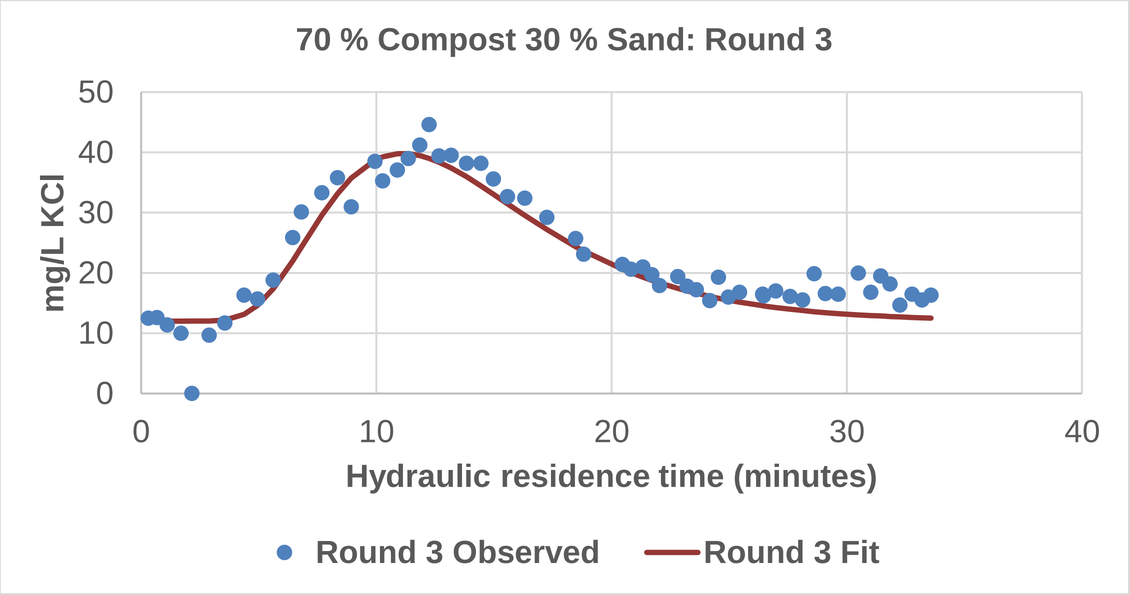
Results from tracer testing on 100 % compost-round 3 (Correlation (r): 99%; RMSE: 1.65 mg/L).



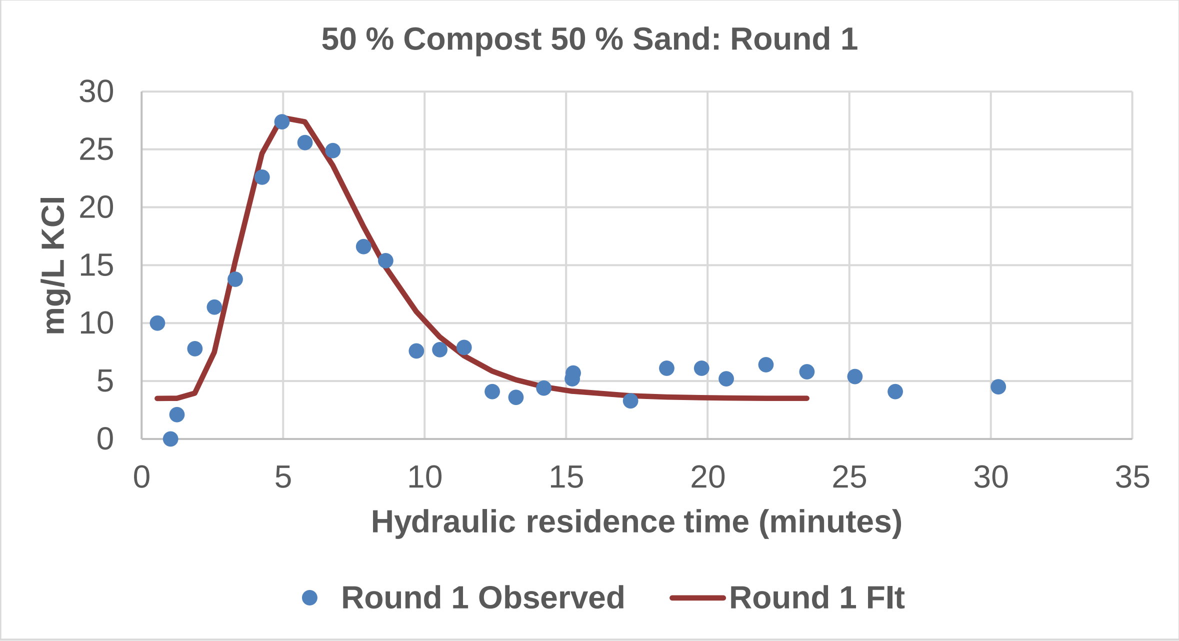
Results from tracer testing on 70 % compost and 30 % sand-round 1 (Correlation (r): 97%; RMSE: 2.48 mg/L).



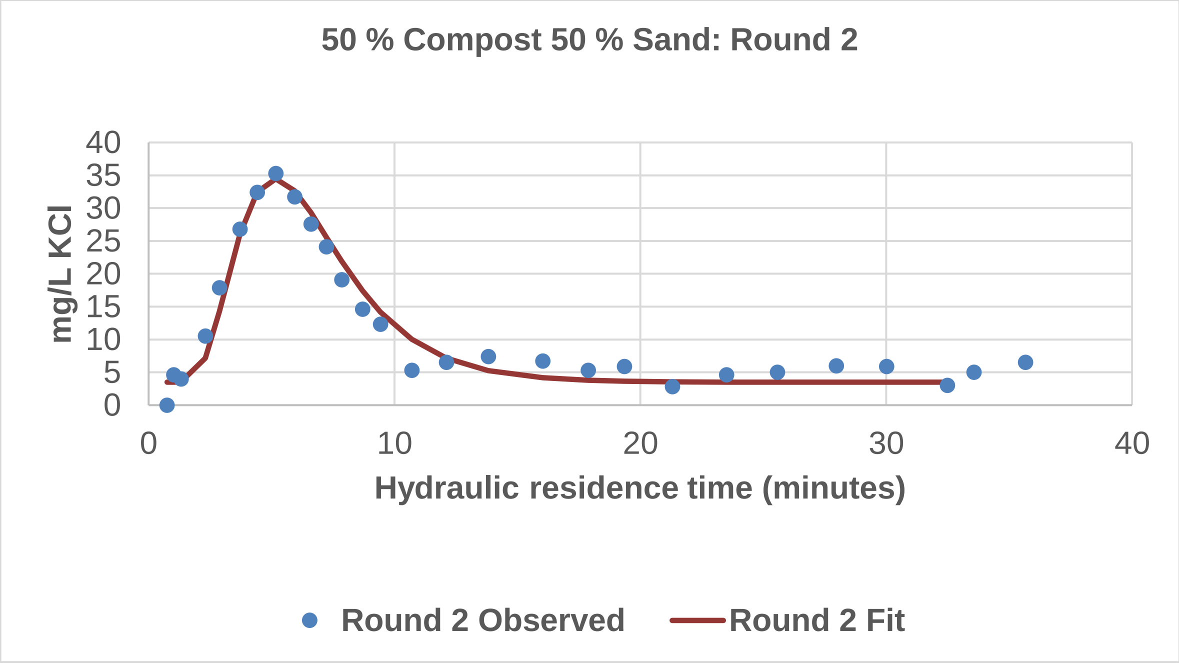
Results from tracer testing on 70 % compost and 30 % sand-round 2 (Correlation: (r) 90%; RMSE: 5.28 mg/L).



