# The VitaSOFT PROCESS: A Sustainable, Long-term Treatment Option for Mining-impacted Water

Report to the

**Water Research Commission**

by

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# EXECUTIVE SUMMARY

The VitaSOFT Process was developed in response to further development requirements that were identified during the development and full-scale demonstration of the Rhodes BioSURE® Process, a biological sulphate-reducing (BSR) process for the treatment of mine-impacted water with a high sulphate concentration.

A consequence of the exploitation of mineral resources is the generation of acid mine drainage (AMD) and acid rock drainage in areas where minerals are associated with sulphide minerals such as pyrite. The occurrence of AMD within South Africa has been reported in a number of areas, with the Western, Central and Eastern Basins of the Witwatersrand Gold Fields being identified as the highest risk. The Rhodes BioSURE® Process was presented as a cost-effective and proven treatment option for the treatment of AMD to mitigate the effect of AMD on water quality.

The development of the Rhodes BioSURE® Process commenced at Rhodes University in the early 1990s, where the hydrolysis of organic material as a carbon source for biological sulphate reduction, with accompanying sulphide production, metal precipitation and alkalinity generation was described. The feasibility of employing primary sewage sludge (PSS) as an electron donor source for biological sulphate reduction was successfully demonstrated in later bench-top studies. Various patents were filed on this process by Rose et al. (US6, 197196, US6,203,700 and EP 1 124 763). The process was successfully scaled up to a 10 Ml/d plant located at ERWAT's Ancor waste water treatment plant (WWTP) in Springs, Gauteng, treating mine water from Grootvlei mine, utilizing PSS as an electron and carbon donor, with final sulphate concentrations of below 250 mg/L. The PSS was supplemented with other carbon sources such as dairy waste and abattoir waste, demonstrating the possibility of utilizing a variety of biodegradable organic waste. Integration of the plant with the Ancor WWTP for effluent polishing and practical access to PSS, as well as the fact that the plant received neutralized water from the high-density sludge (HDS) process at Grootvlei, made this a unique application of the technology.

It was identified by VitaOne8 that further research was required to improve the BioSURE Process for implementation elsewhere, in particular where the AMD has a high acidity, low pH, high concentrations of dissolved metals and a higher than 2000 mg/L sulphate concentration. One of the major criticisms of the BioSURE Process was the reliance on PSS and supplementary material as a carbon source, which may not always be available, as well as the requirement for a continuous supply of iron hydroxide for the removal of sulphides from the effluent of the BSR process, and the associated disposal requirements for large amounts of iron sulphide sludge. The goal of this study was to address these shortcomings in order to develop a more robust process, with broader and more flexible application potential.

Maize silage was identified as an alternative carbon source, with various advantages over PSS, such as the fact that it can be stored for long periods of time without loss of quality, it has a

higher percentage biodegradability when compared with PSS, and it has a lower nitrogen content. It was hypothesized that sufficient alkalinity could be generated in the BSR process to neutralize AMD and to precipitate contaminating metals as metal sulphides, without the need for an upstream HDS process. Biological iron oxidation was suggested as a means to regenerate the iron hydroxide required for sulphide removal, so that a constant supply would not be required. Finally, it was suggested that the effluent of the BSR process could be softened and stabilized by removal of calcium carbonate as magnesium hydroxide to reduce the salinity of the water to meet the final effluent standards for discharge.

These hypotheses were tested, first at bench scale and then in a 1 $m^3$/d pilot plant. The pilot plant was constructed and operated at VitaOne8's research and development facilities in Pretoria using synthetic water resembling that of the Western Basin of the Witwatersrand.

It was demonstrated that maize silage was a valid alternative to PSS as a carbon source for BSR, which can be applied either as a supplementary carbon source where PSS is available, or as a primary source where there are no alternatives. The lower nitrogen content of silage when compared with PSS resulted in a lower ammonia concentration in the BSR effluent. The implication of this is that there is no requirement for integration of the process with a WWTP as was required for the BioSURE Process at Grootvlei mine, or for an alternative nitrification/denitrification step. It was planned to test the SANI process (sulphate reduction, autotrophic denitrification and nitrification integrated process) as an option for ammonia removal, which was ultimately unnecessary. However, the reactor configuration for the SANI process enabled the fortuitous and unintended occurrence of biological sulphide oxidation, the observation of which led to the integration of a biological sulphide oxidation reactor into the process. Results have shown that a significant portion of the sulphide can be removed biologically as elemental sulphur, further reducing the requirement for iron hydroxide.

It was demonstrated that sufficient alkalinity is generated biogenically between the BSR and sulphide-oxidizing processes to not only neutralize the incoming AMD, but also to precipitate all the calcium in the water as calcium carbonate without the need for lime addition. Lime was demonstrated to only be required for the removal of manganese and magnesium in a two-stage process. The cost saving of lime and limestone is therefore two-fold; there is no requirement for an upstream HDS process, and less lime is required for desalination than would typically be required without the contribution of the biogenic alkalinity.

Biological iron oxidation was successfully demonstrated as a viable means to regenerate iron hydroxide from iron sulphide.

Sufficient information was therefore generated to confirm the validity of the process. The process differs significantly from the original patents by Rose et al. and the BioSURE Process as applied at the Ancor site for Grootvlei mine. In this light, a provisional patent was filed in July, 2014, where the integrated process was described. The novelty and advantages over the prior art are described in more detail in this document. A PCT patent application was filed for

the process in July, 2015, as well as a full patent application in Argentina, which is not a PCT member country.

Because the newly developed process differs sufficiently from the initial patents and original BioSURE Process, the decision was taken to change the name of the process to VitaSOFT, an acronym that refers to the four integrated biological processes.

Based on the results of the pilot study, a preliminary process design for a 200 $m^3$/d demonstration plant has been developed. The technology presents a viable long-term treatment solution for the treatment of mine-impacted water.

The operation of the bench-scale reactors will be continued until steady state is achieved, to determine the kinetic values, and for comparison with the established results where only PSS was used. The operation of the pilot reactor will also be continued in order to produce sufficient effluent to determine the operating parameters of the downstream processes more accurately.

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| ADF | Acid detergent fibre |
| AMD | Acid mine drainage |
| ATP | Adenosine triphosphate |
| BSR | Biological sulphate reducing/reduction |
| CBP | Consolidated bioprocessing |
| CO | Carbon monoxide |
| COD | Chemical oxygen demand |
| DM | Dry matter |
| DO | Dissolved oxygen |
| DWS | Department of Water and Sanitation |
| EFC | Eutectic freeze crystallisation |
| FW | Fresh weight |
| HDS | High density sludge |
| NDF | Neutral detergent fibre |
| OM | Organic matter |
| OUR | Oxygen uptake rate |
| P&ID | Process and instrumentation design |
| PSS | Primary sewage sludge |
| PST | Primary settling tank |
| RO | Reverse osmosis |
| SANI | Sulphate reduction, autotrophic denitrification and nitrification integrated |
| SAOB | Syntrophic acetate-oxidising bacteria |
| SHF | Separate hydrolysis and fermentation |
| SOP | Sulphide oxidizing prokaryote |
| SR | Sulphate reduction |
| SRB | Sulphate reducing bacteria |
| SRP | Sulphate reducing prokaryote |
| SSF | Simultaneous saccharification and fermentation |
| UASB | Upflow anaerobic sludge bed |
| VFA | Volatile fatty acids |
| VS | Volatile solids |
| VSS | Volatile suspended solids |
| WEST | Waste water treatment plant engine for simulation and training |
| WWTP | Waste water treatment plant |

# 1 Introduction

South Africa has benefited from the mining industry for more than a century. A consequence of the exploitation of the mineral resources is the generation of acid mine drainage (AMD) and acid rock drainage in areas where the minerals are associated with sulphide minerals such as pyrite. The occurrence of AMD within South Africa has been reported in a number of areas, with the Western, Central and Eastern Basins of the Witwatersrand Gold Fields being identified as the highest risk (DWA, 2013). The Rhodes BioSURE® Process was presented as a cost-effective and proven treatment option for the treatment of AMD to mitigate the effect of AMD on water quality.

The development of the Rhodes BioSURE® Process commenced at Rhodes University in the early 1990s with observations of a high degree of hydrolysis and utilization of organic matter, sulphate reduction, hydrogen sulphide production and associated metal precipitation in these systems, and increased alkalinity. In follow-up studies, the feasibility of employing primary sewage sludge (PSS) as an electron donor source for biological sulphate reduction was successfully demonstrated. These findings led in turn to the bench-scale studies of what became known as the Rhodes BioSURE® Process. Various patents were filed on this process by Rose et al. (US6, 197196, US6,203,700 and EP 1 124 763).

Following bench-scale studies of the enhanced hydrolysis operation, the process was scaled-up to a 40 $m^3$ pilot plant treating water from Grootvlei mine in Springs, Gauteng Province, treating an AMD stream with a sulphate concentration as high as 2000 mg/L. At this time, studies commenced in the University of Cape Town's Chemical and Civil Engineering Departments in which the kinetics of the process were investigated and described. Based on the outcomes of the pilot study, a technical-scale plant with a treatment capacity of 2 Ml/d was initially constructed at ERWAT's Ancor waste water treatment plant (WWTP) with a 2.4 km pipeline to feed mine water from Grootvlei mine by ERWAT. The water was abstracted after the high-density sludge (HDS) process. It was slightly alkaline and did not contain metals in significant concentrations. During this period, ERWAT was appointed by Grootvlei mine to build and operate a full-scale plant for treating 10 Ml/day of the discharge from the Grootvlei mine, also located at ERWAT's Ancor WWTP, utilizing PSS as an electron and carbon donor. Sulphate was reduced to below 250 mg/L. The PSS was supplemented with other carbon sources such as dairy waste and abattoir waste, demonstrating the possibility of utilising a variety of biodegradable organic waste. By this stage, various aspects of the process already differed from the process patented by Rose et al. The plant was in operation for only a short time after completion of the acceptance tests when it was unfortunately shut down due to financial constraints at the mine.

## 1.1 Description of the 10 Ml/d Rhodes® BioSURE Process implemented at ERWAT's Ancor WWTP

Extraneous mine water at Grootvlei Shaft 3 was pumped from 700 m underground to the surface at a rate varying between 70 and 100 Ml/day. The water was treated in a high-density settling (HDS) process with the addition of lime and air, supplemented with oxygen, to oxidise and precipitate ferrous iron. High concentrations of dissolved calcium, magnesium, sodium, bicarbonate and sulphate contributed to the salinity of the water. Of this treated water, 10 Ml/day was pumped to the Ancor WWTP for further treatment in the Rhodes BioSURE® Process. In addition, approximately 2 Ml/day iron hydroxide sludge was pumped to the Ancor WWTP for sulphide removal in the biological sulphate-reducing reactors.

The Ancor WWTP is a conventional wastewater treatment process with mechanical screens, grit removal units, flow measurement, primary sedimentation tanks (PSTs), trickling filters, humus tanks with sludge recycle back to the PSTs and disinfection of the final effluent. The mixed PSS and humus sludge is thickened in the PSTs and normally stabilized in anaerobic digesters. During the full-scale operation of the Rhodes BioSURE® Process, the anaerobic digesters were used to store the sludge before being transferred to the sludge lands. The sewage sludge flowed into a sump from where it was normally pumped to the digesters. The pump station was modified to transfer the sewage sludge for treatment in the Rhodes BioSURE® Process instead. Other sources of organic material were introduced and mixed into the same sump. The mine water and mixed sewage sludge were blended before being distributed in a division box to each of eight biological sulphate-reducing (BSR) reactors. Old humus tanks were modified and used as BSR reactors. These were designed as upflow sludge blanket reactors with sludge recycle. Surplus stabilized biological sludge was wasted at a predetermined rate to control the sludge age, and lime slurry was dosed into the waste sludge line to raise the pH and eliminate odours.

The overflow from the BSR reactors was collected in a transfer pump station and pumped to a vessel where it was mixed with thickened iron hydroxide sludge. The iron hydroxide reacted with the dissolved hydrogen sulphide to form iron sulphide. Old Dortmund-type PSTs were converted into reactor-clarifiers. The reactor-clarifiers consisted of a mixed reaction zone and a turbulent zone to ensure that the reaction with the dissolved hydrogen sulphide was complete, and to provide sufficient energy to coagulate and flocculate the suspension. A flocculent was used to form a stable, readily settleable sludge. The clear supernatant, which contained residual organic material and ammonia, was mixed with the settled sewage and pumped to the trickling filters for final treatment before disinfection and discharge into the river. The thickened sludge was collected from the bottom of the reactor-clarifiers and applied to sludge drying beds for dewatering, drying and oxidation of the sulphide to sulphate under ambient conditions. The process is illustrated in the simplified diagram below (Figure 1).

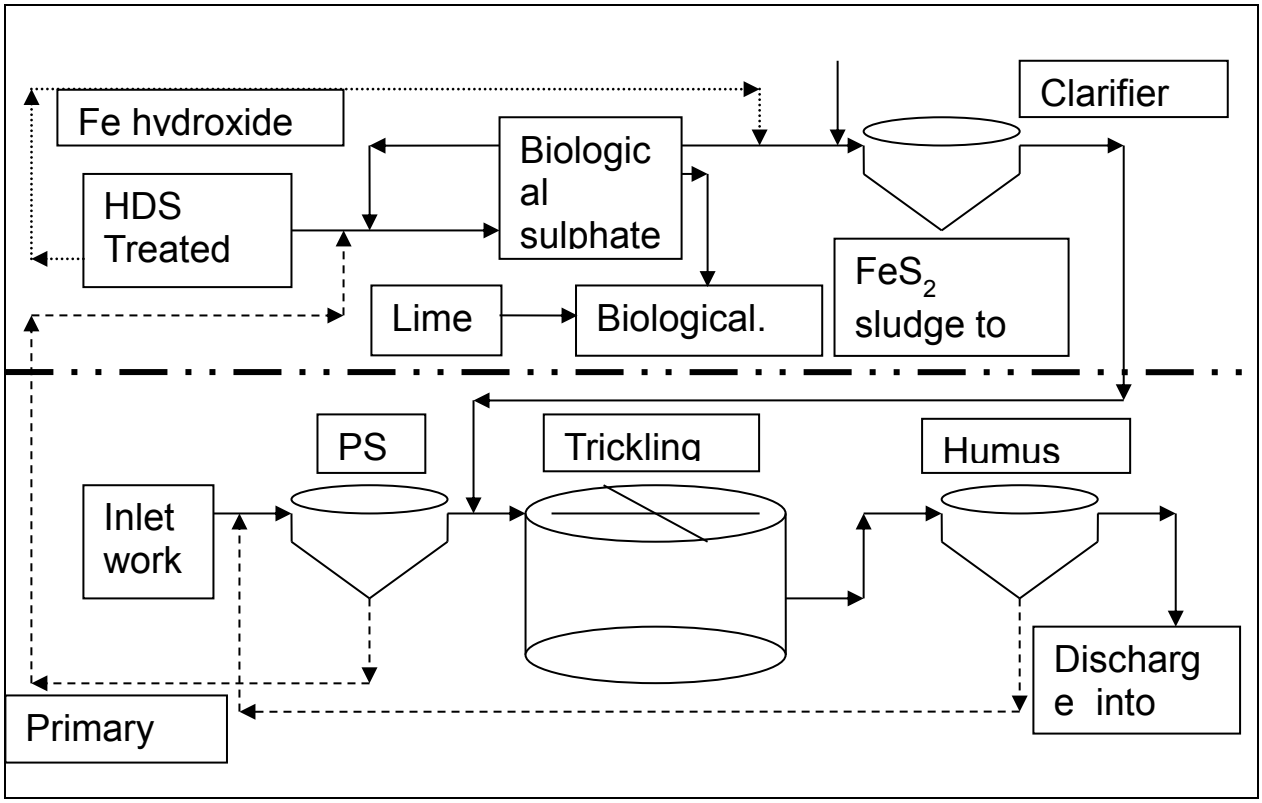


Figure 1 Simplified diagram of the Rhodes BioSURE®Process

### 1.1.1 Rhodes BioSURE® plant performance

The start-up of the reactors was rapid; the results indicated that it took approximately 25 days before a significant fraction of the sulphate was reduced. After the 25$^{th}$ day, a rapid reduction in the sulphate concentration was observed in all the reactors. Although sulphide was present, even after the first day, the rate of sulphide production increased steadily after the 25$^{th}$ day, reaching a peak within 50–55 days. The soluble, biodegradable organic material allowed for the initial production of sulphide, while hydrolysis of the particulate organic material was required before sulphate could be effectively reduced. The plant ran for several months and was signed off after a successful month-long, high-rate performance test. A summary of the discharge concentrations achieved during the high-rate performance test is presented in **Table 1** below.

