

Hydrodynamic performance of a biological sulphate reduction reactor treating high-sulphate acid mine drainage

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ABSTRACT

This master's degree was focused on investigating the evolution of the hydraulic behaviour of a packed bed reactor over time and the effects of these changes on the sulphate removal performance of the reactor. This was achieved by monitoring the hydraulic behaviour over the duration of the study with tracer tests to observe any changes in the flow patterns through the reactor. The physical structure of the packing material was investigated by stabilising and sectioning the packed bed. Computational fluid dynamic modelling of the flow through the reactors was done to further understand the experimental residence time distribution results. This work then enabled the determination of a potential link between the hydraulic behaviour and the sulphate reducing performance of the process, as the structure of the packed bed evolved over time.

Eleven reactors were set up by using packing material that consisted of a specified mix of wood chips, wood shavings, hay, lucerne and kraal manure. The reactors had an average operating volume of 9.7 L. During the 30-day inoculation period, the sulphate concentrations were lowered on average to below 400 mg/L from initial concentrations of 3450 mg/L. The reactors' process parameters after the 30-day inoculation period led to the decision that adequate inoculation occurred, which meant that sufficient biofilms had established to commence continuous operation.

The reactors were continuously operated at a 10-day hydraulic retention time at a constant temperature of 35 °C. The reactors were operated for different durations as several reactors were sent for physical analysis of the packed bed structure. Continuous operation lasted for 364 days, by which the end of the study was reached. The alkalinity, chemical oxygen demand, electrical conductivity, pH, sulphate and sulphide of the effluent were measured twice a week.

The pH readings of the treated water were maintained close to neutral, which was in the expected range for a biological sulphate reduction (BSR) system treating mine water. The oxidation-reduction potential readings of the reactor's effluents were maintained below -240 mV throughout the study, and below -300 mV during the high-performance phase. The electrical conductivity values of the effluent remained relatively constant compared to the feed, which indicated that there was minimal removal of dissolved chemicals/salts and minimal production of carbonates throughout the process.

All the reactors demonstrated similar sulphate removal performance trends exhibiting distinct phases. The lag phase took place from start-up to day 70, the high-performance phase was observed from day 71 to day 120, the crash phase occurred from day 121 to day 130, and the sustained phase lasted from day 131 to day 364.

A peak sulphate reduction rate of 224 g/m³/d with an average rate of 116 g/m³/d was achieved during the high-performance phase. The average sulphate-removal rate in this study was similar to the removal rates achieved in the large-scale integrated management passive (IMPI) and cloSURE® plants, which were 192 g/m³/d and 233 g/m³/d, respectively. The lower removal rates achieved during this study were attributed to the lower amounts of organic carbon in the packing material, and the continuous addition of supplemental substrate in the large-scale tests.

The similarity of the performance indicators of all the reactors indicated high reproducibility and demonstrated the possibility of repeating the results in an upscaling of the process in field operations.

The sequential tracer tests indicated that the tanks-in-series model fitted the experimental tracer responses very well and was therefore used to observe the change of the flow behaviour over the operational periods of the reactors. In general, the mean residence time tended to decrease over time, suggesting that the flow regime developed gradually, indicating the possibility of channelling and bypassing in portions of the reactor. The observed ranges of the residence time distributions among the reactors suggested that the individual packing structures had a significant impact on their respective hydraulic behaviours.

The structure of the packed bed was further investigated through physical analysis and computational fluid dynamic modelling of the process. The images produced by the physical analysis experiments showed the presence of channels and bypassing occurring in some sections of the packed bed. At the top end of the reactors, a relatively even distribution of flow was observed, with a reduction of the flow through the middle of the packed bed, as more flow occurred closer to the walls. The computational fluid dynamic modelled tracer response peaks did not completely overlap, but the shape of the tracer responses exhibited similar behaviours, with the one reactor's residence time distribution peaking earlier with a narrower spread, and the other reactors' residence time distribution curves peaking later with a wider spread. Sensitivity analysis of the diffusion coefficient showed that with a 50-fold increase, similar residence time distribution responses were achieved. These results highlighted the importance of investigating a tracer's properties in new applications, as this could have a large impact on modelling the predicted flows in a packed bed reactor.

The results of this study indicated that the hydraulic behaviour and the permeability of the packed bed during the operational periods of the reactors had a negligible impact on the sulphate removal rates, whereas the availability of organic substrate was the determining factor for attaining high rates of sulphate removal. Providing sufficient organic carbon and ensuring effective transportation of the substrate to the bacteria are major considerations that need to be included as part of the design criteria of a biological sulphate reduction plant to ensure successful implementation of the process at scale.

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NOMENCLATURE

Symbol	Unit	Description
A	-	Pre-multiplier value
α	-	Womersley number
C	mol	Concentration
$E(t)$	-	Dimensionless residence time distribution function
g	m/s ²	Gravity
L	m	Radius
M	-	Evaluation of all momentum, viscous, and body force terms in the momentum conservation equation at the relevant boundary
n	-	Normal vectors
N	-	Number of tanks-in-series
ΔN	mol	Amount of tracer
ρ	kg/m ³	Density
Pe_r	-	Peclet number
σ	d ²	Variance
σ_t	-	Dimensionless variance
τ	-	Tangent vector
t	d	Time
t_m	d	Mean residence time
μ	kg/m/s	Dynamic viscosity
ν	m ² /s	Kinematic viscosity
v	m/s	Velocity
ω	°/s	Angular frequency

ABBREVIATIONS

Abbreviation	Definition
AMD	Acid mine drainage
APHA	American Public Health Association
BSR	Biological sulphate reduction
CFD	Computational fluid dynamics
COD	Chemical oxygen demand
EC	Electrical conductivity
HRT	Hydraulic retention time
KHP	Potassium hydrogen phthalate
ORP	Oxidation-reduction potential
OSHA	Occupational Safety and Health Organization
PPE	Personal protective equipment
R1–10	Reactors 1–10
RO	Reverse osmosis
RTD	Residence time distribution
VCC	Vryheid Coronation Colliery

CHAPTER 1 INTRODUCTION

1.1 Background

Over the past century, mining in South Africa has made a substantial contribution to the country's economy. However, over the past few decades, the closure of coal and gold mines and the legacy of abandoned mine sites have resulted in the discharge of high volumes of acid mine drainage (AMD). The discharge of AMD contaminates freshwater resources that are already stressed, resulting in enduring ecological harm to waterways, a decline in biodiversity, and adverse health effects on communities who depend on these resources (Akciil & Koldas, 2006; Naidoo, 2017; Cunha *et al.*, 2019).

AMD can be characterised as having low pH levels and containing heavy metals and minerals. Typically, the AMD in South Africa can have extremely high concentrations of sulphate, reaching values of up to 7 g/L and above, high salinity, and low concentrations of heavy metals (Coetser *et al.*, 2006). These pollutants introduce difficulties for the cost-effective treatment of AMD to conform to strict target water quality guidelines for end use.

There are currently only two strategies being implemented in South Africa for the treatment of AMD at a large scale, namely, neutralisation and membrane filtration. These treatment methods are effective, but cost intensive, due to continuous chemical and operational costs (Bai *et al.*, 2013; Dhir, 2018). The process of neutralising AMD involves the addition of neutralisation agents, such as lime, sodium hydroxide and sodium carbonate, to elevate the water's pH and facilitate the precipitation of metals as carbonate and hydroxide complexes (Coulton *et al.*, 2003). The continuous utilisation of chemicals increases the operational costs of the neutralisation process. This, coupled with the fact that the process cannot remove sulphate to the required target quality guideline levels, makes this method unsustainable, as it requires additional pre- or post-treatment processes to remove the remaining pollutants. Membrane filtration employs a combination of reverse osmosis (RO) along with nano- and micro-filtration. However, the substantial operational and capital expenses associated with membrane technology systems, along with the costs for disposal or treatment of the large volume of brines generated by these processes, render membrane systems economically unfeasible for the sustainable treatment of AMD over the long term (Simate & Ndlovu, 2014).

To address these challenges, passive treatment systems have been employed, leveraging natural chemical and biological processes to remediate AMD (Skousen *et al.*, 2019). Adopting passive treatment technologies offers an alternative that reduces the overall operational costs that are associated with treating AMD (Dhir, 2018). These systems include processes like alkaline leach beds, constructed wetlands, open lime channels and biological sulphate-reducing reactors (Ziemkiewicz *et al.*, 2003; Skousen *et al.*, 2017). Processes such as these are becoming increasingly

popular due to their cost-effective operational expenses and reduced maintenance requirements compared to active treatment methods (Taylor *et al.*, 2005). These processes are intended to be implemented at smaller mine run-off streams, which require lower maintenance and minimal infrastructure. These systems generally produce irrigation quality water, or water fit for re-use in mineral processing. These passive processes are not intended to be used as a direct replacement for active processes to produce quality drinking water, but to lower the need for active processes where they are not necessarily required.

Biological sulphate reduction (BSR) is a low-cost treatment alternative that has successfully been implemented in several countries to precipitate metals and raise the pH of the treated water, ensuring compliance with the discharge water quality guidelines (Chang *et al.*, 2000; Costa *et al.*, 2009; Sánchez-Andrea *et al.*, 2014). Generally, passive BSR processes have been employed for the treatment of low-sulphate water, as the process has an expected lifetime of over a decade, thus making the process an ideal treatment alternative for mine run-off streams in remote areas (Eppink *et al.*, 2020; Shepherd *et al.*, 2020). A few BSR treatment plants have been piloted and demonstrated in South Africa, with the aim of targeting high-sulphate AMD, but only one field-scale plant is currently in operation (Pulles *et al.*, 2016; Neale *et al.*, 2018; Du Preez, 2021). Under South African conditions, the sulphate removal performance of the process for treating high-sulphate mine waters (ranging from 3 to 4 g/L sulphate) typically exhibits a high-performance lifespan of approximately 300 days (Pulles & Heath, 2009). The decline in the sulphate removal performance after 300 days has not yet been adequately investigated to determine what the exact cause of the phenomenon is.

1.2 Problem statement

The BSR process typically consists of a packed bed that immobilises sulphate reducing bacteria and provides a source of organic carbon, which functions as an electron donor for sulphate reduction. When this electron donor is consumed by the consortium of bacteria that is found within the process, including sulphate reducing bacteria, the packed bed material degrades. The degradation has a negative impact on the hydraulic conductivity of the reactor, resulting in a decline of the sulphate removal performance due to constraints in the transport of the electron donor to the bacteria.

It has been seen in previous studies that the process performance declines after approximately 300 days of operation (Pulles *et al.*, 2016; Neale *et al.*, 2018). It is suspected that the process experiences hydraulic stress at this stage, which can cause hydraulic short-circuiting, limiting the availability of the substrate required for optimal microbial activity and resulting in a dramatic reduction in efficiency of the sulphate removal treatment. Mintek has been developing a BSR process for treatment of mine water (cloSURE®) which was piloted in its second iteration from 2017 to 2018, during which the

packed bed was observed to degrade in such a manner that it caused hydraulic issues, as shown in Figure 1.



Figure 1. cloSURE® biological bed degradation, (a) before operation (large sample), (b) after 18 months of operation (500 mL sample)

It is suspected that one of the major challenges of operating a semi-passive BSR process, treating high-sulphate AMD, is the degradation of the packed bed itself. Typically, these passive systems necessitate mechanical interventions to maintain the process's longevity, such as resetting the hydraulic conductivity of the reactor through churning of the packed bed. This practice is initially effective, but the effectivity declines after each churning event of the bed (Shepherd *et al.*, 2020). This intervention becomes impractical, when high sulphide concentrations are found throughout the packed bed, due to the associated dangers involved; and when the process is scaled up, churning of the packed bed becomes impossible due to the scale of the reactors. It is possible to feed additional substrate materials into the system to ensure the continued availability of organic carbon to the sulphate reducing bacteria to allow sulphate reduction to occur. However, these interventions can be cost intensive and only provide a temporary solution to the inevitable hydraulic conductivity issues.

In addition to understanding the physical attributes of a BSR degrading packed bed behaviour over time, there is also a well-established understanding of the timeline encompassing hydrolysis, lignocellulosic breakdown and substrate availability. Tracer studies were conducted at the Vryheid Coronation Colliery (VCC) BSR plant in South Africa; however, the tests were conducted under unknown operational conditions, which limits the interpretation of the results. After a review of the literature, it does not appear that any computational fluid dynamics (CFD) modelling was done of degrading packed beds in a passive biological sulphate reduction process for high-sulphate laden waters to investigate the hydraulic behaviour of such a process.

A novel application of numerical and computational modelling tools involves investigating the fluid flow characteristics of a packed bed BSR reactor and the susceptibility to changes caused by organic material degradation over time, particularly when treating high-sulphate laden waters. This approach facilitates a more in-depth understanding of the fundamentals of the process.

1.3 Research questions

This study will answer the following research questions:

- How does the flow characteristics of a packed bed structure change over the duration of the operation as it evolves?
- To what extent does the physical structure impact the hydraulic flow of the reactor?
- To what extent does the hydraulic behaviour impact the sulphate removal performance of the process?

1.4 Hypothesis

Microbial activity, which results in biological sulphate reduction, degrades the bed material as it is converted to soluble organic carbon. Sequential residence time distribution experiments, done throughout the study, will show the impact of microbial bed degradation on the flow pattern through the bed. This will decrease the residence time through the reactor as channelling and bypassing occurs. Over time, the bed material will lose its permeability, which will negatively impact on the sulphate reducing performance of the reactor.

1.5 Aim and objectives

The project aims to investigate the changes in a degrading packed bed over time to understand the implications of these changes on the performance of the reactor. The proposed objectives of the project are:

- To assess how the bed hydraulic behaviour changes over time in degrading packed bed reactors.
- To determine whether there is a link between the hydraulic behaviour and the sulphate reducing performance of the process as the packed bed degrades.

1.6 Scope of the study

Mintek is engaged in a project for the development of the cloSURE® process for the treatment of AMD, with a variety of research and design options. These options include microbial characterisation, optimisation of process parameters, pre-treatment of feed water, substrate procurement and optimisation, and packed bed design. Mintek is also busy upscaling the cloSURE®

process and demonstrating it at a larger scale. The work conducted in this study forms part of the supporting laboratory scale research, which will add to the design criteria for a commercial plant.

The primary objective of this research study, as indicated in the problem statement, revolves around assessing how the reactor's hydraulic behaviour affects the sulphate removal performance.

1.7 Delineation of the study

This study will focus on measuring the physical characteristics of the packed bed under controlled conditions using real mine water to provide an accurate composition of what could be found under real-world conditions. It will also include residence time distribution experiments to monitor the flow patterns through the packed bed, its porosity and its structure, over the life of a BSR reactor. Performance parameters of the process will also be monitored, including sulphate, sulphide, alkalinity, pH, oxidation-reduction potential, electrical conductivity and chemical oxygen demand.

CFD modelling will be done using data generated from the physical characterisation of the packed bed as inputs for this work and compared to the residence time distribution experiments.

While the microbial characterisation of the reactors forms a part of the larger Mintek research scope, it falls outside the scope of this master's project. This project will focus on the physical aspects only.

1.8 Study outline

In Figure 2, an illustrative overview of this study is presented. Chapter 1 provides a background on mining, the impact of AMD, and the importance of AMD treatment in South Africa. The problem statement, research questions, hypothesis, aim and objectives, project scope and the delineation of the study are defined.

In Chapter 2, a literature survey provides background on relevant topics regarding the scope of the research. Background is provided on the occurrence and impacts of AMD, the structure of the major rivers, and the locations of the mining provinces and their alignment, showing the risk that AMD can pose. Mine water treatment processes currently being used in South Africa are discussed, including alternative treatment options. BSR is discussed fully, to describe the fundamental characteristics of the process, and to comprehend the factors that influence the behaviour of the bacteria. Furthermore, background is provided of previous and current BSR mine water treatment plants, and the shortcomings experienced during their operation. Hydraulic characterisation techniques are discussed, a background on tracer test procedures is given, and the relevant mathematical equations are provided. Physical characterisation practices for analysing packed bed structures are investigated, outlining the relevant options that could be used for this study. Lastly, a basic overview of CFD modelling is provided.

The experimental methodology is described in Chapter 3. The reactor setup procedure is discussed, including the packing material preparation, operating volume determination and inoculation procedure. The reactor operation, the methods for the measurement of the maintenance parameters, and tracer test and physical characterisation procedures are discussed in full. The sulphide safety measures and the required training and personal protective equipment (PPE) are listed.

The results and discussion of the experimental and work are presented in Chapter 4. Firstly, the reactor setup and inoculation, and the continuous operation maintenance data are discussed to compare and identify the performance stages of the reactors. These results are compared to the behaviour of similar processes, which were found in literature. Chapter 5 discusses the modelling work where the tracer test results of each reactor and tracer test run are compared, and the mixing parameters are discussed to determine whether there were changes in the observed hydraulic behaviour. The sulphate reducing performance is then compared to the hydraulic behaviour, to determine whether there is any link between them. The physical characterisation of the packed bed is described, and the data produced is used in the CFD models. The results from the CFD work are compared to the experimental tracer test work, to aid in understanding the observed flow behaviours.

The conclusions of the study and recommendations for future work are highlighted and discussed in Chapter 6.

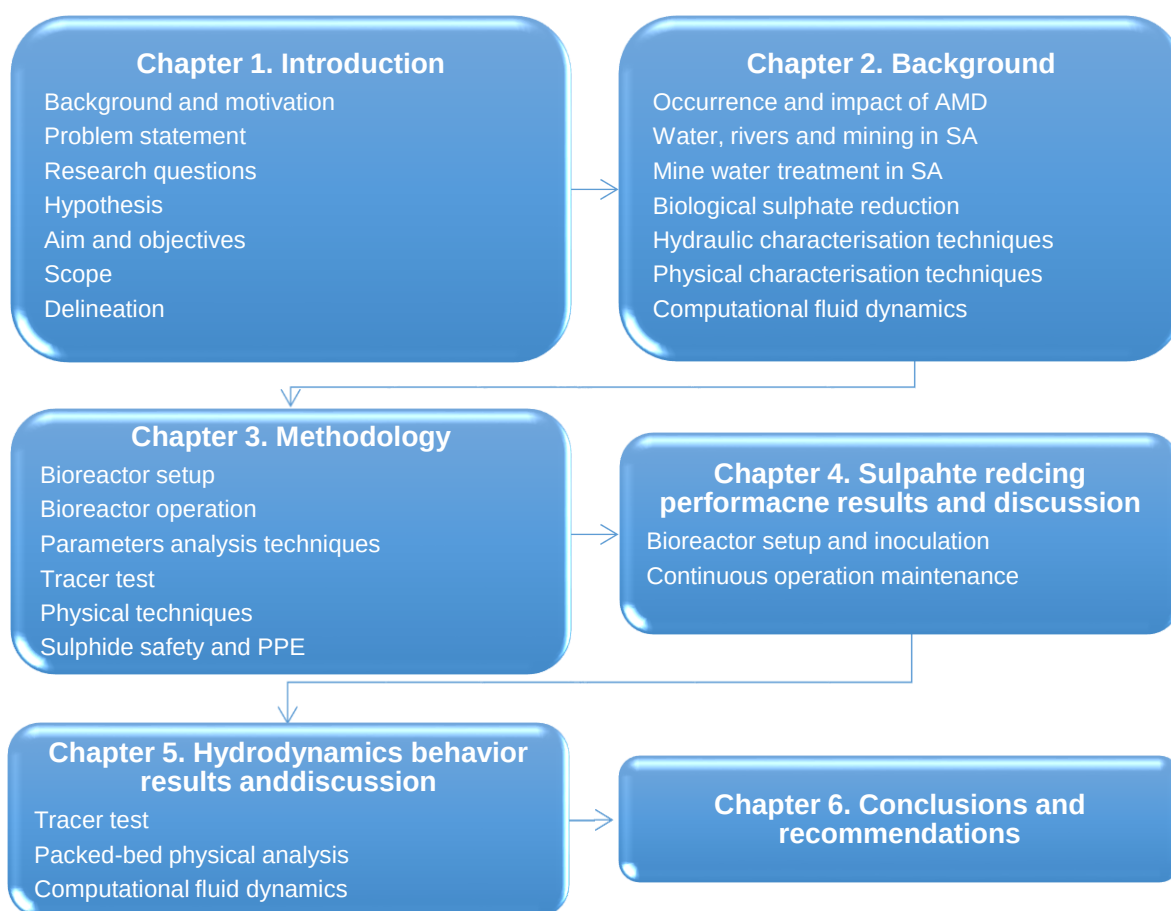


Figure 2. Project outline

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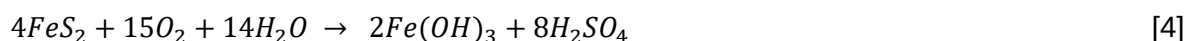
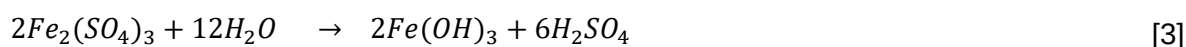
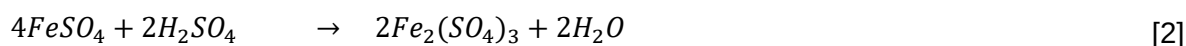
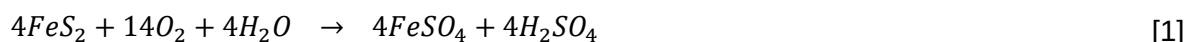
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CHAPTER 2 BACKGROUND

2.1 The occurrence of acid mine drainage (AMD)

Pyrite (FeS_2) is one of the most prevalent sulphide minerals found in nature and is responsible for the formation of most acidic and neutral mine water. Whether the mine water is acidic, sodium-rich, or magnesium/calcium-rich, is determined by the presence of sodium or alkali metals, such as magnesium and calcium carbonates, in the ore (Dama-Fakir *et al.*, 2017).

AMD, which is also referred to as acid rock drainage, is a well-known by-product of mining operations and can be characterised by generally having low pH levels and containing heavy metals and minerals. AMD is formed by an ecological process, as the water levels in underground mines increase and reach higher levels that can bring acidic water and oxygen in contact with sulphide-bearing minerals. The oxidation of pyrite can be expressed by the reactions in Equations [1] – [4] to illustrate the formation of AMD (Barnes & Romberger, 1968):



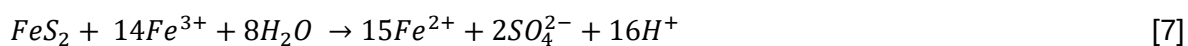
When the pH and bacterial activity in the surrounding environment are correctly balanced, an oxidising environment is formed that oxidises the ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}), as seen in Equation [5] (Johnson & Hallberg, 2003; Akcil & Koldas, 2006):



When the pH values are between 2.3 and 3.5, ferric iron will precipitate as iron (III) oxide-hydroxide ($\text{Fe}(\text{OH})_3$) and jarosite. This reaction will leave small amounts of unreacted ferric iron in solution, while simultaneously lowering the pH of the solution:



The small amount of ferric iron that does not precipitate in Equation [6], is utilised to oxidise additional pyrite, according to Equation [7]:



In all these equations, except for Equation [6], it is assumed that the mineral being oxidised is pyrite, and the oxidant is oxygen. However, other sulphide minerals such as chalcocite (Cu_2S) and pyrrhotite (FeS) have different ratios of metals and metal sulphide than iron and can also be similarly oxidised, due to the acidic nature of the waters, which retain the minerals in solution (Johnson & Hallberg, 2003). There are several other events that can result in the formation of mine impacted water (Maree, *et al.*, 2013):

- The dissolution of limestone/dolomite due to water ingress.
- The partial neutralisation of free acid due to the inherent natural alkalinity of mined and broken rocks.
- Alternating oxidation of pyrite-rich rock when water levels fluctuate in mines due to variable water recharging during seasonal rainfall.
- Dissolution of sulphuric acid and iron sulphates into solution, lowering the pH of the water to a value as low as 1, as mine water levels rise after cessation of mining operations.

2.2 The impact and risks of acid mine drainage

When elements or their complexes, like iron, aluminium, radium, lead, zinc and bismuth, are released into acid water, AMD can become even more toxic (Azarch, 2011; McCarthy, 2011; Naidoo, 2017). If these waters remain untreated, it will cause irreparable environmental degradation, severe health effects in communities who use contaminated water and soil, biodiversity loss and damage to the aquatic system (Azarch, 2011; Han *et al.*, 2017). The impact of AMD on the environment is described in Table 1 (Gray, 1997). The effects of AMD can be so diverse, that the ecological community structure can rapidly collapse so that recovery cannot occur by itself, due to the need to remove the pollutants by physiochemical processes or wait for the pollutants to be biologically degraded, which can take years to happen.

Table 1. The impact of acid mine drainage on the environment (Gray, 1997)

Chemical	Physical	Biological	Ecological
Increased acidity.	Substrate modification.	Behavioural.	Habitat modification.
Reduction of pH.	Increase in stream velocity.	Respiratory.	Niche loss.
Destruction of bicarbonate buffering system.	Turbidity.	Osmoregulation.	Bioaccumulation within food chain.
Increase in soluble metal concentrations.	Sedimentation.	Acute and chronic toxicity.	Loss of food source or prey.
Increase in particulate metals.	Absorption of metals by the sediment.	Death of sensitive species.	Elimination of sensitive species.
	Reduction in turbulence due to sedimentation, increasing laminar flow.	Acid-base balance failure in organisms.	Reduction in primary productivity.
	Decrease in light penetration.	Migration or avoidance.	Food chain modification.

In developing nations, like South Africa, the potential impact of AMD can be considerably larger than would be the case for 1st world countries, due to the lack of infrastructure, capital, and knowledge to treat the pollution. A study conducted by Oskam *et al.* (2021), demonstrated that a considerable number of households in South Africa, particularly those with a lower socio-economic status, face challenges in accessing potable water. Consequently, they have to depend on less safe or distant sources of drinking water, and some households may not have access to clean tap water at all.

The release of AMD places the environment at major risk and raises serious pollution concerns for both present and future generations. With mining operations decreasing globally and more mines closing down, an increased amount of AMD will be produced as rehabilitation of mines and management of mine water post mine closure is a challenge that needs to be addressed

2.3 Water, rivers and mining in South Africa

South Africa is a water-scarce country with a pronounced east-to-west rain-fall gradient, with the western part of the country receiving less than 100 mm of rain per annum compared to the eastern part of the country receiving more than 1000 mm per annum (McCarthy, 2011). Due to the low rain received in the west, combined with high evapotranspiration rates, most of the country has a negative water balance. South Africa also has a very asymmetrical river drainage network, with two major river-flow systems on the eastern and northern escarpments, as shown in Figure 3 (WRC, 2017). The northern part of the country is drained by the Limpopo-Olifants river systems into the Indian Ocean, while on the eastern escarpment the Orange and Vaal rivers rise and flow across the entire country to flow into the Atlantic Ocean. The Vaal River is the major source of water for Gauteng, but also for neighbouring mining districts. Due to the overdependence and overuse of the Vaal River, inter-basin transfers from the Orange and Tugela rivers are required to ensure adequate water capacity for the region (McCarthy, 2011). Increased flows of highly acid water such as AMD into the South African water supply network has put the river systems under even more pressure (Azarch, 2011).

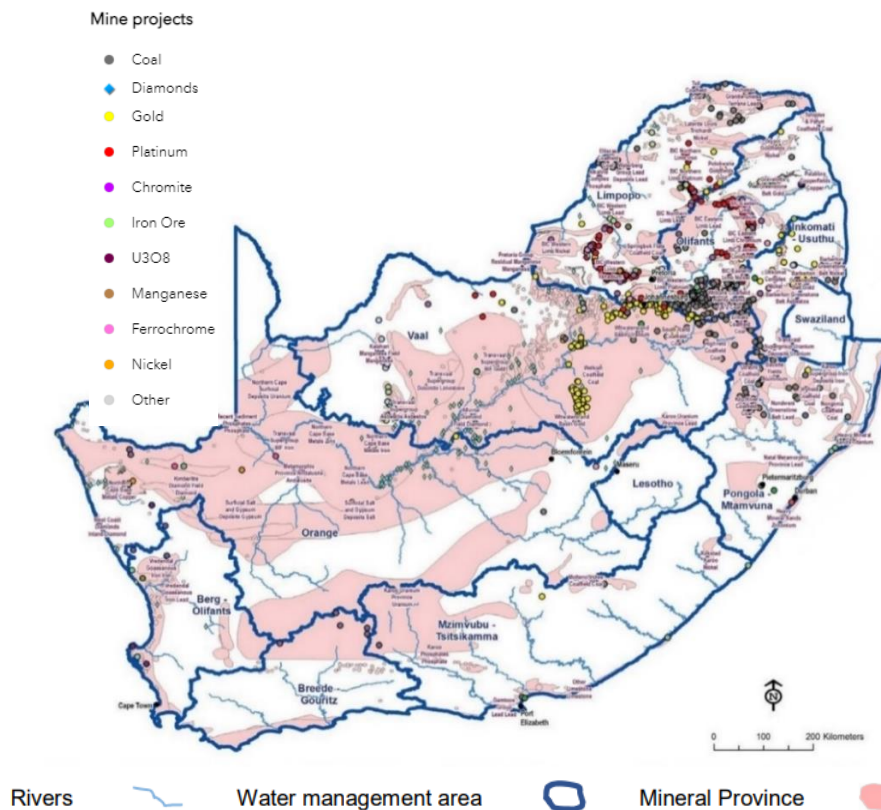


Figure 3. A map showing the water management areas and mining activities in South Africa (WRC, 2017)

Typically, the first type of mine water which is rich in H^+ and Fe^{2+} ions is prone to occur in the Western and Witwatersrand Central basins, and in the Witbank region. The second category

of mine water, which is rich in $\text{Ca}^{2+}/\text{Mg}^{2+}$ ions, is generally found in the far Eastern and Witwatersrand Eastern basins and in the Middelburg region. The third category, which is high in Na^+ , is commonly found in the KwaZulu-Natal and Secunda regions (Du Preez, 2022).

To enhance the management of water resources, the country has been partitioned into nine water management areas, based on catchment regions (DWS, 2014), which are illustrated in Figure 3 by the dark-blue boundary lines marked on the map. The water management systems' boundaries are established based on the division between surface water catchment regions and aquifer limits. The water management areas differ significantly according to water availability and demands, which can be attributed to varying climate conditions, levels and types of economic development, and population characteristics.

The overlap of mining activities, which are divided into mining provinces, indicated by the pink-shaded areas, with the major river systems in the Witwatersrand Basin, as seen in Figure 3, raises even more concerns, as the risks of polluting fresh water sources from mine closure sites, elevate the pollution probabilities. If care is not taken to adequately rehabilitate all the mine and mine closure sites, irreparable damage will be done to South Africa's ecosystems and rivers, which communities, who do not have access to clean tap water, rely on.

2.4 Mine water treatment in South Africa

Many AMD discharge sites in South Africa produces water that consists of sulphates in concentrations of typically above 3 g/l with maximum concentrations of up to 30 g/l, and pH values usually below 4 and containing a variety of dissolved heavy metals (Tutu *et al.*, 2008; Masindi *et al.*, 2018). The high salinity and high sulphate concentrations introduce difficulties for the effective treatment of AMD. Currently, there are two major treatment options being used in South Africa, namely neutralisation and membrane filtration.

The high-density sludge (HDS) process is currently one of the major treatment options that is being used in South Africa for the treatment of AMD (DWS, 2017). This process is one of the most common methods used to treat acidic effluents from mines, and utilises an active treatment approach, which involves the addition of neutralising chemical agents. The process has three steps. Firstly, pH correction and sludge conditioning are done by adding the AMD to a combination of lime and recycled sludge so that partial neutralisation of the water occurs. Secondly, the water goes through a neutralisation and aeration step where the mixture is aggressively aerated and agitated to provide optimal process chemistry performance. Thirdly, the water goes through a solid/liquid separation step where a clarifier is used to separate the

treated effluent from the sludge and the water is then recycled back to the beginning of the process (Sukati *et al.*, 2018).

The HDS process has treatment benefits, including the ability to precipitate iron and other metals, and increase the pH levels and alkalinity, but it does not significantly reduce sulphate concentrations (McCarthy, 2011; Lourenco & Curtis, 2021). When high-sulphate concentrations are present in the AMD, the treated water needs to be diluted, which is not sustainable in a water-scarce country. This dilution is done to achieve the water quality target of a sulphate concentration lower than 200 mg/L. The process also produces large amounts of solid waste, classified as hazardous waste that needs to be disposed of and can become costly over time (Johnson & Hallberg, 2005).

The other option being implemented in South Africa to treat AMD consists of using a combination of neutralisation and membrane technologies to purify polluted waters (McCarthy, 2011). The treated water can reach drinking-water quality standards by utilising reverse-osmosis to remove dissolved solids from saline or brackish feed water. Membranes alone cannot be used for the treatment of AMD, as this requires pre-treatment. The first pre-treatment process involves the use of lime neutralisation for removing metals and raising the pH of the solution. After this process, the solution is filtered using ultrafiltration before reverse-osmosis can occur.

Due to the considerable operational and capital costs associated with membrane technology processes and the additional high cost of disposing or treating the large volume of brine and sludge generated by such processes, membrane systems may not be a sustainable long-term solution (Simate & Ndlovu, 2014).

These treatment options rely heavily on available infrastructure as they require large amounts of electricity, which is not always available where point sources of AMD occur in remote locations. To solve the problem, additional technologies are required for cost-effective and low-maintenance treatment of point-source AMD.

2.4.1 Semi-passive treatment options

In general, passive technologies can be split into geochemical and biological systems (Skousen *et al.*, 2017). Geochemical systems include open limestone channels, limestone leach beds, steel slag leach beds, limestone drains, diversion wells and limestone sand. Biological systems intended for the treatment of AMD include processes like anaerobic constructed wetlands, BSR reactors, vertical-flow wetlands and manganese removal beds. Of all these processes, BSR is one of the most promising options for the treatment of AMD, due

to its joint potential for removing acidity, dissolving metals and lowering the sulphate concentrations in combination with low maintenance costs and no energy requirements. This makes the process an ideal alternative treatment option for use in South African conditions (Hulshoff *et al.*, 2001; Lens *et al.*, 2002).

2.4.2 Biological sulphate reduction (BSR)

Sulphur is found throughout nature in living organisms in several oxidation-reduction states, ranging from a completely reduced form (thiol groups of amino acids) to a highly oxidised sulphate (polysaccharide sulphate) (Nicholas, 1967). Autotrophic bacteria, like *Thiobacilli*, have been found to oxidise reduced forms of sulphur to sulphate to derive their energy for growth. The sequence for oxidation of sulphur and the corresponding energy yield are shown in Table 2 (Nicholas, 1967), and the various oxidation-reduction states of the sulphur cycle, illustrated in Figure 4 (Bos & Kuenen, 1983; Bak & Pfennig, 1987).

Table 2. Oxidation sequence of sulphide to sulphate (Nicholas, 1967)

Oxidation/Reduction	-2	0	+2	+4	+6
State of S	$SH^- \rightarrow$	$S \rightarrow$	$S_2O_3^{2-} \rightarrow$	$S_4O_6^{2-} \rightarrow$	SO_4^{2-}
Energy (kcal)	40	15	5	100	

Assimilation of sulphate into organic sulphur occurs when the sulphate undergoes conversion to the thiol level and becomes incorporated into proteins and amino acids, contributing to the production of organic sulphur (Nicholas, 1967). Desulphurylation can occur through the conversion of organic sulphur into hydrogen sulphide by several aerobic and anaerobic bacteria. The sulphate can be used by the bacteria as the terminal hydrogen acceptor, instead of oxygen, as dissimilation of sulphate to sulphide occurs. Sulphate and sulphide are simultaneously formed when sulphur disproportionation occurs. In this process, elemental sulphur or thiosulphate serves as both an electron donor and electron acceptor. The mechanism of sulphur disproportionation is conducted by highly specialised bacteria, including certain species of sulphate-reducing bacteria (Bak & Pfennig, 1987). The sulphide can serve as an electron donor for phototrophic-chemolithotrophic and phototrophic bacteria. During this process, the sulphide is converted to sulphate and sulphur (Robertson & Kuenen, 2006).

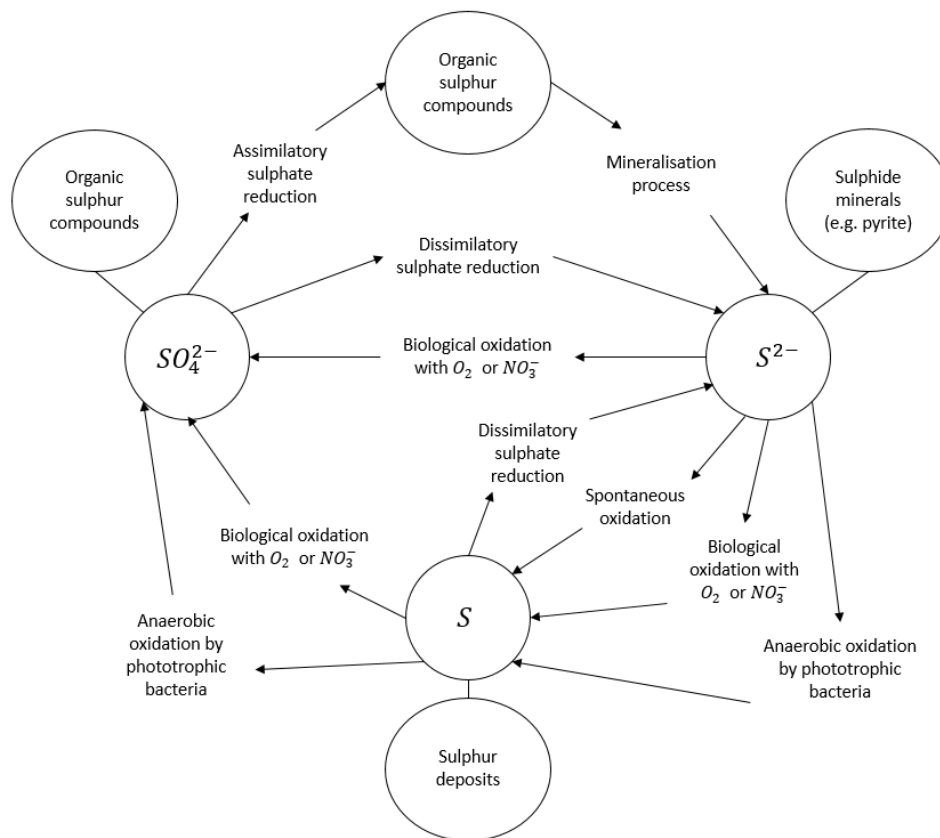


Figure 4. Schematic representation of the sulphur cycle (adapted from Bos & Kuenen, 1983)

Sulphur reducing bacteria can adapt to harsh chemical and physical conditions, enabling them to play a major role within geochemical cycles (Odom & Singleton, 1993; Johnson & Santos, 2020). The high robustness of the bacteria, and the various oxidation-reduction states of sulphur encountered in nature present an opportunity to apply the sulphur cycle in remediation, by manipulating the conditions of the cycle to remove or produce forms of sulphur.