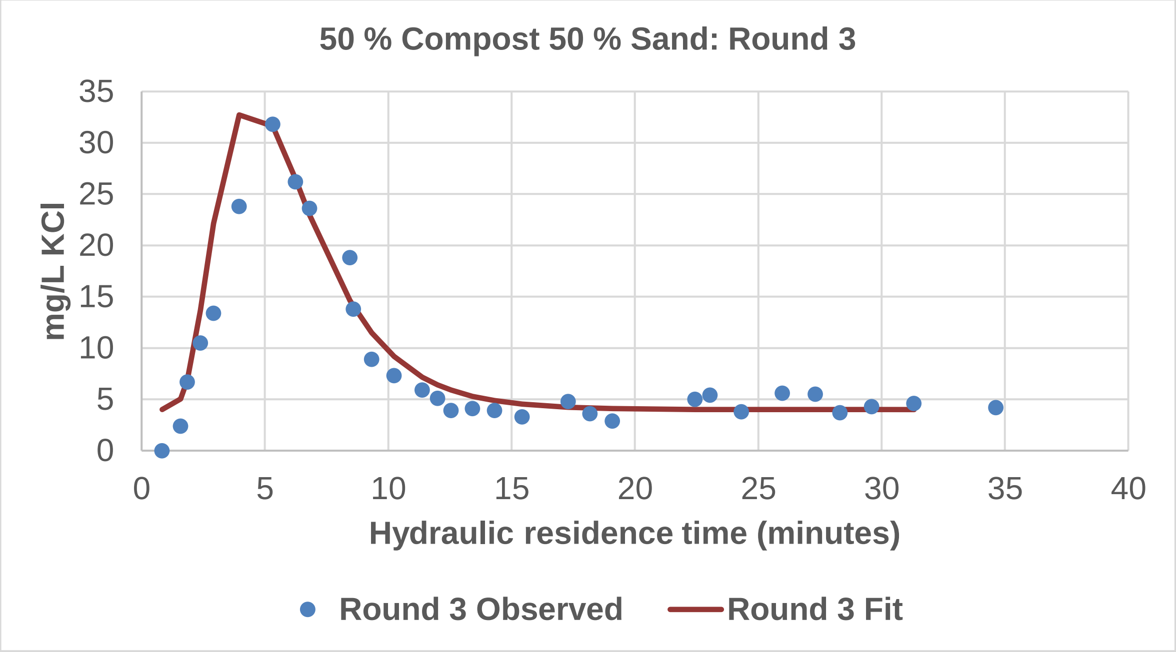
Results from tracer testing on 70 % compost and 30 % sand-round 3 (Correlation (r): 95%; RMSE: 3.32 mg/L).



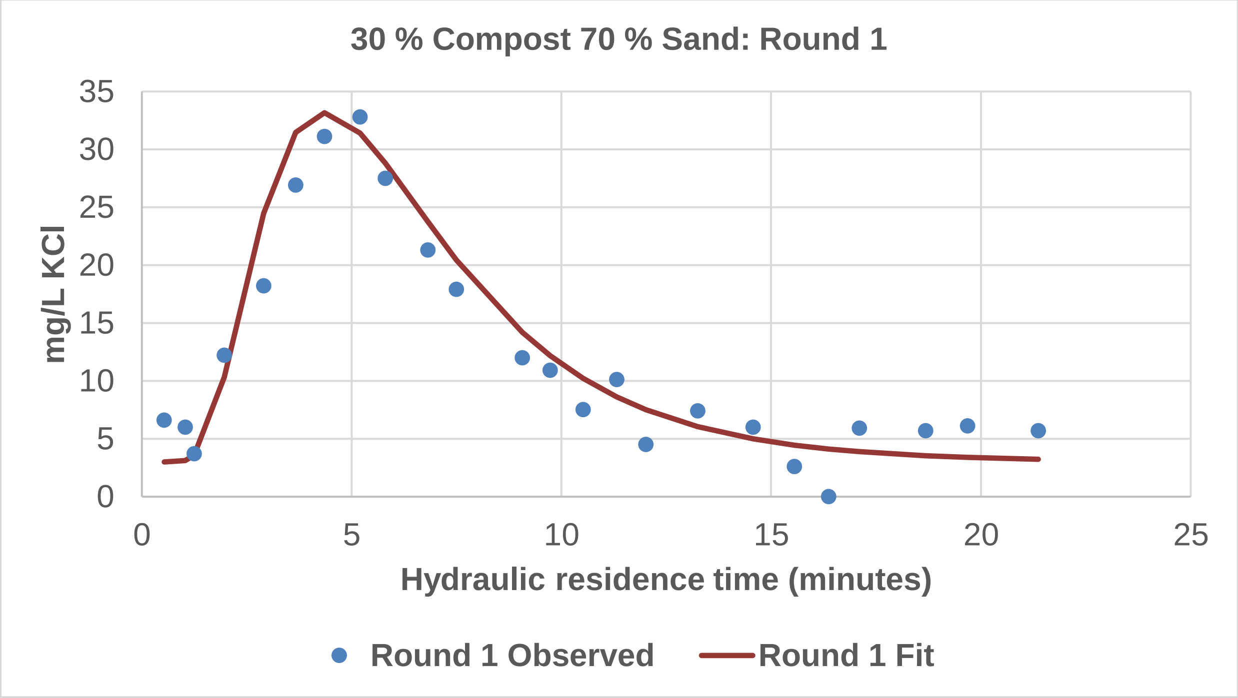
Results from tracer testing on 50 % compost and 50 % sand-round 1 (Correlation (r): 96 %; RMSE: 2.33 mg/L).



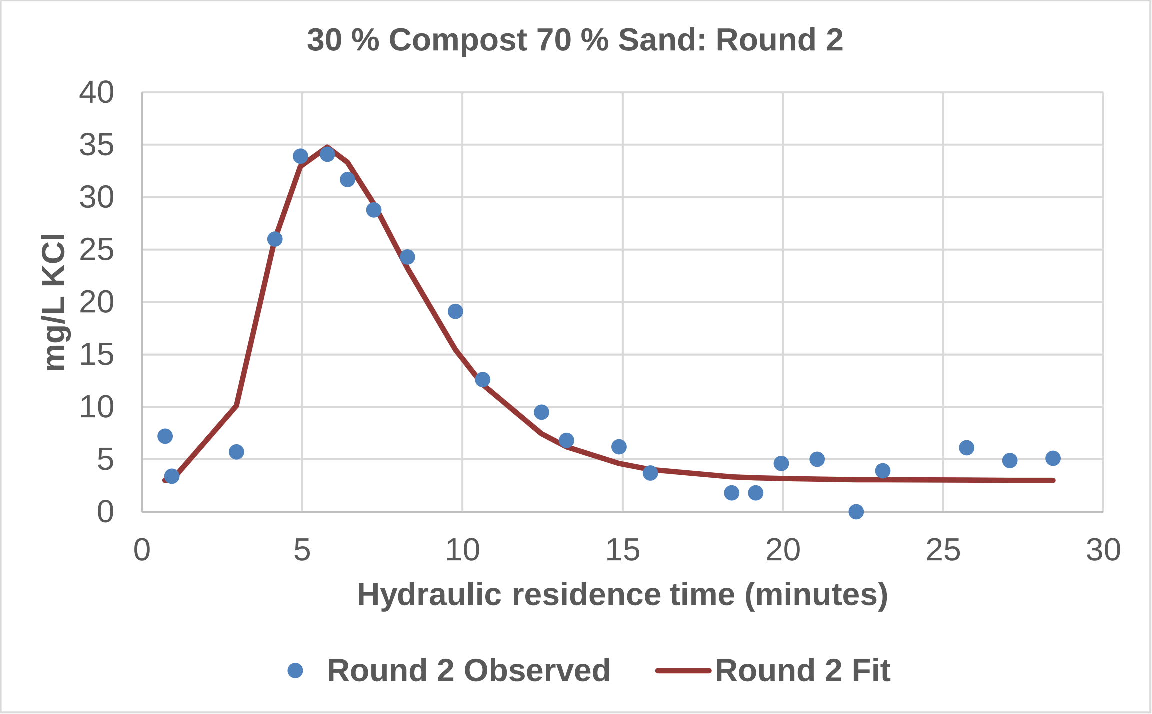
Results from tracer testing on 50 % compost and 50 % sand-round 2 (Correlation (r): 98%; RMSE 2.18 mg/L).