**Table 1 Discharge concentrations achieved during the high-rate performance test of the Rhodes BioSURE® Process**

| Determinant | Units | Final discharge |
|---|---|---|
| **Sulphate as $SO_4$** | mg/L | 215 ± 88 |
| **Total iron as Fe** | mg/ | 0.1 ± 0.05 |
| **Manganese as Mn** | mg/L | 2.3 ± 0.5 |
| **Aluminum as Al** | mg/L | <0.05 |

### 1.1.2 Solid waste disposal

Two sources of solid waste were identified. The surplus biological sludge from the BSR reactors was treated with lime to control odours. The heavy metal content of the sludge was determined by the quality of the PSS. The stability of the sludge was similar to that of anaerobically digested sludge, the only difference being a higher gypsum content, and it was disposed of on the land in a similar manner. Gypsum formed when sulphide, dissolved in the moisture, was oxidised to sulphate in air during the natural drying process. Sulphate reacted with calcium and precipitated as gypsum.

The disposal and drying of the iron sulphide sludge was one of the biggest challenges of the process. The sludge contained unreacted calcium carbonate particles at a concentration of as high as five times that of iron sulphide.

## 1.2 Motivation for this study

It was identified by VitaOne8 that further research was required in order to improve the BioSURE Process for implementation elsewhere, in particular where there was no access to PSS, where the AMD has a high acidity, low pH, high concentrations of dissolved metals and a higher than 2000 mg/L sulphate concentration. In light of this, it was planned to:

1. Conduct bench-scale studies to compare the performance of the biological sulphate-reducing (BSR) reactors using PSS and silage, and combinations thereof, in order to address the concern of a reliable source of organic material;
2. Test the process using a feed of high acidity, low pH AMD. The BioSURE Process generates alkalinity and all indications are that it is sufficient to neutralize the acidity typically measured in the AMD of the Western and Central Basins, without the need for pre-treatment in an HDS process. In addition to acid neutralisation, the hydrogen sulphide in the BioSURE effluent can be recycled to react and precipitate the heavy metals in the AMD as metal sulphides. The challenges are to start up the process with a low pH, acidic feed and to recover and separate the heavy metals as valuable by-products instead of disposing of them as waste;
3. Remove the surplus hydrogen sulphide with iron hydroxide to precipitate the sulphide as iron sulphide, using a biological iron oxidizing process to regenerate iron hydroxide, integrated with the recovery of valuable magnesium;
4. Treat the water for chemical oxygen demand (COD) and nitrogen removal as a polishing step to make it suitable for disposal, or as a pre-treatment for reuse;
5. Soften and stabilise the treated water in order to recover magnesium hydroxide for the removal of surplus hydrogen sulphide and to reduce the salinity of the water in order to meet the final effluent standards. One advantage is that all the scale-forming minerals, such as calcium carbonate and gypsum, are reduced to low enough concentrations to reduce or even eliminate the fouling potential if reverse osmosis

membranes are considered for the removal of mono-valent ions such as sodium, potassium and chloride. The process cannot remove mono-valent ions but, when considered as a pre-treatment step, will extend the lifetime of RO membranes significantly.

A laboratory-scale phase for proof of concept, which was the first phase of the project, commenced in 2013 and was continued throughout 2014 and 2015 to determine design and scale-up factors for a demonstration-scale plant.

The second phase of the project involved the construction and operation of a 1 $m^3$/d pilot plant combining all the unit processes, including the biological sulphate-reducing reactor with effluent recycle, biological sulphide oxidation, iron hydroxide dosing and regeneration and magnesium sulphate dosing and regeneration. The pilot plant was constructed and operated at VitaOne8's research and development facilities in Pretoria using synthetic water resembling that of the Western Basin (see **Table 7** in section 4.2.1).

The goal of the pilot facility was to demonstrate the continuous treatment of AMD using BSR as the core technology, integrated with the other unit processes mentioned earlier, in such a manner as to:

- gain insight into the performance of all the unit processes for process optimization, validation of the design parameters and to train potential operating staff;
- demonstrate the potential to minimize the production of waste products and recover valuable minerals;
- remove sulphide from the effluent of the biological reactor;
- soften water in order to reduce the salinity; and
- reduce the need for water treatment chemicals.

Based on the results of the pilot study, a preliminary process design for a 200 $m^3$/d demonstration plant was developed, and, where possible, the detailed engineering design parameters were indicated.

Sufficient information has now been generated to confirm the validity of the process. It differs significantly from the original patents by Rose et al., and from the BioSURE Process as applied at the Ancor site for Grootvlei mine. A provisional patent was, therefore, filed in July 2014, where the integrated process was described. The novelty and advantages over the prior art are described in more detail in this document. A PCT patent application was filed for the process in July 2015, as well as a full patent application in Argentina, which is not a PCT member country.

Because the newly developed process differs sufficiently from the initial patents and original BioSURE Process, the decision was taken to change the name of the process to VitaSOFT. The development of the new name is discussed later in the document.

The operation of the bench-scale reactors will be continued until steady state is achieved, to determine the kinetic values and for comparison with the established results where only PSS was used. The operation of the pilot reactor will also be continued in order to produce sufficient effluent to determine the operating parameters of the downstream processes more accurately.

# 2 Literature review

## 2.1 Biological sulphate reduction

### 2.1.1 Metabolic activity of sulphate reducers

Many prokaryotes of the two domains, archaea and bacteria, have the ability to utilize sulphate as a terminal electron acceptor while oxidizing organic material to produce carbon dioxide and hydrogen sulphide in anoxic environments. This process is called dissimilatory sulphate reduction (Gottschalk, 1979). They are often referred to as sulphate-reducing bacteria (SRB), but it is more correct to refer generally to them as sulphate-reducing prokaryotes (SRP) since both archaea and bacteria are able to carry out the process (Hansen, 1994). According to Barton & Hamilton (2007), SRP belong to four distinct groups: (i) the mesophilic δ-proteobacteria with the genera *Desulfovibrio*, *Desulfobacterium*, *Desulfobacter*, and *Desulfobulbus*; (ii) the thermophilic Gram-negative bacteria with the genus *Thermodesulfovibrio*; (iii) the Gram-positive bacteria with the genus *Desulfotomaculum*; and (iv) the Euryarchaeota with the genus *Archaeoglobus*. A fifth group, the Thermodesulfobiaceae, has also been described. Although some of the species demonstrate the ability to use electron acceptors other than sulphate, such as sulphur, fumurate, nitrate, dimethylsufoxide and iron (III), dissimilatory sulphate reduction is only possible in strictly anaerobic habitats (Gottschalk, 1979).

Sulphate-reducing prokaryotes are a group of organisms of great economic importance since they play an important role in the environment and in industrial processes where sulphate is present. Most of the experiences are related to the role of SRP in bio-corrosion, degradation of valuable material in the food and petroleum industries, aggressiveness towards concrete structures, odours in potable water and the production of fatally toxic gases in confined spaces, among others. Until recently, SRP have been regarded as nuisance organisms. However, their ability to degrade a wide variety of organic substrates attracted the attention of scientists and engineers to investigate their potential for bioremediation, pollution control and to solve a variety of environmental problems (Barton & Hamilton, 2007). Sulphate is present in industrial waste waters generated by pulp and paper, brewing, edible oil and citric acid production industries. The list can be extended to tanneries and processes that use or produce molasses. All these industries also have high concentrations of complex organic material in their waste water. The use of sulphuric acid in industrial processes is often the source of sulphate in the effluent.

The ability of SRP to utilize organic polymers directly is very rare (Hansen, 1994). They are able to utilize the end products of fermentation of carbohydrates, amino acids, nucleic acids and fatty acids produced during the hydrolysis of polysaccharides, proteins, lipids and other substances (Ristow et al., 2005; Gottschalk, 1979). A large number of energetic electron donors are able to sustain the growth of SRP. The redox potentials of various electron donors vary over a wide range (Table 2). They are able to degrade several complex substrates such as branched-chain fatty acids or aromatic compounds for which they require special

enzymatic reactions, which are not typical of fermentative bacteria. Included in the list of organic substrates utilized by SRP are hydrocarbons, in particular those from crude oil (petroleum) (Barton & Hamilton, 2007). The metabolism of organic substrates by SRP can involve partial removal or modification of a functional group of the substrate, incomplete degradation to form intermediates such as acetate, or complete conversion to $CO_2$ (Hansen, 1994). The term completely oxidizing sulphate-reducing prokaryotes refers to SRP with the ability to oxidize the 2-carbon unit of acetyl-CoA to carbon dioxide. When completely oxidizing SRP are growing on certain readily biodegradable substrates, it is not unusual to observe the excretion of significant amounts of acetate.

The implication is that refractory organic material and accumulation of acetate may often be observed in sulphate-reducing mono-cultures. The process whereby hydrogen reacts with carbon dioxide to from acetate is called homoacetogenesis, and is catalysed by a specific group of bacteria. Their growth is more pronounced at lower temperatures (Siriwongrungson, 2007; Nevatalo, 2010). When homoacetogenic bacteria is the dominant group in a process, high acetic acid concentrations are invariably detected in the effluent, at the expense of methanogenesis and other anaerobic processes.

During fermentation of a large variety of organic molecules, hydrogen is produced and subsequently consumed by many of the chemoautotrophic SRP. They are able to derive their energy from hydrogen and their carbon from inorganic carbon. Du Preez, Maree and Jackson-Moss (1991) successfully demonstrated that producer gas, consisting mainly of hydrogen and carbon monoxide, can be used as a substrate for dissimilatory sulphate reduction. Hydrogen is an excellent energy source for *Desulfovibrio, Desulfomicrobium, Thermodesulfobacterium, Desulfobulbus, Desulfobacteriurn, Desulfosarcina, Desulfomonile, Archaeoglobus* and many *Desulfotomaculum* strains (Hansen, 1994; Van Houten et al., 2006). It is therefore expected that hydrogen will not accumulate in a stable SRP culture when there is an excess of sulphate.

**Table 2 Standard redox potentials ( $E^o$) and Gibbs free energy ($\Delta G^o$) of electron donors (adapted from Barton & Hamilton, 2007)**

| **Redox couple** | **n** | **$E^o$ (mV)** | **$\Delta G°=nFE^o$ (kJ/mol)** |
|---|---|---|---|
| $2CO_2$ + 2acetate/hexose | 8 | -670 | -517 |
| $CO_2$ + acetate/pyruvate | 2 | -660 | -127 |
| $FeS_2/FeS + H_2S$ | 2 | -613 | -118 |
| $SO_4^{2-}/HSO_3^-$ | 2 | -516 | -100 |
| $CO_2/CO$ | 2 | -520 | -100 |
| $Fe^{2+}/Fe^o$ | 2 | -447 | -86 |
| $CO_2$ + acetate/lactate | 4 | -430 | -166 |
| $CO_2$/formate | 2 | -432 | -83 |
| $2H^+/H_2$ | 2 | -414 | -80 |
| $6CO_2$/hexose | 24 | -410 | -949 |
| $S_2O_3^{2-}/HS^- + HSO_3^-$ | 2 | -402 | -78 |
| $CO_2$ + acetate + $NH_3$/alanine | 4 | -400 | -154 |
| Acetate/ethanol | 4 | -390 | -151 |
| $CO_2$/methanol | 6 | -370 | -214 |
| $4CO_2$/succinate | 12 | -312 | -361 |
| $7CO_2$/benzoate | 30 | -300 | -868 |
| 2Acetate/butyrate | 4 | -290 | -112 |
| $CO_2$ + acetate/glycerol | 6 | -290 | -168 |
| $2CO_2$/acetate | 8 | -290 | -224 |
| $4CO_2$/butyrate | 20 | -280 | -540 |
| $3CO_2$ /propionate | 7 | -280 | -189 |
| $N_2/NH_3$ | 6 | -276 | -160 |
| $S^0/H_2S$ | 2 | -270 | -52 |
| $6CO_2$/hexane | 38 | -250 | -917 |
| $CO_2/CH_4$ | 8 | -244 | -188 |
| **$SO_4^{2-}/HS^-$** | **8** | **-217** | **-167** |
| $SO_3H^-/HS^-$ | 6 | -116 | -67 |
| Glycine/acetate+$NH_3$ | 2 | -10 | -2 |
| Fumarate/succinate | 2 | +33 | 6 |
| Trimethylamine N-oxide/trimethylamine | 2 | +130 | 25 |
| Dimethylsulfoxide/dimethylsulphide | 2 | +160 | 31 |
| $Fe(OH)_3 + HCO_3^-/FeCO_3$ | 1 | +200 | 19 |
| $NO_2^-/NH_3$ | 6 | +330 | 191 |
| $NO_3^-/NH_3$ | 8 | +360 | 278 |
| $Mn^{4+}/Mn^{2+}$ | 2 | +407 | 79 |
| $NO_3^-/NO_2^-$ | 2 | +430 | 83 |
| $2NO_3^-/N_2$ | 10 | +760 | 733 |
| $O_2/2H_2O$ | 4 | +818 | 316 |
| $2NO/N_2O$ | 2 | +1175 | 227 |
| $H_2O_2/2H_2O$ | 2 | +1350 | 261 |
| $N_2O/N_2$ | 2 | +1360 | 262 |

### 2.1.2 The role of microbiological consortia in sulphate-reducing reactors and competition of SRP with methanogens

A consortium of micro-organisms in a culture may be able to degrade a wide variety of complex substrates, with one group interdependent on other groups to produce intermediates. The synergy amongst the various groups is important for near complete biodegradation of organic substances (Hamilton, 1998). The steps of anaerobic degradation of organic material are illustrated in Figure 2. Heterotrophic fermentative organisms are able to excrete hydrolytic enzymes to hydrolyze particulate organic material and high molecular weight molecules. The hydrolysis products are then fermented to produce a range of fatty acids, alcohols, glycerol, hydrogen and carbon dioxide with the simultaneous release of ammonia-nitrogen and ortho-phosphorous. This step is generally referred to as acidogenesis. In typical anaerobic processes, a range of fatty acids are fermented to acetic acid, carbon dioxide and hydrogen gas. This process is typically referred to as acetogenesis. Methanogenic bacteria are able to utilize acetic acid, formic acid, carbon dioxide and hydrogen to produce methane. This process, known as methanogenesis, is performed by the activity of two main groups of methanogens, the hydrogenotrophic and acetoclastic methanogens, or by syntrophic acetate-oxidising bacteria (SAOB) operating in cooperation with hydrogenotrophic methanogens (Westerholm et al., 2011). In the presence of sulphate, SRP and methanogens compete for the same substrate, i.e. acetate and hydrogen or carbon dioxide. SRP typically outcompete the methanogens due to several interacting factors: (i) anaerobic respiration with sulphate as the final electron acceptor yields more energy for growth when compared with carbon dioxide; (ii) SRP have a higher affinity for both hydrogen and acetate, enabling them to consume substrates below concentrations possible for use by methanogens; and (iii) SRP generally have a higher specific growth rate than methanogens (Moestedt et al., 2013). Capable of growing on more varied substrates than methane-producing bacteria, SRP are able to compete effectively with acetogenic bacteria by utilizing fatty acids. Under these conditions, not enough substrate will be available for growth by methanogenic bacteria. While methanogenic bacteria are pH-sensitive, SRP are able to tolerate a wider range of pH values, adding to their competitive advantage. Both sulphate reduction and methanogenesis can be the final step in the degradation process of sulphate-fed anaerobic reactors, due to SRP being capable of utilizing many of the intermediates formed during methanogenesis. Competition for substrate in such systems is possible on two levels: competition between SRP and acetogenic bacteria for volatile fatty acids (VFA) and a carbon source, and competition between SRP and methanogenic bacteria for acetate and hydrogen. Methane bacteria are not eliminated in a sulphate-reducing process, and may compete with the completely oxidizing SRP by utilizing the residual acetic acid (O'Flaherty et al, 1998; Van Houten et al., 2006). The result is that stable sulphate-reducing processes with mixed cultures may produce good quality effluents.