To enable BSR as a treatment method for AMD, a reactor can be used that consists of an organic packed bed functioning as a medium to immobilise the sulphate reducing bacteria and supply organic carbon to the bacteria. Several organic substrates, ranging from simple substrates, such as glycerol, ethanol, methanol and lactate to complex substrates such as wood, hay, lucerne and kraal manure can serve as electron donors for the bacteria (Jiménez-Rodríguez *et al.*, 2009; Oyekola *et al.*, 2010). These substrates together with the sulphate the bacteria consume through respiration produce bicarbonates and sulphide as a result. The bicarbonate produced during the BSR process, increases the pH of the solution, while the sulphide found in the solution binds with the dissolved metals to form metal sulphide precipitates (Drury, 1999) – see Equations [8] – [12]:



where M^{2+} refers to the metal(s) in solution.

2.4.3 Biological sulphate reduction performance parameters

The extent of sulphate reduction that can be attained relies significantly on several factors, including the pH and temperature of the solution being treated, the chemical oxygen demand (COD) to SO_4^{2-} ratio, hydraulic retention time (HRT), and metal and sulphide inhibition due to toxicity (De Smul *et al.*, 1999; Brahmacharimayum *et al.*, 2019).

Exposing sulphate reducing bacteria to a low-pH environment, impedes their growth to the extent of inactivity or mortality, leading to a decline in the system's sulphate reduction potential (Kolmert & Johnson, 2001). Research by Pulles & Heath (2009) showed that the large-scale IMPI plant treating AMD, experienced inhibitory effects at pH values below 4.5, with the sulphate removal performance declining. As a consequence, implementing a pre-neutralisation step becomes necessary to elevate the pH of the AMD entering the reactors to an optimal range of between 5 and 5.5, facilitating the bacteria's optimal reduction capabilities (Gyure *et al.*, 1990; Koschorreck, 2008). In combination with this ideal pH range, optimal operational conditions for metabolic function can be achieved by operating the process at mesophilic temperatures ranging from 30 °C to 37 °C (Isa *et al.*, 1986; Neba *et al.*, 2007).

COD represents the oxygen equivalent of the organic carbon substances found in aqueous samples. The amount of sulphate reduction that can occur in a BSR system is highly dependent on the amount of COD that is available to the sulphate-reducing bacteria. The COD/ SO_4^{2-} ratio is the key factor in the reduction of sulphate, as it regulates the competition between sulphate-reducing and methanogenic bacteria for monomeric and H_2 or acetate compounds (Sarti *et al.*, 2009). This ratio should be in the region of 0.67 to 1.5 g COD/g SO_4^{2-} for optimal sulphate reduction to occur (Velasco *et al.*, 2008). If this ratio drops below 0.67, the sulphate-reduction performance declines as the organic matter required for sulphate reduction is not sufficient, and methanogenic reactions will dominate the system. Conversely, the sulphate-reduction competition with methanogenic bacteria increases at higher ratios as shown by the research of Choi & Rim (1991), wherein methanogenic reactions were more

prevalent at a higher ratio of 2.7 while sulphate reducers dominated again when the ratio was lowered to 1.7.

The performance of a BSR system can be highly affected by the HRT of the process. If the HRT is too low, it can negatively affect the removal performance of the system (Cunha *et al.*, 2019). Plentiful research has been done regarding the effects of the HRT on the performance of a BSR system, focusing on more active systems, which operate at much lower HRT (Kaksonen *et al.*, 2004; Dev *et al.*, 2017). The HRT of a BSR process can range from hours to days, depending on the type of process configuration and the chemistry of the mine water (Greiben & Maree, 2000; Greiben *et al.*, 2000; Oyekola *et al.*, 2010). The BSR process can be configured as an actively mixed fluidised-bed reactor, a passive organic packed bed reactor or as a continuously stirred tank reactor. For this process to be applied as a low-cost treatment for AMD in remote areas with limited infrastructure at low flow rates, the use of a semi-passive organic packed bed reactor is preferred as the maintenance, oversight and energy requirements of the process are minimal. This type of process generally has a long HRT, ranging from 5 to 10 days, depending on the amount of sulphate that needs to be removed (Pulles *et al.*, 2016; Neale *et al.*, 2018; Du Preez, 2022).

The BSR process can remove heavy metals from the AMD by precipitating them as metal sulphides. These heavy metals can have toxic effects on micro-organisms when they react with the functional groups of enzymes, which can decrease or completely stop microbial activity (Cabrera *et al.*, 2006). The toxicity of heavy metals in a variation of the BSR process, as obtained from previous studies, is listed in Table 3 (Brahmacharimayum *et al.*, 2019).

Table 3. Metal toxicity on biological sulphate reduction (Brahmacharimayum *et al.*, 2019)

Metal	Toxic concentrations (mg/L)
Zinc	150
Copper	80
Lead	80
Iron	60
Chromium	60
Nickel	20
Cadmium	20
Manganese	10

The chemical and physiochemical attributes of the environment, encompassing the sulphate reducing bacteria, are of utmost importance in dictating the toxicity related to heavy metal inhibition in the BSR process (Jong & Parry, 2003). The sulphide that is formed during BSR

can lower the theoretical toxicity of the AMD being treated by the process as the metals precipitate throughout the process.

By treating AMD, containing high-sulphate concentrations, large amounts of sulphide can be produced if sufficient organic carbon is available to the sulphate reducing bacteria. When the AMD contains lower amounts of heavy metals, less sulphide will be consumed by the precipitation of metal sulphides and the remainder of the sulphide can become inhibitory to the BSR process. This toxicity/inhibition is achieved when the dissolved sulphide concentrations are in the region of 1000 mg/L (Icgen & Harrison, 2006; Moosa & Harrison, 2006). The toxicity is pH dependant as sulphide exists as a combination of H_2S and HS^- within the pH range of 6 to 8, which is the desired target pH of the treated waters (Moosa & Harrison, 2006). This toxicity is reversible, as normal sulphate reduction rates and microbial activity are attained as soon as the sulphide concentration is lowered (Okabe *et al.*, 1995; Maillacheruvu & Parkin, 1996).

In summary, the optimal performance parameters for a passive BSR system treating AMD, containing high amounts of sulphate, are listed in Table 4.

Table 4. Optimal performance parameters for a passive biological sulphate reduction system

Parameter	Range/Value	Reference
pH	5 – 5.5	Gyure <i>et al.</i> , 1990 Koschorreck, 2008
Temperature	30 – 37 °C	Isa <i>et al.</i> , 1986 Neba <i>et al.</i> , 2007
COD	0.67 – 1.5 g COD/g SO_4^{2-}	Velasco <i>et al.</i> , 2008
HRT	5 – 10 days	Du Preez, 2022 Neale <i>et al.</i> , 2018 Pulles <i>et al.</i> , 2016
Sulphide	< 1000 mg/L	Icgen & Harrison, 2006 Moosa & Harrison, 2006

2.5 Biological sulphate reduction plants treating acid mine drainage in South Africa

A few pilot BSR treatment and demonstration plants in South Africa have been targeting high-sulphate AMD, but only one field-scale plant is currently in operation (Mooruth, 2013; Pulles *et al.*, 2016; Du Preez, 2022). BSR processes have shown the capability of operating at high-sulphate reduction rates under controlled conditions of between 160 and 880 g/m³/d, but the process has not been refined enough for commercial use. This necessitates additional research to optimise its effectiveness in treating high-sulphate AMD, as found under South African conditions (Pulles & Heath, 2009).

BioSURE is an active process, which was developed by Rhodes University (South Africa), wherein primary sewage sludge serving as an electron donor was mixed with HDS-treated mine water before introducing it into an anaerobic sludge blanket/bed reactor. The process aimed to reduce sulphate concentrations from a level of 1500 mg/L to below 250 mg/L. The use of primary sewage sludge was found to be problematic, due to the location of mines in relation to sewage treatment plants and the sheer volume of sewage required to treat the water (Rose, 2013).

From 1994 to 2009, a demonstration-scale plant of the IMPI treatment system was constructed and operated at BHP Billiton's Middelburg operations. The IMPI process is a semi-passive biological process, which was developed for the treatment of sulphate-laden mine water discharges (Mooruth, 2013). The IMPI process consists of a primary degrading packed bed reactor, packed with multiple layers of organic electron donors, such as hay, lucerne and kraal manure. Following on the degrading packed bed reactor, the primary sulphide oxidising reactor comes into play, where the oxidation of sulphide to elemental sulphur occurs. After this, there is a secondary sulphate reduction reactor containing a specifically selected single carbon source to lower sulphate concentrations to the required level. This is followed by a secondary sulphide oxidation step, during which the remainder of the sulphide is oxidised to elemental sulphur.

Since 1995, the Vryheid Coronation Colliery (VCC), which is based on the IMPI process, has been in operation, treating AMD of 50 m³/day. Currently, it serves as a bioneutralisation plant, reducing sufficient sulphate to increase the pH of the AMD from as low as 2.8 to a neutral pH (Pulles *et al.*, 2016). This process design comprised 1000 m³ of bioneutralisation reactors, a dosing station for COD feed, using molasses, three limestone reactors for pH polishing, and an aerobic wetland designed to remove residual nutrients. The plant initially showed stable sulphate reduction performance, as during the high-performance phase, it could achieve a

maximum sulphate removal rate of 192 g/m³/d when molasses was fed into it as a supplemental substrate. During the sustained phase, an average sulphate removal rate of 75 g/m³/d was maintained (Pulles & Heath, 2009). More recently, the VCC site has experienced challenges with the long-term hydraulic conductivity of the packed bed, as compaction occurred particularly within the manure layer, which negatively impacted the hydraulic permeability (Van Hille *et al.*, 2021).

During the lifetime of the plant, five distinct phases of sulphate removal performance were seen, which were directly related to bacterial substrate availability, as shown in Figure 5. The phases were:

1. Lag phase (90 – 150 days): during which the microbial community was established and acclimated to the environment.
2. High-performance phase (from 150 days up to < 8 months): during which high rates of sulphate reduction were maintained due to readily available energy in the lignocellulosic bed material, which was efficiently used by the microbes.
3. Crash phase: during which sulphate reduction rapidly declined once readily hydrolysable electron donors (e.g., lignocellulose, glycerol, ethanol, methanol, lactate, wood, hay and lucerne) had been consumed.
4. Sustained phase: during which low rates of sulphate reduction were observed.
5. Cessation phase: during which sulphate reduction ceased once all the hydrolysable electron donor had been consumed.

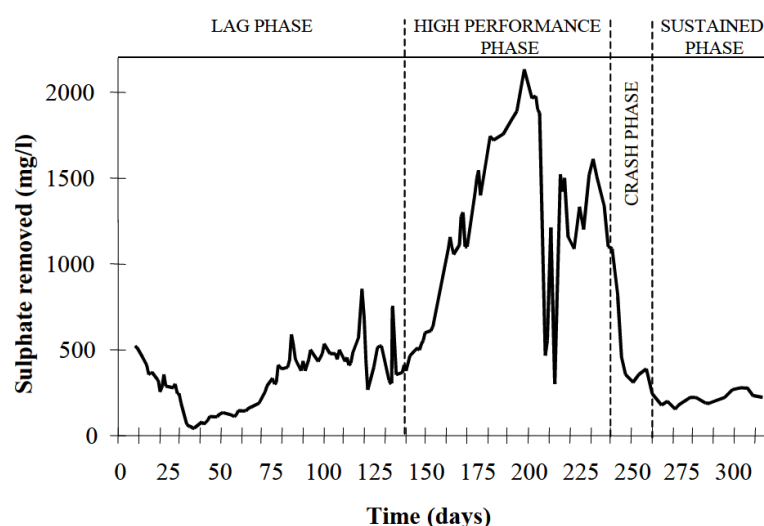


Figure 5. Typical sulphate reduction profile of a passive biological sulphate reduction reactor (Pulles *et al.*, 2004)

Since 2012, Mintek has been engaged in the research and development of the cloSURE® technology for the biological treatment of AMD, intending to reuse the treated water in

agriculture. The work culminated in successful piloting studies from 2016 to 2022 wherein both laboratory and piloting test work demonstrated that the treated water complies with the required water quality guidelines, making it suitable for irrigating various local crops, including maize, wheat, potatoes and sorghum (Neale *et al.*, 2018; Du Preez, 2022). The cloSURE® process utilises a two-stage treatment process. BSR occurs in a packed bed reactor with a supplemental source of organic carbon during the first stage, wherein the bulk of the sulphate is reduced, metal sulphide precipitation occurs, and carbonates are produced that increase the buffering capacity and the pH of the water. The BSR stage is followed by a sulphide oxidation process which oxidises the remainder of the sulphide into sulphur in a floating biofilm. During the third pilot study, the integrated cloSURE® process was operated from 2020 to 2022 treating 500 L/d of AMD per reactor, achieving an average sulphate reduction rate of 233 g/m³/d and a maximum reduction rate of 458 g/m³/d (Du Preez, 2022).

Generally in foreign countries, a typical passive BSR process for the treatment of low sulphate waters has an expected lifetime of over a decade, which makes it an ideal treatment method for AMD point-source streams in remote areas (Eppink *et al.*, 2020; Shepherd *et al.*, 2020). The decline of the hydraulic conductivity of the packed bed occurs long before the expected lifetime is reached, and considerable maintenance is necessary to re-establish the flow and hydraulic conductivity of the reactors. This is achieved by churning the packed bed to re-establish the hydraulic conductivity to allow the process to operate and approach the anticipated lifetime (Denholm *et al.*, 2010; Skousen *et al.*, 2017). Initially, this approach demonstrates efficacy; however, its effectiveness diminishes with each subsequent churning, necessitating more frequent application after each churning event (Shepherd *et al.*, 2020). An additional organic carbon source can also be supplied to the process when the sulphate removal performance declines due to hydraulic conductivity issues, as the packed bed loses its ability to supply the electron donor to the bacteria (Pulles *et al.*, 2016). This process of adding supplementary substrate to the system can be effective until the packed bed encounters severe short-circuiting, which drastically decreases the HRT, minimising contact of sulphate reducing bacteria with the AMD being treated and lowering the sulphate reducing performance of the process.

2.6 Previous studies investigating hydrodynamic behaviour and sulphate removal performance of a biological sulphate reduction process

The extent of research exploring the influence of hydrodynamic behaviour on the sulphate removal performance of a BSR system is currently limited. Bernardez *et al.* (2008) described the hydrodynamic characteristics of an upflow packed bed reactor designed for anaerobic treatment of waste waters using sulphate reducing bacteria. The hydraulic behaviour of the

reactor, containing glass beads as packing material, was investigated at different HRTs of 24.1, 9.8 and 2.6 hours, showing that the reactor behaved in a plug-flow-like regime at high-flow rates, with a prevalence of dispersion and interaction with stagnant zones at lower flow rates. This study did not investigate how these different flow regimes impacted the performance of such a system. Reyes-Alvarado *et al.* (2018) investigated the hydrodynamic behaviour of an inverse fluidised-bed reactor, with low-density polyethylene beads as packing material, at various HRT values ranging from 1 to 0.125 days. It was found that optimal sulphate removal rates occurred at lower HRT values of 0.25 and 0.125 days, with no differences in the observed hydraulic behaviour. Although these studies focused on investigating the hydraulic behaviour of a packed bed reactor, no connections were made between the hydraulic behaviour and the performance of their respective systems. For passive-type treatment systems, the packing material consists of randomised organic materials with non-uniform structures, which can have significant effects on the hydraulic behaviour of a system, compared to a uniform packing structure as seen in the previously mentioned studies.

Botes (2016) investigated the change in permeability and the sulphate removal performance of a variety of bench-scale degrading bed reactors for a 90-day period with a synthetic AMD feed mixture. These reactors contained singular species-packing materials, including manure, straw, compost, wood shaving, chicken litter and a composite test using a layered packing structure. It was found that permeability had a much larger effect on the sulphate removal performance than the type of substrate used. As a fixed volume of each packing material was added to each reactor, the amount of COD added to each of the reactors was likely highly variable; as such, the sulphate removal performance and the duration of sustained removal would mainly be dependent on this factor. Although the permeability was extensively investigated, the type of the flow regime through the packed bed was not investigated. Also, not investigated, was whether the reduction in performance was due to the depletion of the COD as a whole, or whether channelling or bypassing occurred, limiting the availability of COD to the bacteria.

The above-mentioned studies did not consider growth, the establishment of biofilms on the substrate, or the degradation of the packing material over a long-term operation as liberation of COD occurs. Such changes in the physical structure of the packed bed could affect the flow behaviour and have an impact on the sulphate removal performance of the reactor if bypassing or channelling occurs.

2.7 Hydraulic behaviour characterisation methods

The porosity of a BSR reactor can vary greatly, depending on the composition of the packing material. This is due to the primary packing material being used in these systems, of wood chips mixed with other organic materials, such as hay and lucerne. If more of one or the other is used, an uneven porosity distribution can be experienced throughout the packed bed. Uneven porosity of a packed bed can significantly impact the flow regime through the bed.

The degrading packed bed can introduce uncertainty as the porosity of the reactor can be constantly evolving, affecting the porosity of the packed bed which can introduce phenomena like channelling and bypassing of the packed bed to occur if the porosity becomes too low. These occurrences and the general flow characterisation of a packed bed reactor can be investigated by using residence time distribution experiments.

2.7.1 Residence time distribution (RTD)

The process of reactor design and operation can be simplified by better understanding the hydraulic behaviour through a packed bed. Real-world, continuously operated reactors exhibit a hydraulic behaviour that falls in between the two ideal reactor types: the continuously stirred tank reactor (CSTR) and the plug flow reactor (PFR). To better understand the different behaviours of these processes in real-world applications, RTD studies serve as a method to characterise the flow within a reactor (Fogler, 2016). Employing RTD experiments can aid in identifying issues encountered during reactor operation and forecasting conversion or effluent concentrations in existing reactors when a new chemical reaction is employed.

In an ideal plug-flow reactor, all the particles entering the reactor spend exactly the same time inside the reactor. Under all other circumstances, the various particles spend distinct durations within the reactor, leading to a dispersion of the material's residence time spent in the reactor. The RTD of a reactor is a characteristic of the mixing that occurs within the reactor. This means that no axial mixing occurs in a plug-flow reactor, which is reflected by its RTD. The RTD of a reactor can yield distinctive characteristics according to the type of mixing that occurs within the reactor and is one of the most informative characterisations of a reactor (Fogler, 2016).

The RTD of a reactor is determined experimentally by the addition of an inert chemical, molecule, or atom, at time zero, which is referred to as a tracer. The tracer must be non-reactive and easily measurable and should have physical properties similar to those of the reactor mixture and must be completely soluble. The most common types of tracers are coloured, radioactive materials and inert gases (Fogler, 2016). Sodium bromide, sodium fluorescein and sodium chloride have been used as tracers in AMD and biological AMD

treatment processes (Kusin *et al.*, 2010; Weidner *et al.*, 2011; Genty *et al.*, 2018). Due to the nature of the BSR process, wherein the organic packed bed is degraded over time and a release of nutrients and salts can be experienced, the use of a coloured or fluorescent dye is favoured as less interference should be experienced during the degradation process. Fluorescein sodium salt is a favoured fluorescent tracer as it is considered to be an ideal, nearly conservative tracer and has been widely used in hydrological research (Kass, 1998; Leibundgut *et al.*, 2009). Furthermore, this tracer was shown not to be readily biodegradable, which makes it ideal for this study, as sequential tracer tests could be conducted at long hydraulic residence times (Gutowski *et al.*, 2015).

Tracer tests can be conducted in two ways, either through a pulse input where a small dose is added, or through a step input where the tracer is fed constantly to the reactor. In the present study, the focus was on investigating the hydraulic behaviour over the course of the reactors' lifetime, which makes the use of a pulse input is easier to conduct sequentially, as minimal tracer is added to each of the RTD test runs, which decreases the possibility of interference by the tracer with the next RTD test (Fogler, 2016).

2.7.1.1 Pulse input residence time distribution tests

A pulse input RTD test is done by taking an amount of tracer N_0 and suddenly injecting the tracer in one shot into the feed stream in the shortest time humanly possible. An increment of time Δt is chosen, which is small enough that the concentration of the tracer $C(t)$, is the same between time t and the time $(t + \Delta t)$ (Fogler, 2016). The amount of the tracer, ΔN , leaving the reactor between t and $t + \Delta t$ can be expressed by Equation [13],

$$\Delta N = C(t)v \Delta t \quad [13]$$

with v representing the effluent volumetric flow rate. By dividing by the total amount of material that was injected into the reactor, N_0 , Equation [14] is obtained:

$$\frac{\Delta N}{N_0} = \frac{C(t)v}{N_0} \Delta t \quad [14]$$

which represents the fraction of material that has a residence time in the reactor between t and $t + \Delta t$. For a pulse injection, we divide $E(t)$ the residence time distribution function in Equation [15].

$$E(t) = \frac{C(t)v}{N_0} \quad [15]$$

so that

$$\frac{\Delta N}{N_0} = E(t)\Delta t \quad [16]$$

The quantity $E(t)$ describes quantitatively how much time different elements spend in the reactor.

Writing Equation [13] in differential form, yields Equation [17].

$$dN = C(t)v dt \quad [17]$$

By integrating, Equation [18] is obtained.

$$N_0 = \int_0^{\infty} vC(t) dt \quad [18]$$

If the volumetric rate, v , is assumed to be constant then $E(t)$ is defined as shown in Equation [19] (Fogler, 2016).

$$E(t) = \frac{C(t)}{\int_0^{\infty} C(t)dt} \quad [19]$$

2.7.1.2 Mean residence time

The mean value of the variable is equal to the first moment of $E(t)$; the mean residence time, t_m , is described by Equation [20] (Fogler, 2016; Levenspiel & Levenspiel, 2012).

$$t_m = \frac{\int_0^{\infty} tE(t)dt}{\int_0^{\infty} E(t)dt} = \int_0^{\infty} tE(t)dt \quad [20]$$

The second moment commonly used is called the variance, σ_t^2 , which is described by Equation [21].

$$\sigma_t^2 = \int_0^{\infty} (t - t_m)^2 E(t)dt \quad [21]$$

The magnitude of the variance of an RTD distribution, indicates the spread of the distribution, where the greater the value, the longer the time particles will spend in the reactor.

2.7.1.3 Peclet number

The Peclet number (Pe_r) uses the characteristic length that determines the flow behaviour of the packed bed. The Pe_r can be used as an indicator for the extent of axial back-mixing, namely, the lower the number the stronger the back-mixing. If the Pe_r is ≤ 5 , significant back-mixing occurred. This quantifies the dominant liquid flow regime, namely whether it is an advective or diffusive type of flow, whereby, if it is large, advective liquid flow will be the dominant flow regime within a reactor's packed bed. The Pe_r can be calculated using Equation [22] (Fogler, 2016),

$$\sigma^2 = \frac{\sigma_t^2}{t_m} = \frac{2}{Pe_r} - \frac{2}{Pe_r^2} (1 - e^{-Pe_r}) \quad [22]$$

where σ^2 is the dimensionless variance of the RTD.

2.7.1.4 Tank-in-series (TIS) model

The ideal CSTR and PFR are the most basic flow models that are used in the prototyping of chemical reactors. These models represent the ideal case, which is rarely achievable in real-world applications. The TIS model is another popular residence time distribution function for use in non-ideal continuously stirred tank reactors, as shown in Equation [23] (Fogler, 2016).

$$E(t) = A \frac{1}{\tau} \left(\frac{t}{\tau} \right)^{n-1} \frac{n^n}{(n-1)!} e^{\left[\frac{-nt}{\tau} \right]} \quad [23]$$

where t is time (days), n is the number of each tank in a series, τ is the residence time (days) and A a pre-multiplier constant, which allow the curve to be scaled up and down.

The total number of tanks-in-series (N) describes the flow pattern of the reactor, which indicates the significance of back-mixing. If $N \rightarrow \infty$, the flow pattern of the reactor tends to be that of a plug flow reactor, and if $N \leq 3$, the flow of the reactor approaches that of a CSTR and significant back-mixing will occur (Fogler, 2016). N can be calculated by using the dimensionless variance of the TIS model, as shown in Equation [24].

$$N = \frac{1}{\sigma^2} \quad [24]$$

2.7.1.5 Physical characterisation of a packed bed

It is important to understand how the BSR packed bed will change over time and how that will impact on the HRT and flow patterns through the packed bed for effective AMD treatment. Characterisation of the morphology of a packed bed in its operational state can provide

additional information on why certain types of flow behaviour are experienced as observed by RTD tests. The characterisation of these spatial voidage distributions through a packed bed is significant for modelling flow through a reactor.

In recent years, high-resolution X-ray micro-tomography (MCT) machines have been utilised to investigate the morphology of the inner volumes and structures of ores and materials. Work has been conducted to investigate the structure of wood pieces, volumetric renderings of *Pinus taeda* seedlings and scanning of concrete to investigate the quality challenges with road asphalt surfaces (Brodersen, 2013; Du Plessis *et al.*, 2019). This scanning method provides a 3D image of the structure but can be very cost intensive. These types of machines are scarce, and they usually have a limitation in the sample size they can accommodate. If the scanning facility is not nearby, the reactor needs to be transported to the machine, which can drastically change the bed structure because of vibration and compaction during transportation.

A conventional technique has been developed to determine the radial void fraction of packed beds that is based on destructive methods of using wax or a resin to fill the reactor to stabilise the bed structure. The porosity of the packed bed can be determined by weighing the liquefied wax before addition or by machining the bed with a lathe and weighing sections of the bed at each step (Roblee *et al.*, 1958; Benenati & Brosilow, 1962; Goodling *et al.*, 1983; Benyahia, 1996). After the resin or wax has set and dissections have been made, the bed structure is analysed using image analysis (Montillet & Le Coq, 2001; Hamel & Krumm, 2008). The drawback of these methods, excluding the costs associated with the destructive nature of the technique, is that the voids within the reactor need to be empty. For this to occur, the reactor will first need to be drained and then filled with wax or resin. By doing this, the structure of the packed bed can collapse, and the data would not be representative of the reactor under operational conditions. To combat these drawbacks, a method was adapted for the current study, by using gelatine to stabilise the packed bed in its operational state.

2.8 Computational fluid dynamics (CFD)

CFD is a method of numerically solving differential equations governing transport of momentum, mass and energy in moving fluids with computer-based simulations, using the finite volume method (Versteeg & Malalasekera, 2007). The finite volume method consists of three steps: firstly, the governing equations of fluid flow over all the control volumes of the domain are integrated. Secondly, discretisation of the system is done by converting the resulting integral equations into a system of algebraic equations. Lastly, the algebraic equations are solved by an iterative method (Versteeg & Malalasekera, 2007).

Over the last decade, the use of CFD modelling has increased significantly due to the development of new, efficient algorithms to solve fluid flow equations, reducing the need for using empirical information which is only applicable to a limited range of scales of fluid velocity, length, time and temperature. To achieve a computational model that accurately represents the behaviour of the system of interest, experimental measurements are preferred to provide data for CFD modelling. This is not always feasible, and various assumptions have to be made to enable analytical solutions of the differential equations; but, if too many assumptions are made, the validity of the solution is questioned. Hence, a good understanding of the applicable range and limitations of CFD tools is essential to enable the correct use of these tools (Tu *et al.*, 2023).

2.8.1 Computational fluid dynamics process

The CFD modelling process can be divided into five distinct stages: generation of the process geometry, meshing, case setup, solving and post-processing of the data (Tu *et al.*, 2023; Versteeg & Malalasekera, 2007).

2.8.1.1 Process geometry and meshing

To start the modelling process, the physical objective, and the purpose of the model need to be established. From these assumptions, the requirements of the model can then be decided upon, for example: 2D or 3D, the number of phases or processes present, and the detail at which the results are expected. Based on these decisions, the physical domain of the case is constructed with computer-aided design tools, to which the numerical approximations of the governing equations are applied. During this stage, any experimental data gathered can be included in the model. In the current study, this was done to provide an accurate representation of the packed bed, as a change in the structure of the packing material would have had a significant effect on the flow behaviour through the packed bed (Tu *et al.*, 2018).

This step is followed through the spatial discretisation of the domain, namely, meshing the structure into a finite number of control cells or volumes. This step is a key factor in the pre-processing stage for achieving reliable results, as the generation of a mesh can determine success or failure in attaining a computational solution. The accuracy of the CFD solution is governed by the number of cells (mesh elements) within the computational field (Tu *et al.*, 2018; Versteeg & Malalasekera, 2007). For this reason, it is important that a sufficiently fine mesh is created that does not significantly increase the computational resources needed to solve the problem at the required level of accuracy.

2.8.1.2 Case setup

To set up a CFD case, the physics, fluid properties and the boundary conditions are specified in preparing the model for numerically solving the case. These parameters determine how the liquid behaves in the model and must be correctly defined, or inaccurate results will be achieved (Zikanov, 2019).

The flow physics, namely turbulent or laminar flow, can have a considerable influence on the overall fluid dynamics experienced. The flow physics is different for each case and is dependent on the function of the flow rate and the geometry of the structure through which the liquid moves.

One of the fluid properties that has a significant effect on the fluid behaviour is the viscosity (Blazek, 2015). Fluids can be classified according to their behaviour when a shear rate is applied to them. If the viscosity is not related to the shear rate condition of a linear relationship between the shear rate and shear stress, the liquid is classified as a Newtonian fluid; otherwise, it is classified as a non-Newtonian fluid.

With the low HRT, of around 10 days, required for a semi-passive BSR reactor, a constant flow stream is not easily obtainable. With these low flow rates, the phenomenon of pulsatile flow is experienced where the flow drips continuously into the reactor. Pulsate flow in pipe and channel structures affects the wall shear stress where at lower flow rates (higher Womersley numbers), the shear stress becomes low, and the parabolic laminar velocity profile becomes flatter with higher velocities nearer to the wall (Womersley, 1995). In the case of high Womersley numbers, the wall's effect on the bulk flow is negligible and emulates a full-slip boundary condition. The Womersley number (α) is defined by Equation [25],

$$\alpha^2 = \frac{\text{transient inertial force}}{\text{viscous force}} = \frac{\rho \omega U}{\mu U L^{-2}} = \frac{\omega L^2}{\mu \rho^{-1}} = \frac{\omega L^2}{\nu} \quad [25]$$

where L is the radius of the structure through which the flow occurs, ω is the angular frequency of the oscillations, and μ , ρ , ν are the dynamic viscosity, density, and the kinematic viscosity of the fluid, respectively (Fung, 1990).

2.8.1.3 Solvers

The solving step is the main part of the CFD algorithm, where the governing equations are solved, providing the fluid dynamics results (Blazek, 2015). The solving process consists of the following steps (Tu et al., 2018):

- Initialisation and solution control: The user specifies some initial conditions, such as the velocity, pressure, and temperature, together with any parameters related to the numerical solution procedure (equation discretisation description, iterative solution limits, accuracy requirements).
- CFD calculation: The user chooses an appropriate algorithm that solves the governing equations for acquiring a fluid-dynamic solution.
- Evaluating convergence in the CFD modelling calculation: The user monitors the mass and flow conservation equation imbalances until the convergence criteria are met.

To investigate the possible degradation of a packed bed, the use of a conventional solver will have difficulties solving the case as the packing material has a complex structure, with an uneven porosity that varies greatly. An extended multiphase porous media solver (“porousInter”) was developed at the Council for Scientific and Industrial Research (CSIR, South Africa) by Oxtoby *et al.* (2013) (. It is based on the interFoam solver available in OpenFOAM® (ESI-OpenCFD, 2021), and explicitly addresses accuracy and stability issues resulting from sharp discontinuities in the porosity field, without introducing instabilities or oscillations into the solution. The porousInter family of solvers specifically addresses multiphase flow in porous media but can also be used for simpler single phase flow problems. The solvers are published as open-source code and are freely available on GitLab (Oxtoby 2022).

2.8.1.4 Post-processing

The post-processing stage in the CFD modelling process can provide meaningful insights through the use of data analysis and visualisation. In the open-source community, visualisation is provided by programs, such as ParaView, which is a widely used, open-source program that can visualise the data in the form of images to display the flow and other variables at different points of the case (Ayachit, 2015). Quantitative data can also be collected from the CFD model for post-processing analysis. This can be done using a variety of tools, such as the Python programming language. Together with programs, such as Jupyter Notebook, which is an open-source web-based application, these tools provide a rapid and flexible framework to manipulate and interpret data obtained from computational models (Fangohr, 2015).

2.8.2 Computational fluid dynamics studies on the biological sulphate reduction process

CFD modelling tools have been used to improve the understanding of the hydrodynamics of porous media by comparing experimental residence time distributions to the modelled results, as seen in the work done by Bujalski *et al.* (2003), where the CFD predictions demonstrated

strong alignment with the experimental data obtained from a heap leaching column. In this study, stagnant liquid regions within the porous medium were included during tracer simulation, allowing the model to anticipate discrepancies in the RTD curves resulting from fluctuations in particle porosity and particle size distributions. Pawlowski *et al.* (2018) modelled flow patterns, tortuosity and RTD in monolithic porous columns, by utilising X-ray tomography to reconstruct the porous media in the CFD model. The CFD models were successfully validated against the experimentally obtained RTD data, which showed high potential for designing target materials. These studies indicated that CFD tools can be used as a diagnostic tool to better understand the flow through a packed bed by replicating experimental RTD data with CFD models. The structure of the porous media must be accurately represented to allow for accurate and comparable results.

At the time of this study, no research was found investigating the hydraulic behaviour of biological sulphate reduction processes using CFD tools. This suggests some novelty of the work in this study, and the need to further explore the integration of computational modelling methods into the experimental analysis and engineering design processes for biological reactors. BSR processes are gaining popularity as treatment alternatives, but with minimal fundamental understanding of the physical behaviour of the process, the risk of obtaining inaccurate or under-performing designs is significant.

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CHAPTER 3 EXPERIMENTAL METHODOLOGY

3.1 Packed bed material preparation

To prepare the homogenous packing material for the reactors, specific quantities of lucerne, hay, wood chips, wood shavings and kraal manure, as listed in Table 5, were mixed with a skid steer loader to ensure thorough mixing of all the bed materials, as shown in Figure 6. A smaller sample was then taken from the mixed material and mixed again to ensure an evenly distributed mixture of the packing material, as shown in Figure 7.

Table 5. Bed material composition

Ingredient	Percentage (volume)
Wood chips	30
Wood shavings	30
Hay	20
Lucerne	10
Kraal manure	10



Figure 6. Photograph showing the mixing of the bed material with a skid steer loader



Figure 7. Photograph of bed material: a) before re-mixing; b) after re-mixing

3.2 Reactor design

The reactors for the current study were designed with a size limitation in mind, as it was planned to scan the reactors using the micro CT-scanner facility at Stellenbosch University (South Africa) to measure the topography of the packed bed. Because of this size criterion, the dimensions for the reactors were determined to be 200 mm in diameter and 500 mm in height, ensuring the largest possible size that would still fit into the scanning frame of the CT-scanner.

The reactors were custom designed and built by *Polimatrix SA cc*. Polypropylene was chosen as the construction material due to its low density, which would allow for micro-CT scanning of the reactors' packed beds without significant interference from the reactors' walls. The design drawing is shown in Figure 8.

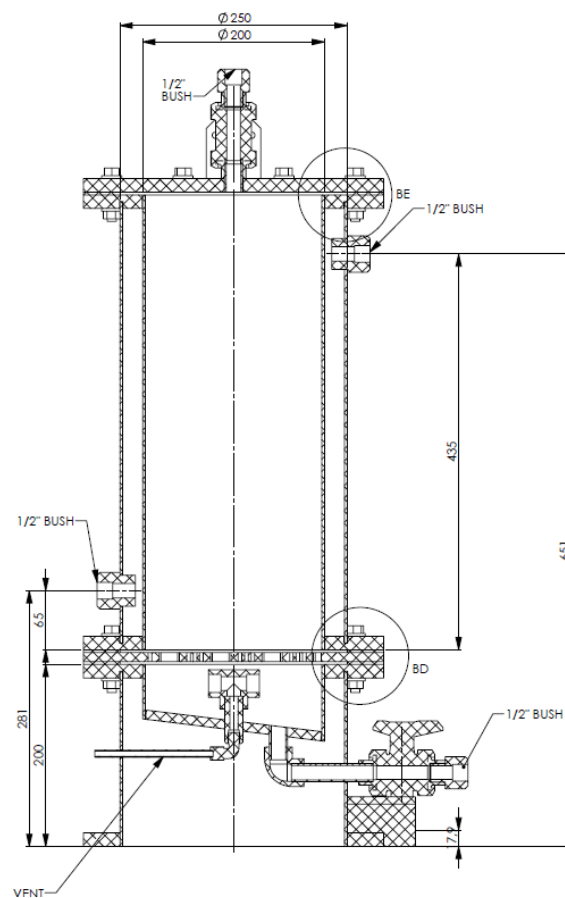


Figure 8. Design drawing of experimental reactors

3.3 Setup of the reactors

Eleven reactors were constructed and filled with 2.5 kg of pre-mixed packing material. The liquid levels for all the reactors were set at the same height by fixing a T-piece at the specified height close to the outlet of the reactors, since the reactors were operated in a down flow configuration. A typical reactor used in the project is shown in Figure 9. The reactors had water jackets to allow for constant temperature control inside the reactors during their respective operational periods.