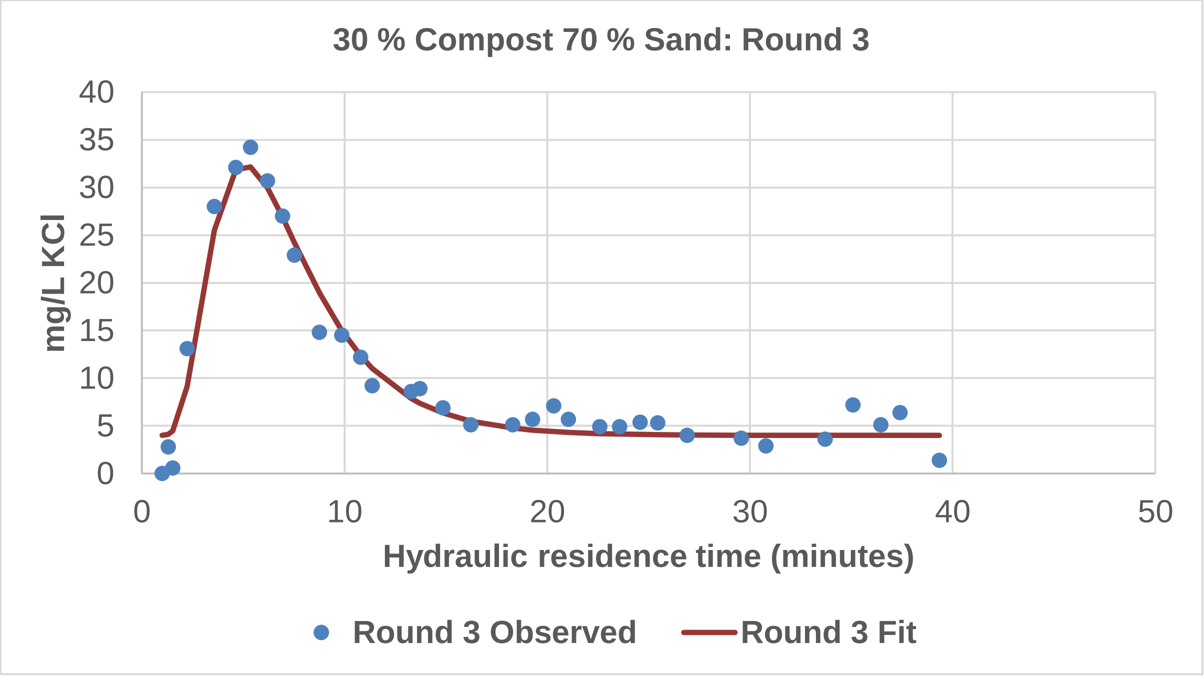
Results from tracer testing on 50 % compost and 50 % sand-round 3 (Correlation (r): 95%, RMSE: 2.77 mg/L).



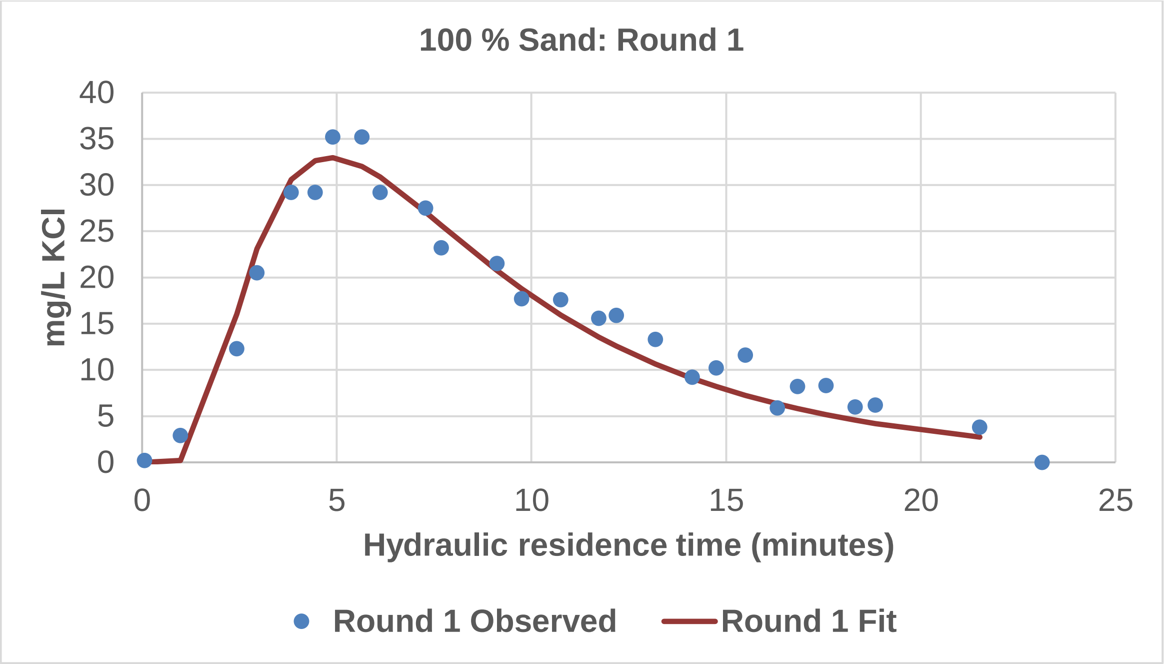
Results from tracer testing on 30 % compost and 70 % sand-round 1 (Correlation (r): 97%; RMSE: 2.71 mg/L).



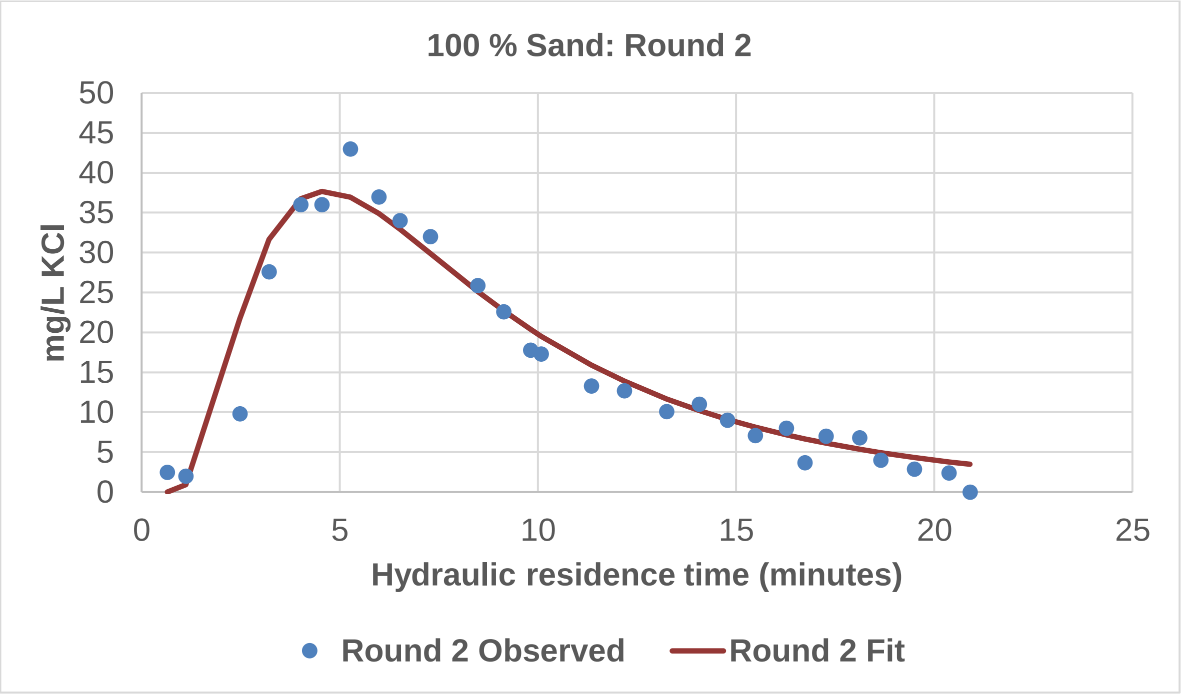
Results from tracer testing on 30 % compost and 70 % sand-round 2 (Correlation (r): (98%; RMSE: 3.08 mg/L; RMSE: 2.05 mg/L).



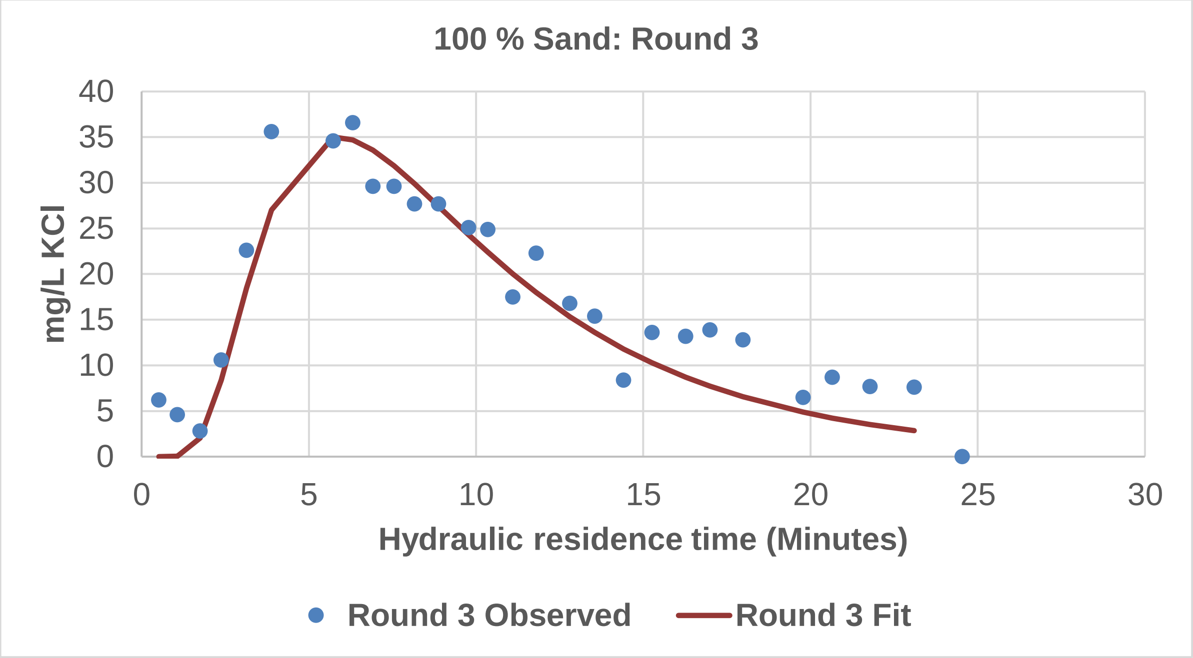
Results from tracer testing on 30 % compost and 70 % sand-round 3 (Correlation (r): 98%; RMSE: 1.92 mg/L).