Sulphate-reducing prokaryotes have been isolated from a wide range of habitats. They occur at the bottom of the sea, in polar areas where they are able to survive in freezing conditions, volcanoes, hot water geysers, the intestines of mammals, including a large population of humans, and a range of other habitats (Hansen, 1994). The presence of SRP in human and animal intestines is, for example, one of the reasons why the start-up of biological sulphate-reducing processes using manure or sewage sludge is so rapid compared to methanogenic processes. The reactor is continuously inoculated when substrate is introduced. Their ability to degrade a wide variety of organic substrates, to survive by using electron acceptors other than sulphate, to tolerate a wide range of temperatures and pH, and the ability to survive in fresh water and highly saline water allows them to adapt easily to highly variable conditions.

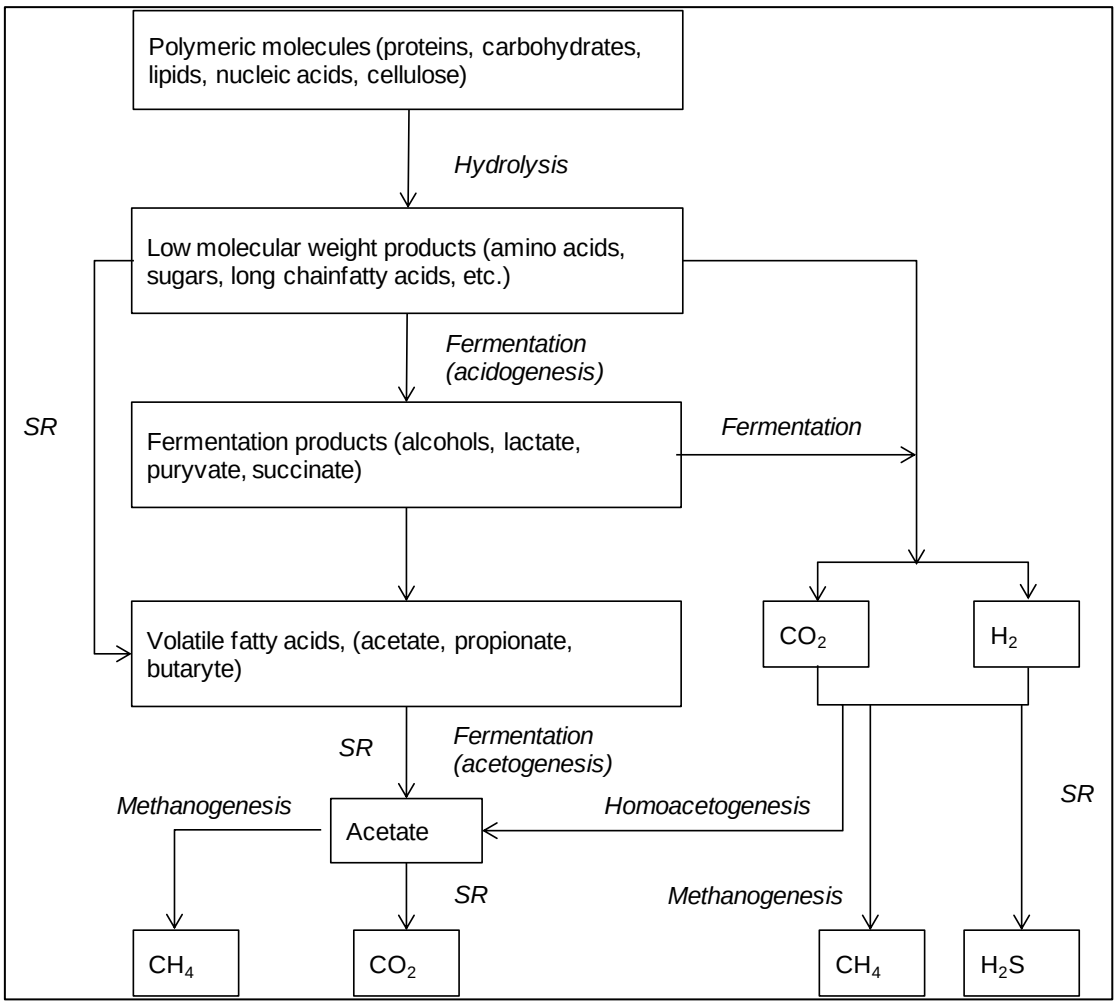


Figure 2 Step-wise anaerobic degradation of organic material and consumption by methanogenic bacteria and SRP (adapted from Nevatalo, 2010). SR = sulphate reduction.

### 2.1.3 Trace metal requirements

It is clear from the above discussion that the performance of high-rate anaerobic reactors depends on retention of highly active mixed culture biomass to metabolize complex organics using available electron acceptors. The methanogenic bacteria and SRP present in mixed

culture require various metal ions for enzymatic activities and growth. Trace metals have been identified as cofactors of enzymes and their bioavailability affects overall functioning of anaerobic digestion systems due to their role in enzymatic activity, membrane stability, nutrient transport, and energy conservation, in both methanogenic bacteria and SRP. Iron (Fe) is involved in energy metabolism and is present in cytochromes, ferredoxins, hydrogenase, methyl transferase, adenosine phosphosulphate (APS) reductase, bisulfite reductase, formylmethanofuran dehydrogenase, formate dehydrogenase, ethanol dehydrogenase, lactate dehydrogenase, carbon monoxide (CO) dehydrogenase, and aldehyde oxidoreductase. Cobalt (Co) has been found to be present in corrinoids, which are also involved in the activity of methyl transferase and CO dehydrogenase, and adenosine triphosphate (ATP) sulphurylase. Nickel (Ni) is a component of methyl-coenzyme M, factor F420, factor F430, hydrogenase and CO dehydrogenase. Zinc (Zn) has been found to be present in hydrogenase, CO dehydrogenase, ATP sulphurylase, and formate dehydrogenase. Zn is also reported to be involved in coenzyme M activation during formation of methyl-coenzyme M. Other trace metals, molybdenum (Mo), selenium (Se) and tungsten (W) are also reported to be present in various coenzymes (Patidar et al., 2006).

Supplementation of Fe, Ni, Co, Mo and other nutrients has shown positive effects on substrate utilization, bacterial activity and process stability of sulphidogenesis. Sulphide precipitation and complexation also have a significant influence on availability of essential trace metals. In view of this, Patidar and Tare (2006) assessed the effect of four critical metals – Fe, Zn, Ni and Co – or their combinations, on sulphidogenic activity in a mixed culture growing in sulphidogenic conditions. The most effective nutrient combination for activity of sulphate reducers was found to be supplementation with Fe and Co. Supplementation with Zn alone showed results similar to that of the control, indicating no Zn limitation but deficiency of other nutrients. Supplementation with the combinations Ni-Co, Ni-Zn-Co and Fe-Ni-Zn showed sulphidogenesis lower than the control, possibly due to antagonistic effect of Ni and/or deficiency of Fe.

Gustavsson et al. (2013) evaluated the effect of Ni and Co supplementation on methane production, volatile solids reduction, pH and VFA in a biogas reactor. Despite extensive Co- and Ni-sulphide precipitation, with Ni being almost totally absent from the solution, the supplementation of Co and Ni had stimulatory effects on process performance. This indicated microbial uptake of Ni from its association to sulphides, challenging the paradigm that metal sulphide precipitation limits trace metal bioavailability in bioreactors. This was in agreement with Jansen et al. (2010), who suggested that Co- and Ni-sulphides may act as metal sources in anaerobic digesters.

For the purposes of this study, a synthetic AMD solution was used. There may be a need to supplement the synthetic solution with metal trace elements, depending on the carbon source used. Based on the findings of Foucher et al. (2001), who found a 30–40% improvement in the sulphate reduction rate when replacing synthetic media with actual AMD

due to the large range of oligo-elements provided by the mine water, it is expected that a higher sulphate reduction efficiency will be observed in the biological reactor whenever the synthetic solution is replaced with actual AMD.

### 2.1.4 Need for active biological sulphate-reducing systems

Recent studies have highlighted the potential of sulphate-reducing passive bioreactors for the treatment of AMD (Sheoran et al., 2010; Choudhary & Sheoran, 2012; Bai et al., 2013). Various organic wastes have been reported as the electron donors for the sulphate reducers in the treatment of AMD. Higher sulphate reduction rates have been obtained with reactive mixtures containing more than one organic carbon source. Generally, these mixtures contain relatively biodegradable sources (poultry manure, cow manure or sludge) and more recalcitrant ones (sawdust, hay, alfalfa or wood chips). Hedin et al. (1991) and Wildeman et al. (1994) reported that many systems have been constructed using compost or other organic wastes to generate an anaerobic environment and provide a source of organic carbon. Complex organics present in the substrate are microbiologically degraded to simpler organics, which are utilized by the SRP. Although wetland plants are sometimes present, many systems have been built without them. Johnson and Hallberg (2005) observed that sulphate reduction in passive bioreactors is confirmed by lower concentrations of sulphate in the effluent than in the influent waters, the presence of free sulphide (depending on metal concentrations and water pH) and lower redox potentials in the effluent waters.

Many of the early studies were conducted for only one to two years (Hedin et al., 1991; Cohen and Staub, 1992; Dvorak et al., 1992; Wildeman et al., 1994). Initially, the results were impressive; pH increased from <4 to >7, and typical trace metal removal exceeded 90%. The bioreactors were originally considered passive because they were believed to be able to function on the order of 20 or more years before requiring major maintenance (i.e. replacement of the treatment media).

Estimates made of the lifetime on the total carbon in these passive systems suggest that their lifetime should exceed 20 years. However, data from field and laboratory studies showed that the rate of sulphate reduction decreases with time, and after several years, rates decreased substantially. To maintain acceptable treatment, either additional organic material must be added or the total metal and acid load to the system would need to be reduced substantially (Choudary et al., 2012). Sulphate reduction is dependent on a continued supply of sulphate and organic compounds produced by the decomposition of the organic matter in the substrate.

Choudary et al. (2012) observed a maximum of 54% reduction in sulphate concentration with a 10-day retention time, with sulphate concentrations reducing from above 4000 mg/L to 1951 mg/L, when manure was applied as the carbon source. In terms of metal removal, 51.49–99.32% of Fe, 84.95–99.97% of Cu, 35.11–99.78% of Zn, 17.87–99.14% of Ni, 63.55–99.02% of Co and 12.68–73.86% of Mn were removed in a maximum retention period of 10

days. Bai et al. (2013) reported that in bench-scale passive tests the concentration of sulphate decreased from 20 800 mg/L in influent AMD to 8 200 mg/L in effluent, with 61% sulphate reduction. The effluent pH was improved from 2.75 to 6.20 during the operation. Ninety-nine percent of $Cu^{2+}$ was removed with the effluent concentration at 0.2 mg/L, and $Fe^{2+}$ was decreased from 545 mg/L to 75 mg/L in the effluent. $Mn^{2+}$ removal was typically low with removal efficiency at 53% due to its higher solubility product of $2.5 \times 10^{-13}$. While these are impressive results in terms of percentage removal, the effluent sulphate concentrations still far exceed what is acceptable for discharge into a water resource, or for commercial reuse. While many metal sulphides have low solubility products, for example $2.93 \times 10^{-25}$ for zinc sulphide and $6.3 \times 10^{-36}$ for copper sulphide, passive systems have a limit to the amount of precipitated colloidal material that can be stored. Ultimately, breakthrough of these sulphides will result in exposure to air and eventual oxidation back to sulphate.

An active sulphate-reducing system that is constantly fed with a readily biodegradable carbon source to ensure a maximum sulphate reduction rate, where the VFA and sulphide concentration can be controlled, would therefore overcome many of the problems associated with passive systems. Furthermore, in passive systems where the metal concentration of the AMD is insufficient to remove all the hydrogen sulphide as metal sulphide precipitates, additional sulphide removal needs to be considered. This could be overcome in an active system such as the BioSURE Process, where iron hydroxide is dosed stoichiometrically to ensure effective control of sulphide in the effluent. This study focused on the development of a new process for the recovery of sulphate and recycling of the iron hydroxide to preclude the need for a constant source. This is discussed in more detail in section 3.2.

### 2.1.5 Carbon and energy sources

When sulphate is reduced to hydrogen sulphide, 8 moles of electrons are consumed for each mole of sulphate reduced. The reduction half-reaction ($R_a$) is as follows:

$$\frac{1}{8}SO_4^{2-} + \frac{19}{16}H^+ + e^- \leftrightarrow \frac{1}{16}H_2S + \frac{1}{16}HS^- + \frac{1}{2}H_2O \qquad 1$$

Expressed on a mass basis, 12 g of sulphate is reduced per mole of electrons consumed. Similarly, when oxygen is reduced, 8 g oxygen is reduced per mole of electrons consumed. The equivalent COD of sulphate is 0.667 g COD/g sulphate.

$$\frac{1}{4}O_2 + H^+ + e^- \leftrightarrow \frac{1}{2}H_2O \qquad 2$$

During dissimilatory sulphate reduction, biomass is produced. The yield coefficient, $Y_H$, is approximately 0.113 g COD biomass/g COD organics hydrolyzed, of which acidogens contribute more than 77% of the biomass (Poinapen, Ekama & Wentzel, 2009). The same authors suggested the composition of PSS as $C_{3.5}H_7O_2N_{0.196}$. It is worth noting that a mixture of other sources of organic material, such as carbohydrates, proteins or fats may change the

relative ratios of C, H, O and N. The reader is referred to the paper of Poinapen and Ekama (2010) for a thorough discussion. Using this information, and following the approach as described by Grady et al. (1999), the redox half-reaction for PSS as an electron donor ($R_d$) is as follows:

$$\frac{1}{16.412}C_{3.5}H_7O_2N_{0.196}+\frac{5}{16.412}H_2O\rightarrow\frac{3.5}{16.412}CO_2+\frac{0.196}{16.412}NH_4^+ +\frac{16.216}{16.412}H^+ +e^- \quad 3$$

The typical composition of biomass is $C_5H_7O_2N$ (Grady et al., 1999). The redox half-reaction for bacterial cell synthesis ($R_c$) is as follows:

$$\frac{1}{20}C_5H_7O_2N+\frac{9}{20}H_2O\leftrightarrow\frac{1}{5}CO_2+\frac{1}{20}HCO_3^- +\frac{1}{20}NH_4^+ +H^+ +e^- \quad 4$$

The overall stoichiometric equation (R) is the sum of the half-reactions:

$$R=R_d-f_eR_a-f_sR_c \quad 5$$

where $f_e$ is the fraction of the electron donor that is coupled with the electron acceptor and $f_s$ is the fraction captured through synthesis of biomass. Ammonia-nitrogen is used as a source of nitrogen, therefore $f_s$ equals $Y_H$ (Grady, et al., 1999).

$$f_s+f_e=1 \quad 6$$

and therefore $f_e$ equals 0.887.

The overall stoichiometric reaction (R) is obtained simply by inserting the half-reactions in Equation 6:

$$C_{3.5}H_7O_2N_{0.196}+1.82SO_4^{2-} \rightarrow 0.093C_5H_7O_2N+0.91H_2S+0.91HS^- +0.977HCO_3^- +2.05\ CO_2+0.103NH_4^+ +0.187H_2O \quad 7$$

From reaction 7 above, the ratio between primary sludge, sulphates and biomass ($C_5H_7O_2N$) is 1:9.67 0.532. This is a molar ratio. Therefore, the mass ratio, in grams volatile suspended solids (VSS) will be 455:929:60. Taking into account that the COD equivalent of primary sludge and biomass is 1.55 g COD/g VSS and 1.42 g COD/g VSS respectively, the ratio in terms of COD values is 705:929:85. Therefore 0.667 g COD is required to remove 1 g $SO_4^{2-}$ from the mine water.