Figure 9. Photograph of a typical reactor used in this study

The reactors were inoculated by mixing 7 L of inoculum, gathered from the then 1 m³/d operational cloSURE[®] BSR plant and 3 L of AMD to provide a large amount of seed culture and a high concentration of sulphate for the bacteria during the commissioning phase of the system. The microbial consortia found in the inoculum was a mixed culture that has been developed over the past 8 years from previous laboratory and piloting test work. The results from the characterisation of the microbial consortia present in the inoculum at the time of the study were still underway due to the considerable lead times from the service supplier. After the mixture was instantaneously added to the reactors, they were left overnight for the bed material to absorb the liquid. The reactors were topped up with inoculum the following day to the specified liquid level to enable calculation of their operating volumes by weighing each reactor before and after the filling process. The process conditions of the reactors was monitored during the inoculation phase by measuring the pH, oxidation-reduction potential (ORP) and sulphate concentration. When these parameters achieved the optimal conditions as listed in Table 6, continuous operation commenced.

Table 6. Optimal conditions to commence continuous operation

pH	ORP (mV)	Sulphate (mg/L)
> 6.5	< -350	< 600

3.4 Reactor operation

AMD was collected in two batches of 2000 L from a mining site in eMalahleni (Mpumalanga, South Africa), to ensure the AMD used for this process stayed relatively constant over the course of the study and to minimise the impact that varying water qualities could have on the process. The pH of the AMD feed was kept constant at ± 5.5 through neutralisation with lime and separation from the solids that were formed during this process. Pre-neutralisation of the AMD was done to avoid the effects of lime passivation inside the reactor and to prevent solids from entering the reactor. The

typical composition of the AMD used in this study is listed in Table 7. The calculated HRT (10 days) was kept constant by fixing the feed flow rate. The flow rate of each reactor was monitored by weighing the effluent buckets twice weekly and adjusted, if needed, to maintain a constant effluent throughput rate. The reactors were kept at a constant temperature of 35 °C, using the water jackets, to allow for optimal microbial growth. The reactors, water bath and pump layout are shown in Figure 10 and the process flow diagram for the experiments is shown in Figure 11. The operating conditions are summarised in Table 8. Sampling of the reactors was done twice a week throughout the study to monitor the process conditions of the reactors. The performance parameters that were monitored, and the summarised methods used, are listed in Table 9 and described in full in Appendix A. It was assumed that the biological degradation primarily stemmed from microbial activity; consequently, the start-up phase and the initial tracer tests were used as the control case

Table 7. Typical acid mine drainage composition

Element	Na	Ca	Mg	Al	Fe	Mn	Ni	Zn
Concentration (mg/L)	51	410	194	77	378	1.84	0.52	2.34

Table 8. Reactor operating conditions

Parameter	Value
pH	5.5
Temperature	35 °C
HRT	10 days ($\pm$ 1 L/d)



Figure 10. Layout of reactors

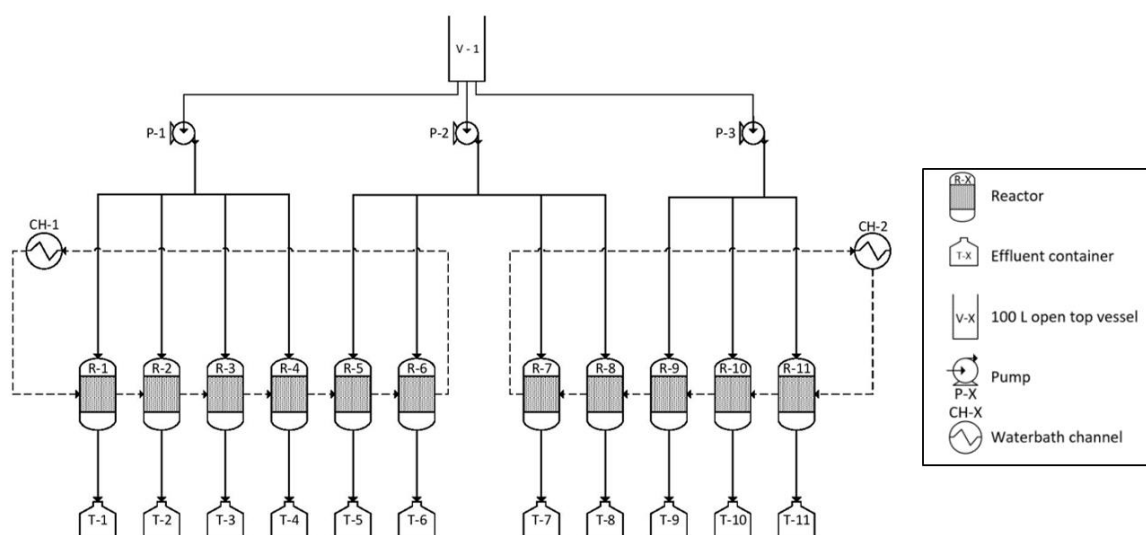


Figure 11. Process flow diagram of the reactors' operation

3.5 Sampling and performance parameters

Table 9. Process parameters and methods

Parameter	Method	Performance Indicator
pH, ORP, Electrical conductivity (EC)	Measured by using calibrated probes and meters.	pH indicated the general performance of the system. ORP showed the reductive nature of the environment and the level of reduction that occurred. EC showed the presence of dissolved salts.
Alkalinity	Determined by using the <i>APHA standard method 2320</i> by titrating samples with 0.02N HCl solution and using bromocresol green/methyl red mixed indicator solution to determine the endpoint.	Alkalinity showed the amount of carbonates that formed according to Equation [8], and the buffering capacity of the treated water.
Chemical oxygen demand (COD)	Determined by using the <i>APHA standard method 5220 D – Closed reflux, Colorimetric Method</i> .	COD showed whether there was sufficient electron donor for microbes to perform BSR by observing the excess found in the effluent.
Sulphate	Determined by using the <i>APHA standard method 9038 – turbidimetric method</i> .	Sulphate concentration was the key performance indicator for the BSR system.
Sulphide	Determined by using the <i>APHA standard method 9215 – potentiometric determination of sulphide in aqueous samples and distillates with ion-selective electrode</i> and the <i>Application Bulletin 1335/3</i> of Metrohm and the <i>Ti Application Note No. T-32</i> by using a Thermo Scientific Titrando machine to titrate with silver nitrate.	Sulphide concentrations gave an indication to what extent BSR occurred and confirmed that sulphate was not consumed/reduced by other reactions.

3.6 Tracer test methodology

To achieve replicable tracer tests, the methodology underwent rigorous validation which is described in the following section.

3.6.1 Equipment

3.6.1.1 Photometric measurements

The photometric analysis of sodium fluorescein tracer was done using SpectraMax Plus 384 Microplate Reader, as shown in Figure 12. The samples were read at 490 nm due to maximum extraction occurring at this wavelength.

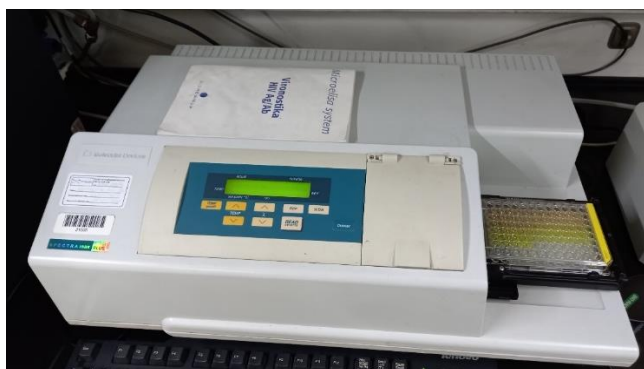


Figure 12. SpectraMax Plus 384 Microplate Reader

3.6.1.2 Fluorescent testing

Fluorescent testing of samples was done with a Synergy™ Mx Monochromator-Based Multi-Mode Microplate Reader, as shown in Figure 13. The sample was read at an extraction wavelength (λ_{ex}) of 460 nm and an emission wavelength (λ_{em}) of 515 nm, according to the manufacturing specification from Merck.



Figure 13. Synergy™ Mx Monochromator-Based Multi-Mode Microplate Reader

3.6.2 Step input

3.6.2.1 Procedure

To show proof of concept, a tracer test was done on a trial reactor. Due to the unknown flow patterns, including the possibility of channelling and of bypassing the reactor bed, a step input tracer method was followed to prevent any miss-sampling of the tracer. A 0.3 g/L sodium fluorescein mixture was fed into the reactor. Samples were taken over 28 days at regular intervals.

3.6.3 Results

As seen in Figure 14, the culture plate of the samples clearly shows when the tracer started to exit the reactor and the concentration progressively increased until the feed concentration was achieved as the samples turned to a green/yellow colour. The effluent samples from A1 to C12 were brown in colour, which could interfere with photometric analysis.

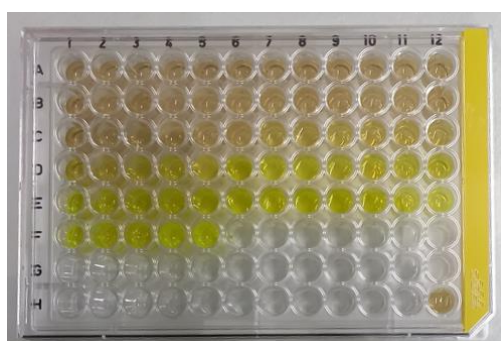


Figure 14. Culture plate with tracer samples

As seen in Figure 15, the data up and until day 15 (A1 to C12) showed a spike in the photometric readings. This was due to the brown colour of the samples, as the appearance of the tracer could only be seen from cell D1. This is problematic as the colour of the process's effluent stream can change when metals precipitate, and sulphur forms as a by-product from the process. The results indicated the need for an alternative analysis method of the samples.

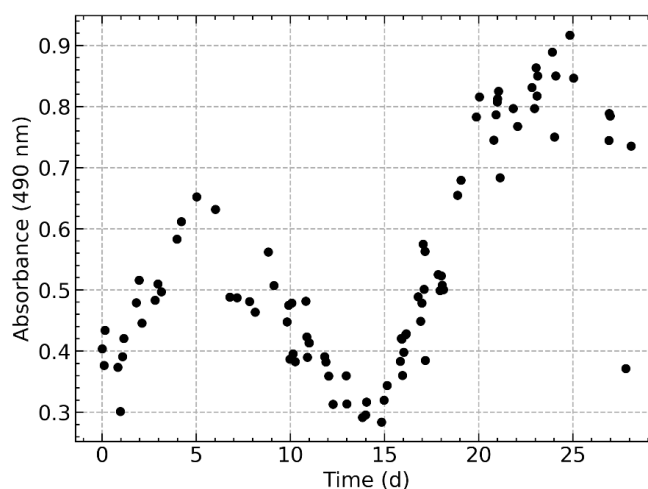


Figure 15. Photometric determination of step input

As already indicated, the samples collected in the test were analysed for fluorescence. By comparing Figure 15 and Figure 16, it can be seen that there was no interference experienced from background fluorescence or colour, as in Test 2.

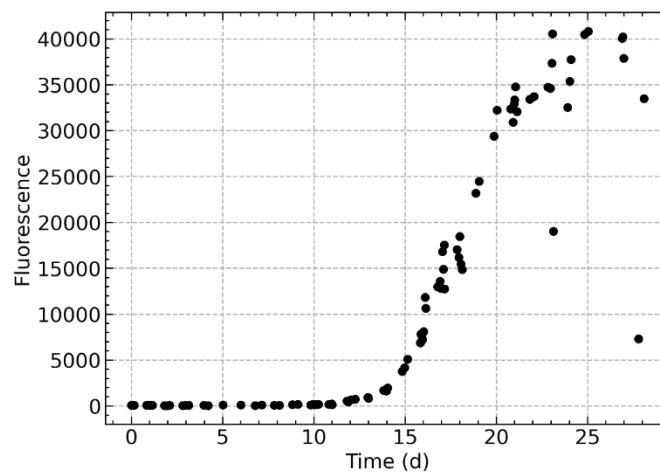


Figure 16. Fluorescence testing of step input.

The step input data is reliable and repeatable when analysed for fluorescence, as shown by Figure 16. This project requires sequential RTD tests of the reactor over the course of the project. Due to the slow residence time of the reactors, the time required to flush the tracer completely out of the reactor to run the next sequential RTD test would have taken too much time, which made the step input method undesirable for this project. A pulse input method was preferred as less tracer would be used and in turn the tracer would be flushed out of the reactor more quickly.

3.6.4 Pulse input 1

To trial a pulse input, a tracer concentration of 0.5 g/L was used in a 50 ml dose.

The fluorescent results of the test are shown in Figure 17. No significant trend in the data could be used to plot an RTD curve for the reactor. By comparing Figure 17 and Figure 18, it could be seen that the tracer pulse concentration of 0.5 g/L, 50 mL was undetectable, and thus a higher concentration pulse was required to get the desired results achieved in the previous experiments.

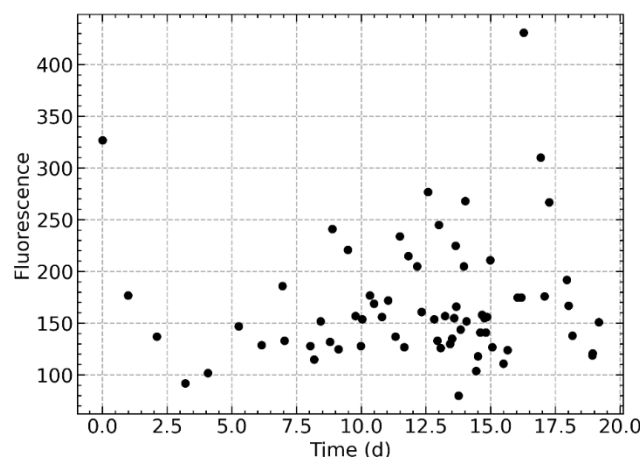


Figure 17. Fluorescent analysis results from 0.5 g/L tracer pulse

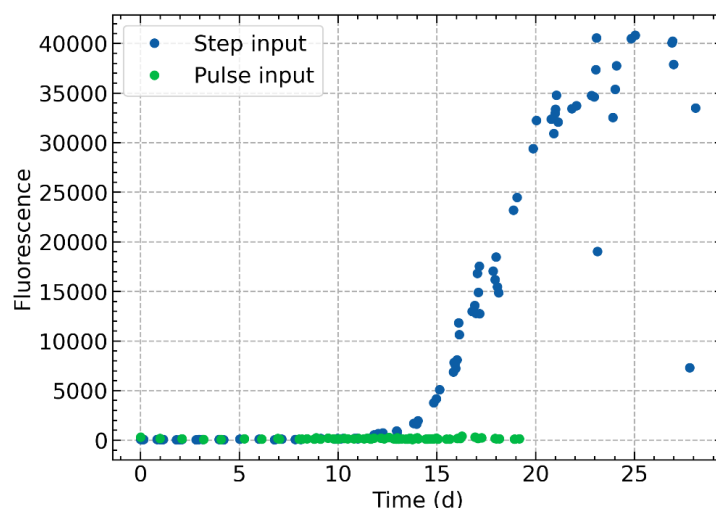


Figure 18. Comparison between Test 3 and Test 4 data

3.6.5 Pulse input 2

A 10 g/L, 100 mL dose was used as a pulse for the tracer test to ensure high enough fluorescence for accurate readings.

With an increased concentration of the tracer pulse, the fluorescence could be measured adequately, as shown in Figure 19. The process produced a good initial plug-flow-like pattern when the tracer exited the reactor. The tail end of the tracer seemed to be long, which could have been due to mixing issues and dead zones within the reactor.

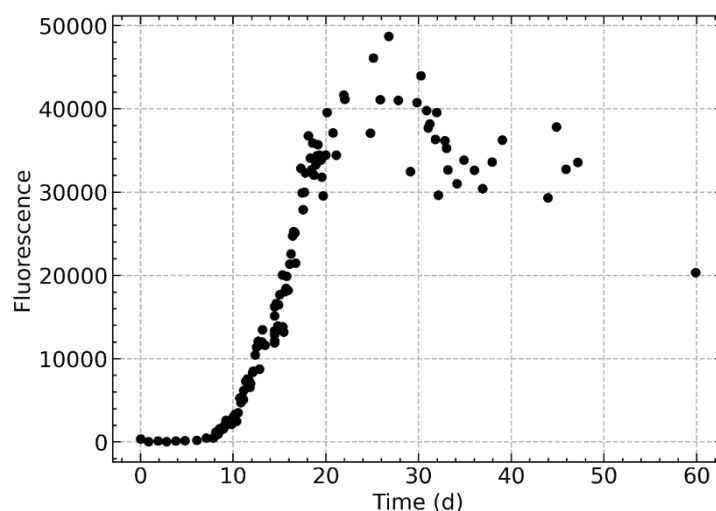


Figure 19. Fluorescent analysis of 10 g/L, 100 mL pulse tracer input

3.6.6 Conclusions

Due to the interference experienced during photometric analysis, it was recommended that only fluorescent testing of samples should be done to ensure accurate and repeatable readings throughout the study.

Due to the long tail experienced in the second pulse test, which could produce interference with the next sequential test, sufficient time should be allowed between tests to ensure minimal interference.

3.6.7 Tracer test final parameters

To start a tracer test, a 90 mL, 7 g/L sodium fluorescein mixture was simultaneously added to each of the reactors to start the residence-time distribution tests. Samples were taken over a 30-day period to get sufficient data for accurate post-processing.

To interpret the data produced from the fluorimeter, a fluorescein fluorescence standard curve was constructed to convert the readings obtained from analysis to molar concentration of the tracer.

3.7 Physical characterisation of the packed bed

3.7.1 Reagents

- Fluorescein sodium salt (used as fluorescent tracer); (Product number: F6377, CAS- No: 518 47-8, Sigma-Aldrich, Missouri, United States).
- Ethylene glycol (Product number: saar2233040, CAS-No: 107-21-1, Merck Group, Darmstadt, Germany).
- Gelatine powder from bakery supply store.

3.7.2 Equipment

- Hotplate magnetic stirrer (Heidolph instruments GmbH & Co. KG, Schwabach, Germany).
- Magnetic stirrer bar.
- PolyScience Whispercool circulating chiller (PolyScience, Illinois, United States).
- Watson-Marlow 520s peristaltic pump (Watson-Marlow, Massachusetts, United States).
- 5 L glass container.
- Thermometer.
- 0 to 6200 g analytical balance (Product no: UX6200H, Shimadzu, Kyoto, Japan).
- 12 L water-jacketed polypropylene reactor.
- Handheld ultraviolet flashlight (Prod no: ZA-493, Zartek, Johannesburg, South Africa).
- DSLR camera.

3.7.3 Stabilisation of the packed bed

3.7.3.1 Blooming of gelatine

Powdered gelatine, which can be bought at any supermarket or baking supplies store, was used. To make the gelatine solution, a ratio of 80 g/L gelatine powder to water was used to make the setting agent. To allow the gelatine to fully dissolve, the gelatine had to be allowed to bloom before it came into contact with the high-temperature water for dissolving the gelatine. This was achieved by adding 150 mL room temperature water per 80 g gelatine to a large container, which was left to bloom for 5 minutes to absorb the water and to swell, as shown in Figure 20.



Figure 20. Blooming of gelatine

3.7.3.2 Making the gelatine mixture

After the blooming process, the remainder of the 80 g/L ratio of water (850 mL for blooming) was added at a temperature of 60 °C to dissolve the gelatine to make the setting agent. If the temperature of the water being added was too low, all the gelatine would not dissolve. The mixture was stirred until all the gelatine was dissolved. Subsequently, 0.5 g/L of fluorescein salt was added to the mixture to add luminescence to the setting agent to better visualise the structure of the packed bed during physical analysis.

3.7.3.3 Feeding of gelatine into reactor

The temperature of the reactor water jackets was kept at 30 °C to prevent sudden congealing when the gelatine mixture was fed into the reactor. The gelatine mixture was heated to maintain the temperature at 60 °C with a hot plate stirrer while the mixture was continuously fed through the reactor. To ensure adequate stabilisation, 120% of the reactor volume worth of the stabilisation mixture was fed through the reactor to prevent significant dilution of the gelatine mixture. The gelatine mixture was fed into the reactor over an 8-hour period to minimise disruption of the packed bed structure. This dosing time period was chosen as it was infeasible to feed the reactor at its HRT of 10 days.

3.7.3.4 Setting of the gelatine mixture

After the gelatine mixture was fed into the reactor, it was rapidly set by cooling the reactor with a chiller to 2 °C. The reactor was left overnight to ensure complete setting of the gelatine mixture. Once the mixture set, it was critical to ensure that the reactor was kept below 4 °C; otherwise, the mixture would liquefy.

After this stage, the three reactors were shipped to the micro CT-scanner facility at Stellenbosch University (South Africa) in an attempt to conduct the characterisation of the bed. The initial results obtained from micro-CT scanning a reactor, which contained only packing material, seemed promising as seen in image (a) in Figure 21. Because of these results, it was planned to scan several reactors at distinct stages of the operation to gain information on the evolution of the packed bed as

it degraded over time. Approaching the end of the study, three reactors were sent for analysis. Each was stopped at different times and kept in a cold room to stop any further reactions from occurring. The results achieved from the scanning of the three reactors were not usable, as the scanning process could not distinguish between the packing material and the liquid inside of the reactor, as seen in image (b) in Figure 21. During the scanning of the three reactors, one reactor was drained in an attempt to achieve better results but did not yield a significant improvement in resolution. These difficulties were attributed to the similarities in densities of the stabilisation liquid (gelatine) and the packing material, and to be mainly due to the CT scanner machine having a faulty power cable so that the machine could not operate at full power. From these results, the methodology that follows was developed.

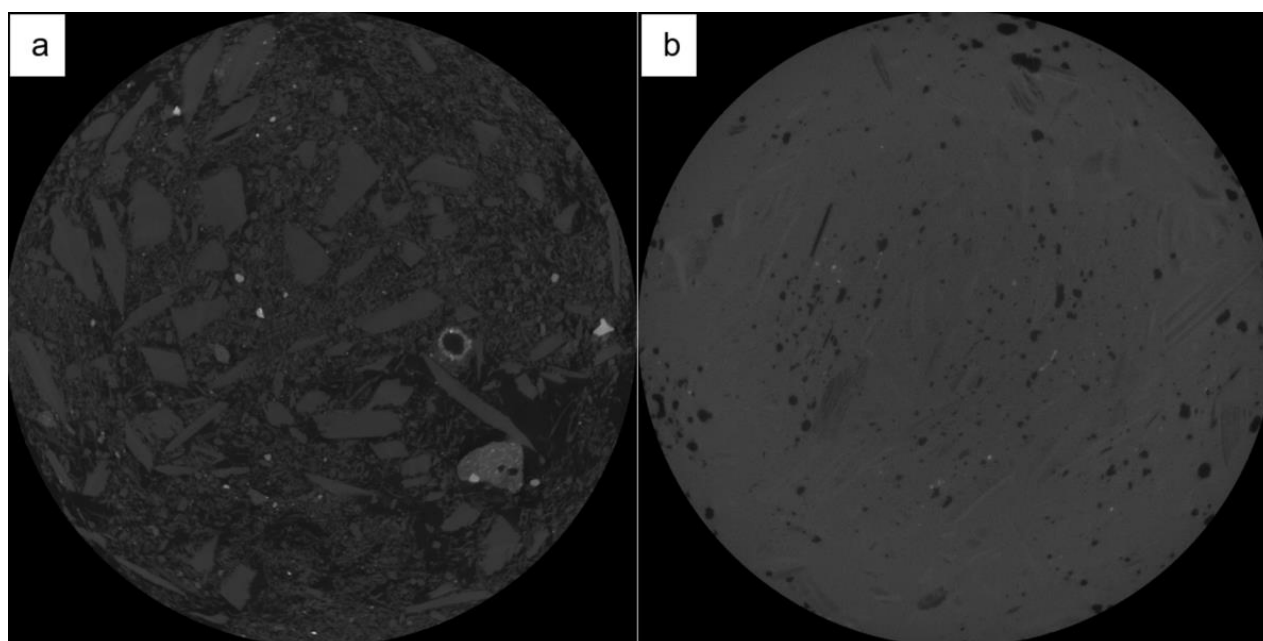


Figure 21. Micro-CT scanning results, (a) initial results from a reactor containing no liquid, (b) final results from a reactor containing the stabilisation mixture

3.7.3.5 Camera setup

The physical characterisation of the bed could only be conducted after at least 24 hours at 2 °C. To ensure that each of the images generated by this process at different positions through the packed bed can be compared to one another reactor's slices, the reactors were set in the same position and orientation for each image. The camera had to be put in manual mode to ensure that the same settings were used for each image to ensure accurate post-processing. The camera settings were as follows: shutter speed of 1"3 and an aperture of F18. A longer shutter speed was used to capture the luminescence of the fluorescein, while an ultraviolet light was pointed at the reactor to clearly differentiate the voids from the packing material. The camera setup is shown in Figure 22.



Figure 22. Camera setup for physical characterisation of the packed bed

3.7.3.6 Sectioning of the packed bed

To enable removal of the packed bed from the reactor for imaging, it needed to be left at ambient temperature for 20 minutes. With the temperature change, the packed bed could slide out of the reactor without compromising the integrity of the bed. The first image was taken when the base of the reactor was removed, which exposed the bottom of the packed bed. The packed bed was slid out of the reactor 4 cm at a time, then cut off with a knife to expose the next part of the bed structure, as shown in Figure 23. After the cut was made, the next image was taken. This process was repeated until the entirety of the packed bed was sectioned and imaged.

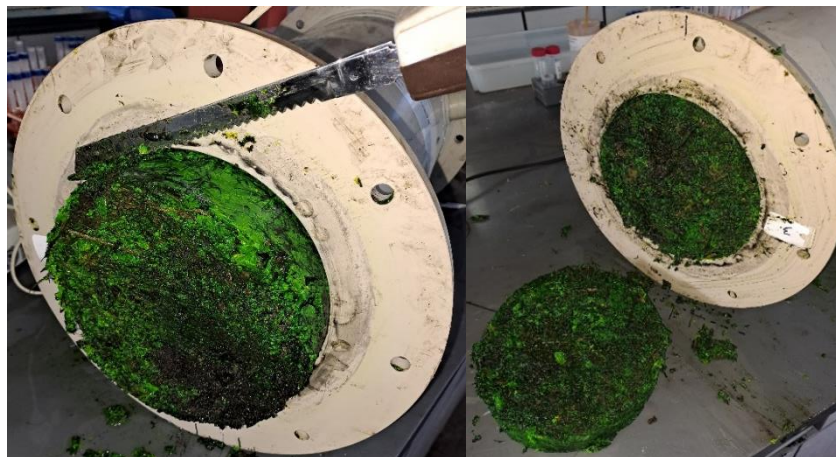


Figure 23. Sectioning process of the packed bed

3.8 Waste handling

The effluent produced throughout the process from the reactors was neutralised at the Mintek liming plant.

3.9 Personal protective equipment (PPE) and safety training

3.9.1 General personal protective equipment

At all times during the experiments, the following PPE had to be worn:

- Acid-resistant overalls.
- Safety glasses.
- Hard hat (when working in the bay areas).
- Steel toe safety boots.
- Latex gloves.

3.9.2 Sulphide safety training and relevant personal protective equipment

3.9.2.1 Personal protective equipment

- Personal H₂S Detector (Pac[®] 6500, Dräger, Lübeck, Germany).
 - This detector was calibrated according to the manufacturer guidelines.
- Full-face respirator (X-plore 6300 full-face respirator mask with Rd40 thread connection, NIOSH-approved, 40 mm filter connection, Dräger, Lübeck, Germany).
- Respirator filter (Combination filter X-plore[®] 1140 A2B2E2K2HgP3 R D, Dräger, Lübeck, Germany).

3.9.2.2 Sulphide properties

- Sulphide gas (H₂S) is a colourless, corrosive, flammable and explosive gas.
- It is highly toxic.
- Due to its higher density than air, it tends to accumulate at the bottom of inadequately ventilated areas.
- It has an odour of “rotten eggs”.
- While initially highly pungent, olfactory desensitisation occurs, leaving potential victims unaware of its presence until it is too late.
- A mixture of H₂S and oxygen is explosive.

3.9.2.3 Sulphide levels and resulting health effects.

- The odour and recognition threshold for the compound is 0.00047 ppm (0.47 ppb), signifying the concentration at which 50% of a human panel can detect its distinctive “rotten egg” smell.
- The Occupational Safety and Health Administration (OSHA) has established a permissible exposure limit of 10 ppm over an 8-hour time-weighted average.

- The concentration range of 10 – 20 ppm represents the borderline level for eye irritation. Prolonged exposure to 20 ppm or higher, lasting over 1 minute, can lead to severe damage to the nerves of the eye.
- OSHA has established the acceptable ceiling concentration at 20 ppm.
- Exposure to 30 ppm results in a complete loss of smell and injury to the blood-brain barrier via the olfactory nerves.
- For an 8-hour shift, the acceptable maximum peak above the ceiling concentration is 50 ppm, with a maximum duration of 10 minutes.
- At 100 ppm, loss of consciousness occurs within 15 minutes, followed by respiratory paralysis in 30 – 45 minutes.
- Exposure to 100 – 150 ppm paralyses the olfactory nerve after a few inhalations, leading to a loss of smell and loss of awareness of danger.
- 200 ppm can cause serious eye injury, with permanent damage to the nerves of the eye.
- Exposure to 320 – 530 ppm may lead to pulmonary oedema with the possibility of death.
- Concentrations between 530 – 1000 ppm result in strong stimulation of the central nervous system and rapid breathing, leading to asphyxia.
- 800 ppm is the lethal concentration for 50% of humans with a 5-minute exposure (LC50).
- Concentrations above 1000 ppm cause immediate collapse with loss of breathing, even after inhalation of a single breath. Although respiratory paralysis may be immediate, it can also be delayed for up to 72 hours.

3.9.2.4 Safety guidelines

- Ensure fan/air conditioning is on to disperse fumes and ensure that roller doors are open during working hours to ensure a well-ventilated space.
- At all times, wear the appropriate PPE for laboratory/bay areas, including H₂S Respirators and H₂S detectors, when working on H₂S reactors and samples.
- All test-work involving the production of H₂S must be performed in/under a fume-hood.
- A H₂S detector must be used in the laboratory area utilised for the related test-work.
- The following liquids contain H₂S:
 - Continuous BSR reactor product.
 - BSR column liquid wastewater.
 - Any samples taken from the BSR reactors.
- If liquids containing H₂S must be stored, they must be stored in a sealed container and placed in a fume-hood.

CHAPTER 4 SULPAHTE REDUCING PERFORMANCE RESULTS AND DISCUSSION

4.1 Setup of reactors

4.1.1 Operating volume determination

The operating volume and the calculated packed bed porosity of the reactors (listed in Table 10), were determined by following the procedure, discussed and demonstrated in Section 3.3. The standard volume deviation between the reactors was 0.166 L, which equated to a < 2% variation of the average reactor volume. This value indicated that the packing mixture was comparable and meant that the behaviour of the reactors could be compared over the course of the study.

Table 10. Operating volume of reactors

Reactor	Volume (L)	Packed bed porosity
R1	9.586	0.443
R2	9.687	0.436
R3	9.672	0.437
R4	9.770	0.430
R5	9.342	0.461
R6	9.803	0.428
R7	9.812	0.427
R8	9.773	0.430
R9	9.780	0.429
R10	9.385	0.458
R11	9.763	0.430

4.1.2 Inoculation of reactors

The sulphate concentrations over the course of the inoculation process for each reactor are shown in Figure 24, and a comparison between the reactors' sulphate concentrations is shown in Figure 25. At the initiation of the inoculation process, the sulphate concentration was measured as 3450 mg/L across all the reactors, which declined to less than 400 mg/L on average within 30 days. All the reactors followed the same overall trend with slight deviations, which were attributed to minimal mixing occurring within the reactors as the inoculation process occurred in semi-batch operation where on each sampling day, 500 mL of the reactor contents were drawn from the outlet and reintroduced at the top of the packed bed. Reactor R1 had a significantly quicker sulphate reduction time, occurring within 20 days of inoculation. This was attributed to increased amounts of organic carbon being present in the packing material which allowed for a higher rate of sulphate reduction, resulting in a larger amount of biofilm formation. The similarity of the sulphate reduction curves indicates that similar amounts of carbon were present in the packing material of each reactor. This

procedure was followed, as previous work at Mintek at laboratory and pilot scale used this method with great success.

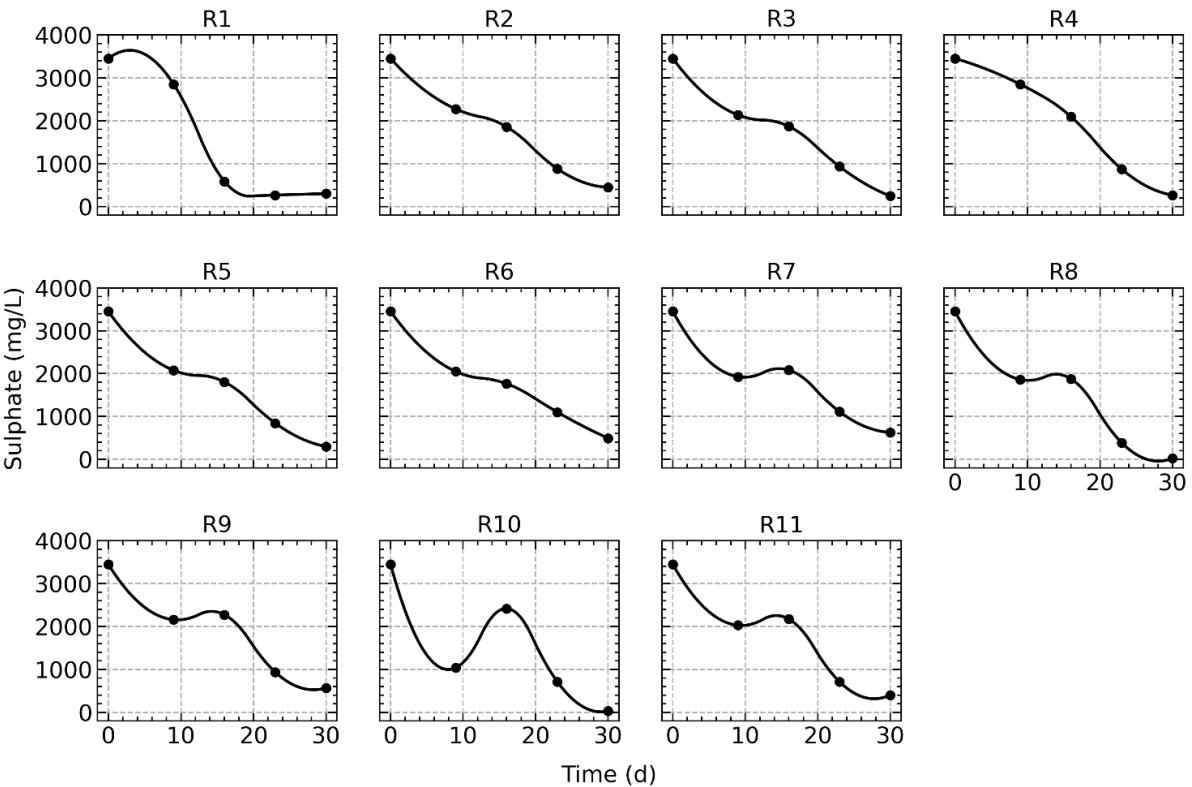


Figure 24. Sulphate concentrations during inoculation period of reactors

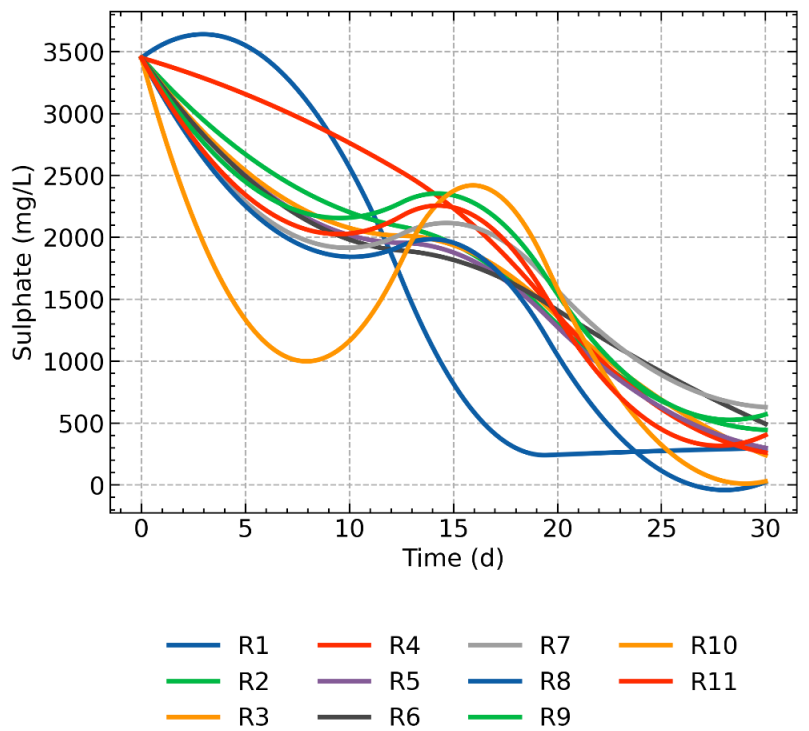


Figure 25. Comparison between reactor inoculation data

The reactors' process conditions parameters at the end of the inoculation process are listed in Table 11. The pH values of the reactors were all close to a value of 7.10, which was in the desired range for BSR effluent targets. The ORP readings of the reactors were in the region of –400 mV, which indicated a highly reductive environment due to the occurrence of sulphate reduction. The sulphate concentrations were all reduced by at least 80%, which demonstrated that an adequate amount of bacteria was present in the system for effective BSR to occur, which was confirmed by the presence of elevated amounts of sulphide in the reactors. These results indicated that continuous operation could commence.