Results from tracer testing on 100% sand-round 1 (Correlation (r): 98; RMSE: 2.3 mg/).



Results from tracer testing on 100% sand-round 2 (Correlation (r): 98%; RMSE: 3.11 mg/L).



Results from tracer testing on 100% sand-round 3. (Correlation (r): 96%; RMSE: 3.83 mg/L).

R^2=0.5948

APPENDIX I: Kinetic Modelled Data

Modelled sulphate and acetate utilisation with varying sulphate concentrations

500 mg/L Sulphate				700 mg/L Sulphate				900 mg/L Sulphate				1200 mg/L Sulphate			
HRT Days	**C_Acetate**	**HRT Days**	**C_Sulphate**	**HRT Days**	**C_Acetate**	**HRT Days**	**C_Sulphate**	**HRT Days**	**C_Acetate**	**HRT Days**	**C_Sulphate**	**HRT Days**	**C_Acetate**	**HRT Days**	**C_Sulphate**
0,20	818,98	0,20	480,54	0,20	819,15	0,20	680,81	0,20	819,32	0,20	881,16	0,20	819,57	0,20	1181,80
0,40	796,02	0,40	460,00	0,40	796,19	0,40	660,27	0,40	796,35	0,40	860,62	0,40	796,60	0,40	1161,30
0,60	772,23	0,60	438,73	0,60	772,39	0,60	639,00	0,60	772,56	0,60	839,35	0,60	772,80	0,60	1140,00
0,80	747,61	0,80	416,72	0,80	747,77	0,80	616,98	0,80	747,93	0,80	817,33	0,80	748,18	0,80	1118,00
1,00	722,16	1,00	393,97	1,00	722,32	1,00	594,23	1,00	722,48	1,00	794,57	1,00	722,72	1,00	1095,20
1,20	695,89	1,20	370,50	1,20	696,05	1,20	570,75	1,20	696,21	1,20	771,09	1,20	696,45	1,20	1071,70
1,40	668,83	1,40	346,32	1,40	668,98	1,40	546,56	1,40	669,14	1,40	746,89	1,40	669,37	1,40	1047,50
1,60	640,98	1,60	321,45	1,60	641,13	1,60	521,67	1,60	641,29	1,60	721,99	1,60	641,52	1,60	1022,60
1,80	612,38	1,80	295,92	1,80	612,54	1,80	496,12	1,80	612,69	1,80	696,43	1,80	612,91	1,80	997,06
2,00	583,09	2,00	269,78	2,00	583,23	2,00	469,94	2,00	583,38	2,00	670,24	2,00	583,60	2,00	970,87
2,20	553,14	2,20	243,07	2,20	553,28	2,20	443,20	2,20	553,43	2,20	643,48	2,20	553,64	2,20	944,09
2,40	522,61	2,40	215,88	2,40	522,75	2,40	415,94	2,40	522,89	2,40	616,20	2,40	523,10	2,40	916,80
2,60	491,59	2,60	188,28	2,60	491,73	2,60	388,26	2,60	491,86	2,60	588,49	2,60	492,07	2,60	889,06
2,80	460,18	2,80	160,40	2,80	460,31	2,80	360,24	2,80	460,44	2,80	560,43	2,80	460,64	2,80	860,98
3,00	428,50	3,00	132,38	3,00	428,63	3,00	331,99	3,00	428,75	3,00	532,15	3,00	428,94	3,00	832,67
3,20	396,69	3,20	104,42	3,20	396,81	3,20	303,66	3,20	396,93	3,20	503,75	3,20	397,11	3,20	804,24
3,40	364,93	3,40	76,86	3,40	365,04	3,40	275,39	3,40	365,15	3,40	475,40	3,40	365,32	3,40	775,85
3,60	333,39	3,60	50,36	3,60	333,49	3,60	247,36	3,60	333,60	3,60	447,27	3,60	333,76	3,60	747,67
3,80	302,29	3,80	26,89	3,80	302,39	3,80	219,77	3,80	302,49	3,80	419,55	3,80	302,64	3,80	719,88
4,00	271,86	4,00	11,68	4,00	271,95	4,00	192,85	4,00	272,04	4,00	392,43	4,00	272,18	4,00	692,70
4,20	242,34	4,20	5,84	4,20	242,43	4,20	166,84	4,20	242,51	4,20	366,16	4,20	242,64	4,20	666,35
4,40	214,01	4,40	3,68	4,40	214,08	4,40	142,00	4,40	214,16	4,40	340,95	4,40	214,27	4,40	641,04
4,60	187,10	4,60	2,66	4,60	187,17	4,60	118,61	4,60	187,23	4,60	317,03	4,60	187,34	4,60	617,02

500 mg/L Sulphate				**700 mg/L Sulphate**				**900 mg/L Sulphate**				**1200 mg/L Sulphate**			
4,80	161,87	4,80	2,09	4,80	161,92	4,80	96,98	4,80	161,98	4,80	294,64	4,80	162,08	4,80	594,51
5,00	138,53	5,00	1,72	5,00	138,58	5,00	77,40	5,00	138,63	5,00	273,95	5,00	138,71	5,00	573,68
5,20	117,25	5,20	1,48	5,20	117,29	5,20	60,23	5,20	117,34	5,20	255,13	5,20	117,41	5,20	554,70
5,40	98,16	5,40	1,31	5,40	98,20	5,40	45,78	5,40	98,23	5,40	238,26	5,40	98,29	5,40	537,68
5,60	81,30	5,60	1,19	5,60	81,33	5,60	34,35	5,60	81,36	5,60	223,40	5,60	81,41	5,60	522,65
5,80	66,66	5,80	1,09	5,80	66,68	5,80	25,95	5,80	66,71	5,80	210,52	5,80	66,74	5,80	509,60
6,00	54,13	6,00	1,02	6,00	54,15	6,00	20,20	6,00	54,17	6,00	199,52	6,00	54,20	6,00	498,44
6,20	43,59	6,20	0,97	6,20	43,60	6,20	16,41	6,20	43,62	6,20	190,28	6,20	43,64	6,20	489,04
6,40	34,83	6,40	0,93	6,40	34,84	6,40	13,91	6,40	34,85	6,40	182,62	6,40	34,86	6,40	481,24
6,60	27,64	6,60	0,90	6,60	27,65	6,60	12,25	6,60	27,66	6,60	176,35	6,60	27,67	6,60	474,85
6,80	21,82	6,80	0,87	6,80	21,82	6,80	11,10	6,80	21,83	6,80	171,28	6,80	21,84	6,80	469,66
7,00	17,14	7,00	0,86	7,00	17,14	7,00	10,30	7,00	17,15	7,00	167,21	7,00	17,15	7,00	465,50
7,20	13,41	7,20	0,84	7,20	13,41	7,20	9,72	7,20	13,42	7,20	163,97	7,20	13,42	7,20	462,18
7,40	10,46	7,40	0,83	7,40	10,46	7,40	9,30	7,40	10,47	7,40	161,41	7,40	10,47	7,40	459,56
7,60	8,14	7,60	0,82	7,60	8,14	7,60	8,99	7,60	8,14	7,60	159,40	7,60	8,14	7,60	457,49
7,80	6,32	7,80	0,82	7,80	6,32	7,80	8,76	7,80	6,32	7,80	157,83	7,80	6,32	7,80	455,87
8,00	4,90	8,00	0,81	8,00	4,90	8,00	8,59	8,00	4,90	8,00	156,60	8,00	4,90	8,00	454,61
8,20	3,79	8,20	0,81	8,20	3,79	8,20	8,46	8,20	3,79	8,20	155,64	8,20	3,79	8,20	453,63
8,40	2,93	8,40	0,80	8,40	2,93	8,40	8,36	8,40	2,93	8,40	154,90	8,40	2,94	8,40	452,86
8,60	2,27	8,60	0,80	8,60	2,27	8,60	8,28	8,60	2,27	8,60	154,32	8,60	2,27	8,60	452,27
8,80	1,75	8,80	0,80	8,80	1,75	8,80	8,22	8,80	1,75	8,80	153,88	8,80	1,75	8,80	451,81
9,00	1,35	9,00	0,80	9,00	1,35	9,00	8,18	9,00	1,35	9,00	153,53	9,00	1,35	9,00	451,46
9,20	1,04	9,20	0,80	9,20	1,04	9,20	8,14	9,20	1,04	9,20	153,26	9,20	1,04	9,20	451,18
9,40	0,81	9,40	0,80	9,40	0,81	9,40	8,12	9,40	0,81	9,40	153,06	9,40	0,81	9,40	450,97
9,60	0,62	9,60	0,80	9,60	0,62	9,60	8,10	9,60	0,62	9,60	152,90	9,60	0,62	9,60	450,81
9,80	0,48	9,80	0,80	9,80	0,48	9,80	8,08	9,80	0,48	9,80	152,78	9,80	0,48	9,80	450,68
10,00	0,37	10,00	0,80	10,00	0,37	10,00	8,07	10,00	0,37	10,00	152,68	10,00	0,37	10,00	450,58