From the same VSS-mass ratio above, it can be demonstrated that 0.49 g VSS in primary sludge will be consumed in order to reduce 1 g $SO_4^{2-}$. Only 64% of the primary sludge is biodegradable and available for sulphate reduction. Therefore, a maximum quantity of 1.31 g $SO_4^{2-}$ can be reduced by using 1 g PSS.

#### 2.1.5.1 Maize silage as an alternative carbon source

Notwithstanding the success using PSS and the reported additional sources of organic material to support the BSR process, limited supply has the potential to restrict the application of the BioSURE Process. There is therefore a need to consider other sources of organic material that can be supplied cost effectively and in large quantities. It is important to consider the composition of an organic source and how it contributes to the production of alkalinity and weak acid and/or bases in the process and their effect on pH buffering. For example, when carbohydrates are degraded during methanogenesis, alkalinity must be added to the process to control the pH.

Volatile fatty acids (acetate, propionate, butyrate) and short chain fatty acids (lactate, pyruvate and malate) are among the main substrates for SRP. Carbon and long chain fatty acids and certain aromatic compounds are occasional substrates. Fermentative products such as methanol, ethanol and acetate are additional sources, and polymers such as cellulose are not degraded by known SRP. The polysaccharide must first be degraded by hydrolytic fermentative anaerobes into sulphidogen-supporting fatty acids and, once these substances are depleted, hydrolytic fermentation is the rate-limiting step because the products of fermentation are completely used up. Similarly, proteins, carbohydrates and lipids or even simple sugars are generally not accessible to SRP and depend on other heterotrophic bacteria to supply them with degradation and fermentation end products (Sheoran et al., 2010).

Coetser et al. (2006) performed a study on chemical characterization of organic substrate with the following conclusions. The most readily biodegradable carbon source should be high in protein content and low in lignin content. The higher the carbohydrate content and crude fat content of a carbon source, the higher the capacity to drive sulphate reduction.

Alkalinity and saline ammonia are produced in sufficient quantities to buffer the effluent when proteinaceous material is degraded in methanogenic systems. In sulphate-reducing processes, alkalinity is produced as a result of the reduction of sulphate to sulphide, while the hydrogen sulphide and bisulphide weak acid/weak base pair plays a significant role to buffer the effluent. Consequently, no additional alkalinity is required when carbohydrates are used as raw material. When proteinaceous material is used, additional alkalinity and saline ammonia is produced. It is of no concern when AMD with a low pH is produced, except that the ammonia-nitrogen content in the effluent will be high. It will require a higher investment in capital costs and higher energy consumption to remove nitrogen from the effluent.

Compared to other lignocellulosic substrates, maize has a low degree of lignification. Maize silage is maize (whole harvested plant) chopped into pieces and fermented with a process dominated by lactic acid bacteria. Ensiling is an interesting solution to store the maize, providing a seasonal buffer to the supply of animal feed, particularly that of dairy cows. Even though losses might take place during the silage storage, these can be controlled and minimized with good management.

The composition of maize silage, as reported by various authors, is summarised in

Table **3**. The average dry matter content is approximately 35%, with organic matter comprising approximately 95% of the dry mass. Crude protein content of maize silage is low, with average values of 7% of dry mass, and starch content averages approximately 30% of dry mass. Organic matter digestibility is reported to be approximately 72%, although Resch et al. (2008) have reported a degradation rate of 94%. The acid detergent fibre (ADF) and neutral detergent fibre (NDF) contents averaged 25% and 43% of dry mass respectively. NDF consists of the cell wall material, including cellulose, hemicellulose, lignin and silica, and is approximately 60% digestible by the rumen micro-organisms. It is important in lactating cows for the following reasons: stimulation of rumen motility, which promotes VFA absorption; stimulation of chewing which results in the secretion of salivary buffers; formation of a rumen mat that entraps small particles, increasing their ruminal digestibility; and providing a consistent source of fuels to the microbes in the rumen which functions to provide a steady supply of fuels to the liver and mammary gland over time (Allen, 2009). Some of these characteristics of NDF in cows also apply to the use of silage as a substrate in the BioSURE Process, providing a slow-release source of carbohydrates and forming a mat to function as a surface for SRP attachment. ADF includes the largely digestible cellulose, indigestible lignin and inorganic silica, and is important because it is negatively correlated with digestibility of silages. As the ADF increases, the silage becomes less digestible, primarily because of the increase in the amount of indigestible lignin.

The typical values of the digestibility of maize silage range from 65%–95% (see

Table **3**) and are based on *in vitro* testing with rumen fluid. Different methods are used when the digestibility is measured in laboratories. The length of fermentation, for example, varies between 24, 36 and 48 hours. The longer the fermentation, the greater is the value of digestibility (Marten et al., 2008). The holding time in the rumen of a cow is approximately 30 hours or less. The value for complete biodegradability is therefore higher than the reported digestibility. The sludge age in a typical BioSURE Process is in the order of 20 days and higher, as opposed to the 48 hours or less fermentation times. Chandler et al. (1980) reported degradability values of 77.2% for maize stalks, 71.8% for maize leaves and 84.8% for maize meal. The plant, excluding the roots, is made of grain (40–45% of dry matter (DM)), stover (55–60% of DM), stem (20–25% of DM) and the cob, shank and husk (20% of DM) (Marten et al., 2008). Chandler et al. (1980) compared the degradability of various other substrates and found a strong linear correlation ($r^2$ = -0.94) between the degradability, expressed as % of volatile solids (VS) and the lignin content of the substrates, expressed as % of VS. The following equation describes the best-fit line to predict the degradability:

$$y = 83\% - 2.8(x) \quad (8)$$

where
y = biodegradability (% of VS)
x = lignin content (% of VS)

The maximum expected biodegradability, in the absence of lignin, is 83%. The microbiological decomposition of substrate is associated with the production of by-products, including biomass, which themselves are not readily biodegradable (Haug, 1993). The reported values for lignin vary between 1.57 and 4.4% expressed as % lignin of dry mass (

Table **3**). This is equivalent to 1.65–4.63% of VS, assuming 95% VS of dry material. Using these figures, the estimated biodegradability of the silage varies between 70 and 78.4%. The composition of the silage, including its lignin content, depends on several factors, the most important being the maturity of the maize plant at harvesting (Bal et al., 1997; Di Marco et al., 2002; Filya, 2004; Jensen et al., 2005; Marten et al., 2008), fermentation temperature, chop length and fermentation period (Mohd-Setapar et al., 2012). Clearly, the different parts of the plant and the grain vary with respect to lignin content and will be hydrolyzed at different rates.

Van Dyk and Pletschke (2012) reviewed the literature related to the bioconversion of various sources of lignocellulose using enzymatic hydrolysis for biofuel production. In broad terms, the bioconversion process is based on two steps. The first step is the hydrolysis of lignocellulose to produce glucose monomers, hexoses and pentoses. The second step is the fermentation of the hydrolysis products to produce ethanol. The authors differentiated between three different types of processes for bioconversion and fermentation of lignocellulose, namely separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF) and consolidated bioprocessing (CBP). When the hydrolysis and fermentation processes are conducted in two separate reactors, it is referred to as SHF. When the two processes are conducted in a single reactor, it is referred to as SSF. When a single organism is used to conduct both processes simultaneously, it is referred to as CBP. Clearly, when a consortium of organisms is used to conduct the hydrolysis and fermentation processes in a single reactor, such as the biological sulphate reducing process, it is regarded as a SSF process.

Lignocellulose is a complex substrate and consists of lignin, carbohydrates such as cellulose and hemicellulose, pectin, proteins and various minerals. Lignin is very resistant to degradation. The function of lignin is to provide rigidity and strength to the plant cells, retard water and protect the plant against pathogens. Cellulose consists of chains of glucose linked by β-1,4 linkages arranged in crystalline microfibrils, and is very recalcitrant to degradation.

Some parts of the cellulose structure are amorphous, and easier to degrade. Hemicellulose, another group of structural compounds, includes xylan, mannan, galactan and arabinan polymers. Xylan is the most abundant hemicellulose and makes up a considerable fraction of the plant dry weight. Heteroxylan is the most abundant fraction of xylans. Heteroxylan serves as a backbone substituted with acetate, arabinose and glucose. Pectin is a hydrophilic polysaccharide. It forms a part of the intercellular network and plays a role in the expansion and shrinkage of cells, depending on the water activity.

The presence of lignin, the degree of crystallinity, the degree of polymerisation of the carbohydrates, available surface area and moisture content play a significant role in resistance to biodegradation. In addition to the lignin content of the plant material, the amount of ferulate cross-linking in lignin, the subunit composition of the lignin and the degree of ester linkages between lignin and carbohydrates determines the recalcitrance.

To produce biofuel from lignocellulose, pre-treatment is essential to achieve effective hydrolysis of the substrate. Pre-treatment assists with partial removal of lignin in order to improve the porosity of the substrate, and disrupt the lignin structure and linkages with other substances. It improves access of cellulase to cellulose, disrupts the hemicellulose structure, reduces the crystallinity and degree of polymerization of cellulose and results in particle size reduction. Pre-treatment aimed at removal and disruption of lignin has the largest benefit. Lignin limits access of enzymes to their substrate, adsorbs and inactivates cellulase enzymes and directly inhibits hydrolytic enzymes. Residues of lignin linked to the polysaccharide chains interfere with the complete breakdown of the chains. Different types of lignin, and their distribution in the plant material, also affect the hydrolysis of the hemicellulose.

Physical, chemical and enzymatic methods are used for the pre-treatment of hemicellulose. Mechanical size reduction is an important method used during harvesting and ensilage of maize silage. The silage is cut into predetermined size ranges and crushed to improve the porosity of the substrate. During fermentation of the material, hydrolytic enzymes are produced to hydrolyze starch, oligosaccharides and sugars, and the pH is reduced as VFAs are produced. However, a large variety of enzymes are required to degrade the lignocellulose components. Three types of enzymes are required to hydrolyze cellulose into glucose monomers. A much larger variety of enzymes is needed to degrade hemicellulose. One group of enzymes degrades the backbone of the substrate and another group of enzymes removes the substituents and improve accessibility of the enzymes to the substrate. Similarly, several enzymes are involved with the degradation of pectin. A variety of anaerobic bacteria, including predominantly *Clostridia*, produce saccharolytic multi-enzyme complexes known as cellulosomes to degrade the components of lignocellulose. During this process, inhibitory products may be formed. It is expected that a synergy exists between the various groups of micro-organisms, where the product of one group of bacteria is consumed as a substrate by the following group at steady state. It is therefore important to understand the factors that may inhibit the process.

Based on this information, it can be concluded that the choice of the type of reactor for the process is important. In an upflow sludge blanket, for example, the more recalcitrant fibrous material will be retained in the sludge bed and accumulate for periods longer than the mean sludge age. Soluble reaction products may be washed out and the inhibitory effect may be reduced. In completely mixed reactors without solids separation, the mixed liquor will be homogenous with a fixed residence time for soluble and particulate material. Inhibitory compounds formed during the process may accumulate. During start-up of the reactors and before achieving steady state, the population of micro-organisms is not stable, which may also result in the accumulation of inhibitory intermediates.

**Table 3: Composition of maize silage as reported by various authors**

| Constituent | Browne et al., 2005 | Cone et al., 2008 | Valencein et al., 1999 | De Boever et al., 2005 | Khan et al., 2012 | Marten et al., 2008 | Pirmohammadi et al., 2006 |
|---|---|---|---|---|---|---|---|
| **Dry mass (DM) (g/kg fresh weight (FW))** | 306 | 312 | 305.2 | 339 | 342 | | 429 |
| **Organic matter (g/kg DM)** | 954 | | | | | | 936 |
| **Organic matter (OM)digestibility (g/100g OM)** | | | | 73.2 | 75.2 | 70-95 | 65.4 |
| **Ash (g/kg DM)** | | 44 | 43.8 | | 38 | 51.4 | |
| **Total N (g/kg DM)** | 15.4 | | | | | 14.1 | |
| **Crude protein (CP) (g/kg DM)** | | 77 | 80.5 | 72 | 71 | 88 | 76 |
| **Crude fat, oil and grease (CFOG) (g/kg DM)** | | | | | | 27-32 | |
| **Crude fibre (g/kg DM)** | | | 207 | | | | |
| **Acid detergent fibre (ADF) (g/kg DM)** | 226 | | 234.8 | | 278 | 270 | 260 |
| **Neutral detergent fibre (NDF) (g/kg DM)** | 419.5 | 368 | 446.6 | 388 | 493 | | 463 |
| **Starch (g/kg DM)** | 291 | 324 | 311.4 | 303 | | 230 | |
| **Lignin (g/kg DM)** | | | 31.1 | | | 15.7-29.3 | 44 |
| **Sugars (g/kg DM)** | | 13 | | | 13 | | |

Browne et al. (2005) compared the digestibility of grass and maize silages. The mean DM content of the grass and maize silages was 256 and 306 g/kg fresh weight (FW), respectively. The grass silage contained less organic matter than maize silage but larger quantities of NDF. In contrast, maize silage contained more non-structural carbohydrate than grass silage, as starch, which comprised 291 g/kg DM. Therefore, with increasing maize silage inclusion within the forage mixtures, there was a commensurate reduction in fibre concentration and increase in starch concentration. Replacing grass silage with maize silage reduced N content of the forage mixture from 18.8 g N/kg DM in grass silage to 15.4 g N/kg DM in maize silage.

Bruni et al. (2010) investigated the use of maize silage as the only substrate for biofuel production, to determine whether it would cause problems connected to long-term process stability. Maize is relatively poor in several necessary growth factors and previous studies on mono-digestion of maize silage in batch showed lower biogas production for batches without dosage of iron, nickel and cobalt. They found that where digested manure was used as an inoculum, the batch tests of this study did not show signs of inhibition or malnutrition of the anaerobic microbes involved in the biogas process. The manure contained a broad spectrum of different trace elements. In continuous operation of reactors only fed with maize, the authors concluded that these trace elements, especially iron, nickel and cobalt should be supplied with the feed, for example by co-digestion with manure, to prevent acidification of the reactors and poor biogas yield.

In mono-digestion biogas plants, the availability of nutrients is insufficient, as these substrates do not provide adequate amounts of trace elements (Hinken et al., 2008). Table 4 shows the composition of typical maize silage in comparison to the nutrient demand of anaerobic micro-organisms. A lack of certain elements like calcium and iron can destabilize the anaerobic digestion process and reduce biogas production. Hinken et al. (2008) analysed different agricultural anaerobic biomasses with special regard to their trace element content. Based on these results, the influence of three trace elements (iron, cobalt and nickel) on anaerobic digestion was studied in anaerobic batch tests at different sludge loading rates and for different substrates (maize silage and acetate). Biogas production was found to be 35% higher for maize silage than in the reference reactor. Similarly, Nges et al. (2012) attributed the good methane yield of 319 $m^3$/ton total solids and low VFA concentration of <0.8 g/l in their continuously stirred pilot biogas reactor fed with maize silage, to supplementation with both macro (N, P, S) and micronutrients (Fe, Co, Mo and Ni).