Table 11. Reactor process conditions parameters at the end of the inoculation process

Reactor	pH	ORP (mV)	Sulphate (mg/L)	Sulphide (mg/L)
R1	7.10	-400	297.73	306.56
R2	7.14	-398	444.64	90.18
R3	7.13	-399	240.93	135.47
R4	7.18	-394	262.47	111.25
R5	7.16	-397	299.69	122.85
R6	7.15	-398	493.61	103.69
R7	7.15	-389	628.76	116.26
R8	7.04	-395	23.99	93.94
R9	7.11	-388	570.00	70.40
R10	7.14	-413	29.87	53.49
R11	7.15	-418	403.51	116.80

4.2 Continuous operation

4.2.1 Hydraulic residence time (HRT)

The average HRTs of the reactors over their respective operational periods are listed in Table 12 and illustrated in Figure 26. The average HRTs of the reactors were close to the target value of 10 days. Occasionally, blockages were observed in the feed lines which slowed the HRTs in several reactors, which were rectified on the same day. The blockages are represented by the spikes seen in Figure 26. The decrease of the HRT, up to 100 days of continuous operation in most of the reactors, was due to degrading of the marprene tubing that was used in the peristaltic pumps. The tubing was regularly replaced to minimise stoppages. However, on day 130, all the pumps failed due to load shedding. Reactor R7 had a higher average HRT than the other reactors due to its relatively short duration of operation (see Table 12), as the decrease in the flow rate due to blockages had a bigger impact. These occurrences were expected to have a negligible impact on the sulphate reducing performance of the reactors, as the overall trend of the HRT remained relatively constant.

Table 12. Average hydraulic residence time of reactors over operational period

Reactor	HRT (d)	Reactor	HRT (d)
R1	9.91	R7	10.63
R2	10.02	R8	10.46
R3	10.31	R9	10.08
R4	9.85	R10	9.49
R5	9.74	R11	10.16
R6	10.24		

The plan, at the start of the study, was to send several reactors, at various stages during continuous operation, for physical analysis at the MCT scanning facility at Stellenbosch University (South Africa). After 140 days of operation, reactor R7 was picked at random to be sent for micro-CT scanning and was placed in a cold room at 4 °C to stop any microbial activity that could further degrade the packing material until it was couriered to the testing facility. After 224 days of operation, another two reactors were planned to be sent for analysis. To determine which reactors to stop and send for analysis, the sulphate removal performance and the hydraulic behaviour were investigated at that time. Reactor R1 had lower sulphate and higher sulphide concentrations than the other reactors, which required further investigation over the long-term operation. Reactor R4 had reasonable sulphate removal performance and the RTD curve was evolving, which also required further observation. As COD spikes were picked up in the effluent of reactor R6, suggesting microbial degradation was occurring, its impact on the hydraulic behaviour required observation. As reactors R2, 3, 8, 9 and 11 all exhibited similar behaviour, it was decided to pick two of these reactors at random, R3 and R11, and send them for micro-CT scanning along with reactor R7. Reactor R9 was kept running as another data point, and reactors R2 and R8 were stopped as their performance ceased. The water jacket of reactor R5 failed after 55 days of operation and the reactor had to be stopped due to water leakage into the reactor. The respective operational periods of the reactors are listed in Table 13.

Table 13. Operational periods of reactors

Reactor	Operational days	Reason for stoppage
R1	364	Physical analysis
R2	224	Removal performance ceased
R3	224	Micro-CT scanning analysis
R4	364	Physical analysis
R5	55	Water jacket broke and compromised reactor
R6	364	End of study
R7	140	Micro-CT scanning analysis
R8	224	Removal performance ceased
R9	364	End of study
R10	350	Physical analysis trial
R11	224	Micro-CT scanning analysis

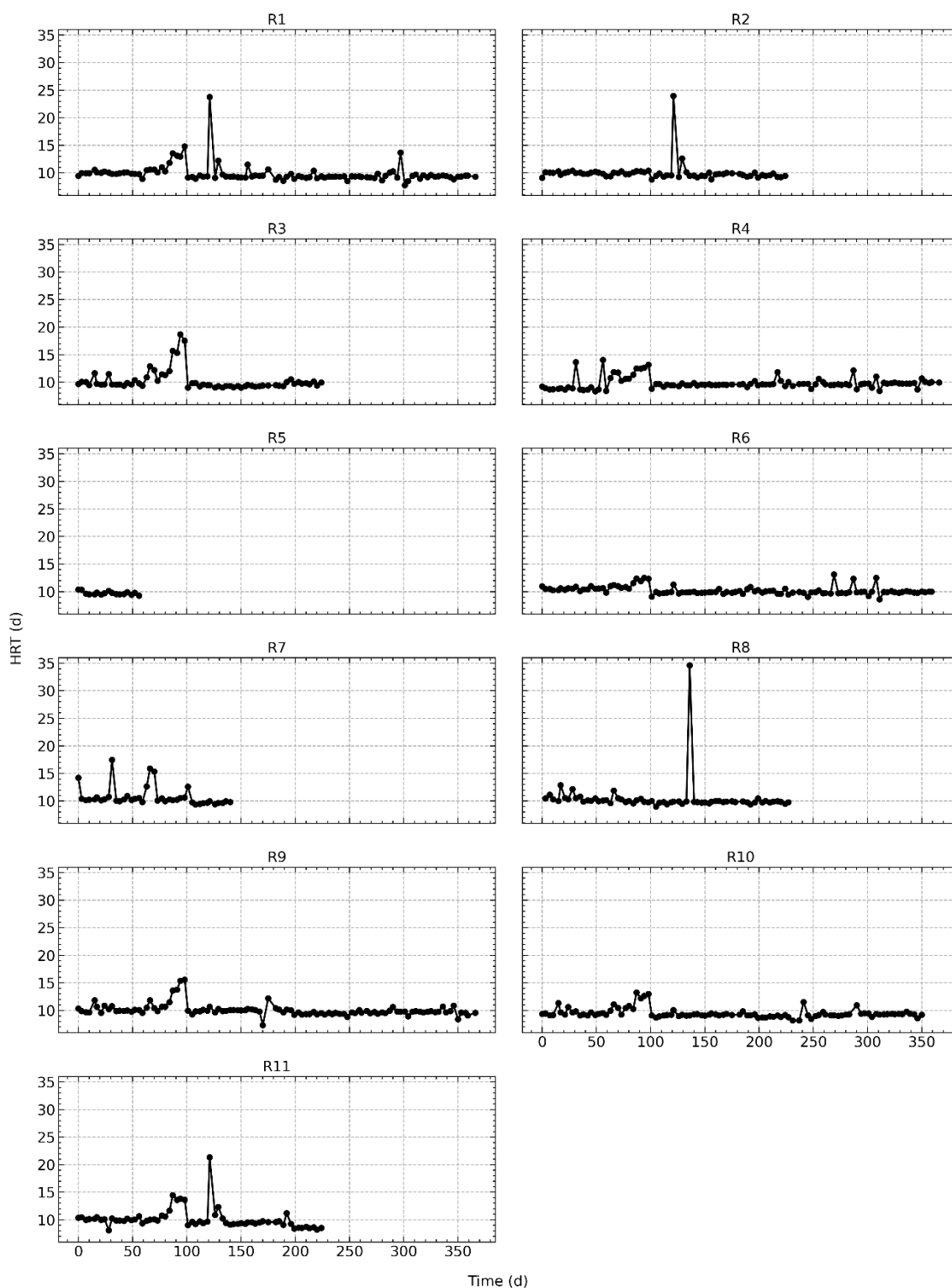


Figure 26. Hydraulic retention time of reactors over operational periods

4.2.2 Alkalinity

The alkalinity of the reactors' effluents is shown in Figure 27. The alkalinity of the mine water was measured when each batch of AMD was received: a sample was neutralised to a pH value of 5.5 and the alkalinity, that was measured, varied between 50 and 55 mg CaCO₃/L. The alkalinity of all the reactors was initially high at around 4000 mg CaCO₃/L after the inoculation process and declined

within the first month of operation to an average range of 100 – 150 mg CaCO_3/L , after which it stayed constant for the remainder of the reactors' operational periods. This indicated that minimal bicarbonates were produced as lower sulphate reduction was occurring, according to Equation [8], due to low amounts of organic carbon available to the bacteria.

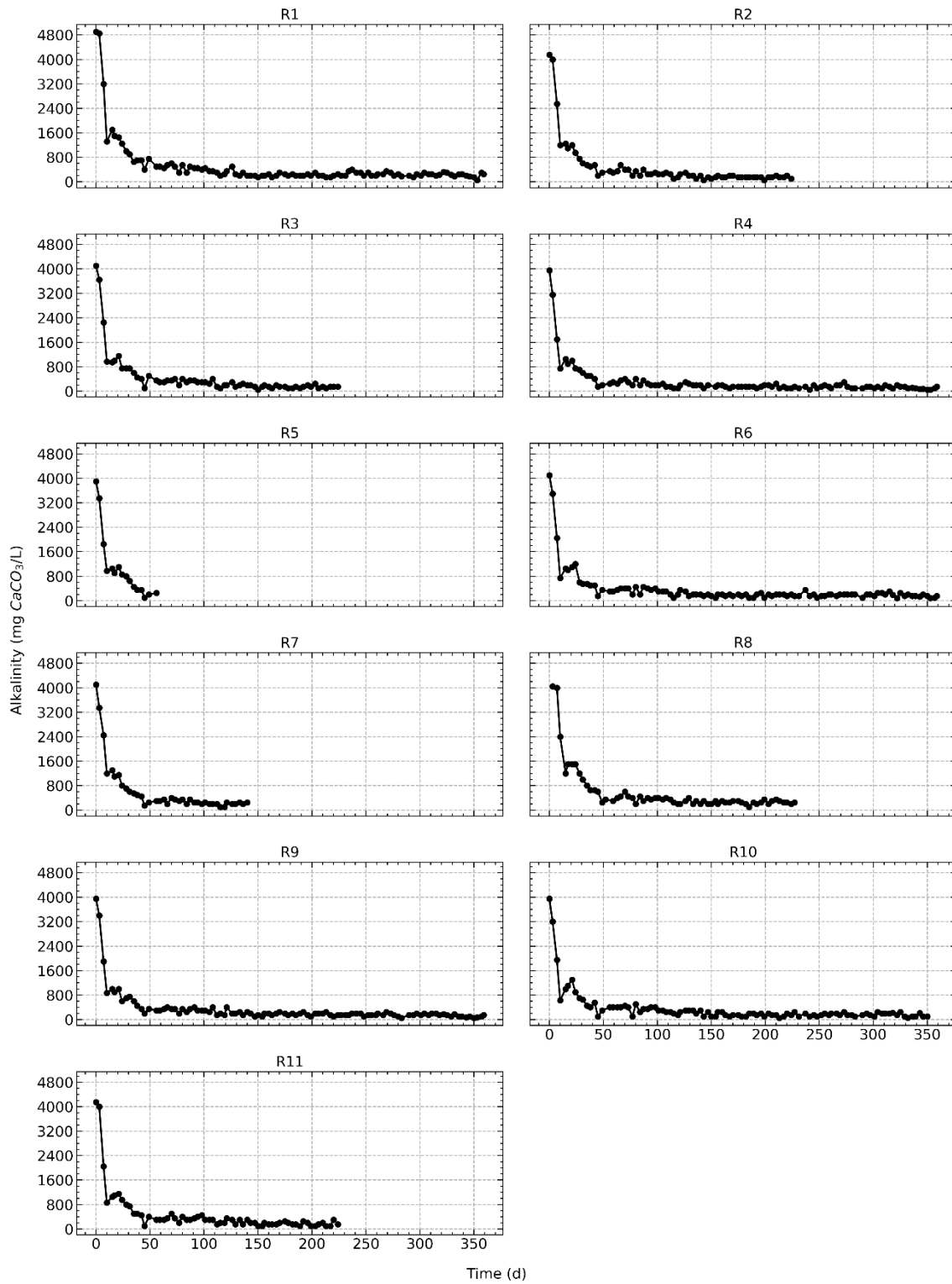


Figure 27. Alkalinity values of reactors over operational periods

4.2.3 pH, oxidation-reduction potential and electrical conductivity values of reactors

In Figure 28, it can be seen that the feed pH values were kept constant at a value of 5.5 over the course of the study, as the AMD was pre-neutralised with lime in batches of approximately 70 L at a time. This constant pH value meant that the variations seen in the reactor's effluents were due to biological activity, and not due to fluctuations of the feed pH. The pH of the reactor's effluents had minor fluctuations, maintaining their pH values between 6.6 and 7.0. This was similar to the results observed at the VCC treatment plant (Pulles *et al.*, 2004), which were within the desired range for the treated water. The increase in pH compared to the feed pH was attributed to the formation of bicarbonates, as evident from Figure 27.

The ORP of the feed varied significantly, with the majority of the values tending to negative, as seen in Figure 29. The negative ORP values of the feed water can be explained by the formation of metal precipitates during the liming process. When metal precipitation occurs through an oxidation reaction to form metal hydroxide precipitates, oxygen will be consumed by the reactions. The decrease in dissolved oxygen levels resulting from these precipitation reactions, in combination with the formation of the reduced compounds, will lead to a decrease in the ORP values (Turingan *et al.*, 2020).

In Figure 30, the ORP of the treated water increased gradually over time but maintained an ORP value of below -240 mV. At the later stages of the study, the ORP values started to increase more rapidly, indicating a less reductive environment, which was attributed to a decline in the sulphate reducing performance. Approaching day 330, the ORP values increased significantly to values between -50 and -80 mV, suggesting that the BSR process was starting to fail while the performance of the system was declining.

The EC values of the feed water can be seen to change at day 280, when the EC increased from values ranging between 0.2 and 1.5 mS/cm to values ranging between 3.0 and 4.5 mS/cm, as is evident from Figure 31. This was due to a new batch of AMD being used. The effluent EC values remained relatively unchanged compared to the feed water, which indicated that there was minimal removal of dissolved salts and formation of carbonates.

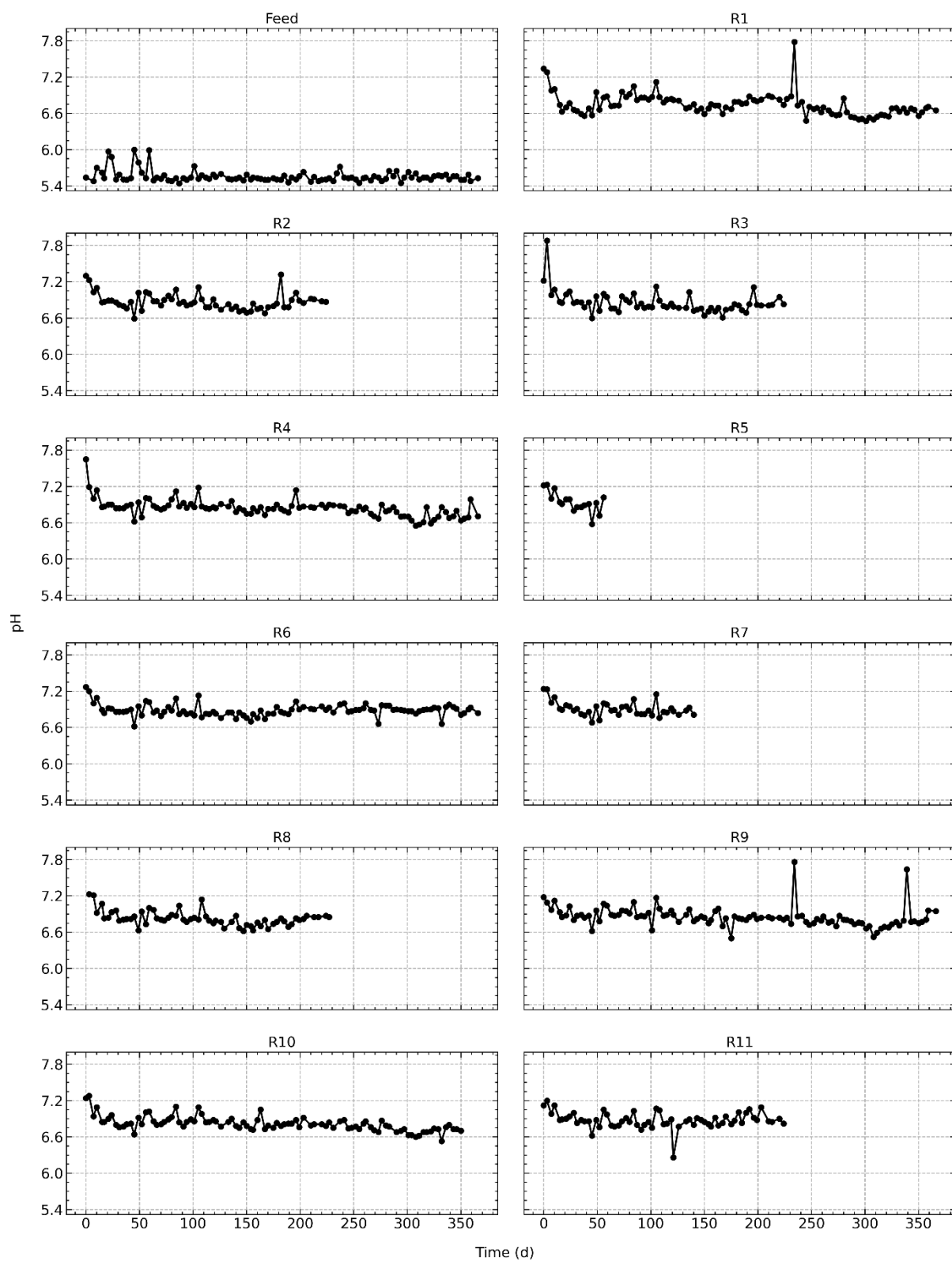


Figure 28. pH values of reactors and feed over operational periods

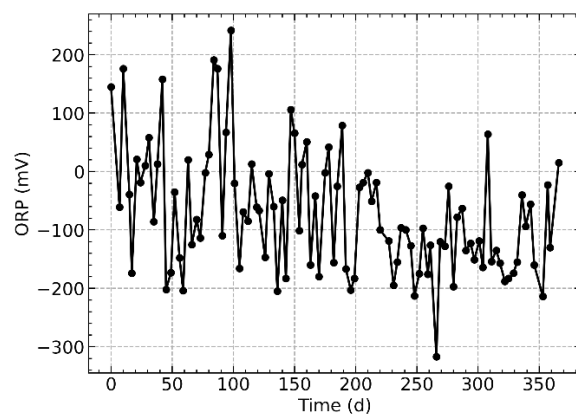


Figure 29. Oxidation-reduction potential values of feed over project duration

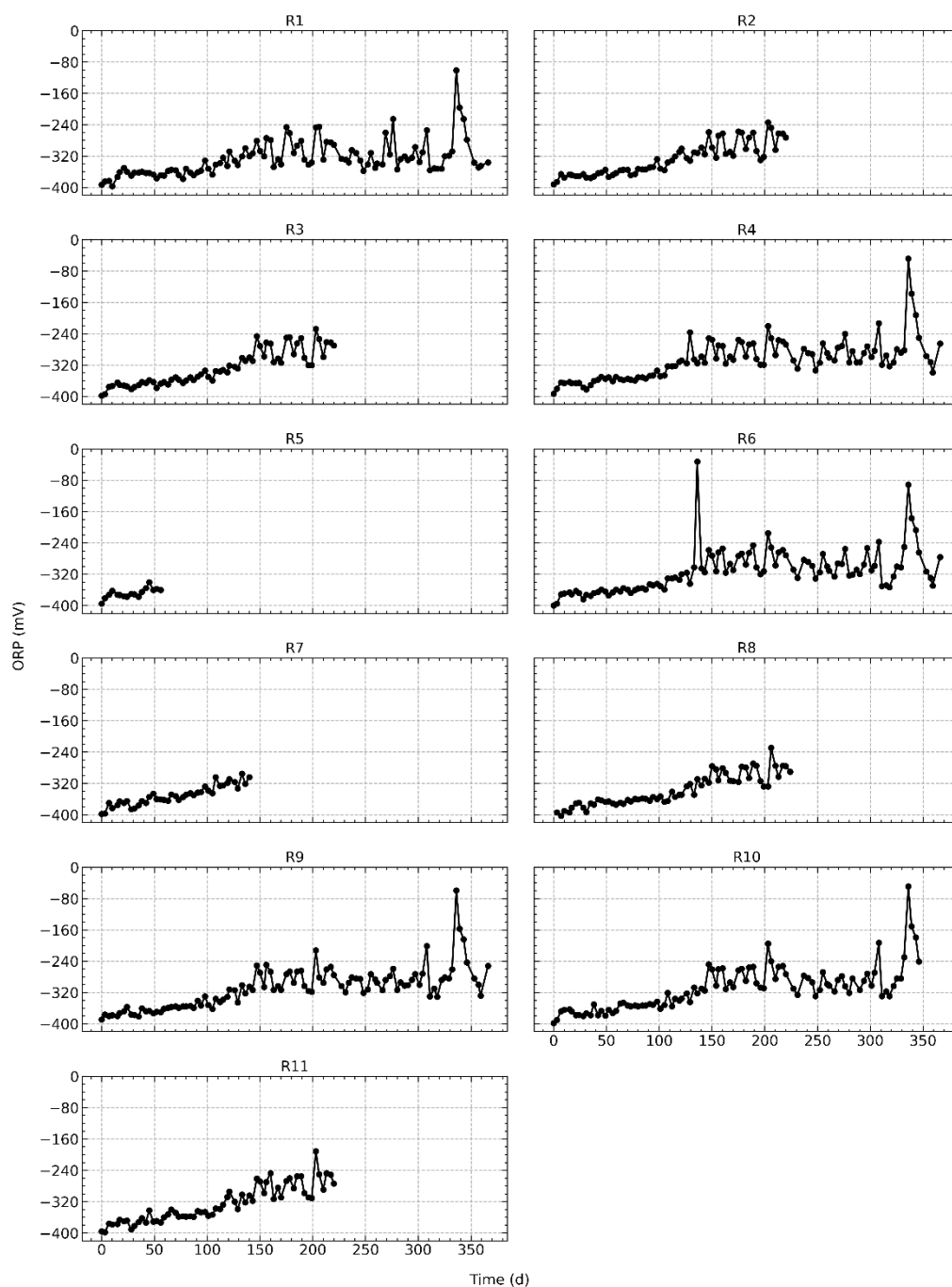


Figure 30. Oxidation-reduction potential values of reactors over operational periods

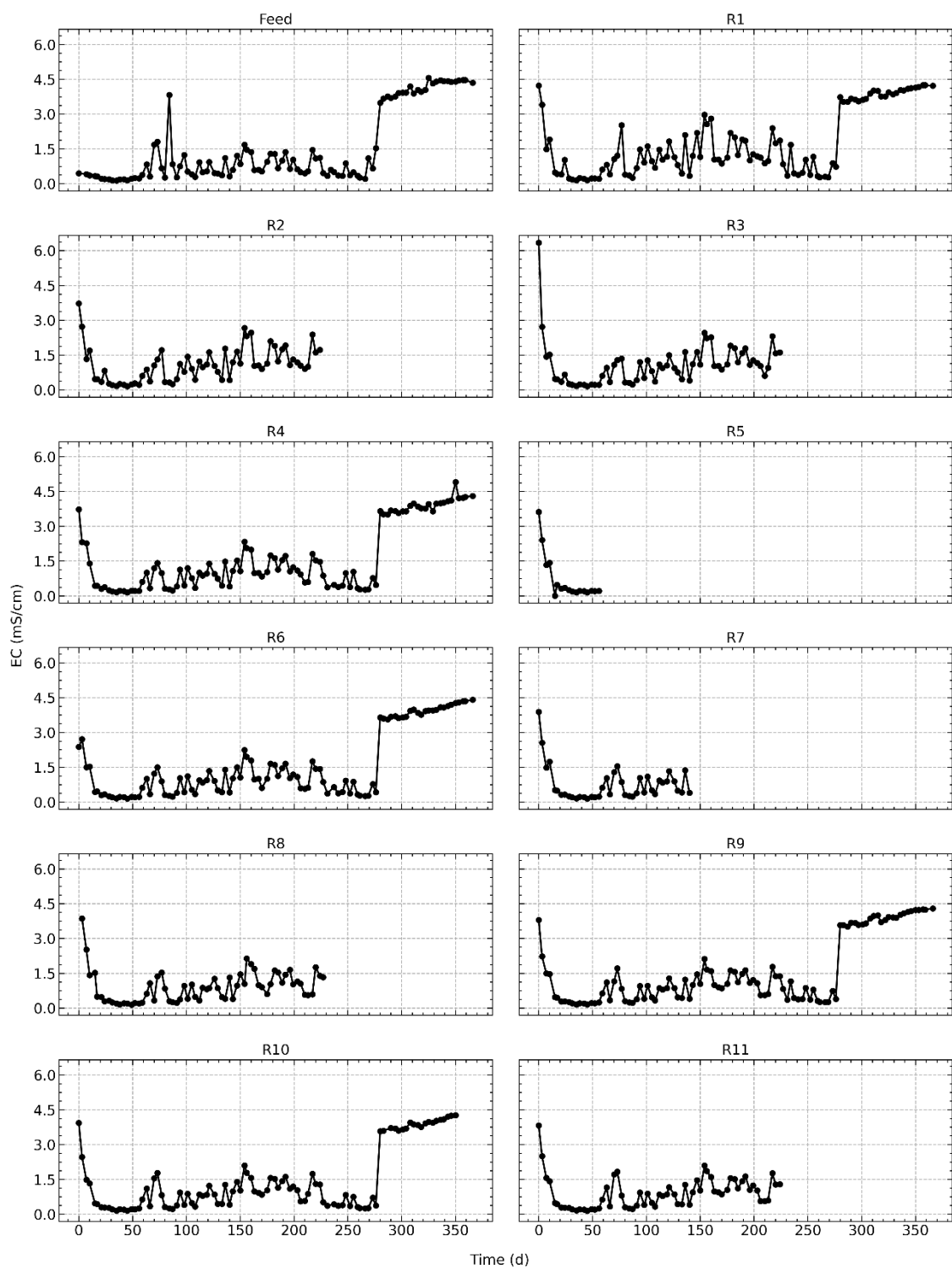


Figure 31. Electrical conductivity of reactors over operational periods

4.2.4 A comparison between the chemical oxygen demand and sulphate concentrations

The COD values of the reactors, during the first 28 days of continuous operation, are shown in Figure 32. The CODs of all the reactors at the commencement of continuous operation were in the region of 1600 mg O₂/L, except for reactor R1, which had an elevated value of 4650 mg O₂/L. These COD concentrations were attributed to the hydrolysis of the packing material, mainly of kraal manure, into solution. The kraal manure was quickly consumed by the bacteria within the first 28 days of continuous operation, while the COD concentrations decreased to values ranging from 100 to 150 mg O₂/L. This decrease indicated that the bacteria were consuming all the available COD, and that the concentrations found in the effluent were unavailable for consumption.

The COD values of the treated water during continuous operation compared to the sulphate concentrations over the reactors' respective operational periods are shown in Figure 33. The concentration scale for the COD in the figure was reduced to allow for better visualisation of the data to compare the increase or decrease in the COD, as the first data point was extremely high compared to what was measured after the first 28 days. The COD concentrations increased to values between 50 and 75 mg O₂/L from days 70 to 120. The slight increase in the COD values during this time did not directly show how much COD was available to the bacteria but indicated that excess amounts of COD remained in the effluent after BSR. During this period, increased rates of BSR occurred, which directly correlated with the elevated COD values. This event was associated with the liberation of organic carbon from the bed material as degradation of the packing material occurred. After this period, the COD concentrations of the reactors remained constant at values of approximately 25 mg O₂/L, as no readily available organic carbon was left in the packed bed. After the above-mentioned high-performance stage, low sulphate reduction performance was experienced and maintained for the remainder of the operation periods. This pointed to the fact that the packing material did not have sufficient available organic carbon for long term high-rate sulphate reduction. This result was similar to the result in the large-scale VCC plant (Pulles *et al.*, 2004).

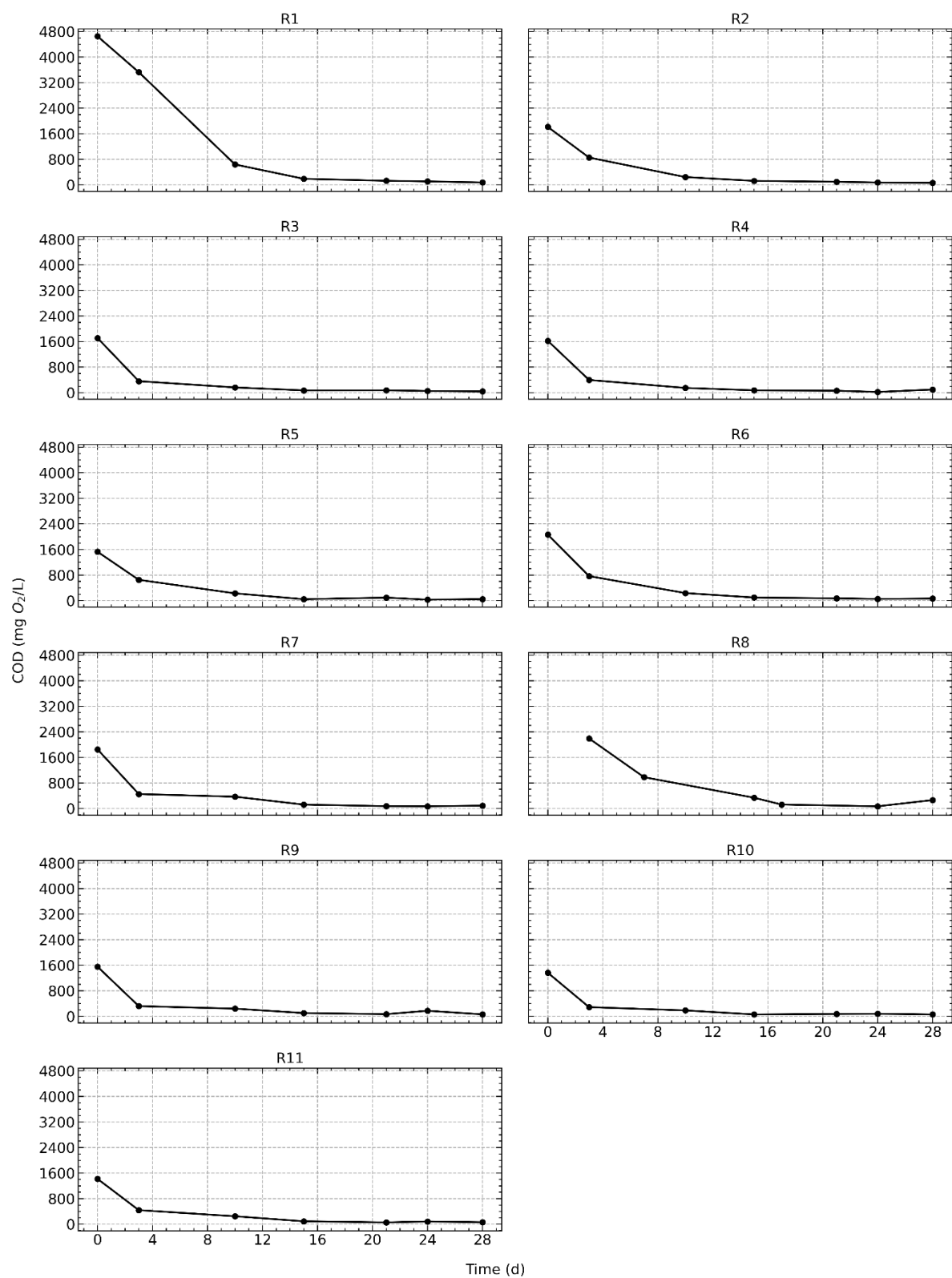


Figure 32. Chemical oxygen demand values of reactors during first 28 days of continuous operation

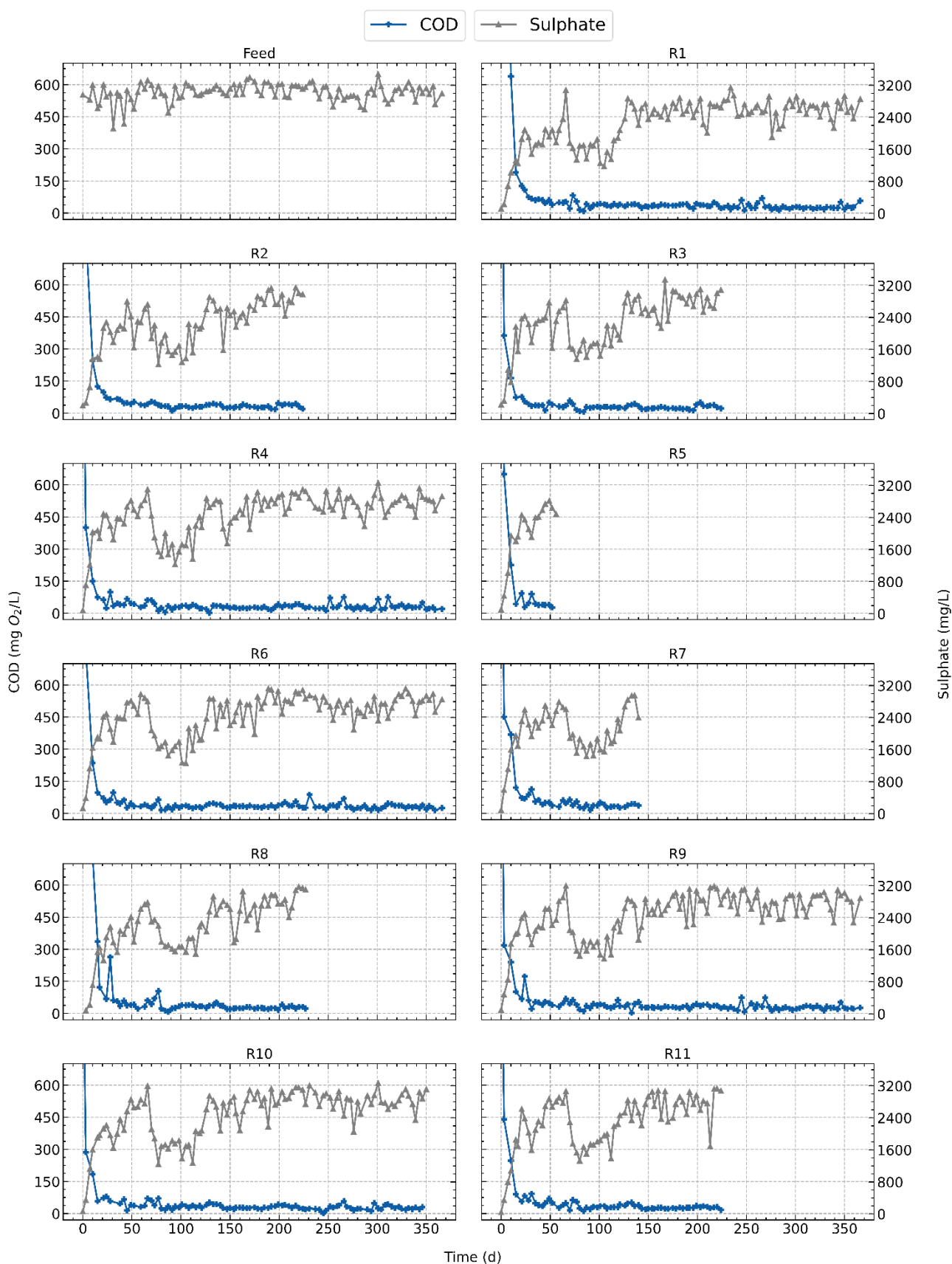


Figure 33. A comparison between the chemical oxygen demand and sulphate concentrations over the reactors' respective operational periods

4.2.5 A comparison between the sulphate and sulphide concentrations

The sulphide concentrations, in terms of S^{2-} , and the sulphate concentrations of the treated water over the reactors' respective operational periods, are shown in Figure 34. The sulphate concentrations of the AMD feed remained constant at around 3000 mg/L over the course of the study, removing the possibility of fluctuations of feed concentrations impacting the reactors' sulphate-reducing performance. After the high-performance stage, from days 70 to 120, during which the sulphide concentrations were maintained at 100 mg/L, they decreased to below 50 mg/L due to the decline in sulphate reduction. The sulphide concentrations closer to the end of the study declined even more, which confirmed sulphate reduction ceased.