Modelled sulphate and acetate utilisation with varying acetate concentrations

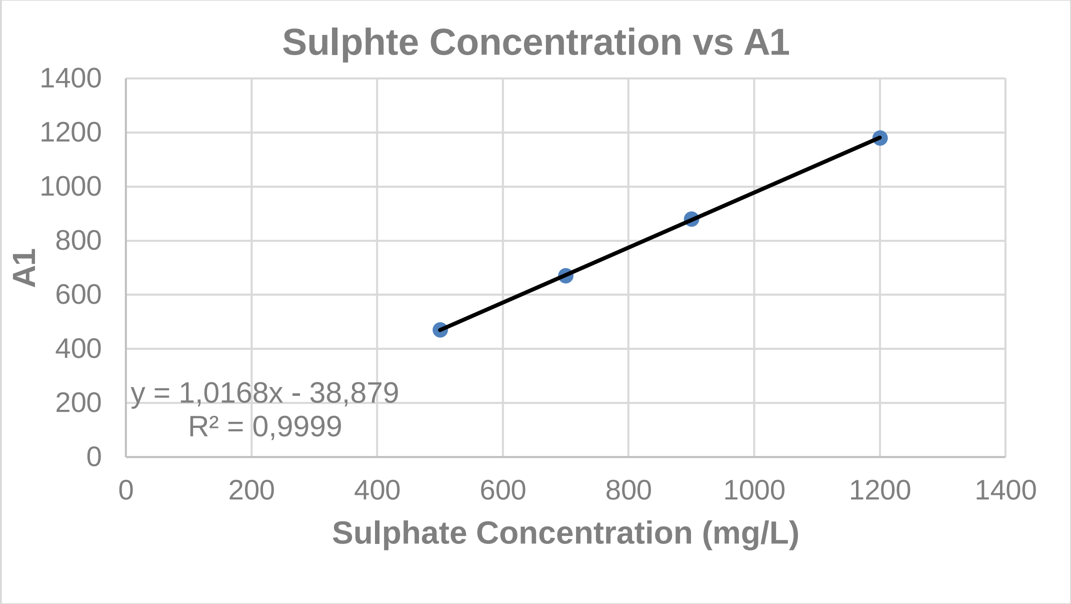
200 mg/L Acetate				500 mg/L Acetate				700 mg/L Acetate				800 mg/L Acetate			
HRT Days	**C_Acetate**	**HRT Days**	**C_Sulphate**	**HRT Days**	**C_Acetate**	**HRT Days**	**C_Sulphate**	**HRT Days**	**C_Acetate**	**HRT Days**	**C_Sulphate**	**HRT Days**	**C_Acetate**	**HRT Days**	**C_Sulphate**
0,20	188,55	0,20	489,65	0,20	481,92	0,20	483,55	0,20	679,88	0,20	481,52	0,20	779,20	0,20	480,79
0,40	177,13	0,40	479,44	0,40	462,94	0,40	466,58	0,40	658,24	0,40	462,17	0,40	756,59	0,40	460,56
0,60	165,92	0,60	469,42	0,60	443,58	0,60	449,27	0,60	635,93	0,60	442,22	0,60	733,18	0,60	439,64
0,80	154,97	0,80	459,62	0,80	423,88	0,80	431,65	0,80	612,96	0,80	421,69	0,80	708,99	0,80	418,01
1,00	144,29	1,00	450,08	1,00	403,87	1,00	413,77	1,00	589,35	1,00	400,58	1,00	684,03	1,00	395,70
1,20	133,94	1,20	440,82	1,20	383,62	1,20	395,66	1,20	565,13	1,20	378,94	1,20	658,30	1,20	372,70
1,40	123,94	1,40	431,88	1,40	363,16	1,40	377,38	1,40	540,32	1,40	356,77	1,40	631,82	1,40	349,05
1,60	114,33	1,60	423,29	1,60	342,57	1,60	358,98	1,60	514,98	1,60	334,13	1,60	604,63	1,60	324,77
1,80	105,13	1,80	415,06	1,80	321,91	1,80	340,53	1,80	489,14	1,80	311,06	1,80	576,77	1,80	299,89
2,00	96,37	2,00	407,23	2,00	301,27	2,00	322,09	2,00	462,88	2,00	287,62	2,00	548,27	2,00	274,46
2,20	88,05	2,20	399,80	2,20	280,73	2,20	303,75	2,20	436,26	2,20	263,87	2,20	519,21	2,20	248,54
2,40	80,21	2,40	392,79	2,40	260,38	2,40	285,59	2,40	409,39	2,40	239,91	2,40	489,66	2,40	222,21
2,60	72,85	2,60	386,21	2,60	240,32	2,60	267,69	2,60	382,34	2,60	215,82	2,60	459,70	2,60	195,55
2,80	65,97	2,80	380,06	2,80	220,67	2,80	250,17	2,80	355,26	2,80	191,72	2,80	429,46	2,80	168,68
3,00	59,56	3,00	374,34	3,00	201,53	3,00	233,10	3,00	328,26	3,00	167,74	3,00	399,04	3,00	141,74
3,20	53,64	3,20	369,04	3,20	183,00	3,20	216,60	3,20	301,49	3,20	144,03	3,20	368,61	3,20	114,92
3,40	48,17	3,40	364,16	3,40	165,20	3,40	200,76	3,40	275,12	3,40	120,77	3,40	338,32	3,40	88,47
3,60	43,16	3,60	359,68	3,60	148,23	3,60	185,68	3,60	249,32	3,60	98,16	3,60	308,36	3,60	62,82
3,80	38,58	3,80	355,58	3,80	132,18	3,80	171,42	3,80	224,27	3,80	76,47	3,80	278,95	3,80	38,96
4,00	34,41	4,00	351,86	4,00	117,14	4,00	158,08	4,00	200,17	4,00	56,10	4,00	250,29	4,00	19,64
4,20	30,62	4,20	348,48	4,20	103,16	4,20	145,70	4,20	177,19	4,20	37,73	4,20	222,64	4,20	9,17
4,40	27,21	4,40	345,43	4,40	90,28	4,40	134,32	4,40	155,52	4,40	22,72	4,40	196,21	4,40	5,22
4,60	24,13	4,60	342,68	4,60	78,54	4,60	123,97	4,60	135,30	4,60	12,91	4,60	171,25	4,60	3,55
4,80	21,37	4,80	340,21	4,80	67,92	4,80	114,64	4,80	116,66	4,80	7,96	4,80	147,95	4,80	2,69
5,00	18,89	5,00	338,00	5,00	58,42	5,00	106,30	5,00	99,70	5,00	5,57	5,00	126,52	5,00	2,17