**Table 4: Nutrient requirements of anaerobic bacteria in comparison to their content in maize silage (adapted from Hinken et al., 2008)**

| | Calculated nutrient requirement (mg/kg COD) | Nutrient content of average data from substrate (mg/kg COD) |
|---|---|---|
| Nitrogen | 7 410 | 14 737 |
| Phosphorus | 1 710 | 2 411 |

| | | |
|---|---|---|
| Potassium | 1 140 | 12 823 |
| Calcium | 456 | 2 082 |
| Magnesium | 342 | 1 534 |
| Iron | 205 | 1 111 |
| Zinc | 7 | 38 |
| Manganese | 2 | 32 |
| Copper | 1 | 4 |
| Nickel | 11 | <1 |
| Cobalt | 9 | <1 |
| Molybdenum | 7 | <1 |

Resch et al. (2008) studied the mono-digestion of whole crop maize silage compared to that of a mixture of maize and grass+clover silage without supplementation. Due to the high VS concentration of the utilized energy crops, a certain dilution was required to guarantee sufficient mixing. The process liquid for dilution was composed of the liquid fraction of separated digestion residue, surface and bunker silo run-off and the intense rainfall run-off. No input of manure or other liquid substrate was provided. For 100% maize input, a methane potential of 6.34% of $CH_4$ production (94% substrate degradation rate) was observed. The mixture of maize and grass+clover silage achieved an 89% degradation rate.

As discussed previously, methanogenic bacteria and SRPs are similar in terms of their enzyme and trace element requirements. However, when using maize silage as a carbon source for sulphate-reducing bacteria treating acid mine drainage, it can be expected that all the required trace elements will be supplied in the AMD. For the purposes of this study trace element supplementation may be required when using silage as the sole carbon source when treating the synthetic AMD solution. This should not be necessary when combining the silage with sewage sludge.

In summary, we propose the use of maize silage as an alternative carbon source in the BioSURE Process for the following reasons:

1. Maize silage consists predominantly of carbohydrates such as cellulose and starch, with a low crude protein content;
2. Maize silage has a high biodegradability, in excess of 90%, based on in situ rumen tests, compared to 48% of PSS;
3. Its ash content of less than 5% on DM basis is very low compared to that of PSS in excess of 15%;
4. It has a high yield, in the order of 50 t/ha for dry land production and 100 t/ha when irrigated;
5. The moisture content of silage is approximately 65% compared to approximately 82% of dewatered sewage sludge;
6. Silage can be stored for several years, generally more than three years, without loss of quality, with records of up to eight years;

7. The processing of silage as cattle fodder is a well-established process. Specialized equipment is used to harvest and cut the material in predetermined sizes as small as 8 mm. It is an important consideration when process equipment is selected, to prevent blockages. The particles are also forced through rollers with an aperture size smaller than 1 mm to fracture them and make the fibres easily accessible to bacteria to accelerate hydrolysis;
8. The major mining areas associated with AMD coincide with high maize production areas.

It may be argued that any attempt to use maize silage as a source of organic material may risk the security of food supply. Treating AMD to acceptable discharge standards may result in higher yields of crops by downstream water users that exceed the demand for AMD treatment. The problem with silage is the requirements of energy, land and fertilizers during its production. As with the production of all crops, it has an environmental impact in terms of surface run-off and consequent pollution of the water courses and seepage of contaminants into the groundwater. In future, when municipal solid waste separation is implemented in South Africa, the biodegradable organic solid waste fraction can be considered as an organic source for the treatment of AMD.

## 2.2 The SANI process for removal of ammonia

The SANI process (sulphate reduction, autotrophic denitrification and nitrification integrated process) was developed for saline sewage treatment to alleviate freshwater scarcity in coastal areas by reducing the required amount of freshwater, by replacing a part of non-potable demand by direct use of saline water (Van den Brand et al., 2013). Sea water is an excellent source of secondary quality water as it is an almost infinite water resource (Ekama, Wilsenach & Chen, 2010) and requires minimal treatment with screening and chlorination for safe usage (Lu et al., 2011). Although this approach has been applied successfully in Hong Kong for some time, it has not been adopted worldwide due to several disadvantages, like the need for a dual water supply system, requirement for use of non-corrosive materials, issues with $H_2S$ formation, impact of increased salinity on biological wastewater treatment, reduced reuse options due to saline effluent, and a lack of proper cost-benefit analysis comparing traditional systems with such novel alternative approaches. However, the SANI process addresses most of the above challenges in a holistic manner, providing a sustainable and cheaper set of solutions in comparison to business as usual practice (Van den Brand et al., 2013).

In Hong Kong, urine diversion toilets were introduced which allowed for selective on-site urine collection, partial treatment (nitrification), and controlled discharge of nitrate-rich (instead of ammonia) urine to the sewer system. The nitrate presence in the sewer system hindered $H_2S$ formation and therefore addressed the issue of odour, safety and corrosion in the sewer system. Furthermore, it allowed for (partial) denitrification to take place in the sewer and reduce aeration requirements at the centralized WWTP.

Direct usage of saline water results in salty and sulphate-rich wastewater, and the latter was recognized not as a barrier but as an opportunity, which led to development and pilot application of the SANI process (Lu et al., 2009; Tsang et al., 2009; Wang et al., 2009). The SANI process consists of two reactors: an upflow anaerobic sludge bed (UASB) reactor, and a reactor with anoxic and aerobic filters (Wang et al., 2009). The UASB reactor is designed to remove organic matter by SRP. Consequently, sulphate reduction leads to production of sulphide. The anaerobic reactor is followed by a second reactor with (1) an anoxic zone for autotrophic denitrification of nitrate with dissolved sulphide generated from sulphate reduction in the previous step, and (2) an aerobic zone to nitrify ammonia and reticulate nitrate to the anoxic zone for denitrification (Wang et al., 2009). A schematic diagram of the process is illustrated in Figure 3. The SANI process combines three important advantages: heavy metals removal and recovery potential due to production of sulphide, comparatively better pathogen removal, and substantially lower excess sludge production over conventional wastewater treatment system processes, while retaining excellent and stable COD and N-removal efficiency of >95%, (Tsang et al., 2009; Lu et al., 2011).

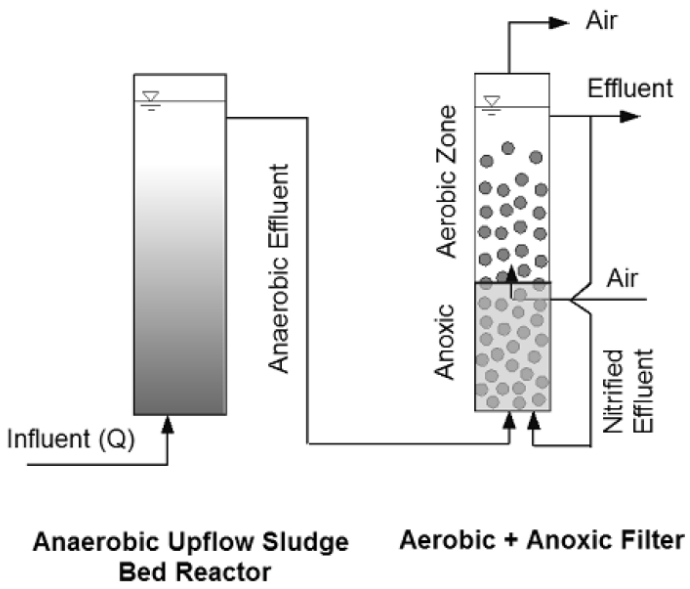


Figure 3 A schematic diagram of the experimental setup of the sulphate reduction, autotrophic denitrification and nitrification integrated (SANI) system (Van den Brand et al., 2013)

In the BioSURE Process, the anaerobic upflow sludge bed reactor of the SANI process is effectively replaced by BSR. It was planned that the sulphide-rich effluent of the sulphate reduction process would be fed to an anoxic upflow reactor packed with growth media to effect autotrophic denitrification, in order to remove the ammonia produced as a by-product of sulphate reduction without the need for an integrated WWTP. The overflow from this reactor would then flow into the iron hydroxide reactor for sulphide removal, where it would be removed as a solid iron sulphide precipitate. The sulphide free effluent, containing high ammonia and residual COD, would then be fed into the final reactor, also filled with media

and aerated to reduce the COD and nitrify the ammonia. This effluent would then be recycled to the autotrophic denitrification reactor to bring it into contact with the sulphide-rich effluent to effect autotrophic denitrification. The idea was that the nitrogen concentration would be relatively low compared to the sulphide concentration, so little sulphide will be oxidized but all the nitrate would be depleted. As is discussed later in the report, very little ammonia was found in the effluent of the biological sulphide oxidation reactor in practice when using maize silage as a carbon source, as opposed to PSS. However, including a SANI reactor step enabled the discovery of feasible biological sulphide oxidation, which is also discussed later.

## 2.3 Biological sulphide oxidation

### 2.3.1 Sulphide oxidizing prokaryotes (SOP)

#### 2.3.1.1 Photosynthetic sulphur bacteria

Anoxygenic photosynthesis is restricted to green and purple bacteria and the heliobacteria. Sulphur is the end-product of photosynthesis in green and purple bacteria, using reduced sulphur compounds as substrates (Lengeler, 1999). Due to its dependence on light, this group of bacteria is not considered for the treatment of AMD (Van Hille et al., 2012).

#### 2.3.1.2 Colourless sulphur bacteria

Colourless sulphur bacteria are so-called for their lack of photo-pigments, although in dense culture they may appear pink or brown due to the presence of large amounts of cytochrome (Johnson, 2000). The chemotrophic sulphide oxidising prokaryotes (SOP) use chemical energy from the oxidation of reduced sulphur compounds for growth, and are thus light independent, unlike the phototrophic SOP. Further subdivision of the chemotrophs is possible based on their ability for autotrophic growth and energy utilisation during sulphur compound oxidation. Here, four subgroups exist. Two of these include organisms capable of autotrophy using sulphur compounds as an energy source, one group are obligate chemolithoautotrophs, while the other are facultative autotrophs (able to grow both as chemolithoautotrophs and as organoheterotrophs) (Janssen et al., 2008). The other two subgroups are obligate heterotrophs, unable to grow in the absence of an organic carbon source. One of these can still utilize energy from the oxidation of reduced sulphur compounds under organic carbon-limited conditions; these are known as chemolithoheterotrophic SOP and are mainly found among the Alphaproteobacteria, and produce sulphate as the final oxidation product (Janssen et al., 2008).

In terms of oxidation of sulphide from waste streams, SOP capable of autotrophic growth are most relevant for a number of reasons. These include (1) much higher sulphide oxidation rates in comparison to heterotrophic SOP, (2) very simple growth requirements, and (3) an extremely high affinity for sulphide and oxygen, which allows them to compete successfully with chemical oxidation of sulphide in natural habitats and in oxygen-limited bioreactors. These organisms generally oxidise sulphide to sulphate, generating more metabolically useful

energy, as opposed to partial oxidation to $S^0$ (Lens and Kuenen, 2001). In order to obtain sulphur as a product, sulphide oxidation must occur under stringent conditions, such as high sulphide loads, and within a narrow redox potential and pH window (Figure 4).

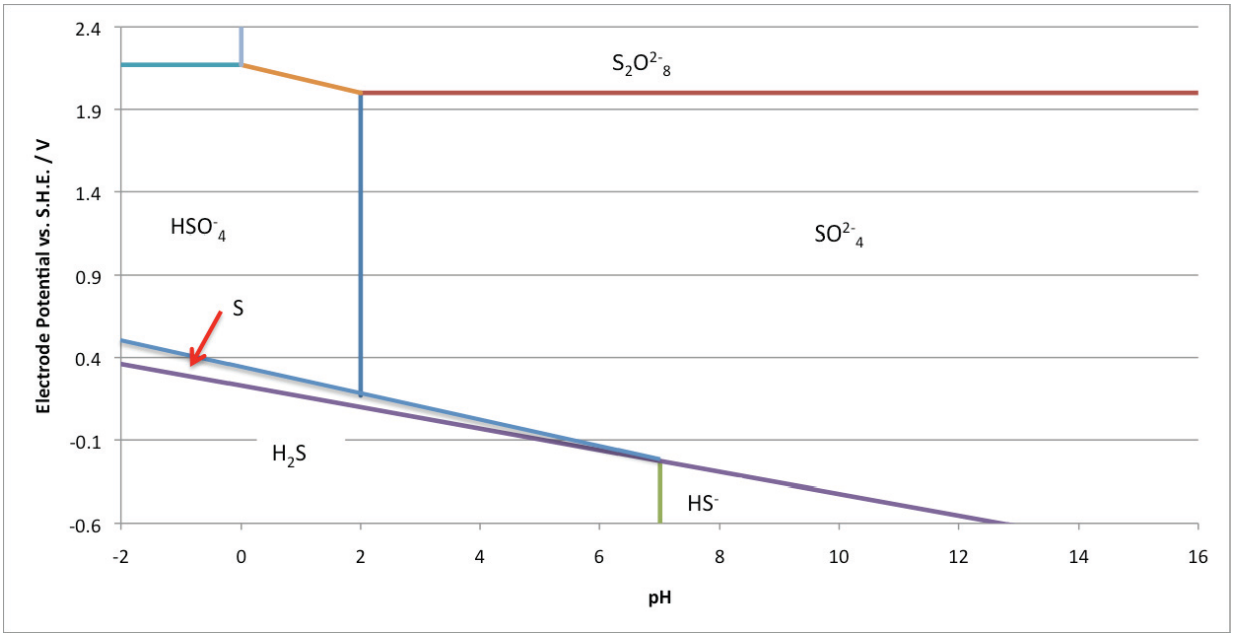

Figure 4: Potential-pH diagram for a sulphur/water system at 298.15 K (Van Hille et al., 2012)

Some SOP deposit elemental sulphur within the cell and use it as an energy source. When the extracellular sulphide is no longer available, these sulphur deposits are rapidly oxidised to sulphate. *Beggiatoa* species convert sulphide into extracellular sulphur which is not auto-oxidisable, thus withdrawing its energy source from the chemical oxidation process. Members of the genus *Thiobacillus* may form intracellular sulphur as an intermediate.

### 2.3.2 Ratio of sulphide to oxygen

The ratio of sulphide to oxygen needs to be carefully controlled in order to achieve partial oxidation to sulphur (Equation 9). If additional oxygen is available, the reaction will proceed to the more thermodynamically stable sulphate, either through the further oxidation of the sulphur intermediate (Equation 10) or the complete oxidation of sulphide (Equation 11).

$$2HS^- + O_2 \rightarrow 2S^0 + 2OH^- \quad (9)$$

$$2S^0 + 3O_2 \rightarrow 2SO_4^{2-} + 2H^+ \quad (10)$$

$$2HS^- + 4O_2 \rightarrow 2SO_4^{2-} + 2H^+ \quad (11)$$

From a bioenergetic perspective, the complete oxidation to sulphate is favoured, as the bacteria derive maximum energy from this reaction. Sulphate production liberates eight electrons whilst sulphur formation only liberates two, hence limiting the energy available for cell proliferation. The oxygen concentration influences the amount of sulphate formed at low

sulphide concentrations. The ratio of sulphide to oxygen should exceed 2:1 to ensure the production of sulphur (Equation 9).

### 2.3.3 pH and redox potential

The pH of the solution in the reactor plays a critical role in the overall distribution of sulphide species. At a pH <9.17, the majority of the aqueous sulphide is present as $HS^-$. If the pH exceeds 9.17, the majority of the sulphide occurs as polysulphides ($S_x^{2-}$). Therefore, an equilibrium exists in the region of pH = 9.17 as described by the following expression (Van den Bosch, 2008).

$$K_x = \left[\frac{[S_x^{2-}][H^+]}{[HS^-]}\right] \left[\frac{\gamma_{S_x^{2-}}\gamma_{H^+}}{\gamma_{HS^-}}\right] \quad 12$$

Control of the total sulphide concentration can be achieved via the measured oxidation reduction potential (ORP). Elemental sulphur formation occurs within a very small pH range. However, this range is dependent on the sulphide and polysulphide concentration as well as the sulphide to oxygen ratio. ORP depends largely on the polysulphide concentration, which is controlled by the total sulphide concentration and pH. However, this relationship is only valid for systems where biologically produced sulphur particles are in excess. As a result, the ORP increases as pH decreases, providing that the total sulphide concentration remains constant.