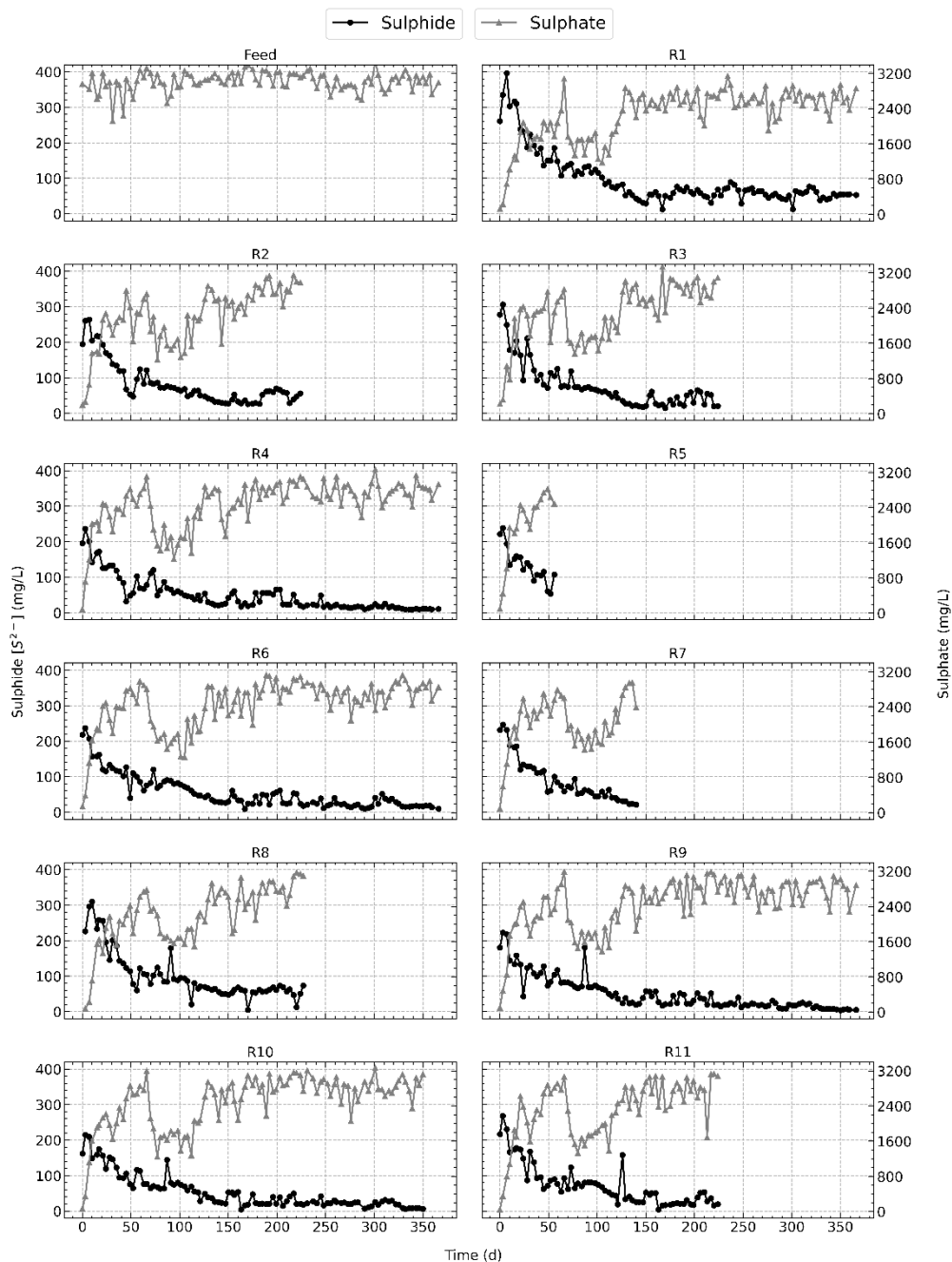


Figure 34. Sulphide concentrations of reactors over the respective operational periods

4.2.6 Sulphate removal performance

The maximum sulphate removal performance of the reactors during the high-performance stage, from days 70 to 120, was on average 40% and a maximum of 60%, as seen in Figure 35. After this high-performance stage, an average sulphate removal percentage of 12% was maintained. This meant that the process only functioned as a bioneutralisation process after day 120.

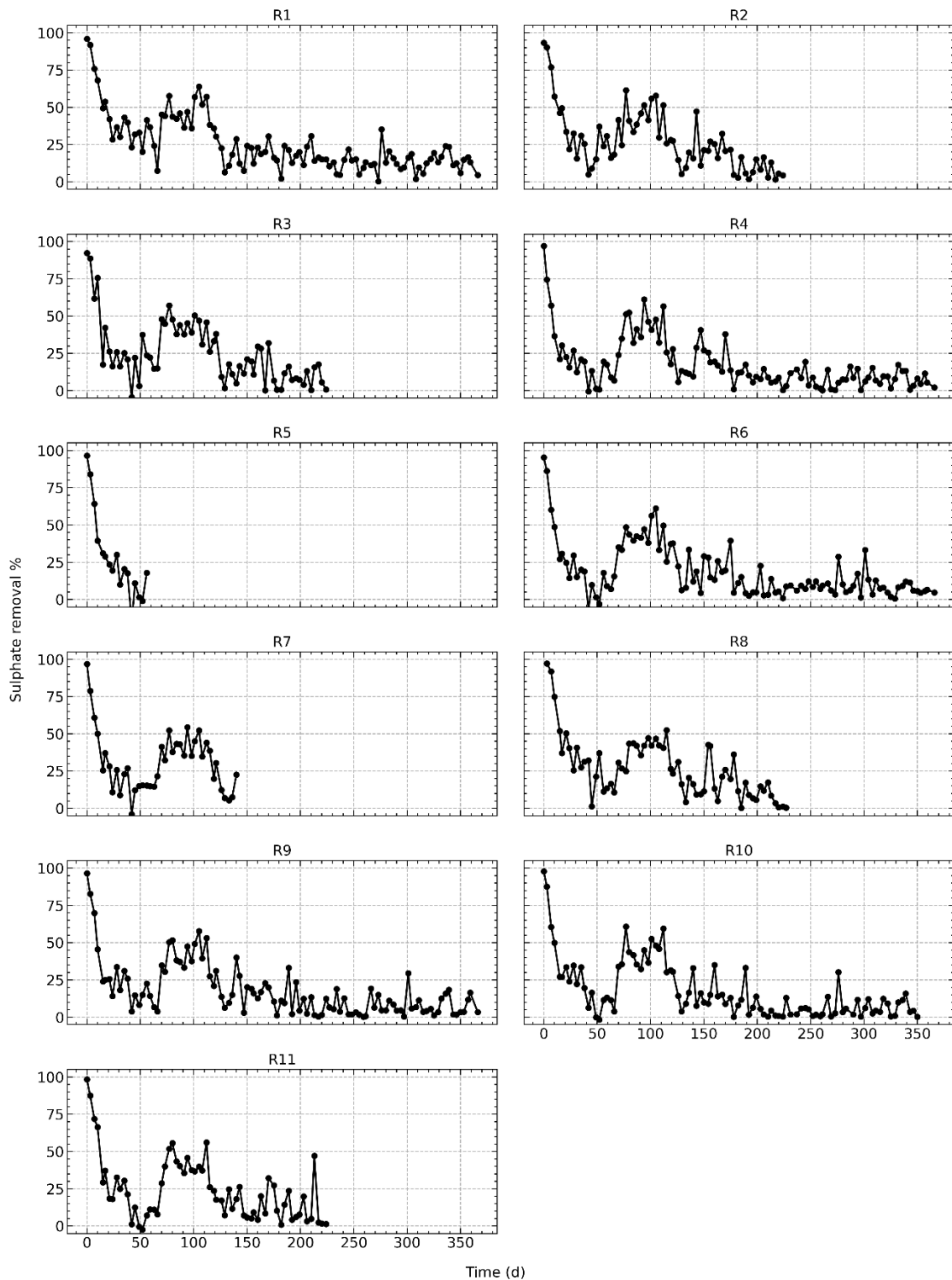


Figure 35. Sulphate removal percentages of reactors over respective operational periods

To compare the results of this study to a typical BSR process, the sulphate-removal performance of the reactors was expressed in the form of average-sulphate-removed, as illustrated in Figure 36. The average could be calculated due to the comparable nature of the packing material as reported in Section 4.1, likeness of the inoculation effectivity as reported in Section 4.1.2, and the similarity of the trends of sulphate removal.

By comparing the average sulphate removal performance in Figure 36 to a typical sulphate reduction profile of a passive reactor, as shown in Figure 37 (Pulles *et al.*, 2004), it can be seen that similar performance stages were experienced. However, in the current study, the high-performance stage occurred much earlier, from days 70 to 120, compared to the VCC plant where the high-performance stage occurred from days 140 to 240 and for twice the time duration. These differences were attributed to a possible variation in the composition of the packing materials, variation in scale, and, mainly, the controlled temperatures of 35 °C in this study, which would increase the rate of reactions, inducing the higher rates of organic carbon liberation from the packed bed.

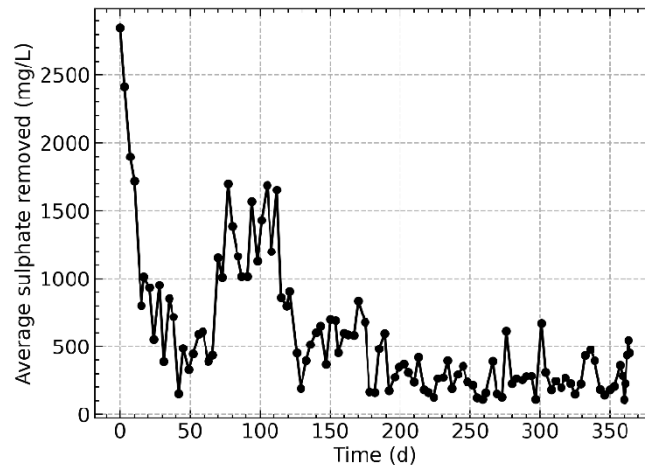


Figure 36. Average sulphate removed in operational reactors

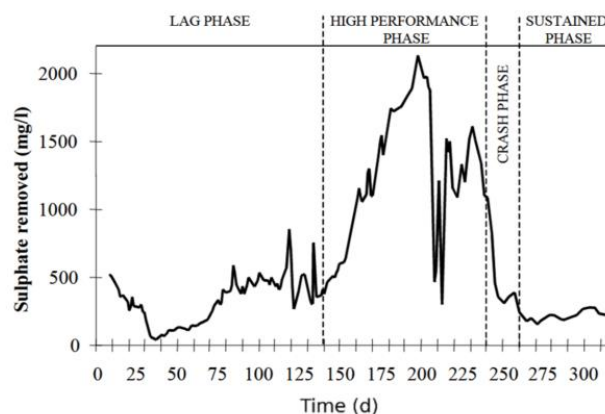


Figure 37: The characteristic sulphate reduction pattern observed in a passive biological sulphate reduction reactor (Pulles *et al.*, 2004)

The sulphate reduction rate of the reactors is shown in Figure 38. During the high-performance stage from days 70 to 120, the average sulphate removal rate was 116 g/m³/d, while a maximum removal

rate of 224 g/m³/d was achieved during this period. The average sulphate removal rate in this study was comparable to the removal rates achieved in the large-scale IMPI (Pulles & Heath, 2009) and cloSURE® (Du Preez, 2022) plants, which were 192 g/m³/d and 233 g/m³/d, respectively. The lower removal rates achieved during this study were attributed to the lower amounts of organic carbon in the packing material, and the continuous addition of supplemental substrate in the large-scale tests.

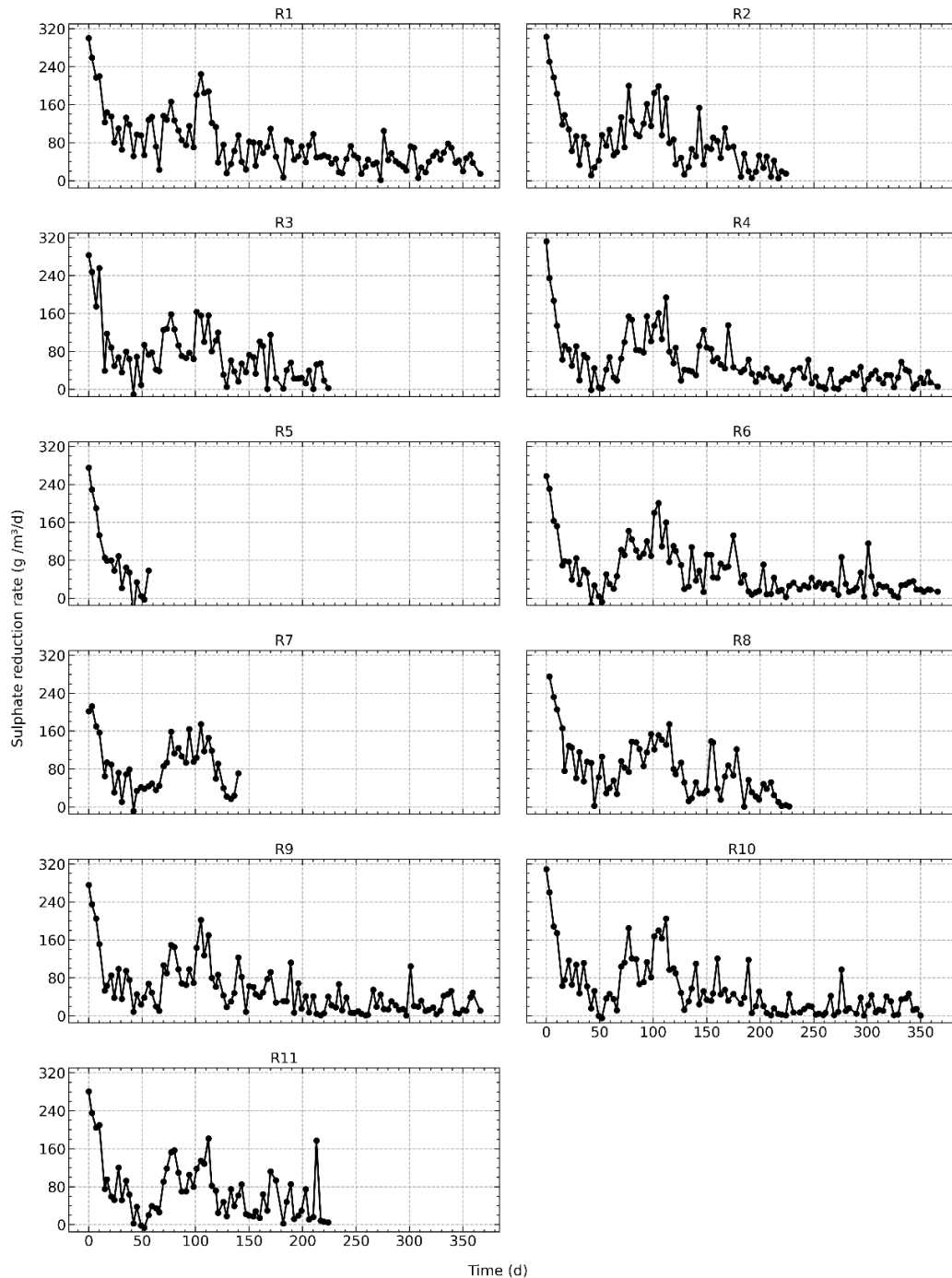


Figure 38. Sulphate reduction rates of reactors over respective operational periods

CHAPTER 5 HYDRODYNAMICS BEHAVIOUR RESULTS AND DISCUSSION

5.1 Residence time distribution analysis

5.1.1 Tools used during modelling processes

The programs and packages used during the data-processing and curve-fitting processes of the RTD results are listed in Table 14. WinPython 3.9.5 was used to run Python on a Windows® operating system.

Table 14. Data-processing environment information

Scripting language	Python 3.9.5
Data analysis platform	Jupyter Notebook 6.4.0 (Kluyver, <i>et al.</i> , 2016)
Packages used	
Pandas (McKinney, 2010)	scipy.optimize.curve_fit (Virtanen, <i>et al.</i> , 2020)
NumPy (Harris <i>et al.</i> , 2020)	Matplotlib (Hunter, 2007)

5.1.2 Tracer test results

The tracer tests were sequentially conducted over the course of a year, comprising five runs. Table 15 shows the days during which the tracer tests were conducted, along with an indication whether the tracer test run was conducted in a particular reactor. During the first tracer test run, no problems were encountered, and all the active reactor tests were conducted. As mentioned previously, the water jacket of reactor R5 failed after 55 days of continuous operation, and the reactor had to be stopped due to water leakage into the reactor. During the second tracer test, a pump failure was experienced due to load shedding, when AMD feed to reactors R1, 2, 8 and 11 stopped for a period of at least 24 hours. Due to this occurrence, the tracer sampling for these reactors was terminated, as the data produced from these tests would be invalid. For the remaining three tracer runs, no difficulties were encountered.

Table 15. Tracer test date of analysis for each reactor

Tracer test run #	Days of operation	Reactor tracer test (Y / N) or (-) for non-operational									
		R1	R2	R3	R4	R6	R7	R8	R9	R10	R11
1	57 – 86	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
2	121 – 155	N	N	Y	Y	Y	Y	N	Y	Y	N
3	188 – 223	Y	Y	Y	Y	Y	-	Y	Y	Y	Y
4	252 – 271	Y	-	-	Y	Y	-	-	Y	Y	-
5	303 – 328	Y	-	-	Y	Y	-	-	Y	Y	-

The standard curve for fluorescence analysis is shown in Appendix B1, with an average standard error of 2.26%. This standard curve was used to convert the fluorescent readings of the tracer samples into tracer concentration, as found in the effluent samples. The results for the first tracer

runs are shown in Figure 39, where it can be seen that low concentration values of the tracer were experienced throughout the test for all the reactors. These low values were attributed to possible degradation of the fluorescein due to extended exposure to light in the sampling tubes after the samples were taken, and to only being analysed at the end of the tracer test for fluorescence. In an attempt to mitigate this occurrence, the sampling tray lids were blacked out to minimise light exposure, and analysis of the samples was done on a weekly basis during the succeeding tracer tests. The majority of these low reading samples occurred below 80 μM across all the tests, and since these low values were not a realistic representation expected from a tracer test to fit a curve to, all values below this set value were removed to allow for accurate post-processing to ensure that the relevant data remained. The tracer test results before and after trimming for the other tracer runs are shown in Appendix C.

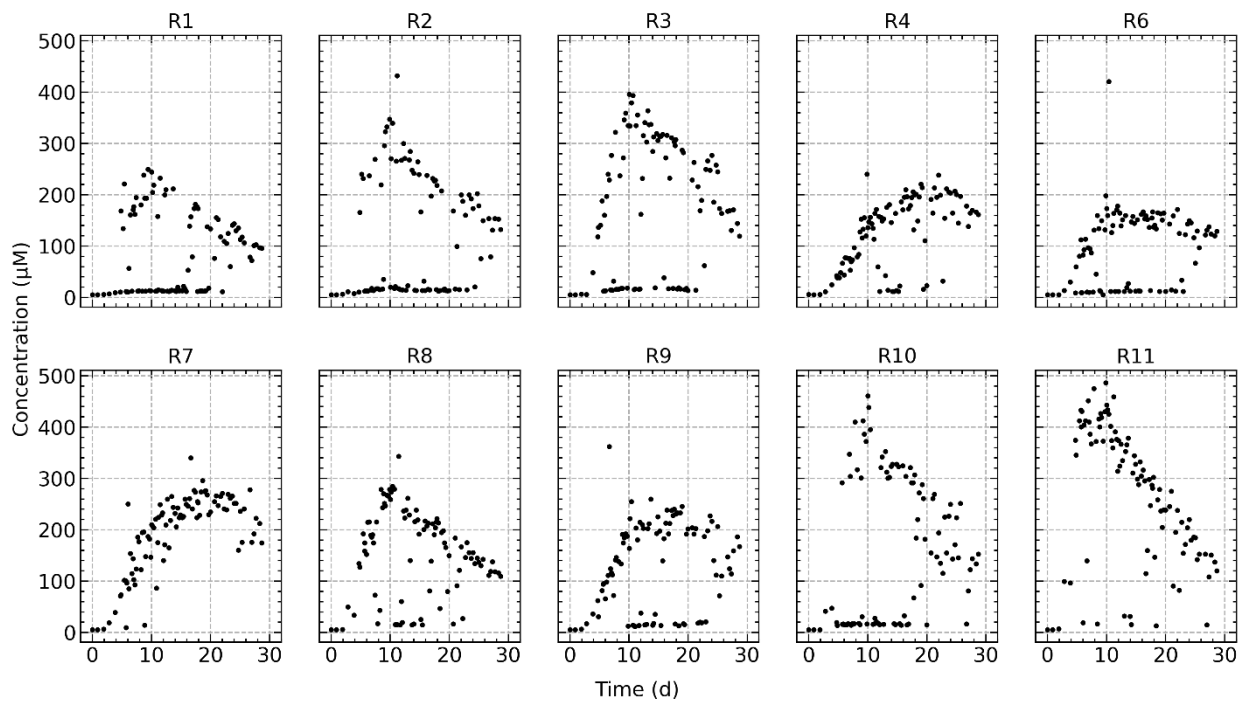


Figure 39. Tracer test results of reactors for Run 1

A comparison between the PFR, CSTR and TIS models fitted to a dataset from the current study is shown in Figure 40. Neither the PFR nor CSTR model followed the shape of the concentration curve that was generated. The accurate fit of the TIS model, however, indicated that it could be used to compare the change in the hydraulic behaviour of the reactors over their respective operational periods. This was a preferred model as it could describe imperfect back-mixing and the non-ideal nature as seen in practical applications.

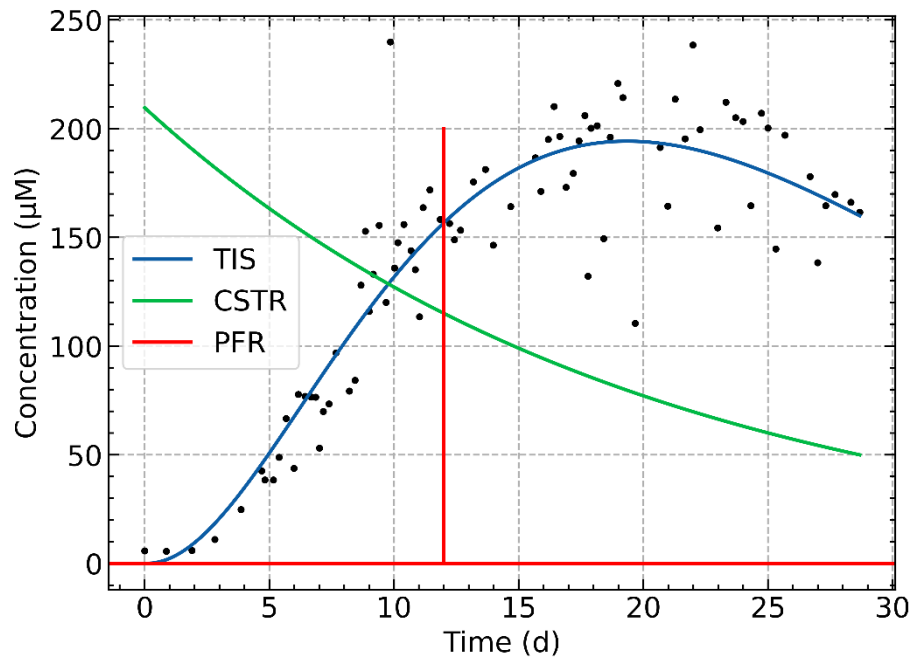


Figure 40. Comparison of flow models fitted to experimental tracer data

The fitment of the TIS model to the tracer data for Run 1 is shown in Figure 41. The model fits the data very well and follows the trend of all the data points during the initial spike, and the long tail after the peak of the tracer as it leaves the reactor. The TIS model fitted to the experimental data of the other runs is shown in Appendix C.

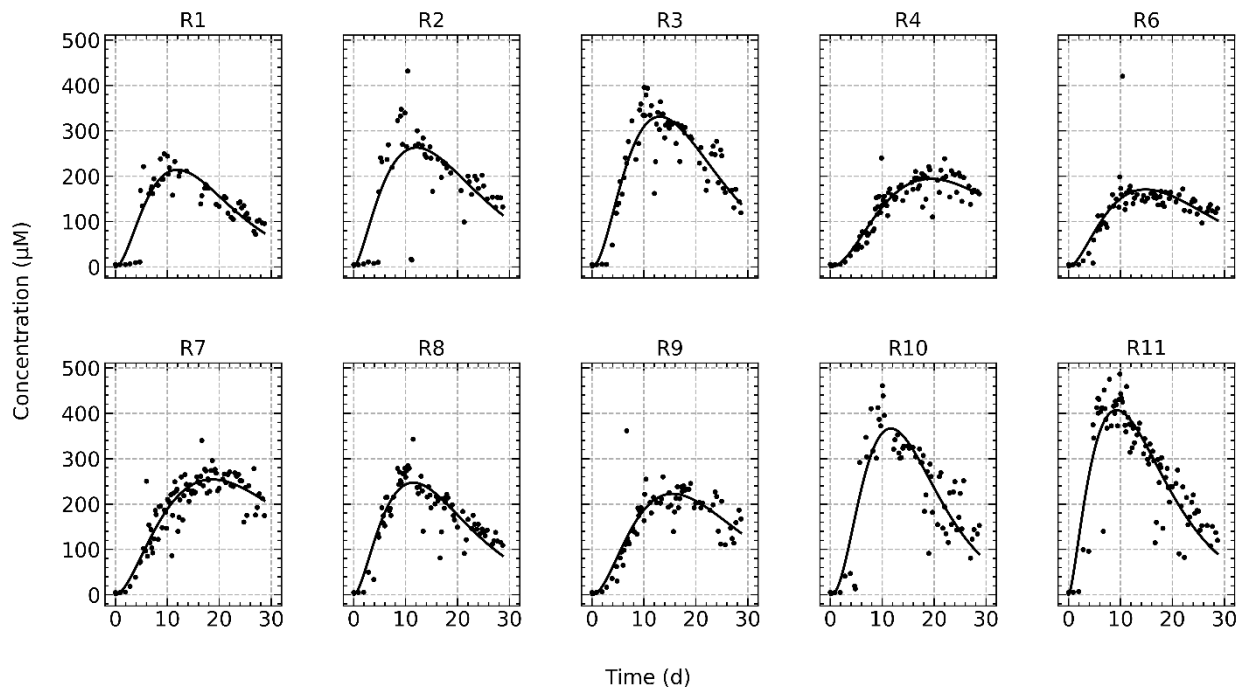


Figure 41. Tanks-in-series curve fitted to tracer data of reactors for Run 1

5.1.3 Comparative analysis

To allow for a comparison between the hydraulic behaviour and sulphate reducing performance, the average sulphate removal percentage for each reactor, during the periods the tracer tests were conducted, was used to compare the performance of the reactors (see Table 16). Reactor R1 maintained a higher average sulphate removal percentage, whereas the rest of the reactors behaved similarly at a lower average percentage. The increased performance of Reactor R1, observed from when the reactor was inoculated, suggested that more available substrate was present in its packing material.

Table 16. Average removal percentage for reactors during the tracer test runs

Tracer run #	Reactor sulphate removal %									
	R1	R2	R3	R4	R6	R7	R8	R9	R10	R11
1	38.83	32.95	35.52	28.86	29.24	31.67	28.78	29.01	29.11	29.71
2	17.85	19.97	14.76	20.12	19.54	14.20	19.67	18.35	15.02	13.65
3	17.99	7.37	8.77	8.64	6.36	-	7.26	9.75	6.37	11.11
4	10.98	-	-	4.72	9.02	-	-	5.25	3.23	-
5	12.03	-	-	8.03	6.50	-	-	5.25	5.76	-

The comparison between the RTD curves achieved during the five tracer test runs is shown for each reactor in Figure 42. Initially (Run 1), most of the reactors had a flatter curve with a long tail, implying a slower mean residence time was achieved with significant back-mixing occurring. In the subsequent tracer test runs, a slight evolution of the RTD curves can be seen in the majority of reactors, experiencing a higher peak much earlier after every run. A decrease in the mean residence time was expected, possibly attributed to the occurrence of channels or bypassing in sections of the packed bed. It was observed that the RTD curves evolved initially, where after the evolution slowed.

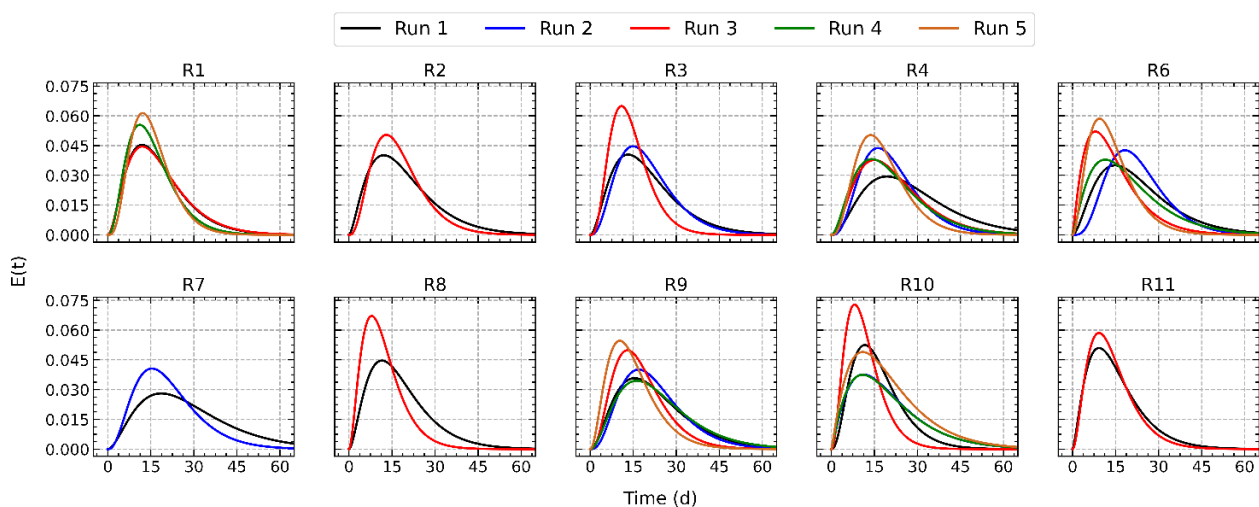


Figure 42. Comparison of residence time distribution curves for each reactor between each tracer test

A comparison between the RTD curves of the reactors during each tracer test is shown in Figure 43. The comparison between the mean residence time and the residence time at the peak tracer concentration is illustrated in Figure 44, and a summary of the RTD parameters is presented in Appendix D.

During the first tracer test, the observed responses showed no correlation with each other when the residence time at the peak varied between 9 and 20 days. At this stage, with this significant variation observed, the individual packing structure of the packed bed could have had a big effect on the hydraulic characteristics. During the period this tracer test was done, a high-performance stage for BSR was experienced, but the difference in the hydraulic behaviour seemed to have no effect on the individual performance of the reactors as similar sulphate removal percentages were achieved.

During the second tracer test, the reactors behaved more comparably, with a decreased spread of the RTD curve at the peak concentrations experienced around day 15 of the tracer test, except for reactor R10, which peaked at day 11 of the tracer test. This test occurred at the end of the performance phase, from days 121 to 155 during continuous operation. During this time, all the reactors experienced a sharp decline in sulphate removal performance from 30% to 18%, on average. With no significant change in the observed hydraulic behaviour, no correlation could be found between the hydraulic behaviour and the declining sulphate reducing performance at this stage.

Further evolution of the RTD curves was seen during the third tracer run from days 188 to 223 of continuous operation, when the spread of the curves reduced, and higher peaks were observed occurring earlier. During this time, the sustained sulphate reduction phase commenced when the average sulphate removal percentage was maintained around 8%, except for reactor R1, which had an elevated removal percentage of 18%. Reactor R1's RTD curve maintained the same shape with a higher peak, showing no indication that the hydraulic behaviour was responsible for the elevated sulphate removal performance as compared to the other reactors.

During the fourth and fifth tracer test runs, the RTD curves of reactors R4, 6, and 9 followed the same trend with the peak value increasing, and the residence time decreasing. This meant that the reactors' flow regime gradually moved in the direction of a more plug-flow-like regime, indicating the possibility of channelling and/or bypassing occurring in sections of the packed bed. Reactor R1's RTD curve remained relatively constant, with the peak increasing and the spread decreasing. Even though the general trend moved towards a less mixed flow, the flow remained well mixed, as indicated by the long tail and wide spread of the RTD curves and the high values of the variance. The evolution of the RTD curves slowed down during the last two tests, suggesting that the change in the packed bed structure ceased at this stage.

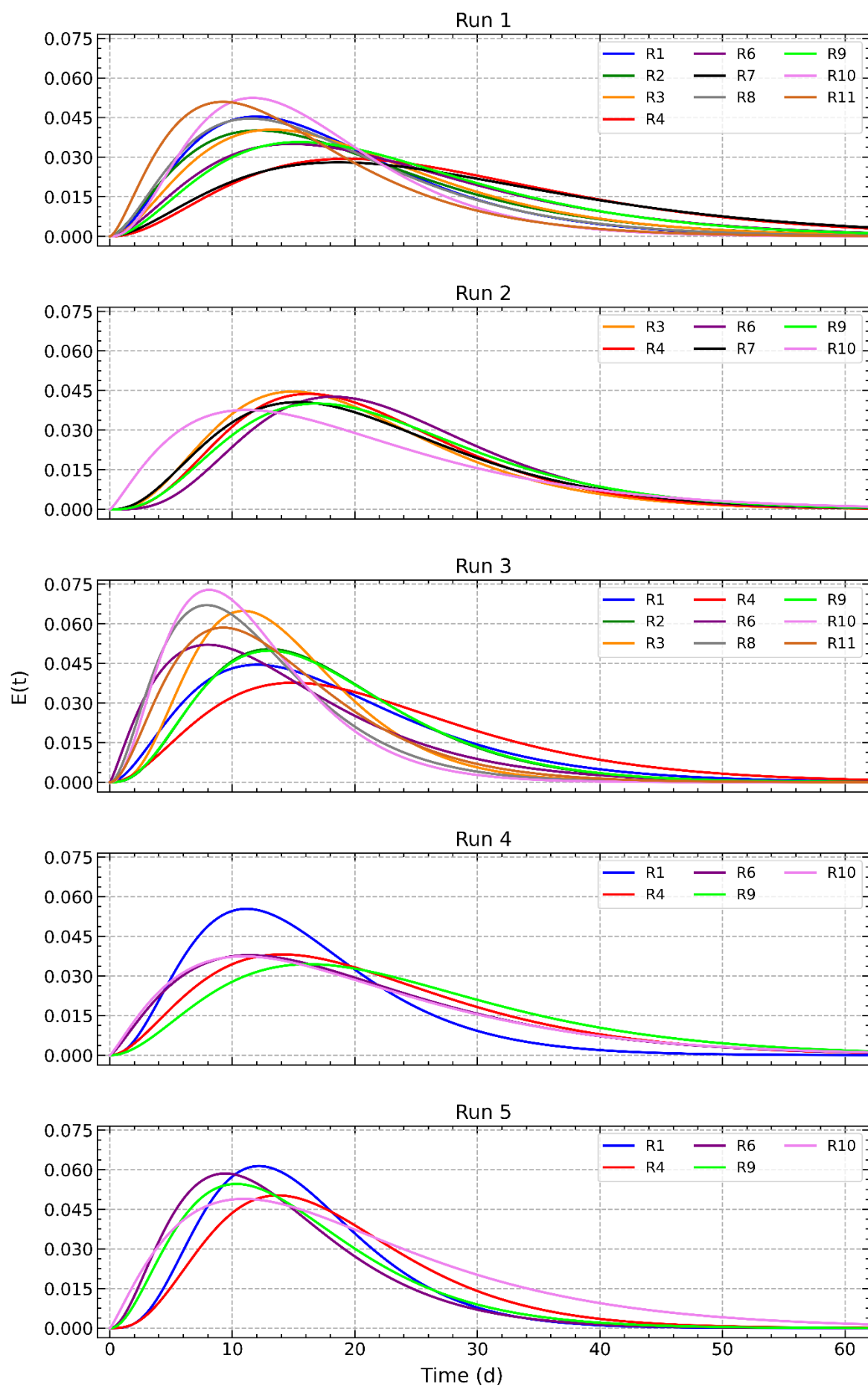


Figure 43. Comparison of residence time distribution curves for each tracer test run of all reactors

In general, the mean residence time decreased over time, as can be seen in Figure 44, where a comparison is shown between the times at which the peak concentration of the tracer was observed and the mean residence time of each reactor. The time differences between these values were attributed to the long tails observed during the tracer tests, when back-mixing occurred, significantly increasing the time the tracer spent in the reactor. The spread of the residence time values between the reactors suggested that the individual packing structures had a significant impact on the overall flow through the packed beds, but over time, all reactors followed a similar trend when the residence times tended to decrease.

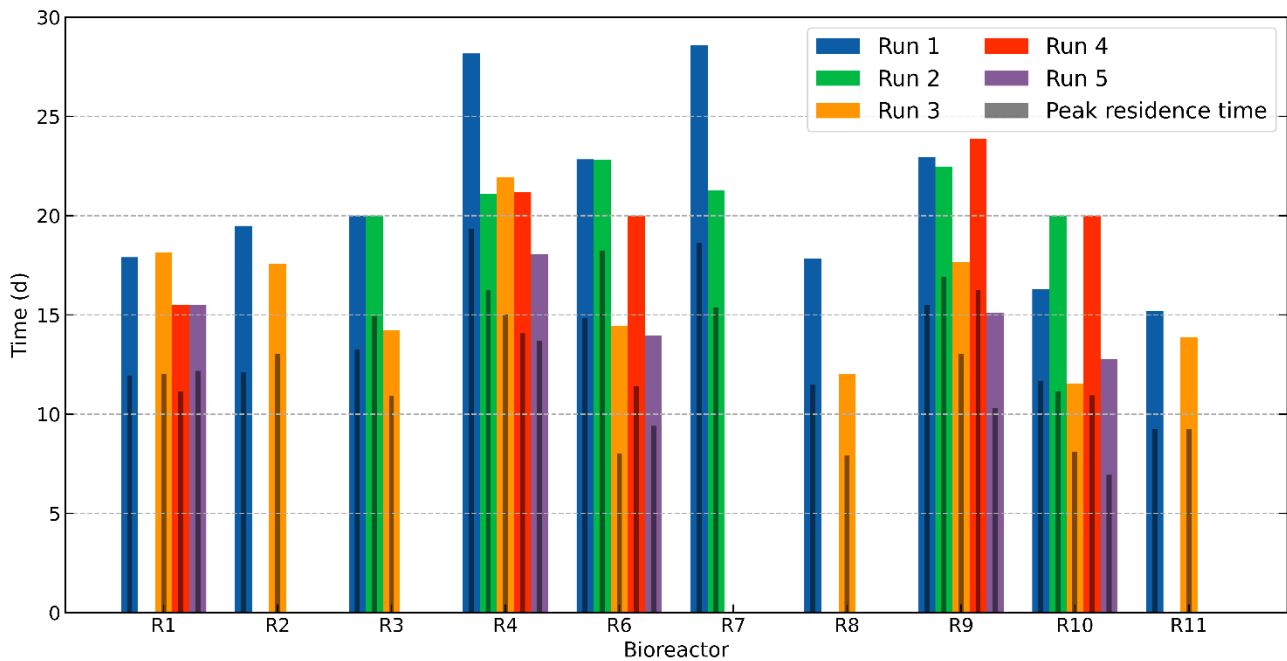


Figure 44. Bar graph comparing the mean residence time and the residence time at the peak of the concentration curve of the reactors for each tracer test run

The magnitude of the variance for the reactors indicated a wide spread of the residence time distributions, which suggested a non-plug-flow regime through the packed bed, as seen in Figure 45. This meant that most of the feed spent significantly more time in the reactor than the pre-determined HRT of 10 days. Over time, a minor trend was observed because in most of the reactors, the variance slightly decreased, suggesting that the structure of the packed bed was evolving, and that bypassing/channelling could be occurring.