200 mg/L Acetate				500 mg/L Acetate				700 mg/L Acetate				800 mg/L Acetate			
5,20	16,68	5,20	336,02	5,20	49,99	5,20	98,93	5,20	84,47	5,20	4,30	5,20	107,06	5,20	1,84
5,40	14,71	5,40	334,27	5,40	42,56	5,40	92,47	5,40	70,97	5,40	3,54	5,40	89,68	5,40	1,62
5,60	12,96	5,60	332,70	5,60	36,09	5,60	86,85	5,60	59,16	5,60	3,05	5,60	74,38	5,60	1,45
5,80	11,41	5,80	331,32	5,80	30,47	5,80	81,99	5,80	48,95	5,80	2,72	5,80	61,11	5,80	1,33
6,00	10,03	6,00	330,09	6,00	25,64	6,00	77,83	6,00	40,24	6,00	2,48	6,00	49,78	6,00	1,25
6,20	8,82	6,20	329,00	6,20	21,51	6,20	74,29	6,20	32,88	6,20	2,31	6,20	40,23	6,20	1,18
6,40	7,74	6,40	328,04	6,40	17,99	6,40	71,29	6,40	26,72	6,40	2,18	6,40	32,30	6,40	1,13
6,60	6,80	6,60	327,20	6,60	15,02	6,60	68,75	6,60	21,63	6,60	2,08	6,60	25,77	6,60	1,09
6,80	5,96	6,80	326,45	6,80	12,51	6,80	66,62	6,80	17,43	6,80	2,01	6,80	20,46	6,80	1,06
7,00	5,23	7,00	325,79	7,00	10,40	7,00	64,84	7,00	14,01	7,00	1,95	7,00	16,17	7,00	1,03
7,20	4,58	7,20	325,22	7,20	8,63	7,20	63,35	7,20	11,23	7,20	1,91	7,20	12,74	7,20	1,02
7,40	4,01	7,40	324,71	7,40	7,16	7,40	62,11	7,40	8,98	7,40	1,87	7,40	10,01	7,40	1,00
7,60	3,51	7,60	324,26	7,60	5,93	7,60	61,08	7,60	7,17	7,60	1,85	7,60	7,84	7,60	0,99
7,80	3,08	7,80	323,87	7,80	4,90	7,80	60,22	7,80	5,71	7,80	1,83	7,80	6,14	7,80	0,98
8,00	2,69	8,00	323,53	8,00	4,06	8,00	59,52	8,00	4,55	8,00	1,81	8,00	4,79	8,00	0,98
8,20	2,36	8,20	323,23	8,20	3,35	8,20	58,93	8,20	3,62	8,20	1,80	8,20	3,74	8,20	0,97
8,40	2,06	8,40	322,97	8,40	2,77	8,40	58,44	8,40	2,87	8,40	1,79	8,40	2,92	8,40	0,97
8,60	1,80	8,60	322,74	8,60	2,29	8,60	58,04	8,60	2,28	8,60	1,78	8,60	2,27	8,60	0,96
8,80	1,58	8,80	322,53	8,80	1,89	8,80	57,71	8,80	1,81	8,80	1,77	8,80	1,77	8,80	0,96
9,00	1,38	9,00	322,36	9,00	1,56	9,00	57,43	9,00	1,44	9,00	1,77	9,00	1,38	9,00	0,96
9,20	1,21	9,20	322,20	9,20	1,28	9,20	57,21	9,20	1,14	9,20	1,76	9,20	1,07	9,20	0,96
9,40	1,06	9,40	322,07	9,40	1,06	9,40	57,02	9,40	0,90	9,40	1,76	9,40	0,83	9,40	0,96
9,60	0,92	9,60	321,95	9,60	0,87	9,60	56,86	9,60	0,72	9,60	1,76	9,60	0,65	9,60	0,96
9,80	0,81	9,80	321,85	9,80	0,72	9,80	56,74	9,80	0,57	9,80	1,75	9,80	0,50	9,80	0,96
				10,00	0,59	10,00	56,63	10,00	0,45	10,00	1,75	10,00	0,39	10,00	0,96

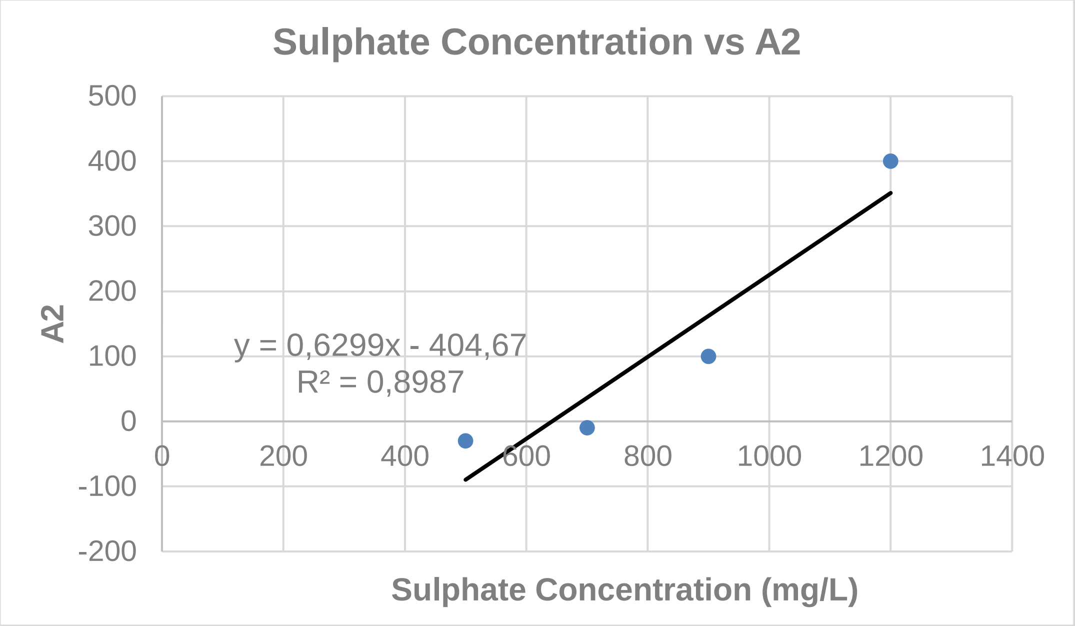
SULPHATE REDUCTION AND ACETATE UTILISATION FUNCTION (model 1)

Fitting function parameters used in generalised mathematical model

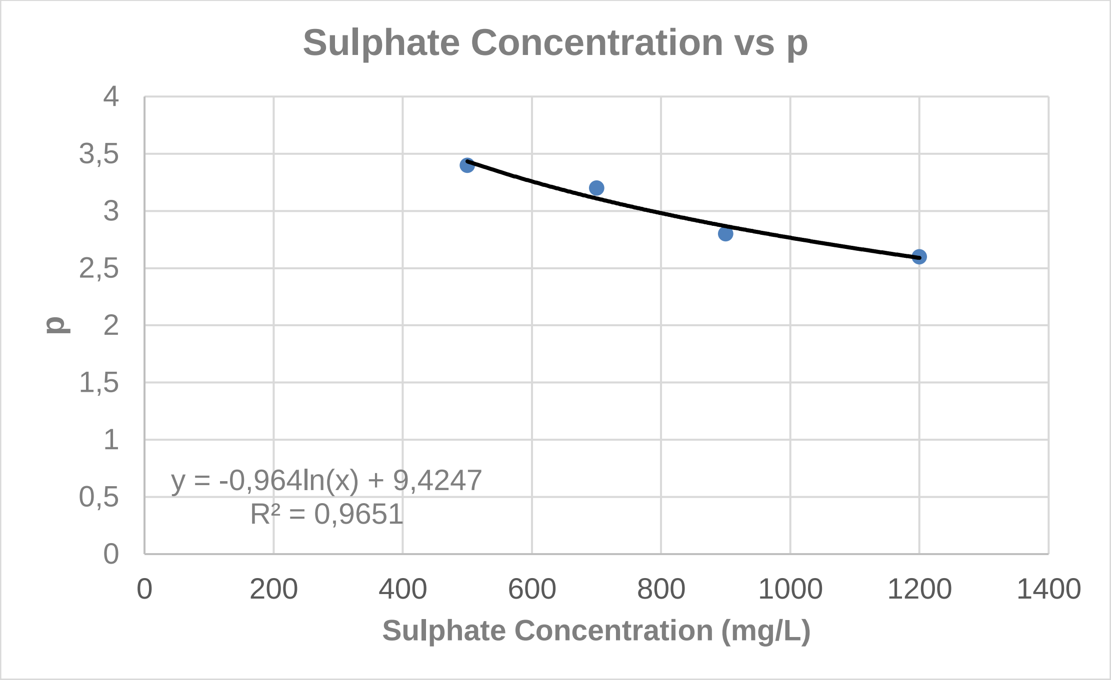
Sulphate concentration →	**500**	**700**	**900**	**1200**
Function parameters ↓				
A1	470	670	880	1180
A2	-30	-10	100	400
p	3,4	3,2	2,8	2,6
x_0	2,5	3	3,3	3,4