Therefore, the ORP can be related to pH and total sulphide concentration via the following equations (Van den Bosch, 2008):

$$S_x^{2-} + xH_2O + 2(x-2)e^- \leftrightarrow xHS^- + xOH^- \quad 13$$

Applying this general equilibrium equation (13) to the Nernst equation (14), the electrode potential can be represented as (15):

$$E_h = E_h^0 - \frac{R.T}{nF}\ \chi \ln \frac{\Pi_j[red]^{nj}}{\Pi_i[ox]^{nj}} \quad 14$$

$$E = E_h^0 - \frac{R.T}{nF}\ \chi \ln \frac{[HS^-]^1[OH^-]^x}{[S_x^{2-}]^1} \quad 15$$

Combining the above equation with the equilibrium constant (16), the following equation (17) relates ORP to pH and $S^{2-}_{tot}$*:*

$$[S_{tot}^{2-}] = [HS^-] + [S_x^{2-}] \quad 16$$

$$ORP=E_h^0-\frac{R.T}{(2x-2)F}\ .\ln\left(\frac{10^{-14}}{(2x-2)F}\right).\ln\left(\frac{[S_{tot}^{2-}].K_x}{K_x+10^{-pH}}\right) \quad 17$$

Therefore, if there is stringent control of the solution redox, it is possible to oxidise sulphide to elemental sulphur by creating an oxygen-limiting environment. Sulphate formation is minimized at the high sulphate loading rate (50 mg/L.h) and sulphide to oxygen ratio (2.6 : 1). Hence, the optimal redox potential range is -142 to -128 mV (Janssen et al., 1998).

Biologically produced sulphur, or biosulphur, is produced from the oxidation of hydrogen sulphide. Polysulphides plays an important role as intermediates in the reaction (Kleinjan, 2005). Once sulphur is formed, it is attacked by hydrogen sulphide to produce polysulphide and is therefore solubilized in a reversed reaction. Dissociated hydrogen sulphide ($HS^-$) is a strong nucleophile, and attacks the sulphur ring and produces polysulphide (Blumentals et al., 1990). Polysulphides are unstable in alkaline solutions. Above pH 8.7, the solubilization of sulphur is accelerated (Kleinjan, 2005). Polysulfides are unstable in acidic solutions (Kleinjan, 2005). It is therefore important to maintain the reactor pH within a narrow range to ensure optimal performance with respect to sulphur production.

Since alkalinity is produced when sulphur particles are formed during the oxidation of hydrogen sulphide, the simultaneous precipitation of calcium carbonate plays an important role in maintaining the reactor pH within the desired pH range.

## 2.4 Calcium carbonate precipitation in the sulphide oxidation reactor and lime softening reactor

Water containing calcium and magnesium bicarbonate can be softened by increasing the alkalinity. The bicarbonates are formed in the biological sulphide reducing reactor. The alkalinity can be increased as a result of the oxidation of sulphide to biosulphur or by adding lime, for example. The reaction to remove calcium is as follows:

$$Ca(HCO_3)_2+2OH^- \leftrightarrow CaCO_3\downarrow+CO_3^{2-}+2H_2O \quad 18$$

Magnesium precipitates as magnesium hydroxide (brucite):

$$Mg^{2+}+2OH^-\leftrightarrow Mg(OH)_2\downarrow \quad 19$$

When sufficient alkalinity is available, calcium carbonate will precipitate with the brucite. Provided that the concentrations of calcium and magnesium match that of the carbonic species, very low concentrations of calcium and magnesium may be achieved at equilibrium. The equilibrium concentrations are determined by the solubility products of calcium carbonate and magnesium hydroxide, and are dependent on water temperature and salinity. However, in the presence of significant quantities of magnesium, magnesium calcite, instead of pure calcite, is precipitated. The equilibrium concentration of calcium and magnesium in

the effluent is therefore dependant on the magnesium concentration of the influent. It was also observed that the presence of dissolved magnesium ions significantly reduces the rate of precipitation (Benjamin et al., 1977). It was further observed that dissolved organic material in wastewater effluents also has a significant impact on the precipitation kinetics and the value of the equilibrium constants and solubility of the solid precipitates.

Since the objective is to separate the contaminants in the AMD from each other, and to recover the high-quality by-products in order to reduce solid waste production and to add value for cost reduction, two tests were conducted to observe the removal of calcite or magnesium calcite in fluidized bed reactors. The first set of tests was conducted on the sulphide-rich effluent from the BSR reactor, where the alkalinity was produced as a result of sulphide oxidation to sulphur. The maximum pH achieved in this reactor was too low to effect any precipitation of magnesium. However, the magnesium concentration was high enough to interfere with the precipitation of calcite, and magnesium calcite was precipitated instead. The second set of tests was conducted in a subsequent fluidized bed reactor where the pH was raised to above 11.2 to effect simultaneous magnesium hydroxide and calcite precipitation. In a typical lime softening process, a slurry with a mixture of magnesium hydroxide and calcite is produced. Since magnesium hydroxide without calcite is required to neutralize the ferric sulphate solution produced in the biological iron oxidation reactor (as explained later), a second fluidized bed reactor was operated to precipitate calcite selectively on the fluidized particles and retain it inside the reactor, while the flocculated suspension of magnesium hydroxide was allowed to leave the reactor and recovered as slurry.

# 3 Findings from the technical and full-scale BioSURE plants treating water from Grootvlei mine

## 3.1 Carbon source

Rajkumar (2009) simulated data from the BioSURE pilot studies using WEST® (Wastewater Treatment Plant Engine for Simulation and Training). It was found that the lower the ratio of COD to sulphate fed, the more sulphate was reduced by a given amount of COD. COD utilization remained effectively constant until the sulphate neared complete removal (see Figure 5). The sulphate removal ratio increased almost linearly up to almost complete removal. This was a consequence of the reactions being limited by the hydrolysis rate. A simulated effluent VFA concentration of 0.07 mg/L was adequate to maintain stable sulphidogenic conditions. The rate-limiting process was the first step of hydrolyzing the particulate COD, and thus the dominant factor determining the model's characteristics. Once the substrate was solubilized, the methanogenic and sulphate-reducing populations of organisms competed for it, and the outcome of this competition determined the second level of characteristics, i.e. how much COD was consumed during sulphidogenesis and how much during methanogenesis. Issues such as sulphide inhibition were not significant under the conditions experienced by the pilot plant. At a COD:$SO_4$ ratio of 1.3, low $SO_4$ removal was predicted by the model, with improved removal up to 3.1:1 ratio. Above 3.1:1, the COD concentration was predicted to be too high to allow continuous inhibition of methanogens, resulting in the generation of methane, which should be avoided.

The BioSURE pilot plant reflected certain important features of the model that emerged while simulating various scenarios:

- The process seems to be quite resilient in the face of upsets. In particular, it did not seem to suffer from the pH-related instabilities typical of methanogenic anaerobic digestion.
- Production of methane was negligible under the operating conditions applied.
- $H_2S$ inhibition was not an important factor under the operating conditions applied.

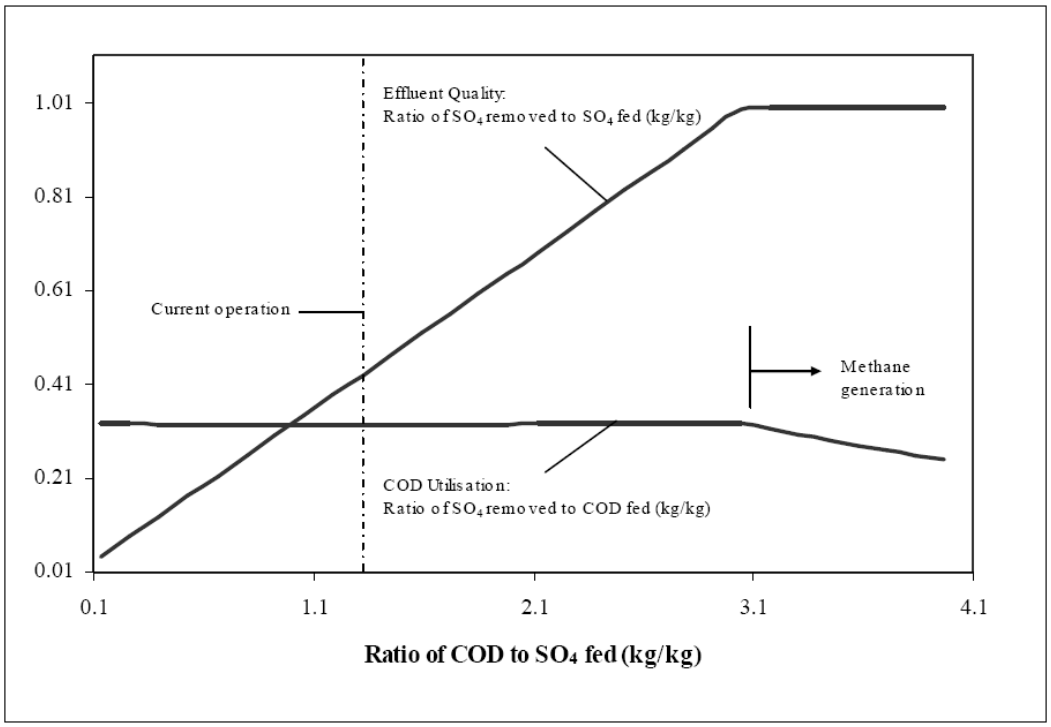


Figure 5 Modelled ratio of COD to $SO_4$ fed (Rajkumar, 2009)

The underflow of the PSTs (primary settling tanks) consisted of primary sludge and humus sludge. The quantity of primary sludge was determined by the load in the influent, the efficiency of the PSTs with respect to solids capturing and thickening, and the quantity of humus sludge recycled and captured in the PSTs. The quantity of humus sludge was determined by the growth yield of heterotrophic and autotrophic organisms in the biofilters. Phosphorus was removed in a chemical removal process, utilizing ferric chloride. The humus sludge and chemical sludge were simultaneously collected in the humus tanks and then recycled to the influent. At an average flow of 25.5 Ml/day, the average total COD load received at the Ancor WWTP was equivalent to 16.7 ton COD/day. The suspended solids were removed at a rate of 52% after primary settling, with a 31% reduction of the COD of the settled sewage. The primary sludge accounted for 5.2 ton COD/day in the underflow. A histogram showing the available PSS, measured as COD, at the Ancor WWTW is presented in Figure 6.

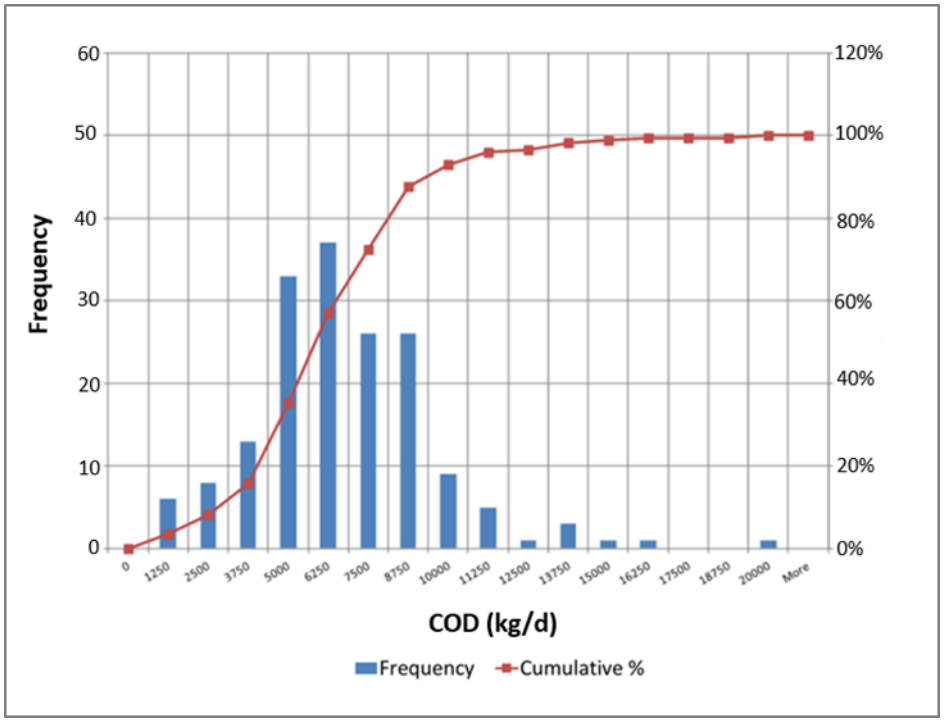


Figure 6 Available primary sewage and humus sludge (as COD) at the Ancor WWTP for the operation of the Rhodes BioSURE® Process

The yield of heterotrophic organisms in the biofilters was 0.5 mg COD biomass generated per mg COD substrate consumed (Grady et al., 1999). The COD concentrations in the settled sewage and the final effluent were 450 mg/L and 41 mg/L respectively; a difference of 409 mg/L. It was suggested that 205 mg/L, expressed in terms of COD, of heterotrophic biomass, or 5.3 ton/day was generated. This was the maximum quantity that could be generated and factors such as sludge age were not accounted for. The inflow to the Ancor WWTP contained an average of 16 mg/L ammonia-nitrogen. This was equivalent to 411 kg/d. No values were available for total nitrogen (TKN). It was generally observed that 75% of the TKN consisted of saline ammonia-nitrogen, implying a total estimated load of 550 kg nitrogen per day. It is generally observed (and in this case, too) that the ammonia-nitrogen concentration in the overflow from the primary sedimentation tanks, as well as laboratory settled samples, does not differ from that of the influent. It suggests that no, or very little, mineralization takes place in the PSTs. The saline ammonia-nitrogen did not account for the particulate material. It was assumed that the total particulate nitrogen fraction equalled 139 kg/d and that between 15 and 20% of the particulate material settled in the PSTs. The TKN concentration of the settled sewage was therefore approximately 20 mg/L or equivalent to 514 kg/day. The average ammonia-nitrogen and nitrate-nitrogen concentrations in the settled sewage and effluent were respectively 4.1 and 2.3 mg/L which amounts to a difference of 13.6 mg/L between the biofilter feed and effluent. The yield of nitrifying organisms equalled 0.1 mg VSS/mg N consumed or 0.148 mg COD/mg N consumed. This suggested a maximum quantity of autotrophic biomass of 52 kg/day that may be formed.

Denitrification may also take place that would result in a lower yield of biomass. This discussion suggests that no more than 10.5 ton COD could be expected in the underflow from the PSTs. Not all the organic material was biodegradable. A histogram of the ratio of COD to $SO_4$ is illustrated in Figure 7, indicating that approximately 17 ± 8 t/day of PSS, measured as COD, was required to remove 10 t/day of sulphate. This implied that an additional COD source was required.