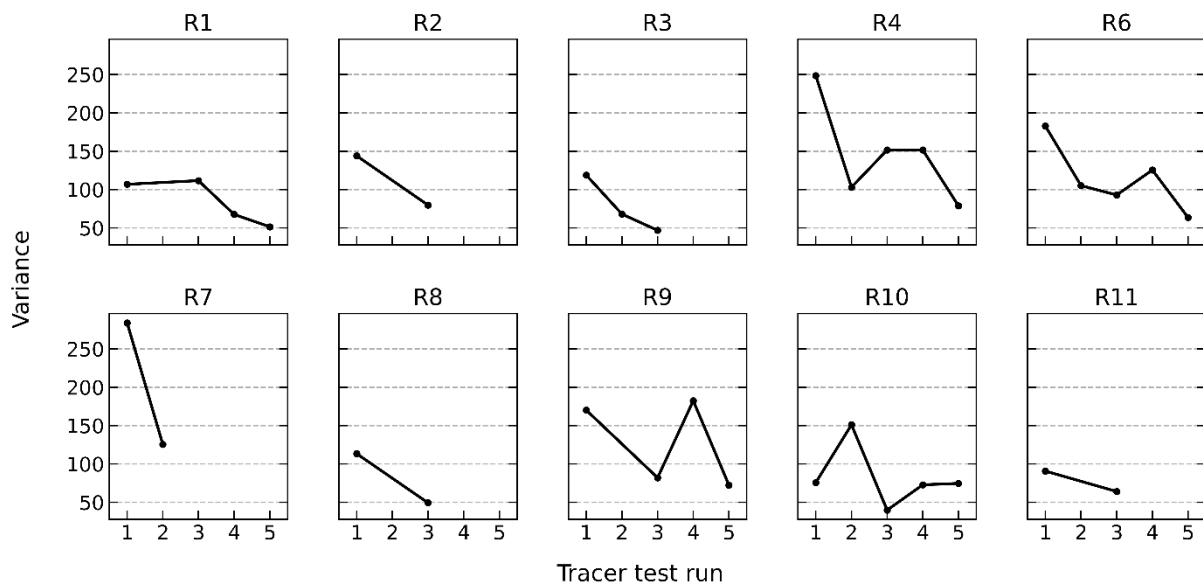


Figure 45. Evolution of the variance of the reactors for each tracer test run

Two mixing indicators, N for the TIS model and the Peclet number, are shown for each tracer test of the reactors in Figure 46 and Figure 47, respectively. During the first tracer test, most reactors conformed to $N \leq 3$ and $Pe_r \leq 5$, indicating a completely mixed flow. The reactors that exceeded these parameters were marginally out of scope, indicating that a mixed flow pattern could still be assumed. Over time, a trend was observed, as these mixing parameters increased, suggesting that mixing through the reactors decreased, and would continue to do so over time. Even though the mixing indicators increased, they remained close to the completely mixed flow limits, and the flow remained well mixed.

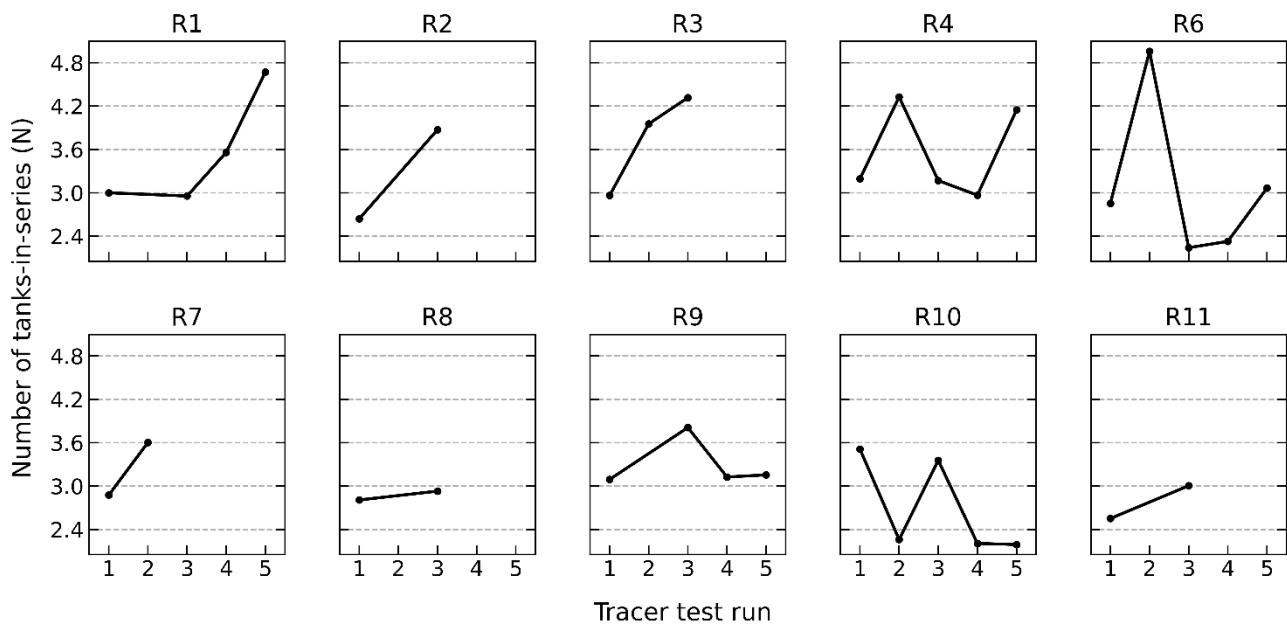


Figure 46. Evolution of the number of tanks-in-series of the reactors for each tracer test run

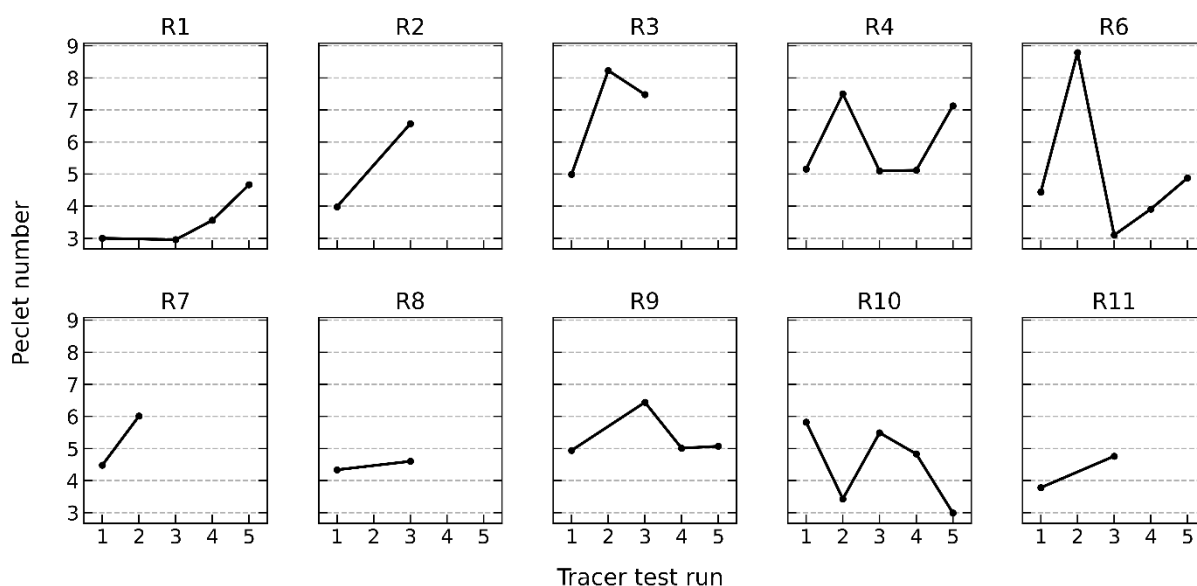


Figure 47. Evolution of the Peclet number of the reactors for each tracer test run

5.2 Packed bed physical analysis

5.2.1 Gelatine stabilisation and sectioning results

To simplify the post-processing of images produced by the sectioning and imaging processes, and to allow for a direct comparison between the reactors' packed beds at distinct positions, the sectioning of the packed beds was done similarly for the reactors that were analysed. The packed beds were sectioned into 4 cm slices, and the positioning of each slice is shown in Figure 48.

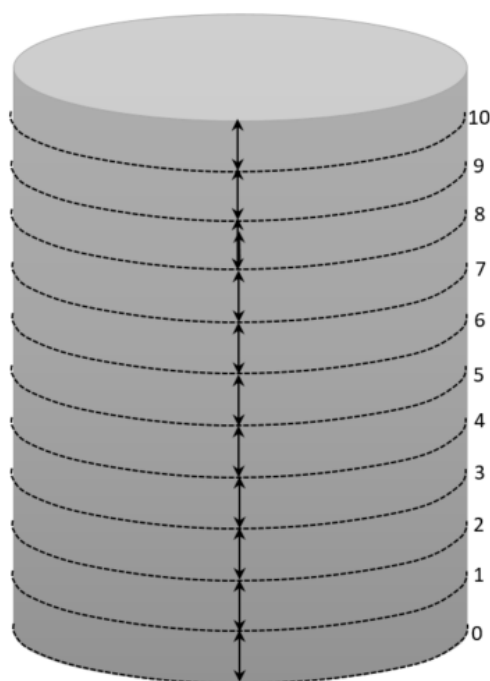


Figure 48. Position of slices made during the sectioning and imaging processes of the reactors' stabilised packed beds

5.2.2 Reactor R1's physical analysis results

The sectioning results for reactor R1, after 364 days of operation, are shown in Figure 49. The images show the occurrence of channels being formed closer to the walls of the reactor, especially in images (1) to (3) where the flow was predominantly occurring on one side of the reactor, and where the darkly coloured areas showed where the stabilisation mixture did not flow. The same occurrence was seen in most of the images, where flow was not evenly distributed across the slices. Images (6) to (9) showed significant amounts of flow that occurred closer to the walls of the reactor, suggesting bypassing of the centre of the packed bed. This could lead to reduced contact with sulphate reducing bacteria and contact with organic carbon, resulting in lower sulphate reduction performance. At the top end of the packed bed (image 10), a relatively even distribution of flow across the slice was seen, where after the flow through the middle of the packed bed reduced as the liquid passed through the packed bed closer to the base. At the base of the reactor (image 0), an even distribution of flow across the slice was seen, which was due to the presence of a liquid section, below the packed bed, which naturally distributed the liquid across the base of the packed bed.

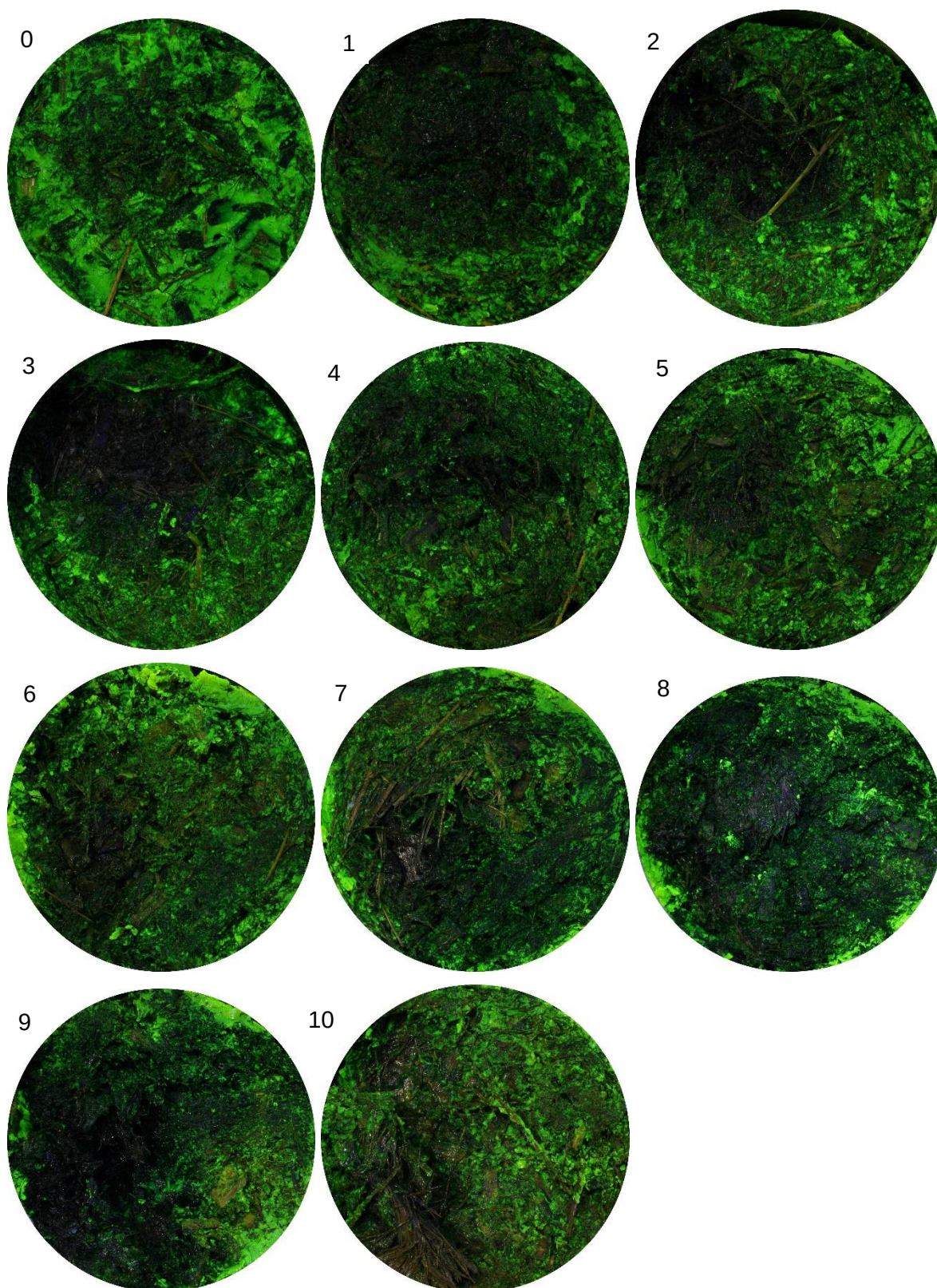


Figure 49. Images produced of each slice during the gelatine stabilisation and sectioning processes for reactor R1, where (0) is at the base and (10) is at the top of the reactor's packed bed

5.2.3 Reactor R4's physical analysis results

The sectioning results for reactor R4, after 364 days of operation, are shown in Figure 50. In image (10), a brown-coloured section was observed in the centre of the slice, due to the presence of solids, probably a form of iron sulphate precipitate, or possibly jarosite that formed in the feed tanks, the contents of which were pumped into the reactor. These solids blocked the centre of the packing material. This bypassing can still be seen in slice (9), where minimal flow was observed in the centre of the slice. This dead space decreased in size as the flow moved down the length of the reactor and disappeared by slice (8). Reactor R4 showed a similar trend to reactor R1, where the flow was spread across the slices closer to the top of the reactor and increased dead spaces were observed at the slices closer to the base of the reactor with the presence of larger channels.

To further understand the impact of the channelling and dead spaces within the reactors, the images were used in CFD models to examine the flow through the packed bed and to evaluate the tracer responses from the CFD models in comparison with what was experienced in experimental tracer tests.

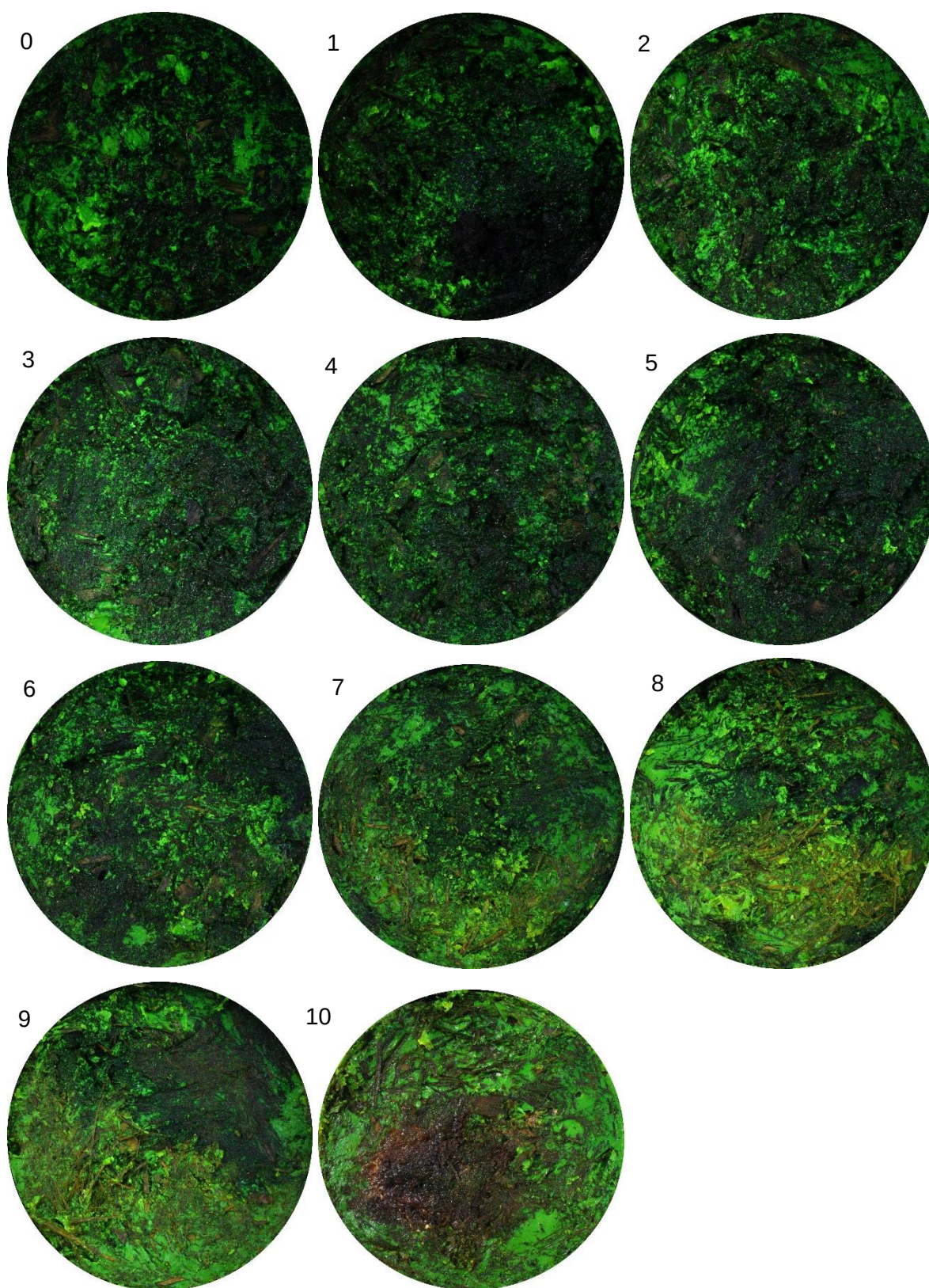


Figure 50. Images produced from each slice during the gelatine stabilisation and sectioning processes for reactor R4, where (0) is at the base and (10) is at the top of the reactor's packed bed

5.3 Computational fluid dynamics

5.3.1 Computational fluid dynamics programs and post-processing tools

The programs and packages used during the CFD modelling process and post-processing of the RTD results, are listed in Table 17. WinPython 3.9 was used to run Python on a Windows® operating system.

Table 17. Computational fluid dynamics data processing environment information

Mesh generation software	Gmsh 4.11(Geuzaine & Remacle, 2009), cfMesh 1.1.1	
CFD simulation software	ESI OpenFOAM® v2212 (ESI-OpenCFD, 2021)	
Visualisation software	ParaView 5.10.0 (Ayachit, 2015)	
Scripting language	Python 3.9.5	
Data analysis platform	Jupyter Notebook 6.4.0 (Kluyver, <i>et al.</i> , 2016)	
Pre- and post-processing packages used		
NumPy (Harris, <i>et al.</i> , 2020)		Scipy (Virtanen, <i>et al.</i> , 2020)
scikit-image (Van der Walt <i>et al.</i> , 2014)		Matplotlib (Hunter 2007)

5.3.2 Process geometry, image processing and porosity field

The reactor geometry was specified directly using coordinates and dimensions in accordance with the design drawing, as shown in Section 3.2, taking into consideration the height of the liquid level and the placement of the outlet T-piece.

The photos produced of the physical analysis were processed using Python code in Jupyter Notebook to segment the images into a porous structure. The segmentation process relied on the green-colour properties of the fluorescent dye used in the stabilisation mixture. This was based on the assumption that a maximum green-colour value (255) indicated a wide open (porous) space, and the absence of green (0) indicated a solid (non-porous) structure. The closer the value to a high of 255, the progressively more porous the structure was considered to be. This process was repeated for each slice while, for consistency, the camera settings remained constant for all the photos.

To construct a porosity map for the packed bed in the simulation, the porosity values generated for each 2D slice, as described in Section 5.3.2, were averaged on a grid corresponding to a computational mesh resolution of 0.005. The averaged 2D-porosity slices were then vertically stacked, as shown in Figure 48, and interpolated linearly between the slices, which resulted in a reconstructed 3D-porosity field. This porosity field was fitted to the initial porosity average of 0.43 due to the repeatability of the results, as presented in Section 3.1, to achieve a representative model.

The reconstructed 3D-porosity field physical structure-equivalent of reactors R1 and R4 is shown in Figure 51. It can be seen that the reconstructed structures of both reactors showed the presence of

dead spots throughout the packed bed, which was more prevalent in reactor R4. These results coincided with the images produced of the physical analysis process wherein the fluorescent setting agent could not penetrate and, therefore, could not illuminate when the ultraviolet light was shone upon the slices. This could mean that these sections were not utilised and could thereby limit the availability and transport of the organic carbon to the bacteria.

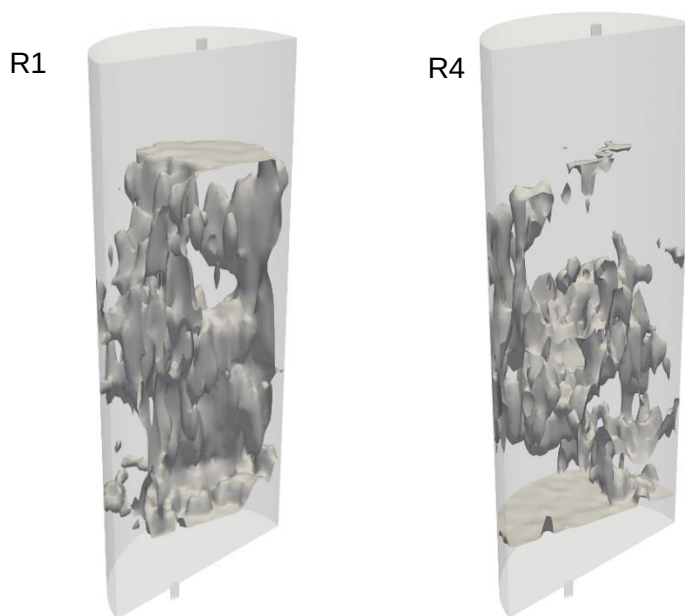


Figure 51. Packed bed structure of reactors, R1 (left) and R4 (right)

The porosity field of the packed bed structures across the vertical slices of the reactors is shown in Figure 52 and of a horizontal slice in the middle of the reactor in Figure 53. It can be seen that the porosity varied across both the vertical and horizontal slices. Both reactors showed increased porosity close to the walls, which represented open sections of the packed bed. This irregular porosity would cause uneven permeability of the packed bed structure that could lead to the formation of channels that would run through the packed bed to form a complex path, increasing the overall distance the liquid travelled through the reactor. Consequently, this could possibly impair the reactor's ability to remove the sulphate as reduced amounts of substrate would be available, in addition to lower amounts of bacteria in contact with the mine water.

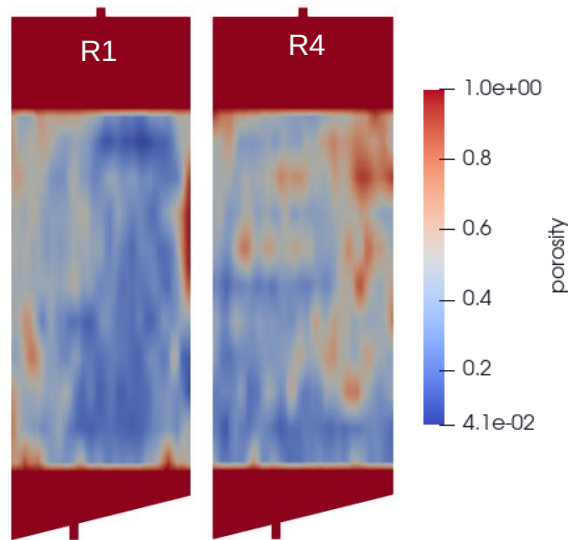


Figure 52. Porosity field of vertical slice down the middle of reactors, R1 (left) and R4 (right)

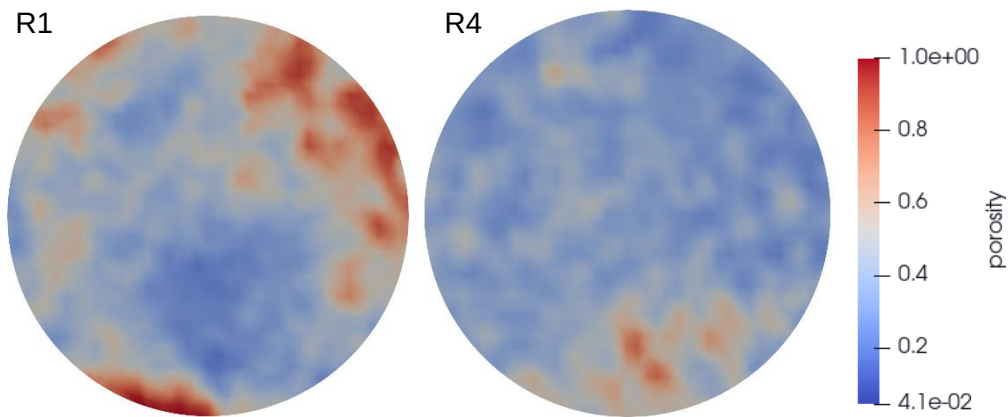


Figure 53. Porosity field of horizontal slice in the middle of reactors, R1 (left) and R4 (right)

5.3.3 Computational fluid dynamics modelling workflow and case conditions

The geometry was meshed using a pair of open-source meshing tools, called Gmsh and cfMesh. Gmsh was used to construct the outer surface of the geometry, and cfMesh was used to fill the space inside the surface with polyhedral elements with a mesh resolution of 0.005. The mesh-dependence study is shown in Appendix E. The mesh generated for the reactor is illustrated in Figure 54, showing that the structure was meshed into predominantly hexahedral elements by the cfMesh tool. It is important to note that in the CFD models, the airspace above the packed bed was not considered, and only the liquid phase was modelled.

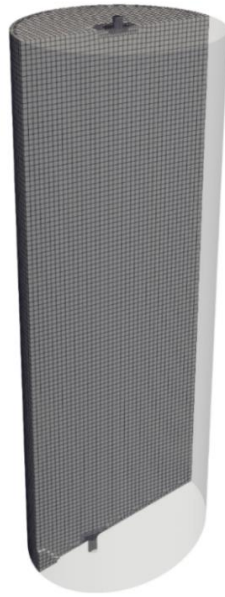


Figure 54. Mesh structure of the reactors

The steady state flow simulation employed the **porousInter** solver, which was developed by Oxtoby *et al.* (2013), to model the flow through the packed bed due to the arbitrary porosity distributions of a packed bed structure. This solver explicitly addresses accuracy and stability issues resulting from sharp discontinuities in the porosity field. The steady state flow fields produced by the steady state flow simulation provided the basis for the tracer dispersion model. The dispersion of the tracer was modelled using a version of the **scalarTransportFoam** solver, available in standard OpenFOAM® distributions, which accounts for the convection and diffusion of a tracer material in the presence of specified velocity fields. The tracer concentration was added to the reactors as a step input, and the concentration of the tracer was measured at the centreline of the outlet of the reactor. Since the flow rate was low, the addition of the tracer did not affect the flow field and the calculations could be decoupled. The measured tracer concentration curve was then differentiated numerically to obtain a pulse input response, to compare the results with what was obtained during the experimental tests. The CFD workflow is illustrated in Figure 55.

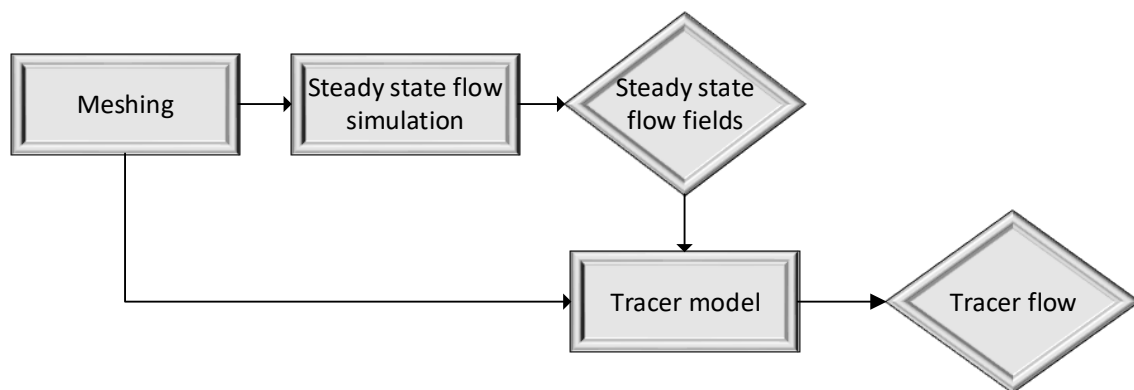


Figure 55. Computational fluid dynamics workflow process diagram

The AMD feed water, consisting mainly of water and small amounts of dissolved metals, which was removed by the lime-neutralisation step, as described in Section 3.3, was considered a Newtonian fluid, which was assumed to be similar to water (density = 1000 kg/m³, viscosity = 0.001 kg/m/s). In the current study, the BSR reactor temperatures were maintained at a constant temperature, and thus, the energy transport equation was not solved as isothermal conditions were assumed. Single-phase flow was considered as minimal amounts of H₂S were produced (< 2%, which was mostly in a dissolved form at the operating pH) during operation. The diffusion coefficient of the tracer in the fluid was specified, based on literature, as 0.42 x 10⁻⁹ m²/s (Casalini *et al.*, 2011). The boundary conditions for the steady state flow model and the tracer dispersion model are listed in Table 18. As mentioned previously, with the low flow rates, pulsatile flow would occur where the wall shear force would be close to zero and the velocity at the wall would be a slip condition (velocity not equal to zero). A summary of the numerical methods, used in the OpenFOAM® models, is listed in Appendix F.

Table 18. Model boundary conditions

Variable	Boundary	OpenFOAM® boundary type	Condition
Velocity	inlet	fixedValue	$(0, 0, v_{z0})$
	outlet	zeroGradient	$\frac{\partial \mathbf{v}}{\partial \mathbf{n}} = 0$
	walls	slip	$\frac{\partial(\mathbf{v} \cdot \boldsymbol{\tau})}{\partial \mathbf{n}} = 0,$ $\mathbf{v} \cdot \mathbf{n} = 0$
Pressure	inlet	fixedFluxPressure	$\frac{\partial P}{\partial \mathbf{n}} = \mathbf{M} \cdot \mathbf{n}$
	outlet	fixedValue	0
	walls	fixedFluxPressure	$\frac{\partial P}{\partial \mathbf{n}} = \mathbf{M} \cdot \mathbf{n}$
Concentration (tracer)	inlet	fixedValue	1
	outlet	zeroGradient	$\frac{\partial c}{\partial \mathbf{n}} = 0$
	walls	zeroGradient	$\frac{\partial D}{\partial \mathbf{n}} = 0$

Under the above-mentioned conditions, v_{z0} represents the inlet velocity which was determined from the cross-sectional area of the inlet and the volumetric feed rate of AMD to the reactor (1 L/d). $\mathbf{n}$ and $\boldsymbol{\tau}$ are the normal and tangent vectors to the boundary surface. $\mathbf{M}$ was the evaluation of all momentum, viscous, and body force terms in the momentum conservation equation at the relevant boundary. At wall boundaries, flow perpendicular to the surface was zero and dominated by the gravitational body force term $-\rho \mathbf{g}$. The fluid momentum and viscous forces at inlets should also be

included. However, in the present study, these additional terms were generally very small due to the low overall velocities in the system.

5.3.4 Computational fluid dynamics results

5.3.4.1 Steady state flow model

Figure 56 illustrates the streamlines through the reactor, obtained from the steady state flow model, and Figure 57 shows the streamlines across the top of the reactor as the mine water flowed through reactors R1 and R4. By comparing the results obtained from the steady state model to the vertical slices of the porosity distributions of both the reactors, in Figure 52, it can be seen that the regions with higher porosities corresponded to the positions of the increased streamlines, as seen from the steady state flow results in Figure 56. These increased concentrations of streamlines through the higher porosity areas emphasised the potential influence of an uneven porosity, which resulted in an irregular distribution of the fluid flow through the packed bed. Abnormal routes of the flow lines were observed at the top of reactor R1, where the length of the path was longer than the height of the packed bed. These effects of the uneven flow distributions through the packed bed were investigated in the tracer dispersion model.

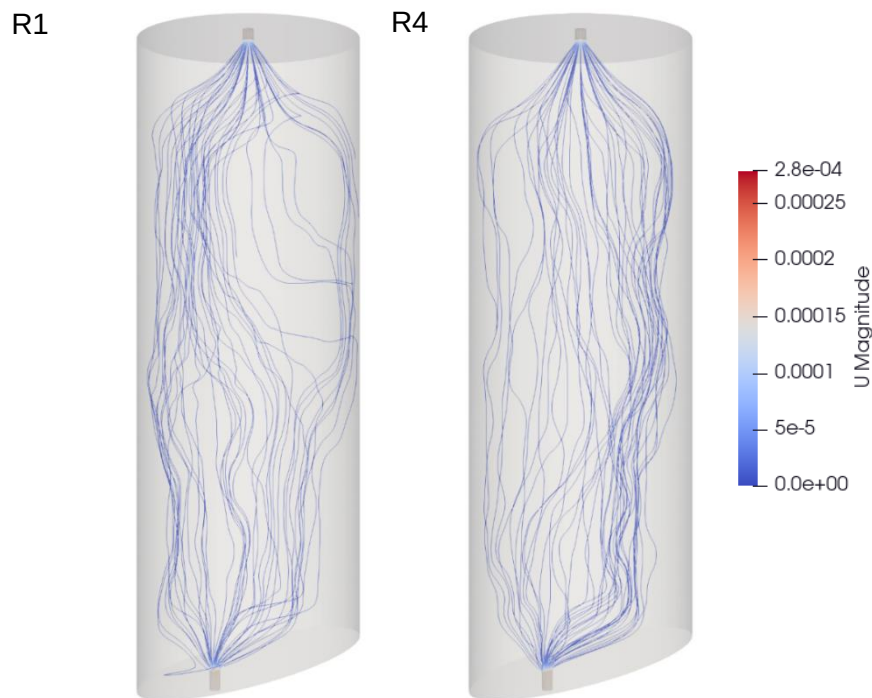


Figure 56. Steady state flow model streamlines through reactors, R1 (left) and R4 (right)

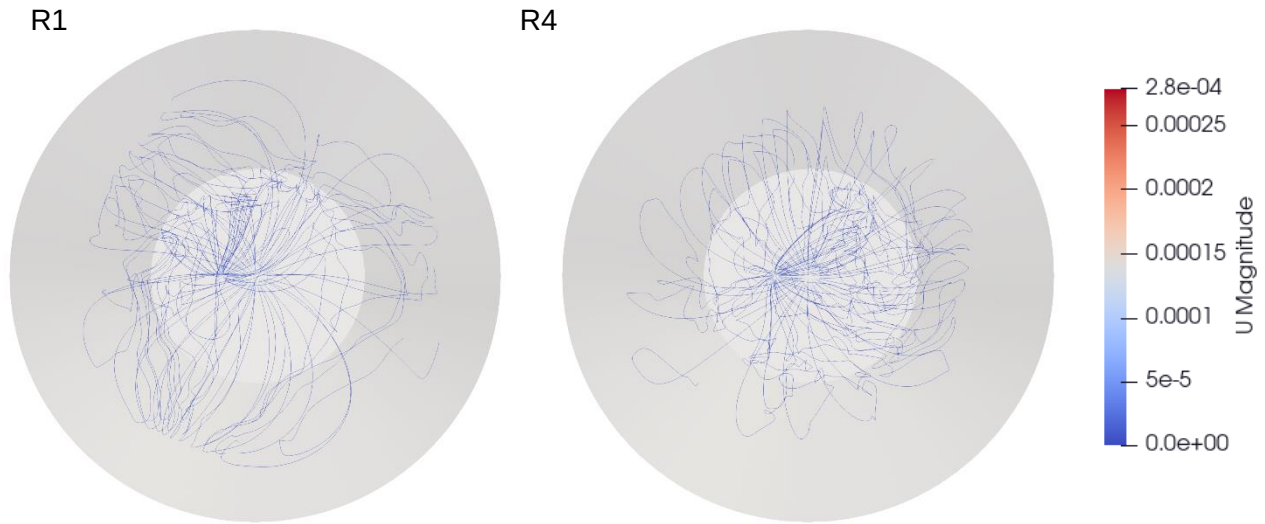


Figure 57. Steady state flow model streamlines across the top of reactors, R1 (left) and R4 (right)

5.3.4.2 Tracer dispersion model

Figure 58 and Figure 59 show the concentration of the tracer at different time intervals as it flowed through the reactors, after it was added as a step input. The initial tracer flow in both cases behaved similarly, with the initial flow preferentially occurring on one side of the reactors. This asymmetric flow followed the higher porosity areas, as seen from the inconsistent porosity distributions in Figure 52. The asymmetry was more prevalent through reactor R1, where the blue coloured areas (no presence of tracer) remained for a longer period of time. With the observed spread of the tracer, it could be assumed that most of the flow of the mine water could not effectively utilise most of the packed bed due to the variation of the permeability.