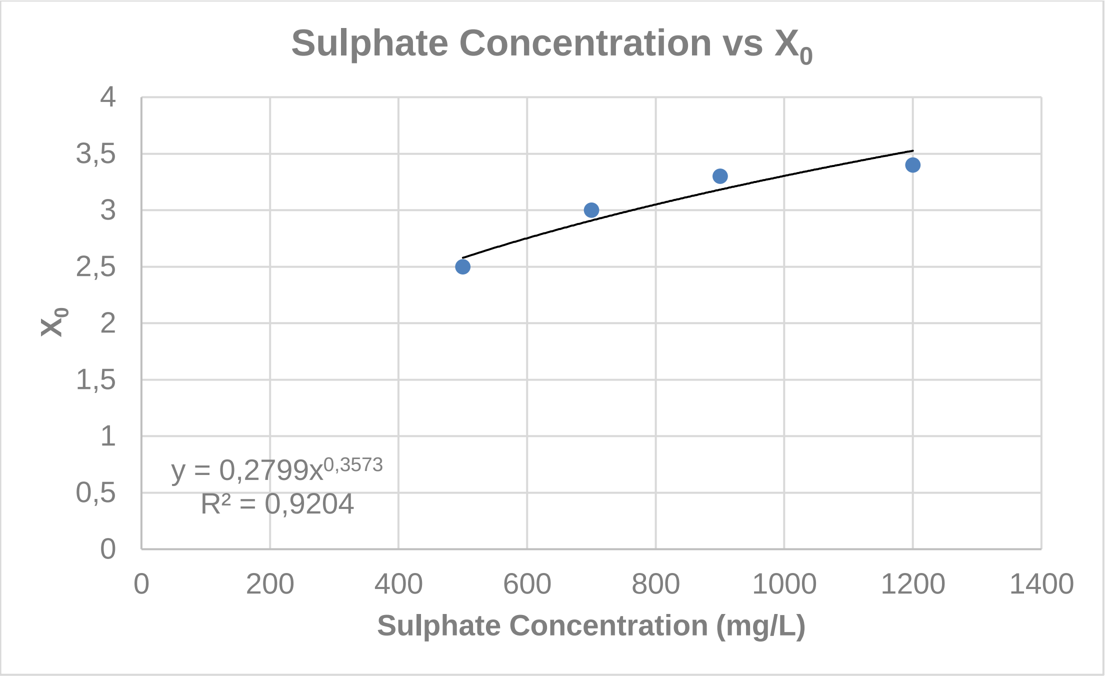
Function for feed concentration (SO_4^{2-}) versus A1



Function for feed concentration (SO_4^{2-}) versus A2



Function for feed concentration (SO_4^{2-}) versus p



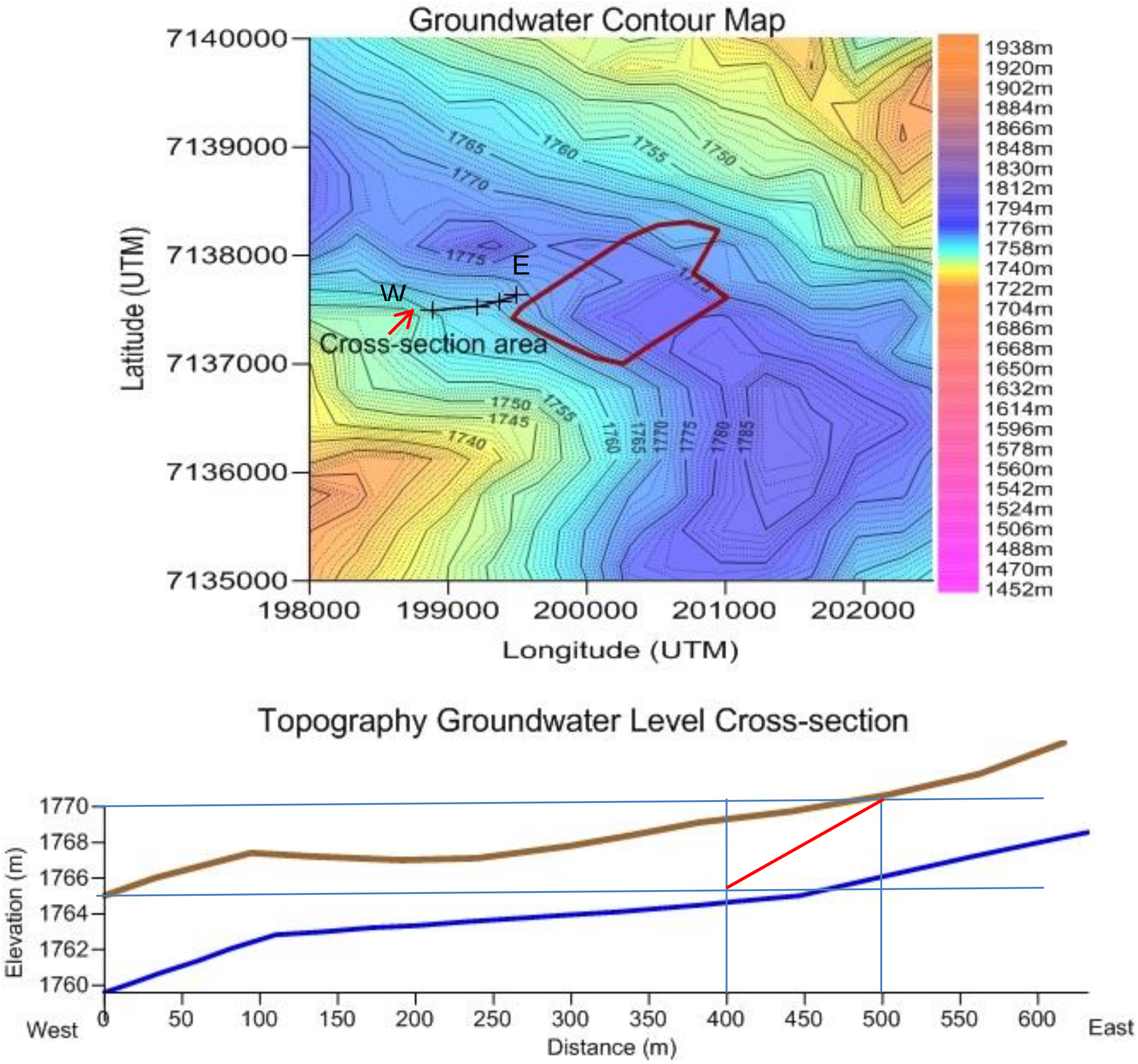
Function for feed concentration (SO_4^{2-}) versus X_0

APPENDIX J: Groundwater Map

Groundwater contour map borehole information

Name	X	Y	z (topo)	z (WL)	WL
1	29.95528	-25.92861	1744,00	1744,00	0,00
2	30.06667	-25.89583	1704,00	1692,00	12,00
3	29.90000	-25.82778	1759,00	1759,00	0,00
4	29.97723	-25.96556	1686,00	1683,87	2,13
5	29.93334	-25.76666	1857,00	1849,00	8,00
6	29.96667	-25.87723	1714,00	1704,00	10,00
7	29.83333	-25.83250	1696,00	1686,00	10,00
8	30.10389	-25.95000	1524,00	1518,00	6,00
9	30.01945	-25.95694	1679,00	1673,21	5,79
10	29.96554	-25.97723	1680,00	1670,00	10,00
11	29.92500	-25.75473	1776,00	1769,00	7,00
12	29.92806	-25.86834	1690,00	1685,40	4,60
13	30.01667	-25.81528	1757,00	1757,00	0,00
14	30.06667	-25.84694	1716,00	1716,00	0,00
15	29.95000	-25.80667	1778,00	1772,00	6,00
16	30.00889	-25.82045	1790,00	1790,00	0,00
17	30.03056	-25.76111	1855,00	1855,00	0,00
18	30.03056	-25.76114	1855,00	1850,00	5,00
19	29.92807	-25.86834	1690,00	1679,34	10,66
20	30.01667	-25.91250	1695,00	1677,00	18,00
21	30.06667	-25.79305	1795,00	1765,00	30,00
22	29.98333	-25.78084	1852,00	1852,00	0,00
23	30.00800	-25.85288	1781,00	1781,00	0,00
24	30.00833	-25.86028	1767,00	1760,30	6,70
25	29.96444	-25.88140	1697,00	1697,00	0,00
26	30.00800	-25.85290	1776,00	1769,50	6,50
27	30.00063	-25.86422	1757,00	1749,90	7,10

Topo: Topography *WL: Water level*



Groundwater topography cross-section at possible excavation area

Red line: Site specific gradient (i)