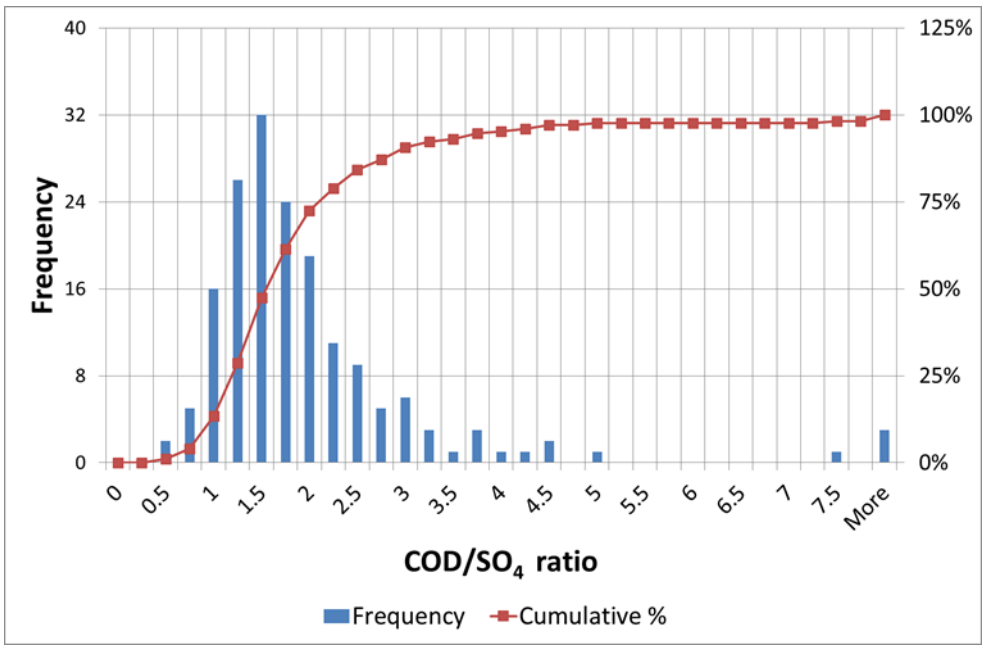


Figure 7 Ratio of primary sewage sludge (measured as COD) to reduce a unit mass of sulphate

A survey of the industries in the region was conducted, and waste from the dairy industries and abattoirs was accepted, after running trials on the waste for a few weeks. Dairy waste is usually disposed on landfill sites and contributes significantly to the moisture content of the solid waste; this is particularly problematic during the rainy season. The waste from the dairy industries included waste ice cream from Nestlé (7.5% mass COD per volume, three loads of 10 $m^3$/week), with a high fat and carbohydrate content, and waste yoghurt (5% mass COD per volume, 6-10 $m^3$/day for 5 days/week), with a high protein content, all of which were readily and completely biodegradable. Although the waste was received regularly, it was delivered intermittently. Due to its biodegradability, it was rapidly consumed in the BSR reactors which led to excursions of high and low sulphate concentrations in the discharge. Providing storage capacity in the primary sludge sumps and separating the waste from the PSS allowed the operators to feed the waste gradually into the BSR reactors. In this manner, there was excellent control of the sulphate concentration in the discharge.

Waste from the abattoirs included blood and stomach contents. Trials were also conducted on condemned material to determine its suitability. Although most of the condemned material was biodegradable, it contained hoofs, pieces of hide and gut that could not be handled by the pumps and it was therefore not accepted. Maceration and screening of the

material could possibly overcome this problem, but the option was not exercised. It is worth mentioning that the trials indicated that the fats could be effectively hydrolyzed and consumed by SRB. The blood, with high protein content, was readily and completely biodegradable. Blood was acquired from Morgan Beef in Springs (5 $m^3$/day) and RTV in Benoni (5 $m^3$/day). The stomach contents contained mostly partially hydrolyzed cellulose fibres. It was observed that this was slowly biodegradable and, as such, dampened the variation of the sulphate concentration in the discharge. Since the hydrolysis of particulate material is the rate-limiting step in the BSR process (Grady et al., 1999), the presence of the hydrolytic micro-organisms in the stomach contents was considered an advantage to assist with the decomposition of the particulate material. Rumen content was acquired from Morgan Beef (5 t/day).

Important lessons were learned with the introduction of additional carbon sources into the process:

- The disposal of large quantities of biodegradable organic waste with a high moisture content creates enormous operational problems for managers and operators of landfill sites, especially with respect to the production and control of leachate. Treating this waste in the BioSURE Process and integrating it with a conventional wastewater treatment process offered an alternative waste disposal option.
- The current practice of disposal of abattoir waste on land and burying the condemned meat has serious consequences with respect to soil and groundwater pollution, odours, scavenging of condemned meat by people, dogs and jackals, and the associated health risks. Again, supplying it as a carbon source to the BioSURE Process is a way to eliminate these risks. A wastewater treatment plant with its operational procedures is designed to contain similar risks.
- The conversion of organic material into bicarbonate in the BSR process, instead of carbon dioxide gas or methane in conventional processes, has an important positive implication with respect to the carbon footprint of these industries.
- Charging industries for solids disposal and treatment in the BioSURE Process offers an opportunity to recover some of the costs and makes the process an attractive long-term option for mine water treatment. This would only be possible if the industrial waste is not already earmarked for disposal in centralised municipal biogas production facilities.

## 3.2 Removal of sulphide from the effluent of the biological reactor

Hydrogen sulphide is produced in biological sulphate removal processes. It is extremely toxic, with very low lethal concentrations in the atmosphere and in the aquatic environment. It has a rotten-egg odour at very low threshold concentrations. It is highly corrosive and may damage steel structures and electrical and electronic equipment. It is therefore essential to remove sulphide from the effluent stream.

The development work relating to biological sulphate-reducing processes in the past has been focused on the biological process, and very little attention was given to the aspects of hydrogen sulphide removal from the effluent stream. It was therefore not surprising that the biggest challenges that were faced during the full-scale implementation were related to this aspect. Various methods can be considered for its removal.

The partial pressure of hydrogen sulphide is a function of dissolved hydrogen sulphide concentration, temperature and pH, amongst others. The hydrogen sulphide concentration in the effluent was in the order of 0.2 to 0.4 g/l. The following important conclusions could be made:

- The partial pressure was too low to allow the formation of bubbles. Gas production and associated mixing is not a concern in the design and operation of the sludge bed reactors
- The pH of the effluent was always close to 7.5, indicating a partial pressure of approximately 0.03 atm. At an ambient pressure of 0.85 atm, the gas contained approximately 3.5% (v/v) of hydrogen sulphide. This is a lethal concentration and entering of confined spaces should be strictly controlled. It is, however, too low to consider gas scrubbing technology to recover hydrogen sulphide from the effluent, even at reduced pH values
- Adjusting the pH to 9 reduced the partial pressure and the associated risks of odours and toxicity.

Precipitate obtained from the HDS (high density sludge) process at Grootvlei mine could react with the hydrogen sulphide to produce an iron sulphide precipitate. This was a waste product that contained ferric hydroxide precipitate. Since it was present as an oxide or hydroxide, it had the added advantage that it did not contribute any undesirable anions to the water such as chloride when using ferric chloride. One disadvantage, however, was the large quantity of calcium carbonate grit in the sludge. It was often five times as much as the iron hydroxide, and caused considerable problems with respect to blockages of tanks and pipes, abrasiveness and associated damage to pumps and valves, and solids handling, dewatering, drying and storage. Most of the downtime was spent on removal of blockages and repair of damaged pump impellers. Most of the calcium carbonate particles have a settling velocity higher than 6 m/h and selection of appropriate equipment can remove the particulate material effectively from the flocculated iron hydroxide suspension. It finally became necessary to add a grit removal system to improve the performance of the plant.

Based on the stoichiometry, the reaction between ferric hydroxide and hydrogen sulphide is as follows:

$$2Fe(OH)_3(s)+3H_2S \rightarrow Fe_2S_3(s)+6H_2O \qquad 20$$

The stoichiometric ratio between iron consumed and hydrogen sulphide removed is in the order of 2:3 on a molar ratio basis, or equivalent to 1.09 mg Fe consumed per mg $H_2S$ on a mass ratio basis. Daily analysis of the samples, and calculations based on mass balances, indicated a ratio of 1:1 which was consistent with this reaction. The sulphide has been oxidized to sulphate and reacted with the particulate calcium carbonate to produce gypsum.

Hydrogen sulphide was removed to less than 5 mg/L in the reactor-clarifiers, provided that the there was a sufficient supply of iron hydroxide. The iron hydroxide concentration varied considerably and was strongly dependant on the operation of the HDS plant at the mine. One of the challenges was coordination between the operations at the mine and those of the BioSURE Process. It was finally necessary to add a thickening step with enough storage capacity on the site to ensure a consistent supply of iron hydroxide sludge on the site.

It was important to increase the iron hydroxide concentration as much as possible through thickening, and to limit the variability of the concentration. The moisture content of the sludge was high and, as explained earlier, contained sulphate at a concentration equal to that of the mine water. The mass of sulphate introduced in this manner was enough to raise its concentration in the effluent. As an example, introducing 1 Ml/day of iron hydroxide slurry with a 1500 mg/L sulphate concentration into a 10 Ml/day flow, will raise the sulphate concentration by 136 mg/L. Considering a discharge limit of 250 mg/L, it is obvious that control of iron hydroxide dosing was very important.

## 3.3 Removal of ammonia

In the full-scale BioSURE Process, the ammonia-nitrogen concentration was typically in the order of 22 mg N/l in the effluent of the reactor-clarifiers after sulphide removal. The effluent was then applied to the trickling filters of the WWTP with which the plant was integrated. The trickling filters reduced the ammonia-nitrogen to acceptable levels (<10 mg/L as N). The effluent from the BioSURE Process was only introduced on three of the nine trickling filters, and comparison of the trickling filters with and without the effluent showed no negative impact on the performance of the WWTP.

It should be realized that the opportunity to integrate the BioSURE Process with a conventional WWTP is rare. Further treatment of the BioSURE effluent, after hydrogen sulphide removal, is required to reduce the COD and ammonia-nitrogen. Trickling filters could be used for this purpose independently of a WWTP, but there is a concern that the effluent nitrate-nitrogen concentration may be unacceptably high due to the lack of available organic material for denitrification. The SANI process, whereby a fraction of the nitrate-rich effluent is recycled to bring it into contact with the sulphide-rich effluent to effect autotrophic denitrification was considered as an alternative option, to be tested in this study.

# 4 Materials and methods

This project consisted of three experimental phases, which included semi-batch reactors for assessment of the carbon source and effect on sulphate reduction, a 40 L/d continuous process including all unit processes, and finally a 1 $m^3$/d pilot plant.

## 4.1 Batch experiment for carbon source assessment and effect on sulphate reduction

The purpose of the first experiment was to determine the effect of temperature and sludge age, with only PSS as carbon source, only silage as carbon source and a mixture of PSS and silage on the performance of the process. The second experiment was operated as a continuous flow-through process in which all the major steps of the BioSURE Process were combined.

One of the objectives of the bench-scale tests was to compare the performance of the BSR reactors when fed with silage as a carbon source instead of PSS. A total of 27 containers, each with an effective capacity of 2.5 L, was used to test the performance of the process at various temperatures, PSS:silage ratio and sludge ages, as indicated in Table 5. The PSS and silage ratios were based on the stoichiometric requirement of COD for sulphate reduction.

The problem of operating a large number of reactors to test all the permutations at the same time was overcome by arranging them in three separate rotating triangles, each operated at a different temperature with different PSS:silage feed ratios and operated at different sludge ages (Figure 8).

Figure 8 Triangular assembly of 9 reactors shown with the dividing baffles

The rotating triangles were driven slowly by a single motor with a common shaft. Each triangle was an assembly of nine containers and was enclosed in a HDPE tank for insulation. The tanks

were partially filled with water and the temperature of the water was controlled using aquarium heaters for the higher than ambient temperatures, while a source of cold water was used to keep the cold tank below ambient temperature. Recycle pumps were used to recycle water through a spray nozzle in each of the tanks to ensure uniform air temperature distribution around the rotating triangles. Each of the 27 containers was fitted with a separating baffle to divide the volume so that a fixed fraction of the volume could be drained to maintain a predetermined sludge age. The reactors of each of the three assemblies were drained every third day.

Feeding and harvesting of the reactors was not practical, and blockages occurred as a result of the small diameter of the sampling ports. This unit was replaced with a more practical design. The modified design consisted of an assembly of three banks, with ten cylindrical containers per bank, mounted on a horizontal shaft (Figure 9). Although only nine containers were required per bank, it was necessary to add an additional container per bank to balance the weight of the rotating assembly.

Figure 9 One of three banks with ten containers mounted on a horizontal shaft

The shaft was driven slowly at approximately 2 rpm by a single 3-phase Bonfiglioli motor, fitted with a speed reducer and a WEG CFW08 vector inverter. Each bank of ten containers was enclosed in an HDPE tank for insulation (Figure 10). The tanks were partially filled with water and the temperature of the water was controlled using 100 W Dophin aquarium heaters for the higher than ambient temperatures, while a refrigeration unit was used to produce a source of cold water to keep the cold tank below ambient temperature. Sunsun HJ-742 recycle pumps were used to recycle water through a spray nozzle in each of the tanks to ensure uniform air temperature distribution in each of the enclosures.

Figure 10 Batch reactors with the cover of one of the three enclosures removed. The temperature in each of the enclosures was independently controlled

Each of the containers was fitted with a tube so that a fixed fraction of the volume could be drained to maintain a predetermined sludge age. The tube had a diameter of 36 mm, which was large enough to contain the largest silage particles. Each tube had a sealed stopper on the outside and was open inside the container. The containers were partially filled with mixed liquor and contained 1800 ml. Each tube had a volume of 180 ml (Figure 11). The reactors of each of the three assemblies were drained every day in order to maintain a 10-day sludge age, or every second day for a 20-day sludge age, and every third day for a 30-day sludge age. When the assembly was stopped with some of the tubes in the downward direction, the liquid level in the containers was slightly below the open top of tube inside the container. When the tubes were drained in this position, only the contents of the tubes were drained. As the assembly rotated, the tubes were filling and emptying during each rotation in order to ensure that a homogenous sample was collected every time. The rotating assembly was stopped with the tubes in the upward position for refilling and feeding with silage. The samples were collected in Tuffy Zipper Bags and sealed immediately, in order to avoid hydrogen sulphide gas or carbon dioxide losses or air entrainment. Baffles were inserted into each of the containers to ensure proper mixing during rotation.

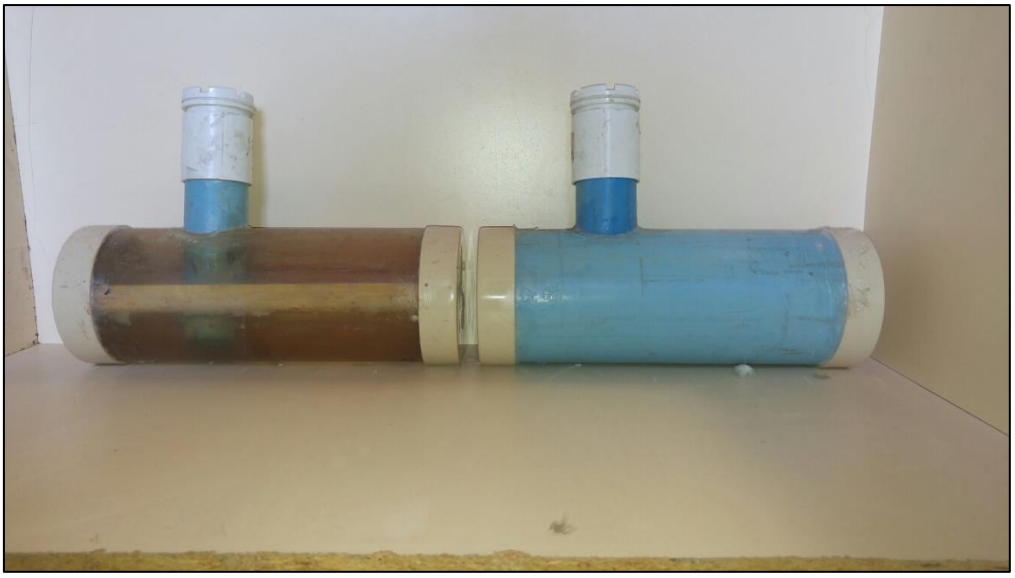

Figure 11 Picture showing the details of the containers. The transparent container on the left-hand side shows how the tube extends into the container. It also shows the baffle inside the container for mixing.

This arrangement is particularly suitable to feed silage without the need to reduce the particle size. Measuring the rate of hydrolysis of the actual particle sizes normally found in the industry is essential for proper scale-up. There was concern that deliberate reduction in particle size would enhance the hydrolysis rate artificially. Using conventional completely stirred tank reactors would not be possible without homogenizing the particulate material.