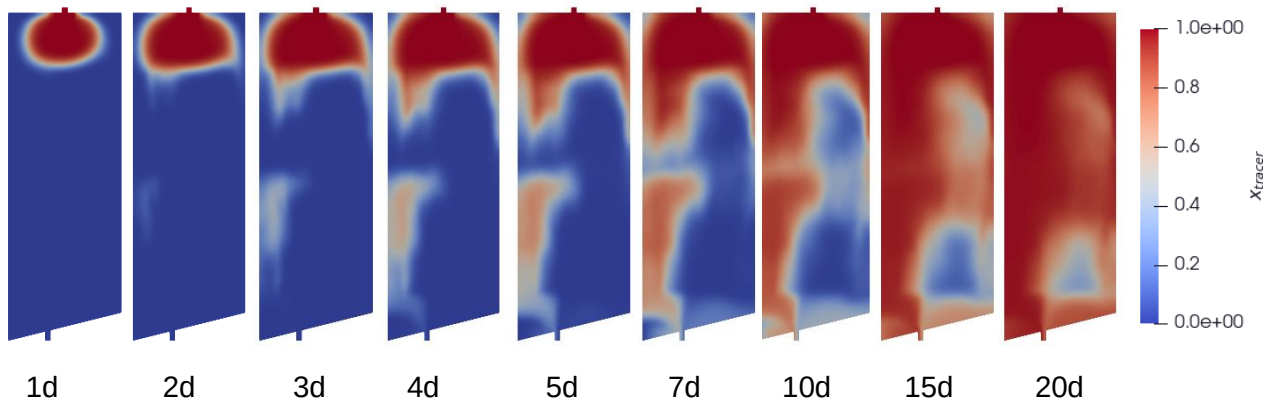


Figure 58. Tracer flow through reactor R1 at corresponding time intervals

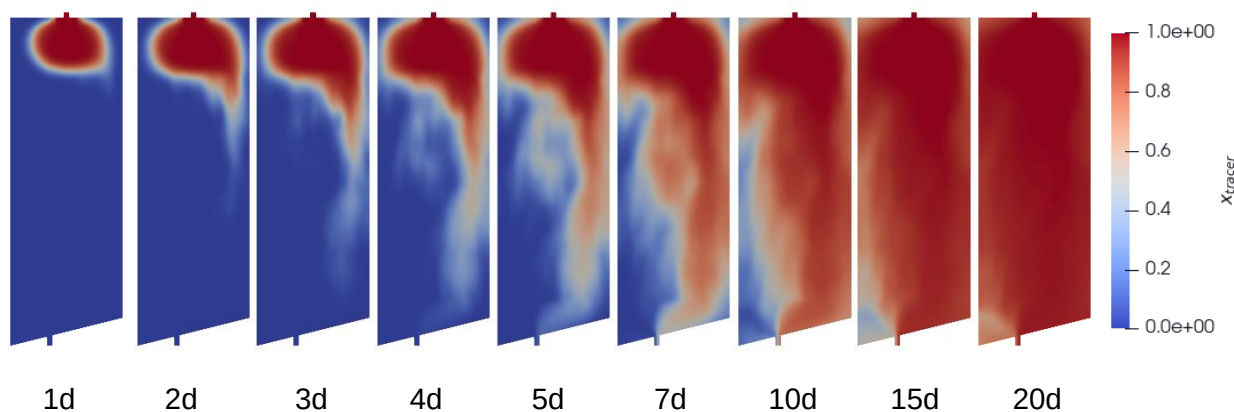


Figure 59. Tracer flow through reactor R4 at corresponding time intervals

5.3.4.3 Residence time distribution and comparison of modelling approach

To investigate the results obtained from the CFD modelling of the process, the modelled RTD results were compared to the experimental RTD curves obtained from tracer Run 5, which are shown in Figure 60. Although the modelled peaks did not align with the experimental data, the shape of the tracer responses exhibited similar behaviour, with reactor R1 peaking earlier and higher with a narrower spread, and reactor R4 having a delayed and lower peak with a wider spread. The earlier peak of reactor R1 was attributed to the bypassing sections of the packed bed, as seen from the tracer dispersion model, in Figure 58, where the tracer at day 20 did not saturate the entire reactor. It showed a reduction in the effective volume of the reactor, which allowed for the peak concentration to be measured earlier. The similarity in the shape of the responses indicated that the observations made regarding the structure of the packed bed during physical analysis and the CFD models could be correlated back to the packed bed structure of the experimental reactors, suggesting the occurrence of channels at the end of the operational periods. The variation in the residence time between the modelled and experimental data was investigated by sensitivity analysis (see Section 5.3.5).

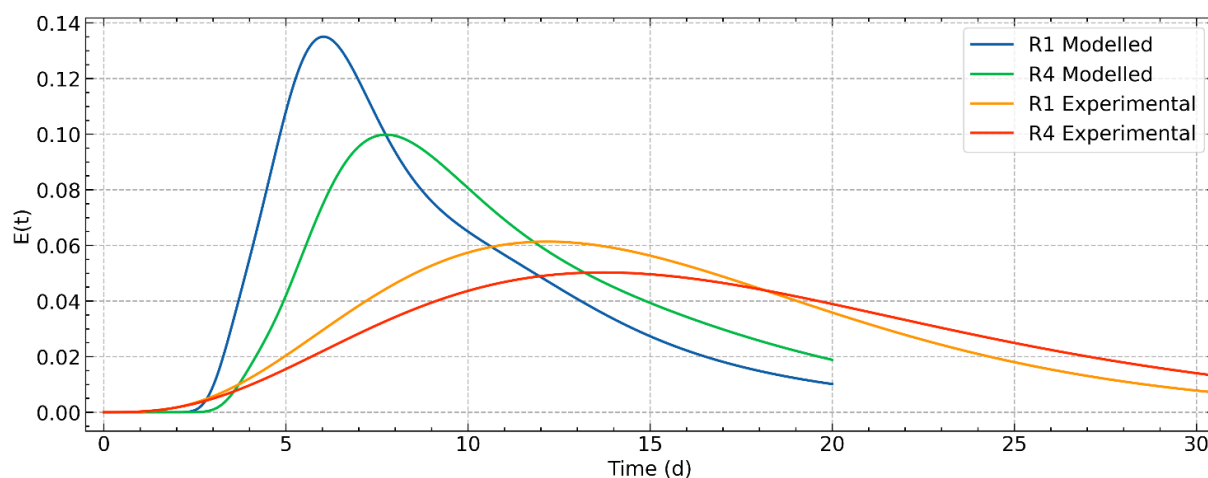


Figure 60. Comparison of the experimental residence time distribution responses with the computational fluid dynamics modelled residence time distribution responses for reactors R1 and R4

5.3.5 Computational fluid dynamics sensitivity analysis

5.3.5.1 Effect of porosity interpretation

The assumptions made during the reconstruction of the packed bed, which determined the porosity of the reconstructed models, were expected to impact the volume of the reactor. The change in the operating volume would impact the time at which the tracer reached the outlet. To investigate the effect the porosity might have on the CFD outputs, the five cases which were assessed are listed in Table 19.

Table 19. The effect of porosity on computational fluid dynamics of investigated cases

Case	Case description	Rationale
R1_0.43	Reconstructed packed bed scaled to an average porosity of 0.43.	Baseline.
R1_0.48	Reconstructed packed bed scaled to an average porosity of 0.48.	Investigating the effect of the fitted porosity value on the modelled RTD results with 10% volume of the packed bed hydrolysed.
Rc_0.43	Constant uniform porosity (0.43) packed bed.	Investigating the effect of a uniform-porosity packed bed.
Rc_0.48	Constant uniform porosity (0.48) packed bed.	
R_Empty	Empty reactor containing only liquid.	Investigating the flow behaviour through a reactor containing no packed bed.

The RTD responses achieved from these cases are illustrated in Figure 61. The RTD curves of the constant porosity cases showed a delayed peak, with a higher peak value and a narrower spread, indicating that with a constant porosity, a more plug-flow-like regime could be obtained as more of the packed bed structure was utilised. The empty case showed a much longer delay before the tracer was measured at the outlet; this was expected due to the larger equivalent volume the tracer had to flow through because of the higher porosity. The peak concentration during this case occurred at around 14 days, lining up accurately with the liquid volume of the reactor of 13.66 L and a flow rate of 1 L/d. This validated the soundness of the assumptions made during the setup of the CFD models.

It was noticeable that, under the assumption that 10% of the kraal manure would be hydrolysed, a small delay of the RTD curve was experienced. This change was not significant, though it was an important factor for increasing the accuracy of the reconstructed case, where, if an unrealistic porosity would be selected, the volume of the reactor could be unrepresentative of a real-world case. For this reason, it is recommended that a reliable method is developed for estimating the operating volume of the reactor so that the porosity of a large-scale plant could be accurately measured.

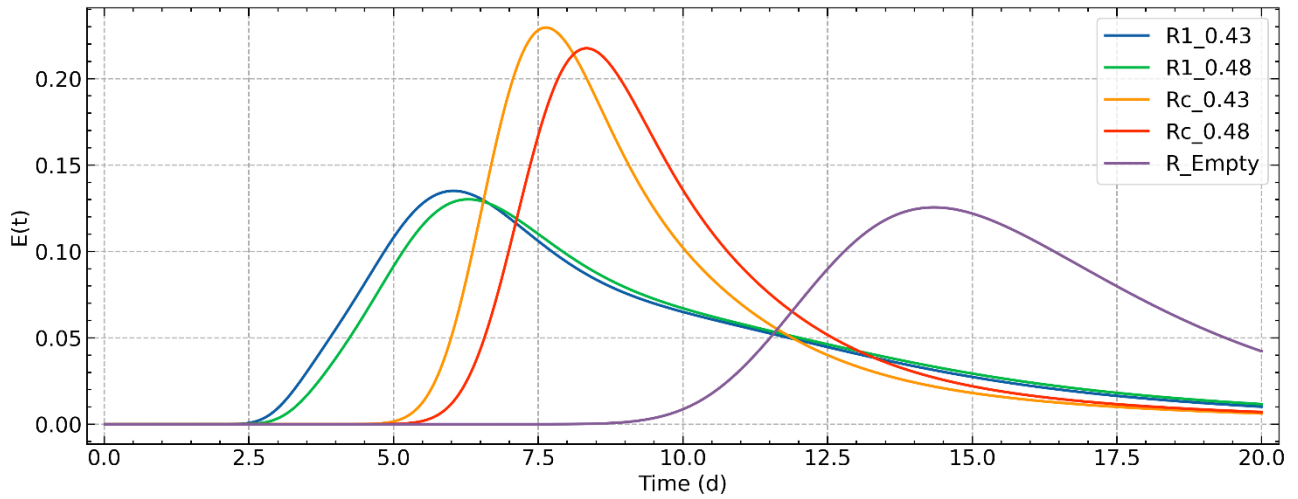


Figure 61. Comparison of different porosity cases using reactor R1

5.3.5.2 Diffusion coefficient

It was possible that the presence of dissolved salts and metals, as found in AMD, and the presence of bacteria, could have influenced the diffusion coefficient in the current study, which would have had a notable impact on the dispersion of the tracer. To investigate the potential impact of a variance in the diffusion coefficient due to the application of the tracer in this study, six cases were examined. The diffusion coefficient of the R1_0.43 case was multiplied by factors of 10, 50, 100, 500 and 1000 (see Figure 62). It can be seen that with an increase, of up to 50 times, the peak was delayed with a longer tail which resembled the experimental results. As the diffusion coefficient was increased further by 1000 times, the peak was experienced earlier with an exceptionally long tail. Thus, when the diffusion coefficient is increased to an exceptionally large number, the flow regime will exhibit a more CSTR-like behaviour where the peak is experienced sooner, and the tail is extended due to extensive mixing.

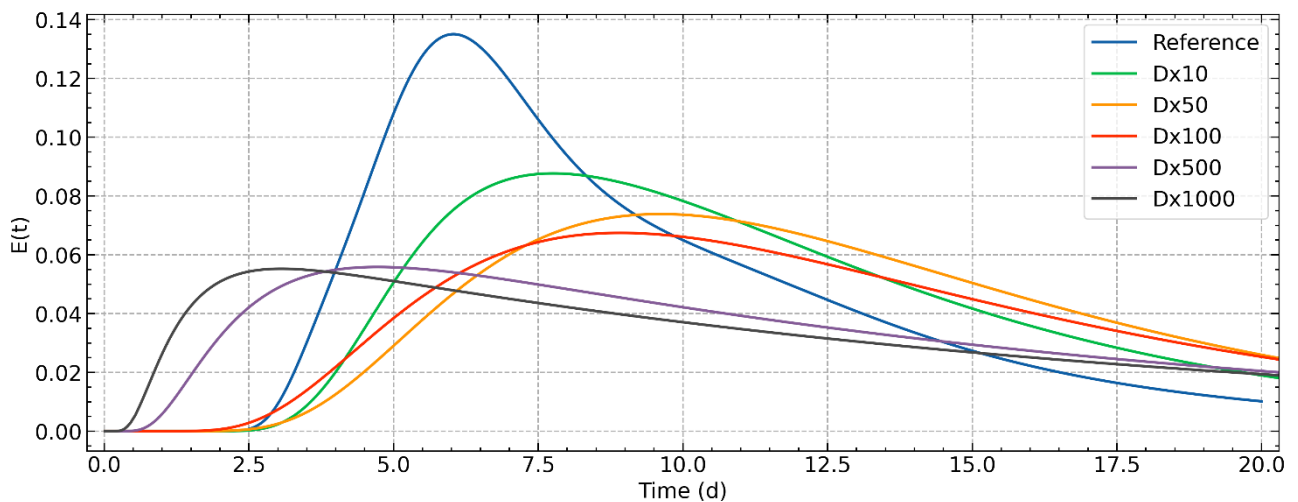


Figure 62. Diffusion coefficient sensitivity analysis on case R1_0.43

A best-case scenario of the diffusion coefficient (Dx50) was chosen for comparison with the experimental RTD results to assess the congruity of the findings (see Figure 63). The tracer

response from the Dx50 case was very similar to the experimental results, where the time at which the peak concentration occurred and the shape of the response, were more comparable. These results indicated the importance of understanding the tracer properties in new applications, as they could have a significant effect on tracer behaviour: with a small change in the diffusion coefficient (Dx10), the peak tracer concentration was delayed by around a day. Although these results were comparable, until the diffusion coefficient is scientifically measured under the current study conditions, the results will remain a best-case scenario.

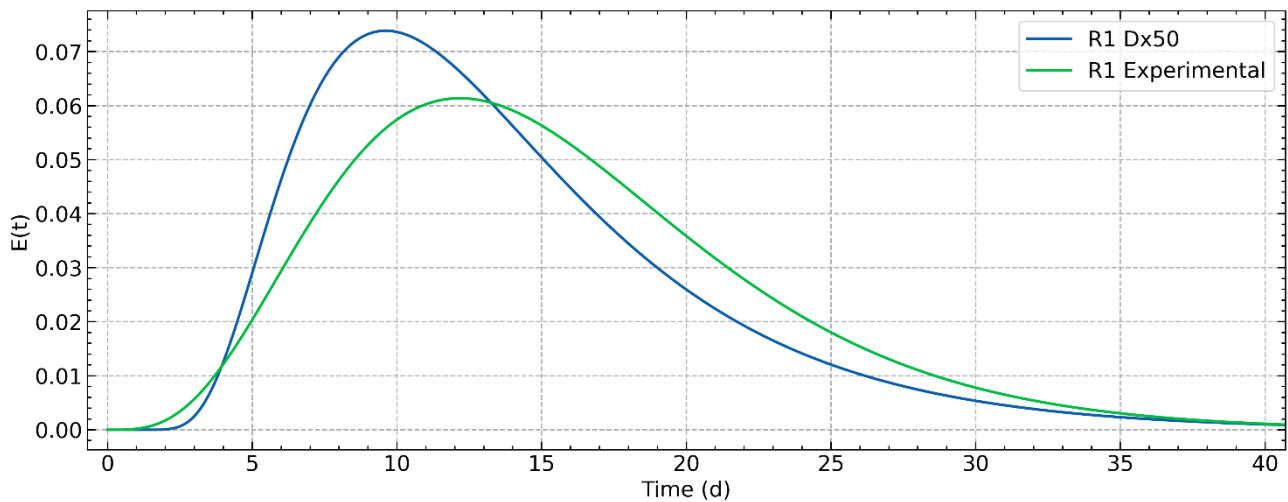


Figure 63. Diffusion coefficient best-case scenario compared to experimental residence time distribution results for R1

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CHAPTER 6 CONCLUSIONS AND RECOMMENDATIONS

This master's degree was focused on investigating the evolution of the hydraulic behaviour of a packed bed reactor over time and the effects of these changes on the sulphate removal performance of the reactor. This was achieved by monitoring the hydraulic behaviour over the duration of the study with tracer tests to observe any changes in the flow patterns through the reactor. The physical structure of the packing material was investigated by stabilising and sectioning the packed bed. CFD modelling was done of the flow through the reactors to further understand the experimental RTD results. This study was done to determine whether there was a link between the hydraulic behaviour of the process and its sulphate reducing performance, while the structure of the packed bed evolved over time.

6.1 Reactor performance

The conclusions related to the reactor performance are:

- The pH values of treated water of all the reactors were similar, with values maintained between 6.6 and 7.0. These values were in the expected range for a BSR system used for treating mine water, as the target pH values for discharge were close to neutral.
- The ORP of the reactors' effluent was maintained below -240 mV throughout the study, and below -300 mV during the high-performance phase of the reactors. These negative values showed that the bacteria created a reductive environment due to the occurrence of sulphate reduction.
- The EC values into and out of the reactors remained relatively constant, which indicated that there was minimal removal of dissolved chemicals/salts. This was due to the limited availability of organic carbon, which led to lower rates of sulphate reduction.
- A minimal increase in alkalinity occurred through the reactors, with an approximate production of 150 to 200 mg CaCO_3/L . This low production of bicarbonates indicated the limited organic carbon availability, which led to lower rates of sulphate reduction, in accordance with Equation [8].
- A slight increase in COD concentrations, between days 70 and 120, was observed. The level of COD did not directly indicate how much organic carbon was available to the bacteria for consumption, but rather indicated that excess amounts of COD remained in the effluent after BSR occurred. This was attributed to the liberation of organic carbon from the bed material as degradation and hydrolysis of the packing material occurred.

- Following the high-performance stage from days 70 to 120, during which the sulphide concentrations were maintained at 100 mg/L, a decrease in the sulphide concentrations occurred to values below 50 mg/L as a result of the decline in sulphate reduction. The sulphide concentrations closer to the end of the study declined even more as sulphate reduction ceased, indicating the process only functioned as a bioneutralisation process after the high-performance phase due to limited availability of organic carbon.
- All the reactors exhibited the same sulphate removal performance trends, with distinct phases, as listed in Table 20.

Table 20. Reactor performance phases

Phase	Operational days	Duration
Lag	0 – 70	70
High-performance	71 – 120	50
Crash	121 – 130	10
Sustained	131 – 364	234

A maximum sulphate removal rate of 224 g/m³/d and an average of 116 g/m³/d were achieved during the high-performance phase from day 71 to day 120. The average sulphate removal rate in this study was similar to the removal rates achieved in the large-scale IMPI and cloSURE[®] projects, of 192 g/m³/d and 233 g/m³/d, respectively. The lower removal rates achieved during this study were attributed to the lower amounts of organic carbon in the packing material, and the continuous addition of supplemental substrate in the large-scale tests.

6.2 Reactor hydraulic observations

The conclusions related to the hydraulic behaviour are:

- The TIS model fit the experimental tracer responses very well and was used to observe the change of the flow and mixing characteristics over the operational periods of the reactors.
- The first tracer test demonstrated that there were significant variations in the tracer responses between reactors. This indicated that the individual packing structures of the reactors had a significant impact on the hydraulic behaviour, with the residence time at peak tracer concentration varying between day 9 and day 20. This difference in the hydraulic behaviour seemed to have no effect on the individual sulphate removal performances of the reactors, as similar sulphate removal performances were achieved.
- During the second tracer test, the reactors behaved more comparably, with a decreased spread of the RTD curve as peak tracer concentrations were experienced around day 15,

except for reactor R10, which peaked at day 11. With no significant change in the observed hydraulic behaviour, no correlation could be made between the hydraulic behaviour and the declining sulphate reducing performance at that stage.

- Further evolution in some reactors' RTD curves was seen during the third tracer run from days 188 to 223, as the spread of the curves reduced, and higher peaks, occurring sooner, were observed. During this time, the sustained sulphate reduction phase commenced, when the average sulphate removal percentage was maintained around 8%, except for R1, which demonstrated an elevated sulphate removal percentage of 18%. For reactor R1, the RTD curve maintained the same shape with a higher peak, showing no indication that the hydraulic behaviour was responsible for the elevated sulphate removal performance as compared to the other reactors.
- During the fourth and fifth tracer test runs, the RTD curves of R4, 6 and 9 followed the same trend, as their peak values increased, and their residence times decreased. This meant that the reactor's flow regime gradually moved in the direction of a more plug-flow-like regime, indicating the possibility of channelling and/or bypassing occurring through the packed bed. For reactor R1, the RTD curve remained relatively constant, with just the peak increasing and the spread decreasing. Even though the general trend was towards a less mixed flow, the flow remained well mixed, as indicated by the long tail and wide spread of the RTD curves and the high values of the variance.
- In general, the mean residence time tended to decrease over time. The time differences between the mean residence time and the residence time at peak concentration values were attributed to the long tails observed during the tracer tests, as back-mixing occurred, significantly increasing the time the tracer spent in the reactor. The spread of the residence times among the reactors suggested that the individual packing structures had a significant impact on the overall flow through the packed bed, but that, over time, a similar trend was followed with residence times tending to decrease.
- The two mixing indicators, namely the number of tanks-in series (N) for the TIS model, and the Peclet number, mostly conformed to $N \leq 3$ and $Pe_r \leq 5$, indicating a completely mixed flow. The reactors that exceeded these parameters were marginally out of scope, indicating that a mixed-flow pattern could still be assumed. Over time, a trend was observed when these mixing indicators slightly increased, suggesting that mixing through the reactors was decreasing, and could possibly continue to do so over long-term operation. Even though the mixing indicators increased, they remained close to the completely mixed-flow limits, and the flow remained well mixed.

- The evolution of the RTD curves slowed down during the last two tracer tests, suggesting that the change in the packed bed structure was slowing during this stage. Over long-term operations, the observed evolution of the RTD curves and the continuous increase in the mixing parameters could continue. This evolution could be ascribed to substantial biofilm growth occurring on the packing material in conjunction with the accumulation of solids (metal sulphide precipitates, and/or sulphur) within the system. This accumulation of solid material within the packed bed, combined with the occurrence of microbial degradation of the packing material, would force the flow through the channels, limiting availability of substrate and contact time with the bacteria, and thus decreasing the sulphate reduction performance. This change in permeability of the packed bed would lead to the inevitable bypassing of the reactor, which in turn will lead to the possibility of complete loss of hydraulic conductivity over a long-term operation.

6.3 Packed bed structure analysis

The conclusions related to the packed bed structure are:

- During physical analysis of R1 and R4 after 364 days of operation, the images showed the occurrence of channels and bypassing of most of the packed bed. At the top end of the packed bed, a relatively even distribution of flow across slices was seen, where after the flow through the middle of the packed bed declined as the liquid passed through the packed bed closer to the base.
- If increased microbial activity occurred closer to the base, more biofilms would be present, which could decrease the permeability. If this increased microbial activity occurred, formation of metal sulphide precipitation could occur which could accumulate in the packing material, causing a further decrease in the permeability.

6.4 Computational fluid dynamics analysis

The conclusions related to the packed bed structure are:

- The packed bed structure could be reconstructed by utilising the physical analysis method, wherein it was possible to distinguish between the packing material and the aqueous phases within the reactor. Further work is required to increase the accuracy of the image processing to determine the optimal slice thickness that is required to capture the majority of the packed bed structure.
- The modelled tracer flows showed that the majority of the flow of the mine water cannot effectively utilise the packed bed due to the variation in the permeability. For this reason, it is critical that the selection of the packing material and the packing method should be done in

such a manner that a relatively constant porosity can be achieved across the entirety of the reactor.

- Although the CFD modelled RTD peaks did not line up with the experimental data, the shape of the tracer responses exhibited similar behaviours. Reactor R1 peaked earlier and higher with a narrower spread, and R4 had a later and lower peak with a wider spread. This suggests that with accurate experimentally determined inputs; the refinement of the physical analysis method, and process parameters such as the diffusion coefficient, more representative results could be expected. This was seen by the best-case diffusion coefficient scenario as the RTD curves were much more comparable.
- By varying the porosity of the meshed structure to assess the impact of the hydrolysis of portions of the packing material, a small impact was observed as the RTD curves were delayed. This showed the importance of developing a reliable method to estimate the operating volume of the reactor enabling an accurate specification of the porosity of a large-scale plant.
- By varying the diffusion coefficient, it was observed that even with a small increase, the residence times and the shape of the tracer responses varied greatly, highlighting the importance of investigating tracer properties in new applications, as a variable diffusion coefficient can have a large impact on modelling the predicted flows through a packed bed reactor.
- The similarity in the shape of the responses indicated that the observations made of the structure of the packed bed during physical analysis and of the CFD models can be fairly correlated back to the packed bed structure of the experimental reactors. This suggested that some channels were present at the end of the operational periods.

6.5 Concluding remarks

The similarity of the performance indicators of all the reactors showed a high reproducibility of the overall results; and, therefore, demonstrates the possibility of achieving the same results while upscaling the process in field operations.

The results of this study indicate that the hydraulic behaviour and the permeability of the packed bed during the operational lifetimes of the reactors had a negligible impact on its sulphate removal performance. This was observed from the comparison of the sulphate removal performance in Table 16 to the hydraulic behaviour illustrated by the residence time distribution curves of the reactors over each tracer test run as illustrated in Figure 43. It was rather the availability of organic substrate that was the determining factor for achieving high sulphate removal rates. The supply of organic carbon to the bacteria is the most important factor in successfully treating AMD. Providing

sufficient organic carbon and ensuring effective transportation of the substrate to the bacteria is a major consideration that needs to be included in the design criteria of a BSR plant to ensure successful implementation of the process at scale for the treatment of high-sulphate AMD.

6.6 Future work

Potential research lines that can be pursued to benefit understanding of the BSR process and further improve the process, are the following:

- Over longer-term operations, when additional substrate is fed to the reactor, and high sulphate reduction occurs, increased amounts of dissolved metals will be found in the AMD. Increased amounts of metal sulphides could clog the reactor. This would significantly impact the hydraulic behaviour and would have a significant impact on the reactor's ability to reduce sulphate concentrations. Future work would include running experiments during which additional substrate is fed to the reactor to assess how high-rate sulphate removal effects solids formation that may cause packed bed fouling and increased degradation.
- Using a significantly up-scaled reactor that better represents the geometry of a large-scale BSR reactor to assess the correlation between the behaviour of the flow regimes and pertinent observations in this study.
- Refinement of the physical analysis method to produce repeatable and accurately reconstructed physical structures of the packed bed.
- Development of an in-situ method for physical analysis of the packed bed structure where the reactor can be run after the analysis, allowing for the measurement of individual changes of the packing structure and porosity.
- Development of a reliable method to estimate the operating volume of the reactor to accurately calculate the porosity of a large-scale plant. This will add significant information to the design criteria for a large-scale plant for optimal hydraulic performance.
- Determination of the diffusion coefficient of any tracer used under specific operating conditions in which the tracer test would be conducted to accurately investigate the hydraulic behaviour over a long-term operation.
- To explore the use of alternative RTD models, such as compartment models, for an in depth understanding of the hydrodynamics of the reactors.

APPENDIX A

CHEMICAL ANALYSIS METHODOLOGIES

APPENDIX A1 pH, OXIDATION-REDUCTION POTENTIAL AND ELECTRICAL CONDUCTIVITY

- The pH of samples was measured by using a Portavo 902 pH meter (Prod no: 1686, Knick Elektronische Messgerate GmbH & Co. KG, Berlin, Germany) and a daily calibrated Profitrode pH probe (Prod no: 6.0255.100, Metrohm, Herisau, Switzerland).
- The oxidation-reduction potential (ORP) of the samples were reported against Ag/AgCl against a 3M KCl electrode. The measurements were done using a Portamess® 911 pH/ORP meter (Prod no: 5774685, Knick Elektronische Messgerate GmbH & Co. KG, Berlin, Germany) and a Liquid-Glass ORP probe (Prod no: 238145, details, Hamilton, Nevada, United States)
- The EC was measured using a Cond 3310 meter and TetraCon® 325 probe set (Prod no: 2CA301, Xylem, Washington, D.C., United States).

APPENDIX A2 ALKALINITY

The alkalinity of the effluent samples was determined with *standard method 2320 B – Titration method*. As the pH values of the samples during the study never rose above 8, phenolphthalein alkalinity could not be measured.

Reagents

- 32% hydrochloric acid (Platinum grade, Product no: H1112CC02500, Associated Chemical Enterprises chemicals, Johannesburg, South Africa).
- Bromocresol green-methyl red, mixed indicator solution (Product no: 318728, Sigma-Aldrich, Missouri, United States).

Equipment

- Hotplate magnetic stirrer (Heidolph instruments GmbH & Co. KG, Schwabach, Germany).
- Magnetic stirrer bar.
- 50 mL burette and stand.
- 50 mL glass Erlenmeyer flask.
- Plastic dripper.
- 1 – 10 mL pipette (Product no: 4642110, Thermo Fisher Scientific, Massachusetts, United States).
- 100 – 1000 µL pipette (Product no: 4642090, Thermo Fisher Scientific, Massachusetts, United States).

Procedure

- 1 mL sample was added to the 50 mL flask followed by 10 mL distilled water.
- 3 drops of the mixed indicator were added to the mixture.
- The magnetic stirrer was placed in the flask and the flask was placed on the stirrer and the solution was titrated from the starting point: blue colour, to the end point: orange colour.

The total alkalinity, as mg CaCO₃/L, was calculated by using Equation [26]:

$$\text{Alkalinity} = \frac{A * N * 50000}{\text{Vol sample (mL)}} \quad [26]$$

where A = volume standard acid used in mL, N = normality of titrant acid.

APPENDIX A3 CHEMICAL OXYGEN DEMAND

The chemical oxygen demand (COD) of the effluent samples was determined by using the *APHA 5220 D – closed reflux, colorimetric method*.

Chemicals

- Potassium dichromate, K₂Cr₂O₇ (Platinum grade, Product no: P2031TT00500, Associated Chemical Enterprises chemicals, Johannesburg, South Africa).
- Concentrated sulphuric acid, H₂SO₄ (Platinum grade, Product no: S2741CC00500, Associated Chemical Enterprises chemicals, Johannesburg, South Africa).
- Mercuric sulphate, HgSO₄ (Platinum grade, Product no: M1475TT00500, Associated Chemical Enterprises chemicals, Johannesburg, South Africa).
- Silver sulphate, Ag₂SO₄ (Platinum grade, Product no: S2415NN00050, Associated Chemical Enterprises chemicals, Johannesburg, South Africa).
- Potassium hydrogen phthalate, KHP (Platinum grade, Product no: P2070NN00500, Associated Chemical Enterprises chemicals, Johannesburg, South Africa).

Equipment

- Standard 10 mL capped glass tubes.
- 0 to 6200 g analytical balance (Product no: UX6200H, Shimadzu, Kyoto, Japan).
- Thermoreactor (Spectroquant® TR 620, 171202, Merck Group, Darmstadt, Germany).
- Spectrophotometer (Spectroquant® prove 300, Product no: 173017, Merck Group, Darmstadt, Germany).
- 1000 mL volumetric flask.
- 1 – 10 mL pipette (Product no: 4642110, Thermo Fisher Scientific, Massachusetts, United States).
- 100 – 1000 µL pipette (Product no: 4642090, Thermo Fisher Scientific, Massachusetts, United States).

Reagents

- Potassium dichromate digestion solution.
 - 500 mL of distilled water, 1.022 g (low COD) or 4.903 g (medium COD) or 10.216 g (high COD) $K_2Cr_2O_7$, previously dried at 150°C for 2 hours, 167 mL concentrated H_2SO_4 , and 33.3 g $HgSO_4$ were added to a 1000 mL volumetric flask and dissolved and cooled to room temperature, and diluted to 1000 mL.
- Sulphuric acid reagent.
 - Ag_2SO_4 was added to concentrated H_2SO_4 at the rate of 5.5 g Ag_2SO_4 /kg H_2SO_4 . The solution was left to stand for 1 to 2 days to fully dissolve.
- Potassium hydrogen phthalate (KHP) standard solution.
 - A 10 g/L COD standard solution was made up by adding 8.503 g of previously dried KHP to a 1000 mL volumetric flask and filled with distilled water.

Standard curve procedure

To measure the COD of the samples, a standard curve of KHP, which provided standard values of COD at specific ranges for the specific concentration (low/medium/high) of the digestion solution, had to be constructed. Standard concentrations to construct the standard curve were achieved by diluting the 10 g/L standard to the values indicated in Table 21. The average standard errors of the low, medium and high concentrations were determined at 8.81, 4.55 and 1.27%, respectively. The standard curve data points are shown in Appendix C3.

Table 21. Dilution samples of standard solution

Low-KHP concentration (g/L)	Medium-KHP concentration (g/L)	High-KHP concentration (g/L)
0.00 (Distilled water)	0.00 (Distilled water)	0.00 (Distilled water)
0.01	0.05	0.10
0.02	0.10	0.30
0.04	0.15	0.40
0.06	0.20	0.50
0.08	0.30	0.60
0.10	0.40	0.80
0.15	0.50	0.90

Analysis procedure

- Treatment of the samples.
 - 2.5 mL of the pre-diluted sample was placed into the glass test tube and 1.5 mL of the digestion solution was added. The sulphuric acid reagent was carefully run down the inside of the vessel to form an acid layer under the sample-digestion solution

layer. The glass test tube was then tightly capped, and the tubes were inverted several times to mix the contents completely.

- The tubes were placed in the thermoreactor which was preheated to 150°C and the samples were refluxed for 2 hours with the protective shield closed.
- To prevent precipitate formation, the samples underwent gradual cooling to room temperature. After cooling, venting was performed, if needed, to release any generated pressure during digestion. The contents of the reaction vessels were mixed to combine condensed water and dislodge any insoluble materials. To ensure a clear optical path, the suspended matter was allowed to settle.
- The spectrophotometer zero was set using a glass test tube that was filled with distilled water.
- The absorption of the samples was measured at 420 nm, including a digested blank (distilled water as sample volume), to validate the quality of the analytical reagents and to establish the COD blank value.
- The COD values of the samples were determined by using the standard curve.

APPENDIX A4 SULPHATE

The sulphate concentrations were determined by using the *APHA standard method 9038 – turbidimetric method*. This method utilises barium sulphate's suspension characteristic under controlled conditions, wherein the turbidity was measured using a spectrophotometer and compared to a standard curve to determine the sulphate concentrations in solution. The average standard error of the method was 2.17%. The standard curve data points are shown in Appendix C2.

Chemicals

- Sodium chloride, NaCl (Platinum grade, Product no: S2471NN00500, Associated Chemical Enterprises chemicals, Johannesburg, South Africa).
- 32% hydrochloric acid (Platinum grade, Product no: H1112CC02500, Associated Chemical Enterprises chemicals, Johannesburg, South Africa).
- Glycerol (Platinum grade, Product no: G0991NN02500, Associated Chemical Enterprises chemicals, Johannesburg, South Africa).
- Ethanol diol (Platinum grade, Product no: E0751TH00500, Associated Chemical Enterprises chemicals, Johannesburg, South Africa).
- Barium chloride (Platinum grade, Product no: B0191TI00500, Associated Chemical Enterprises chemicals, Johannesburg, South Africa).
- Sodium sulphate anhydrous, Na₂SO₄ (Platinum grade, Product no: S2631NN00500, Associated Chemical Enterprises chemicals, Johannesburg, South Africa).
- Distilled H₂O.

Equipment

- 0 to 6200 g analytical balance (Product no: UX6200H, Shimadzu, Kyoto, Japan).
- 25 mm diameter polyethersulfone filter paper (0.45 µm) (GIC Scientific, Roodepoort, South Africa) and filter paper housings (Product no: WHA420200, Whatman International Ltd, Buckinghamshire, United Kingdom).
- 100 mL volumetric flask.
- Spectrophotometer (Spectroquant Prove 300, Product no: 173017, Merck Group, Darmstadt, Germany).
- Standard glass cell tubes.
- 1 – 10 mL pipette (Product no: 4642110, Thermo Fisher Scientific, Massachusetts, United States).
- 100 – 1000 µL pipette (Product no: 4642090, Thermo Fisher Scientific, Massachusetts, United States).
- Vortex mixer (Vortex-Genie 2, Product no: SI-0266, Scientific Industries, Inc., New York, United States).
- 15 mL centrifuge tubes.