APPENDIX K: Detail Designs with Remaining Substrates

100% Compost Substrate

Design Parameters	Value	Unit	Source Term	Error value	Error %
Hydraulic Design					
Volumetric flow (Q) rate	18,33	m^3/day	Decant volume, plus 10% safety factor		
	18333,33	L/day			
Hydraulic Conductivity (K)	41,687	m/d	Experimentally calculated	0,2720	0,6526
Porosity (n)	0,7068		Experimentally calculated	0.0084	1,1948
HRT	4,0	Days	General Mathematical Model		
Hydraulic gradient (i)	0,051		Calculated (site specific)		
Seepage Velocity	2,98	m/d	Calculated	0,055	1,847
Length	11,93	m	Calculated	0,220	1,847
Depth	1,20	m	Design parameter		
Cross-sectional area (A)	8,70	m^2	Design parameter		
Width	7,25	m	Calculated		
Void Volume	103,75	m^3	Calculated	1,917	1,847
Volume compost required (100% of substrate volume)	103,75	m^3	Calculated	1,917	1,847
Volume sand required (0% of substrate volume)	0,00	m^3	Calculated	0,00	1,847
Dry density compost	469,90	Kg/m^3	Experimentally calculated	10,00	2,128
Mass compost required	48754,01	Kg	Calculated	1938,207	3,975
Physicochemical design factors					
	Value	**Unit**	**Source Term**	**Error value**	**Error %**
Molar mass sulphate	96,06	g/mol	Literature	-	-
	96062,60	mg/mol		-	-
Molar mass compost	170,00	g/mol	Literature	-	-
Ratio (compost/acetate)	1,68		Literature	-	-
Useable compost by mass	0,30		Literature	-	-
Dry density compost	469,90	kg/ m^3	Experimentally calculated	10,00	2,128
Bio-physical design (calculate Compost required to reduce sulphate from 700 mg/L to < 250 mg/L)					
Influent sulphate concentration	700,00	mg/L	Site specific	-	-

Design Parameters	Value	Unit	Source Term	Error value	Error %
Target sulphate concentration	250,00	mg/L	DWS (IUCMA) Requirement	-	-
Effluent (product) sulphate concentration	195,20	mg/L	General Mathematical Model with 4 day HRT		
Sulphate removed	504,80	mg/L		-	-
	9254666,67	mg/day		-	-
Moles sulphate removal rate	96,34	mol/day	Calculated	-	-
DOC removal rate	96,34	mol/day	Calculated	-	-
Moles available compost required	161,75	mol/day	Calculated	-	-
Total microbial available compost required over operational time	85730,04	moles	Calculated	-	-
Mass available compost required	14574106,24	gram	Calculated	-	-
Useable and unusable mass compost (simple and complex organics)	48580354,15	gram	Calculated	-	-
Useable and unusable mass compost (simple and complex organics)	48580,35	Kg	Calculated	-	-
Volume compost required	103,38	m^3	Calculated		
Maximum operation time	530,00	days	Design parameter	-	-

70% compost 30% sand substrate

Design Parameters	Value	Unit	Source Term	Error value	Error %
Hydraulic Design					
Volumetric flow (Q) rate	18,33	m^3/day	Decant volume, plus 10% safety factor		
	18333,33	L/day			
Hydraulic Conductivity (K)	41,06	m/d	Experimentally calculated	0,2720	0,6626
Porosity (n)	0,6161		Experimentally calculated	0,0074	1,1948
HRT	4,0	Days	General Mathematical Model		
Hydraulic gradient (i)	0,050		Calculated (site specific)		
Seepage Velocity	3,34	m/d	Calculated	0,062	1,857
Length	13,37	m	Calculated	0,248	1,857
Depth	1,20	m	Design parameter		
Cross-sectional area (A)	8,90	m^2	Design parameter		
Width	7,42	m	Calculated		
Void Volume	119,03	m^3	Calculated	2,211	1,857
Volume compost required (70% of substrate volume)	83,32	m^3	Calculated	1,548	1,857
Volume sand required (30% of substrate volume)	35,71	m^3	Calculated	0,66	1,857
Dry density compost	469,90	Kg/ m^3	Experimentally calculated	10,00	2,128
Mass compost required	39151,98	Kg	Calculated	1560,382	3,985
Physicochemical design factors					
	Value	**Unit**	**Source Term**	**Error value**	**Error %**
Molar mass sulphate	96,06	g/mol	Literature	-	-
	96062,60	mg/mol		-	-
Molar mass compost	170,00	g/mol	Literature	-	-
Ratio (compost/acetate)	1,68		Literature	-	-
Useable compost by mass	0,30		Literature	-	-
Dry density compost	469,90	Kg/ m^3	Experimentally calculated	10,00	2,128
Bio-physical design (calculate Compost required to reduce sulphate from 700 mg/L to < 250 mg/L)					
	Value	**Unit**	**Source Term**	**Error value**	**Error %**
Influent sulphate concentration	700,00	mg/L	Site specific	-	-
Target sulphate concentration	250,00	mg/L	DWS (IUCMA) Requirement	-	-

Design Parameters	Value	Unit	Source Term	Error value	Error %
Effluent (product) sulphate concentration	195,20	mg/L	General Mathematical Model with 4 day HRT		
Sulphate removed mg/l	504,80	mg/L		-	-
	9254666,67	mg/day		-	-
Moles sulphate removal rate	96,34	mol/day	Calculated	-	-
DOC removal rate	96,34	mol/day	Calculated	-	-
Moles available compost required	161,75	mol/day	Calculated	-	-
Total microbial available compost required over operational time	69554,56	moles	Calculated	-	-
Weight available compost required	11824274,88	gram	Calculated	-	-
Useable and unusable mass compost (simple and complex organics)	39414249,59	gram	Calculated	-	-
Useable and unusable mass compost (simple and complex organics)	39414,25	Kg	Calculated	-	-
Volume compost required	83,88	m^3	Calculated		
Maximum operation time	430,00	days	Design parameter	-	-

30% compost 70% sand

Design Parameters	**Value**	**Unit**	**Source Term**	**Error value**	**Error %**
Hydraulic Design					
Volumetric flow (Q) rate	18,33	m^3/day	Decant volume, plus 10% safety factor		
	18333,33	L/day			
Hydraulic Conductivity (K)	20,162	m/d	Experimentally calculated	0,2720	1,3493
Porosity (n)	0,5282		Experimentally calculated	0,0063	1,1948
HRT	4,0	Days	General Mathematical Model		
Hydraulic gradient (i)	0,051		Calculated (site specific)		
Seepage Velocity	1,93	m/d	Calculated	0,049	2,544
Length	7,71	m	Calculated	0,196	2,544
Depth	1,20	m	Design parameter		
Cross-sectional area (A)	18,00	m^2	Design parameter		
Width	15,00	m	Calculated		
Void Volume	138,84	m^3	Calculated	3,532	2,544
Volume compost required (30% of substrate volume)	41.65	m^3	Calculated	1,060	2,544
Volume sand required (70% of substrate volume)	97.19	m^3	Calculated	2,472	2,544
Dry density compost	469.90	Kg/m^3	Experimentally calculated	10,00	2,128
Mass compost required	19571.75	Kg	Calculated	914,429	4,672
Physicochemical design factors					
	Value	**Unit**	**Source Term**	**Error value**	**Error %**
Molar mass sulphate	96,06	g/mol	Literature	-	-
	96062,60	mg/mol		-	-
Molar mass compost	170,00	g/mol	Literature	-	-
Ratio (compost/acetate)	1,68		Literature	-	-
Useable compost by mass	0,30		Literature	-	-
Dry density compost	469,90	kg/ m^3	Experimentally calculated	10,00	2,128
Bio-physical design (calculate Compost required to reduce sulphate from 700 mg/L to < 250 mg/L)					
	Value	**Unit**	**Source Term**	**Error value**	**Error %**
Influent sulphate concentration	700,00	mg/L	Site specific	-	-
Target sulphate concentration	250,00	mg/L	DWS (IUCMA) Requirement	-	-

Design Parameters	Value	Unit	Source Term	Error value	Error %
Effluent (product) sulphate concentration	195,20	mg/L	General Mathematical Model with 4 day HRT		
Sulphate removed mg/l	504,80	mg/L		-	-
	9254666,67	mg/day		-	-
Moles sulphate removal rate	96,34	mol/day	Calculated	-	-
DOC removal rate	96,34	mol/day	Calculated	-	-
Moles available compost required	161,75	mol/day	Calculated	-	-
Total microbial available compost required over operational time	31218,67	moles	Calculated	-	-
Weight available compost required	5307174,54	gram	Calculated	-	-
Useable and unusable mass compost (simple and complex organics)	17690581,79	gram	Calculated	-	-
Useable and unusable mass compost (simple and complex organics)	17690,58	Kg	Calculated	-	-
Volume compost required	37,65	m^3	Calculated		
Maximum operation time	193,00	days	Design parameter	-	-

The 100% sand substrate contains no organic material. No detail design was therefore conducted for 100% sand substrate.