Reagents

- Sulphate standard.
 - Approximately 0.2958 g of Na₂SO₄ was weighed as accurately as possible and mixed with distilled water in a 100 mL volumetric flask to make up a 2 g/L sulphate standard mixture.
- Conditioning reagent.
 - 75 g NaCl, 30 mL HCl (32%), 50 mL glycerol and 100 mL ethanoldiol were dissolved in 300 mL deionised water to make the conditioning reagent.
- Barium chloride reagent solution.
 - 0.2 g weight of BaCl₂ was placed in a 15 mL centrifuge tube per 2 mL distilled water used. This solution was mixed with the vortex mixer for 2 minutes to achieve a super saturated solution. (This was done because adding the powdered BaCl₂ to the reaction mixture did not react adequately, and mixed results were achieved; with the saturated solution, stable results were achieved.)

Standard curve sulphate samples preparation

To conduct the test, a 5 mL sample was required. A standard curve had to be drawn each time the analysis was done. The standard solutions' concentrations that were required were 10, 20, 30, and 40 mg/L. This was achieved in the following way:

- In a 15 mL tube, a 1 + 9 dilution was made by using the 2 g/L sulphate standard to produce a 0.2 g/L sulphate solution (1 mL 2 g/L standard + 9 mL distilled water).

- Five 15 mL tubes were filled with 5 mL distilled water (tubes for 0, 10, 20, 30, 40 mg/L standards).
- To increase accuracy, a fixed volume of water was first removed from each tube and replaced with the 0.2 g/L standard, as indicated in Table 22.

Table 22. Dilution values for sulphate standard solution

Sulphate standard sample (mg/L)	Water removed	0.2 g/L standard added
0	0	0
10	1 x 0.25 mL	1 x 0.25 mL
20	2 x 0.25 mL	2 x 0.25 mL
30	3 x 0.25 mL	3 x 0.25 mL
40	4 x 0.25 mL	4 x 0.25 mL

- Once the samples were prepared, 0.25 mL of the pre-prepared conditioning solution was added.
- To start the reaction, 200 μ L of the saturated barium chloride solution was added, the 1-minute timer was started immediately, the cap was closed, and the solution was placed in the vortex mixer for 1 minute (NOTE: For accurate results, one sample was done at a time to ensure precise testing, as the test was time-sensitive).
- The adsorption of the samples was read at 420 nm. The 0 mg/l sample was used as the first sample in a standard glass test tube to set the zero point for the spectrophotometer.

Sample analysis

The samples were filtered through a 0.45 μ m filter paper to ensure a clear optical path.

The same process was followed for sample analysis as during the standard curve determination:

- 5 mL of appropriately diluted sample was placed in a 15 mL tube.
- Once the samples were ready, 0.25 mL of the pre-prepared conditioning solution was added.
- To start the reaction, 200 μ L of the saturated barium chloride solution was added, the 1-minute timer was started immediately, the cap was closed, and the solution was placed in the vortex mixer for 1 minute (NOTE: One sample was done at a time to ensure precise testing for accurate results, as test was time-sensitive).
- If the photometric reading was above or close to (within 0.50) the 40 mg/L standard solution reading, the dilution was increased, and the sample measurement was repeated. If the reading was below the 10 mg/L standard solution reading, the dilution was decreased, and the measurement of the samples was repeated. If no dilution was used and the sample read low, the value was noted and used as-is.

- The standard curve produced was used to calculate the sulphate concentrations of the samples.

APPENDIX A5 SULPHIDE

The sulphide concentration of the samples was determined with the *APHA standard method 9215 – potentiometric determination of sulphide in aqueous samples and distillates with ion-selective electrode* method by using the *Application Bulletin 1335/3 of Metrohm* and the *Ti Application Note No. T-32*. The sulphide concentration of the samples was determined by titrating with a silver nitrate solution using an Ag titrode for the equivalence-point indication.

Chemicals

- Silver nitrate (Platinum grade, Product no: S2390TC00100, Associated Chemical Enterprises chemicals, Johannesburg, South Africa).
- Sodium chloride (Platinum grade, Product no: S2471NN00500, Associated Chemical Enterprises chemicals, Johannesburg, South Africa).

Equipment

- Automatic Omnis titration system with magnetic stirring capabilities (Product no: 2.1001.0320, Metrohm, Herisau, Switzerland).
- Ag titrode with Ag₂S coating (Product no: 6.0430.100, Metrohm, Herisau, Switzerland).
- 100 mL glass tube.
- Magnetic stirrer bar.
- 0 to 6200 g analytical balance (Product no: UX6200H, Shimadzu, Kyoto, Japan).
- 1 – 10 mL pipette (Product no: 4642110, Thermo Fisher Scientific, Massachusetts, United States).

Reagents

- 0.1 M standard AgNO₃ stock titrant solution
 - 16.988 g of AgNO₃ was added to 1000 mL distilled water in the titrator-provided glass bottle.

Titration standardisation procedure

- Three amounts of 0.2 g of sodium chloride were measured as accurately as possible.
 - The exact amounts were noted down and recorded in the program for increased accuracy.
- Each of the three 0.2 g doses were placed into the glass tubes and 25 mL of distilled water was added, which allowed for the probe to be entirely covered for the titration process and the magnetic stirrer bar was added.

- These samples were then titrated. The program automatically adjusted the titrant concentration during calculation of future sample sulphide concentrations.

Sample analysis procedure

- A known volume (between 5 mL and 20 mL for the current study) of sample was placed into a 100 mL glass tube with an appropriate amount of distilled water to raise the volume to cover the base of the probe.
- The samples were titrated, and the sulphide concentrations were noted.

APPENDIX B

STANDARD CURVE AND METHODS FOR ERROR DETERMINATION

APPENDIX B1 FLUORESCENCE STANDARD CURVE

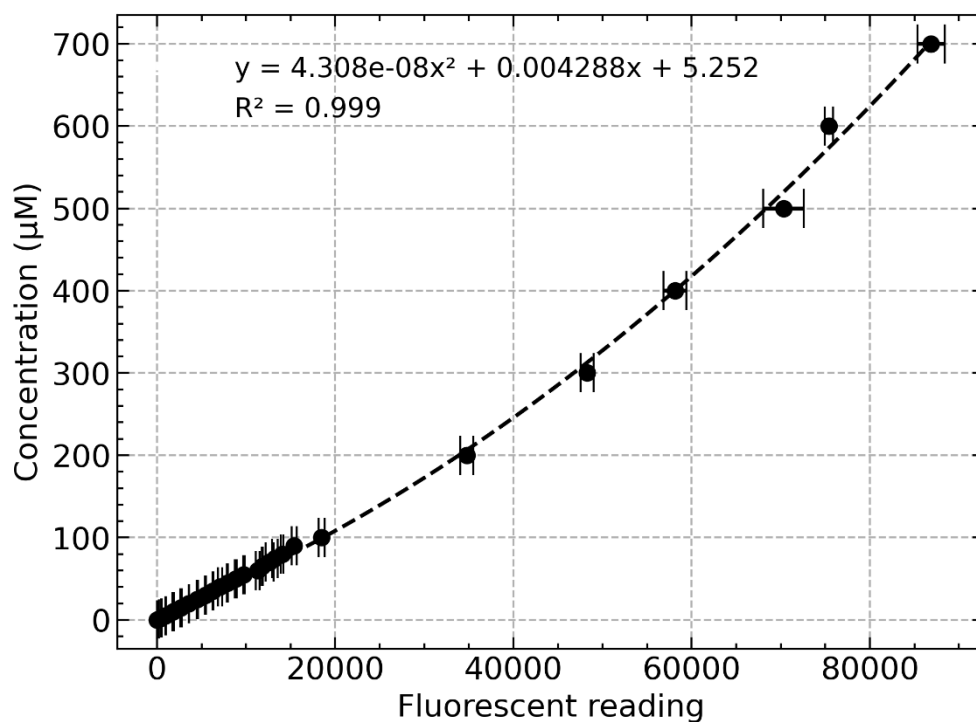


Figure 64. Fluorescence standard curve

APPENDIX B2 SULPHATE ERROR TEST

Table 23. Sulphate method error test data

	Concentration (mg/L)				
	0	10	20	30	40
Absorbance at 420 nm	0.026	0.138	0.267	0.397	0.522
	0.029	0.137	0.266	0.403	0.530
	0.028	0.138	0.258	0.409	0.529
	0.030	0.143	0.263	0.408	0.522
	0.029	0.143	0.262	0.410	0.524
Standard deviation	1.52E-03	2.95E-03	3.56E-03	5.41E-03	3.85E-03

APPENDIX B3 CHEMICAL OXYGEN DEMAND ERROR TEST

Table 24. Chemical oxygen demand method error test data

Concentration (mg/L)	Absorbance at 420 nm			Standard deviation
High range COD				
0.10	2.79	2.83	2.82	1.68E-02
0.30	2.34	2.34	2.33	4.71E-03
0.40	2.04	2.05	2.08	1.81E-02
0.50	1.76	1.80	1.80	2.00E-02
0.60	1.48	1.49	1.49	5.10E-03
0.80	0.85	0.86	0.87	7.93E-03
0.90	0.62	0.57	0.62	2.56E-02
Medium range COD				
0.05	1.38	1.49	1.39	5.01E-02
0.10	1.24	1.26	1.23	1.43E-02
0.15	1.12	1.12	1.10	8.96E-03
0.20	0.93	0.93	0.88	2.29E-02
0.30	0.59	0.52	0.60	3.33E-02
0.40	0.39	0.41	0.34	3.21E-02
0.50	0.18	0.18	0.18	9.43E-04
Low range COD				
0.01	0.22	0.21	0.20	8.58E-03
0.02	0.21	0.17	0.20	1.64E-02
0.04	0.15	0.12	0.12	1.54E-02
0.06	0.07	0.07	0.07	2.87E-03
0.08	0.04	0.04	0.05	3.30E-03
0.10	0.04	0.04	0.04	0.00E+00
0.15	0.04	0.04	0.04	4.71E-04

APPENDIX C

FLUORESCENT READINGS, TRIMMING AND CURVE FITTING PROCESS

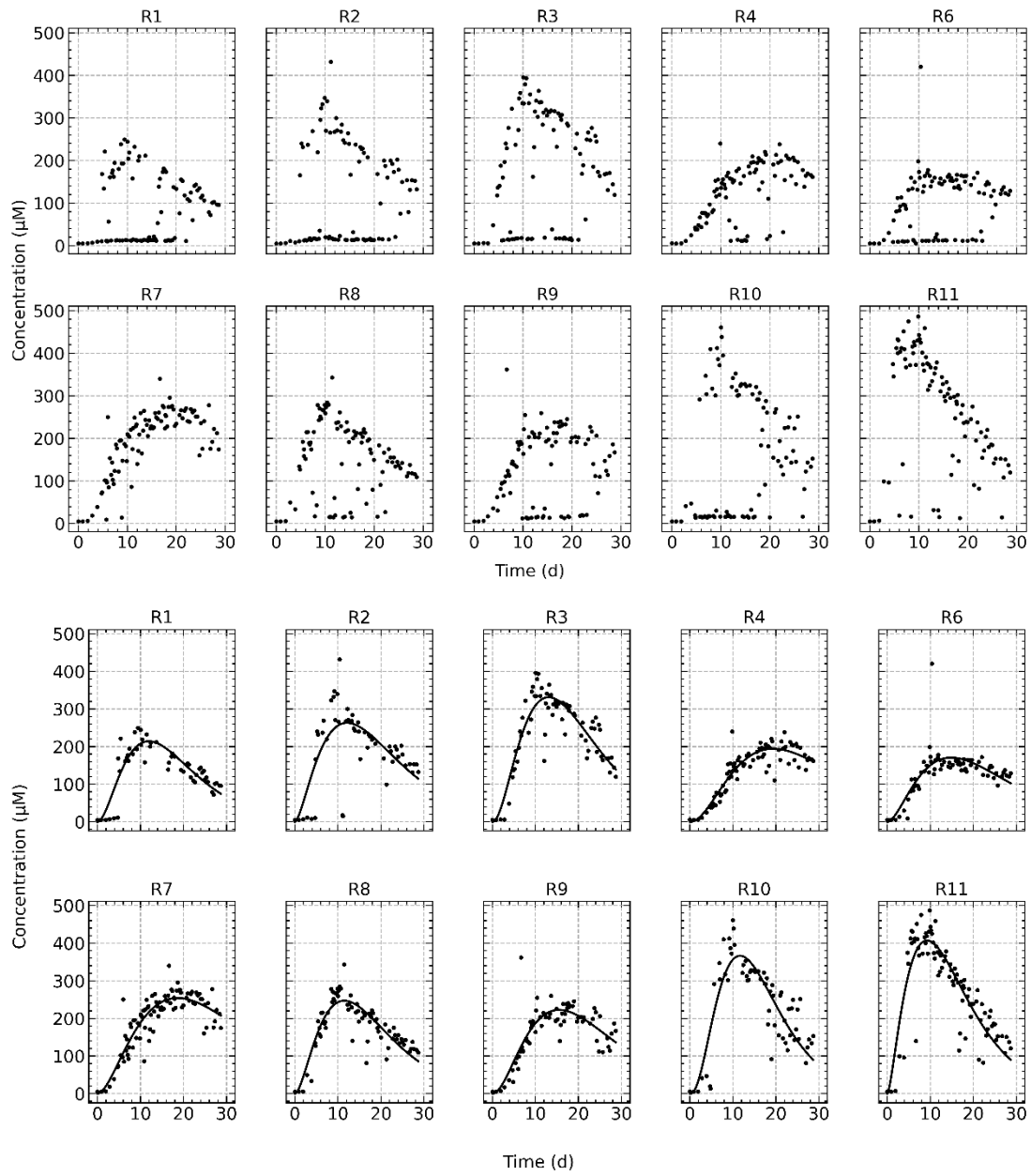


Figure 65. Fluorescent readings before and after trimming process and tanks-in-series curve fit lines for tracer Run 1

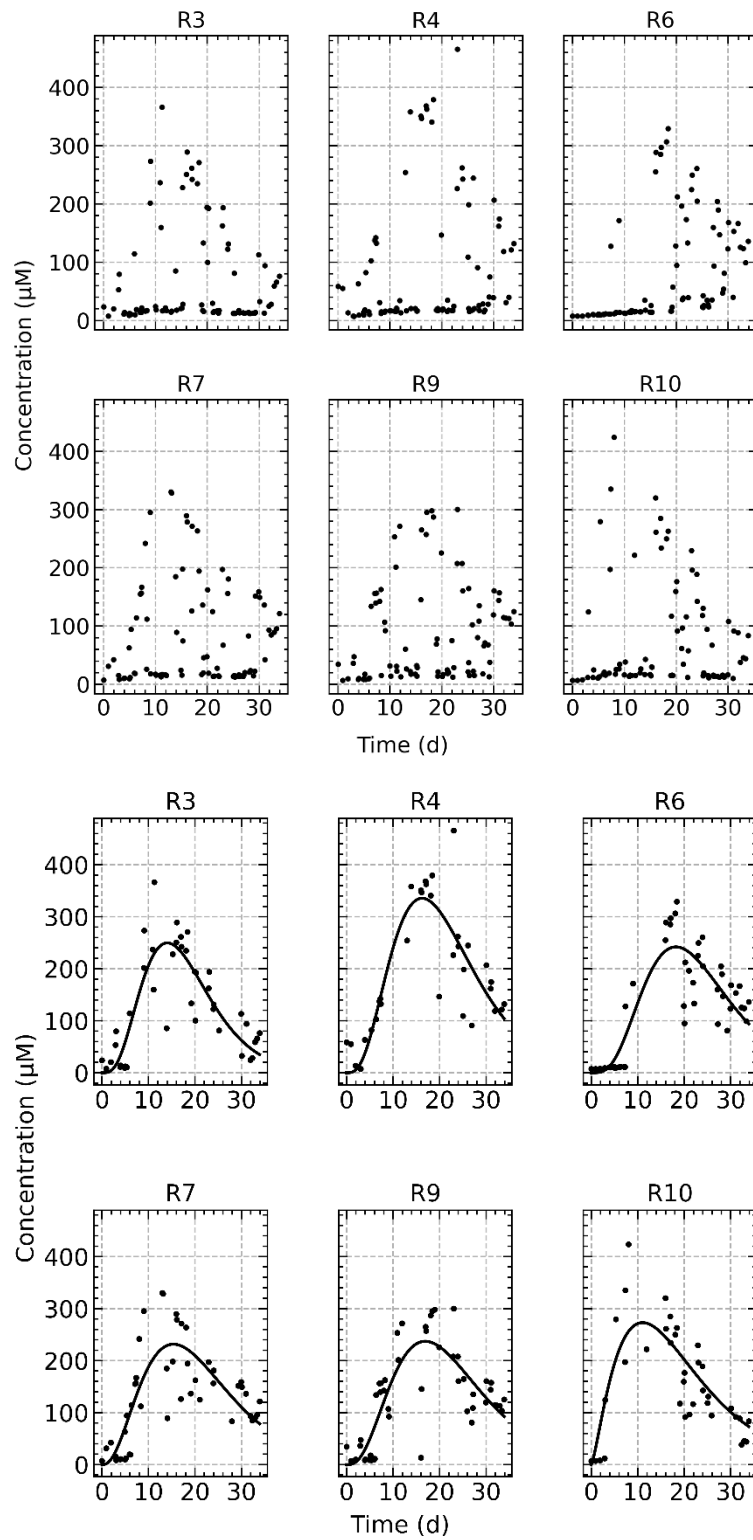


Figure 66. Fluorescent readings before and after trimming process and tanks-in-series curve fit lines for tracer Run 2

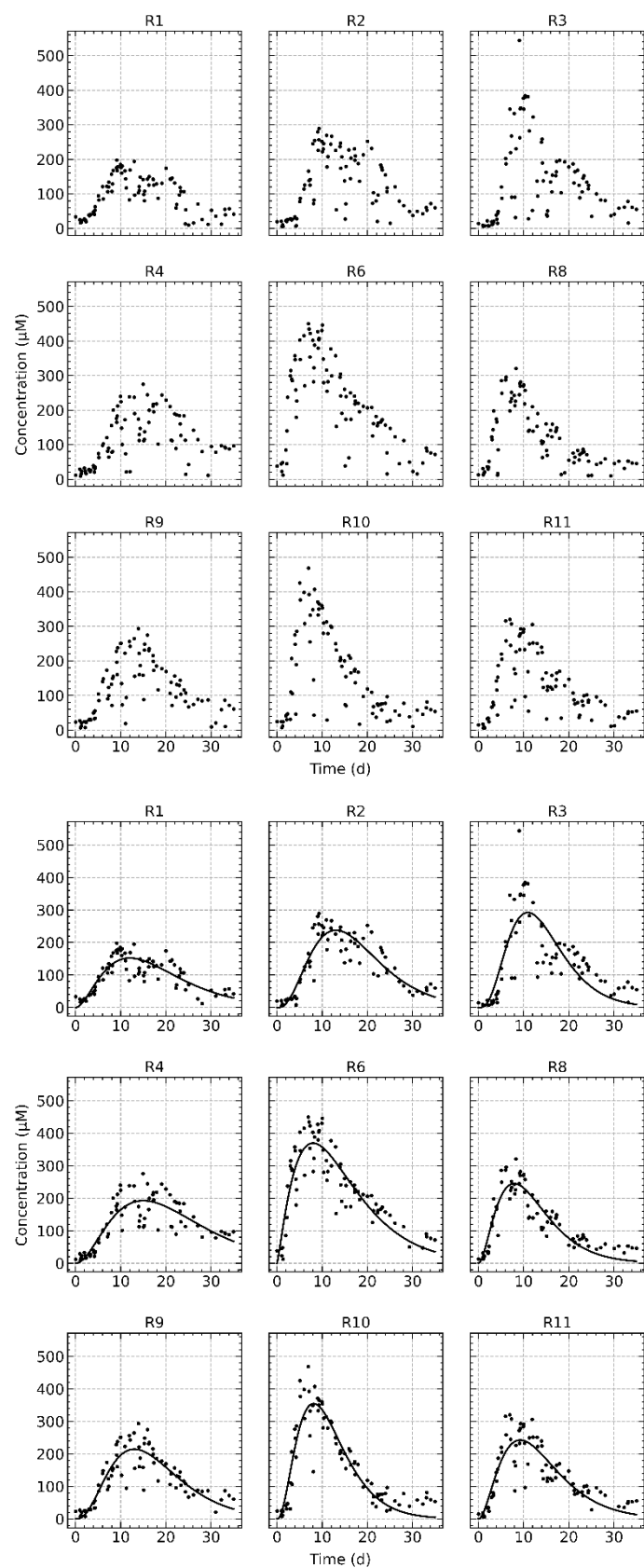


Figure 67. Fluorescent readings before and after trimming process and tanks-in-series curve fit lines for tracer Run 3

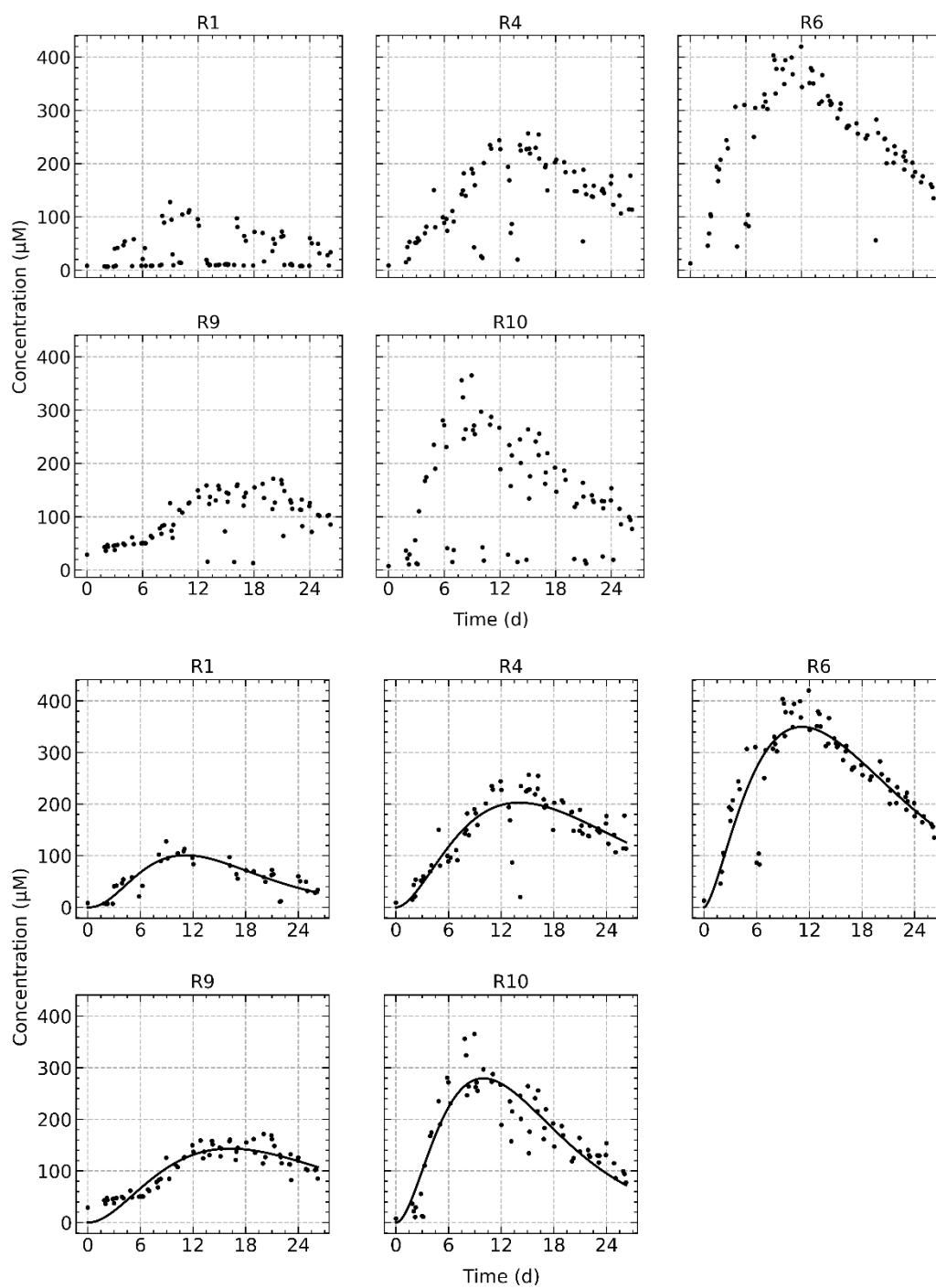


Figure 68. Fluorescent readings before and after trimming process and tanks-in-series curve fit lines for tracer Run 4

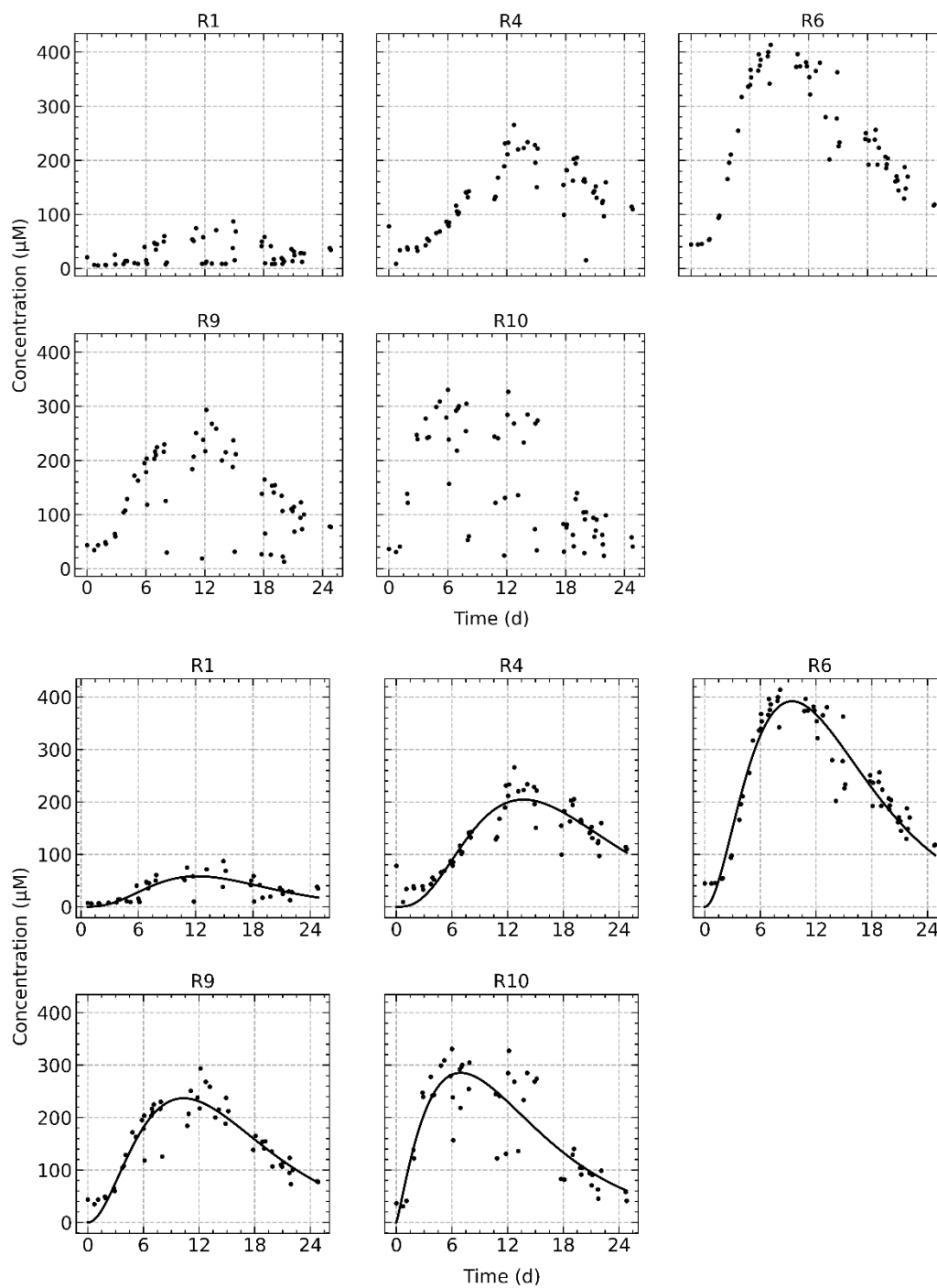


Figure 69. Fluorescent readings before and after trimming process and tanks-in-series curve fit lines for tracer Run 5

APPENDIX D

RESIDENCE TIME DISTRIBUTION PARAMETERS

Table 25. List of residence time distribution parameters of reactors for each tracer run

Reactor										
	R1				R2		R3			
Tracer run #	1	3	4	5	1	3	1	2	3	
HRT T (peak) Tm Var N Peclet	10.41	9.36	9.22	9.29	9.83	9.51	11.06	9.21	9.89	
	11.94	12.00	11.14	11.94	12.10	13.04	13.24	14.94	10.90	
	17.90	18.16	15.50	17.90	19.49	17.57	19.24	17.85	14.21	
	106.83	111.59	67.50	106.83	143.87	79.68	118.80	67.99	46.76	
	3.00	2.96	3.56	3.00	2.64	3.87	3.12	4.68	4.32	
	4.75	6.57	5.92	8.20	3.98	6.57	4.99	8.23	7.48	
	R4					R6				
Tracer run #	1	2	3	4	5	1	2	3	4	5
HRT t (peak) tm Var N Peclet	11.01	9.55	9.84	9.67	9.73	10.83	9.91	10.08	9.77	9.87
	19.34	16.24	15.01	13.70	19.34	14.84	18.24	8.00	11.40	14.84
	28.17	21.11	21.92	18.06	28.17	22.83	22.83	14.42	18.08	22.83
	248.45	102.95	151.58	78.69	248.45	182.73	105.10	92.87	125.40	182.73
	3.19	4.33	3.17	4.15	3.19	2.85	4.96	2.24	2.61	2.85
	5.16	7.50	5.10	5.12	7.13	4.44	8.79	3.10	3.91	4.88
	R7		R8		R9					
Tracer run #	1	2	1	3	1	2	3	4	5	
HRT t (peak) tm Var N Peclet	11.37	9.67	10.24	9.85	10.55	10.01	9.58	9.58	9.68	
	18.64	15.37	11.47	7.90	15.51	16.91	13.04	16.24	15.51	
	28.57	21.26	17.83	12.01	22.93	22.45	17.67	23.87	22.93	
	283.84	125.42	113.28	49.26	170.09	124.59	81.94	182.35	170.09	
	2.88	3.60	2.81	2.93	3.09	4.04	3.81	3.12	3.09	
	4.51	6.01	4.34	4.60	4.94	6.92	6.44	5.01	5.07	
	R10						R11			
Tracer run #	1	2	3	4	5	1	3			
HRT t (peak) tm Var N Peclet	10.09	9.20	8.99	9.13	9.29	10.33	8.84			
	11.67	11.14	8.10	6.94	11.67	9.24	9.24			
	16.30	19.00	11.56	12.78	16.30	15.20	13.87			
	75.69	151.03	39.79	74.61	75.69	90.64	64.01			
	3.51	2.39	3.36	2.19	3.51	2.55	3.00			
	5.82	3.43	5.49	4.83	2.99	3.78	4.76			

APPENDIX E

MESH DEPENDENCE STUDY

The objective of this study was to evaluate the influence of the mesh element size on the tracer results. To achieve this, a mesh dependency study was conducted by systematically reducing the mesh element size from 7 mm, aiming to identify the point at which the tracer curve exhibited minimal variation from the preceding mesh size. The simulations maintained the mesh resolution throughout the entire workflow, starting from the generating the porosity field using the photos, then into the flow model, and finally to the tracer dispersion model. In Figure 70, the resulting tracer curves at mesh element sizes of 4 mm, 5 mm, 6 mm and 7 mm were compared. It was observed that the tracer curves stabilised at a 5 mm mesh element size, indicating that the level of mesh refinement yielded accurate results without incurring further computational costs. Consequently, a 5 mm mesh element size was selected as the optimal choice for the CFD simulations.

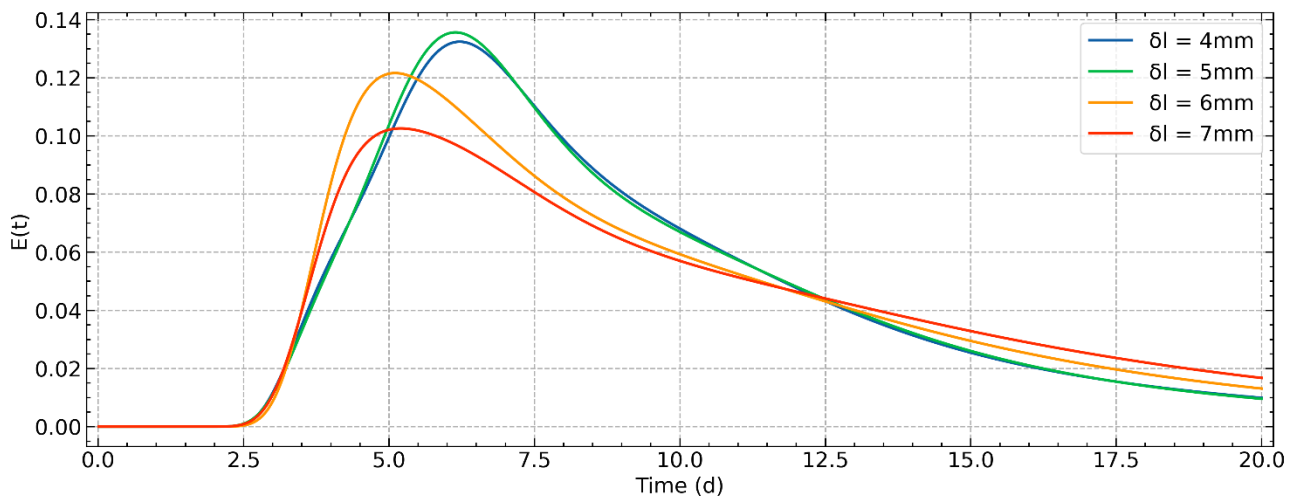


Figure 70. Mesh dependency results

APPENDIX F

COMPUTATIONAL FLUID DYNAMICS NUMERICAL METHODS

APPENDIX F1 STEADY STATE FLOW MODEL fvSchemes

```
ddtSchemes
{
    default Euler;
}

gradSchemes
{
    default          Gauss linear;
}

divSchemes
{
    div(rhoPhi,U)      Gauss wLimitedLinearPorous 1;
    div(phi,alpha)     Gauss vanLeer;
    div(phirb,alpha)   Gauss linear;
    div(U)              Gauss linear;
}

laplacianSchemes
{
    default          Gauss linear corrected;
}

interpolationSchemes
{
    default          linear;
}

snGradSchemes
{
    default          corrected;
}

fluxRequired
{
    default          no;
    p_rgh;
    pcorr;
    alphal;
}
```

APPENDIX F2 STEADY STATE FLOW MODEL fvSolution

```
solvers
{
    pcorr
    {
        solver          PCG;
        preconditioner
        {
            preconditioner  GAMG;
            tolerance       1e-05;
            relTol          0;
            smoother        DICGaussSeidel;
            nPreSweeps      0;
            nPostSweeps     2;
            nFinestSweeps   2;
            cacheAgglomeration false;
            nCellsInCoarsestLevel 10;
            agglomerator     faceAreaPair;
            mergeLevels      1;
        }

        tolerance       1e-05;
        relTol          0;
        maxIter          100;
    }

    p_rgh
    {
        solver          GAMG;
        tolerance       1e-07;
        relTol          0.05;
        smoother        DIC;
        nPreSweeps      0;
        nPostSweeps     2;
        nFinestSweeps   2;
        cacheAgglomeration true;
        nCellsInCoarsestLevel 10;
        agglomerator     faceAreaPair;
        mergeLevels      1;
    }

    p_rghFinal
    {
        solver          PCG;
        preconditioner
        {
            preconditioner  GAMG;
            tolerance       1e-07;
            relTol          0;
            nVcycles        2;
            smoother        DICGaussSeidel;
            nPreSweeps      2;
            nPostSweeps     2;
            nFinestSweeps   2;
            cacheAgglomeration true;
            nCellsInCoarsestLevel 10;
            agglomerator     faceAreaPair;
            mergeLevels      1;
        }

        tolerance       1e-07;
        relTol          0;
    }
}
```

```

        maxIter          20;
    }

    U
    {
        solver            PBiCG;
        preconditioner     DILU;
        tolerance          1e-06;
        relTol             0;
    }

    UFinal
    {
        solver            PBiCG;
        preconditioner     DILU;
        tolerance          1e-08;
        relTol             0;
    }

    alpha.water
    {
        nAlphaCorr         1;
        nAlphaSubCycles 2;
        cAlpha              1;
    }
}

PIMPLE
{
    momentumPredictor    no;
    nCorrectors           3;
    nNonOrthogonalCorrectors 0;
    //pRefCell            0;
    //pRefValue            0;
}

```

APPENDIX F3 TRACER DISPERSION MODEL fvSchemes

```
ddtSchemes
{
    default          Euler;
}

gradSchemes
{
    default          Gauss linear;
}

divSchemes
{
    default          none;
    div(phi,T)       Gauss upwind;
}

laplacianSchemes
{
    default          none;
    laplacian(DT,T)  Gauss linear corrected;
}

interpolationSchemes
{
    default          linear;
}

snGradSchemes
{
    default          corrected;
}
```

APPENDIX F4 TRACER DISPERSION MODEL fvSolution

```
solvers
{
    T
    {
        solver          PBiCGStab;
        preconditioner   DILU;
        tolerance        1e-06;
        relTol           0;
    }
}

SIMPLE
{
    nNonOrthogonalCorrectors 0;
}
```