**Table 5 Ranges of the different variables used to compare their effect on the performance of the biological sulphate-reducing reactors**

| **Variable** | **Range** | | |
|---|---|---|---|
| Temperature | 15°C | 25°C | 35°C |
| PSS:silage ratio | 1:0 | 1:1 | 0:1 |
| Sludge age | 10 days | 20 days | 30 days |

The sulphate-reducing reactors were seeded with anaerobic sludge sourced from the WWTP operated by Zonderwater Correctional Services, that receives only domestic sewage. The reactors were fed every third day with PSS sourced from the same WWTP as a carbon source. Silage was sourced from a local producer in Cullinan to supplement or replace the PSS. Synthetic AMD water was used as the feed, with the composition specified in Table 6. The performance of the reactors was monitored by measuring the VFAs and alkalinity, using the so-called 5-point titration procedure developed by Moosbrugger et al. (1993), total COD (excluding sulphide) and residual sulphate and hydrogen sulphide, according to the Standard Methods for Examination of Water and Wastewater (APHA/AWWA/WEF, 2012). The carbon feed rate was adjusted gradually, based on the production rate of VFAs, to prevent failure of

the reactors as a result of overdosing and accumulation of VFAs. The reactors were operated until a steady state was reached and a constant sulphate removal rate was achieved.

Several practical problems were experienced as a result of blockages caused by the fibrous materials, which affected accurate control of sludge age and introduced air during sampling, causing partial sulphide oxidation. The reactors were modified without loss of continuity of the trials.

**Table 6 Composition of synthetic mine water**

| Determinant | Units | Value |
|---|---|---|
| pH (@ 25° C) | (-) | 7.0-7.5 |
| Total Dissolved Solids (TDS) | mg/L | 2570 |
| Alkalinity as $CaCO_3$ | mg/L | 50 |
| Chloride as Cl | mg/L | 100 |
| Sulphate as $SO_4$ | mg/L | 1750 |
| Sodium as Na | mg/L | 250 |
| Potassium as K | mg/L | 20 |
| Calcium as Ca | mg/L | 250 |
| Magnesium as Mg | mg/L | 200 |

## 4.2 Continuous process

### 4.2.1 Feed water quality

The composition of the AMD pumped from underground at Sibanye Gold in Randfontein and representing the water quality of the AMD in the Western Basin is summarized in **Table 7** below. It is required that the final effluent should be fit for use either as potable water or for environmental release, or for irrigation.

The water quality data indicates that the water quality is not fit for human consumption, except for the mono-valent ions. Sodium should also be removed when it is considered for environmental release. Uranium, not listed in the table, also exceeds the limit for potable water. No attempt has been made to design the plant for uranium removal, but it will be monitored when the actual AMD is used in the pilot study.

The ratio of the $95^{th}/50^{th}$ percentile values for the various parameters suggests a high variability of iron, sodium, manganese and acidity. The variations of the other parameters are within a narrower range. The water quality data was simulated using Visual Minteq, and the composition of the water considered for the simulation is summarized in the table below. The values were adapted from **Table 7** to balance the mass and charge of the parameters.

**Table 7 Western Basin AMD water quality and limits for treated effluent (DWAF, 2012)**

| AMD water quality: Western Basin | | | | | | | | | Ratio $95^{th}/50^{th}$ | Limits | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Parameter** | **Unit** | **$5^{th}$** | **$10^{th}$** | **$50^{th}$** | **$60^{th}$** | **$75^{th}$** | **$90^{th}$** | **$95^{th}$** | | **Potable SANS 241-1:2015** | **Environ-mental release** |
| pH @25°C | | 3.5 | 3.9 | 5.4 | 5.5 | 5.6 | 5.9 | 6 | (-) | >5 to 9.7 | >6.4 to 8.5 |
| Conductivity | mS/m @25°C | 320 | 334 | 385 | 392 | 415 | 434 | 442 | 1.148 | <170 | <100 |
| TDS | mg/L | 3549 | 4031 | 4628 | 4743 | 4890 | 5208 | 5434 | 1.174 | <1200 | <650 |
| Iron | mg Fe/l | 358 | 439 | 662 | 703 | 772 | 890 | 954 | 1.441 | 0.3 | <1 |
| Sulphate | mg $SO_4^{2-}$/l | 2366 | 2687 | 3085 | 3162 | 3260 | 3472 | 3623 | 1.174 | <250 | <350 |
| Sodium | mg Na/l | 65 | 86 | 110 | 118 | 132 | 175 | 227 | 2.064 | <200 | <80 |
| Calcium | mg Ca/l | 424 | 470 | 549 | 558 | 584 | 633 | 703 | 1.281 | NS | |
| Manganese | mg Mn/l | 31 | 38 | 56 | 63 | 70 | 81 | 89 | 1.589 | <0.1 | <2 |
| Acidity | mg $CaCO_3$/l | 794 | 864 | 1039 | 1062 | 1174 | 1406 | 1520 | 1.463 | | |
| Additional information | | Sample 1 | Sample 2 | | | | | | | | |
| Chloride | mg Cl/l | 39.1 | 39.2 | | | | | | | <300 | <75 |
| Potassium | mg K/l | 14.9 | 16.7 | | | | | | | | |
| Magnesium | mg Mg/L | 175 | 193 | | | | | | | NS | |

NS – Not specified

**Table 8 AMD feed water quality used for the simulations**

| Parameter | Unit | Value |
|---|---|---|
| pH @ 25°C | | 3.68 |
| Iron | mg Fe/l | 558 |
| Sulphate | mg $SO_4^{2-}$/l | 3314 |
| Sodium | mg Na/l | 110 |
| Calcium | mg Ca/l | 549 |
| Manganese | mg Mn/l | 56 |
| Chloride | mg Cl/l | 39 |
| Potassium | mg K/l | 17 |
| Magnesium | mg Mg/L | 193 |
| Σ of cations | eq/kg | 4.3993 e-2 |
| Σ of anions | eq/kg | 4.3993 e-2 |
| Ionic strength | mol/l | 0.0841 |

The ionic strength is less than 0.5 M, suggesting that the Davies model can be used to estimate the activities of the ions (Stumm & Morgan, 1981).

### 4.2.2 Design of continuous process

The continuous process was constructed to treat 40 l/d of synthetic AMD (Figure 12, Figure 13)

Figure 12 Picture of the continuous process

It consisted of two upflow sulphate-reducing reactors in series, with a sludge recycle from the top of the second reactor to the bottom of the first reactor. The reactors were seeded with anaerobic digested sludge, and fed with synthetic AMD, with a composition as indicated in Table 6. As it was seeded, no additional carbon source was introduced, with the purpose of depleting most of the biodegradable organic fraction of the digested sludge before the introduction of silage. Only silage was fed to the first of the two reactors. The required quantity of silage was introduced once daily through a tube fitted through the lid on top of the reactor all the way to the bottom of the reactor. A plunger was used to force the silage through the tube. Surplus sludge was wasted from the second reactor via the recycle pump to control the sludge bed in the second reactor to ensure that a clear effluent is discharged from the second reactor. The overflow from the second reactor was fed into the bottom of the anoxic reactor. Graded filter media was used as a growth media to support a fixed biofilm. Humus sludge collected from the domestic WWTP was used to inoculate the anoxic reactor. The sulphide in the effluent from the anoxic reactor was treated with iron hydroxide sludge and flocculated with a polymer in a reactor-clarifier. The iron sulphide sludge was manually wasted and the clear overflow was gravity-fed into the bottom of the aerobic reactor. The air was supplied with a peristaltic pump with a variable speed controller so that the aeration rate could be controlled. Granular carbon, instead of sand, was used as a growth media to limit flow resistance. Humus sludge collected from the domestic WWTP was used to inoculate the aerobic reactor. The treated effluent was collected in a drum from where part of it was recycled to the bottom of the anoxic reactor.

All the pumps were fitted with variable speed drives and flow rates could be adjusted as the process reached steady-state operation. The reactors were operated between 25 and 28°C.

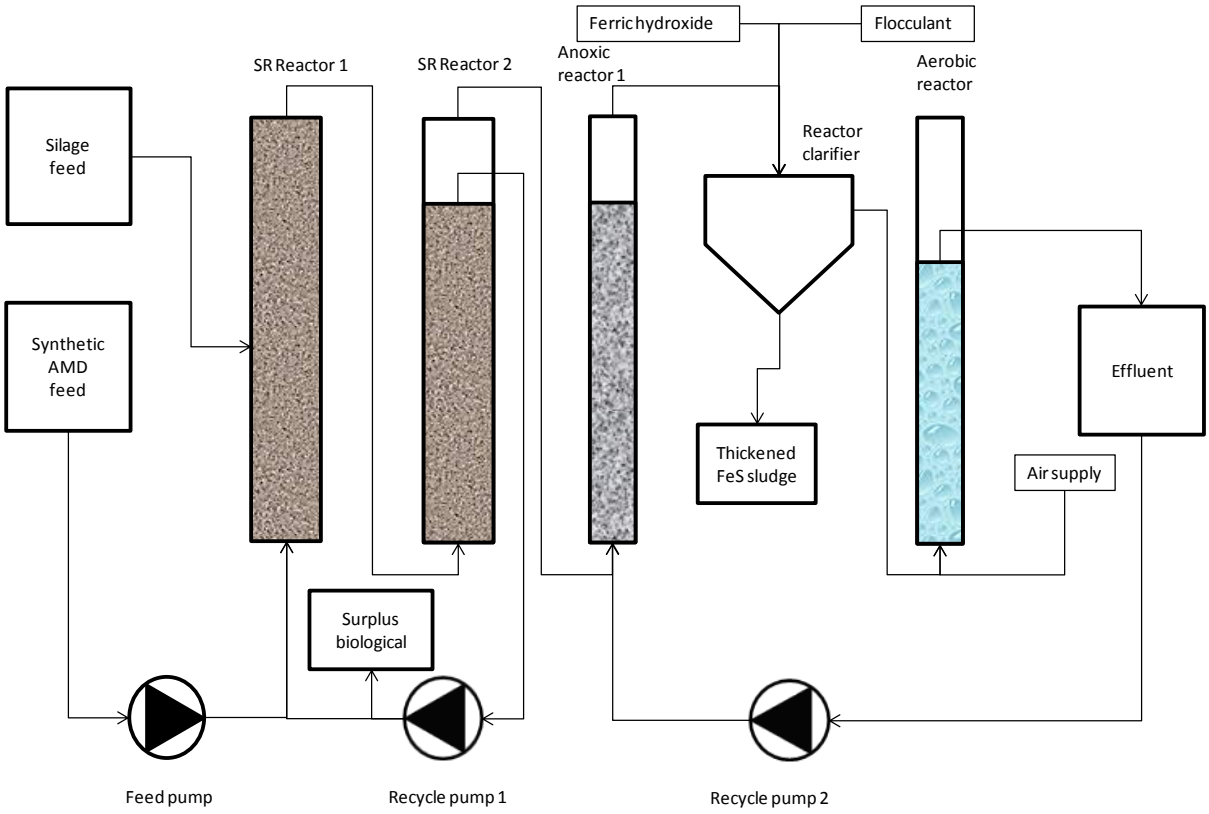


Figure 13 Schematic layout of the 40 L/d continuous process

### 4.2.3 Sulphide removal with iron hydroxide

Ferric hydroxide was precipitated by adding a 0.2 M NaOH solution to a diluted ferric chloride solution. The precipitated iron hydroxide was filtered and washed with demineralised water to remove any sodium chloride. The dewatered ferric hydroxide was suspended and diluted to provide a stock solution of 1 g/L as Fe. Sodium sulphide solutions with a hydrogen sulphide concentration of 300 mg/L were prepared in de-aerated water by adding sodium sulphide. The iron hydroxide was dosed in incremental dosages and the residual hydrogen sulphide and alkalinity was measured.

### 4.2.4 Measurement of oxygen uptake rates of iron sulphide sludge

Under certain circumstances, it may be necessary to dispose of the iron sulphide sludge on drying beds, for example. This was the case during the operation of the full-scale BioSURE Process when there was no shortage of iron hydroxide. It was observed that the dewatering capacity of the sludge was improved by more than four times when the iron sulphide was stabilized by aeration. Tests were conducted to measure the oxygen uptake rate for the chemical oxidation of the precipitated iron sulphide sludge. These tests were conducted in the laboratory in 2 L stirred jars. Aquarium pumps with air stones were used to introduce air into the jars. A jar stirrer was used to ensure completely mixed systems. The following factors were tested:

- Different solids concentration

- Different mixing speeds
- Different number of aerators

The temperature of the samples varied between 23 and 26°C. The pH of the sludge samples varied from 7.4–7.9.

The oxygen uptake rate may be expressed by the following relationship:

$$r_{O_2}=kLa([O_2]_{sat}-[O_2]_{diss})-OUR \quad (21)$$

where

$r_{O_2}$ = rate of change of dissolved oxygen (mg/L.h)

$kLa$ = coefficient of oxygen transfer

$[O_2]_{sat}$ = dissolved oxygen at saturation

$[O_2]_{diss}$ = measured dissolved oxygen concentration

$OUR$ = oxygen uptake rate (mg/L.h)

The rate of change of dissolved oxygen (DO), $r_{O2}$, is the slope of the graph of DO versus time. From the graph below (Figure 14), one may observe the rapid initial DO increase from 0 to 2.8 mg/L. After a few minutes, a steady-state concentration of 2.8 mg/L was reached. It remained constant for about 4 hours, implying that the rate of change of DO was 0 for this period. When $r_{O2}$ = 0, the oxygen transfer rate equals the OUR and was at a maximum:

$$0=kLa([O_2]_{sat}-[O_2]_{diss})-OUR \quad (22)$$

$$\therefore kLa([O_2]_{sat}-[O_2]_{diss})=OUR \quad (23)$$

When the aeration was stopped for a few moments, while keeping the suspension mixed with the paddle stirrer, the dissolved oxygen was depleted at a constant rate. By measuring the slope of the curve during this period, the instantaneous OUR was measured. Frequent measurements of the OUR were made during the course of the experiments in this manner. At the end of the experiment, when no further consumption of oxygen was observed, the sample was saturated with dissolved oxygen. With the values of $[O_2]_{sat}$ and OUR known, the value of kLa could easily be determined during the steady-state period, using the following ratio:

$$\therefore kLa= OUR/([O_2]_{sat}-[O_2]_{diss}) \quad (24)$$

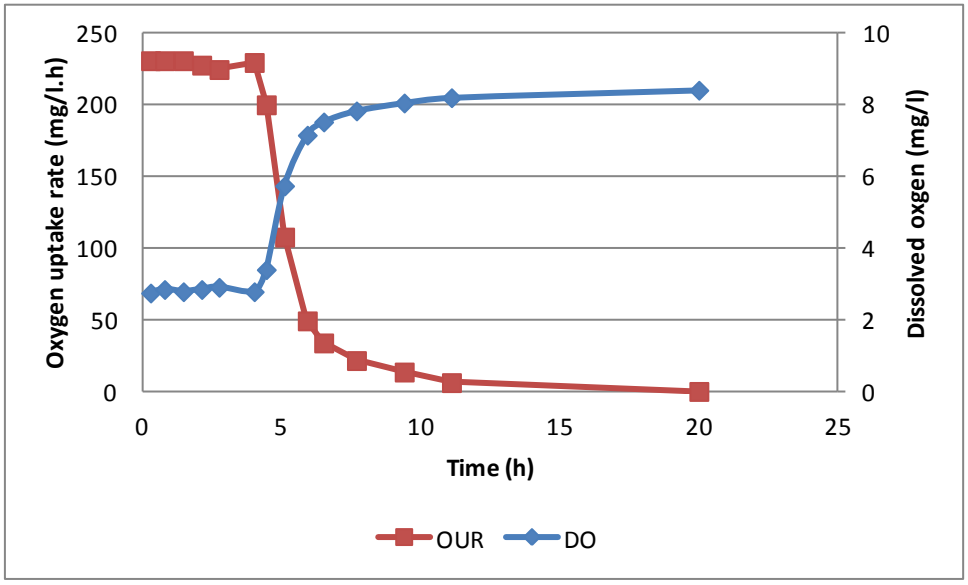


Figure 14 Dissolved oxygen and measured OUR during the chemical oxidation of FeS

The results were used to calculate the cumulative mass of oxygen consumed during the aeration of a sample containing 15 g of precipitate (Figure 15). This information was used to compare the results with all the variables tested.

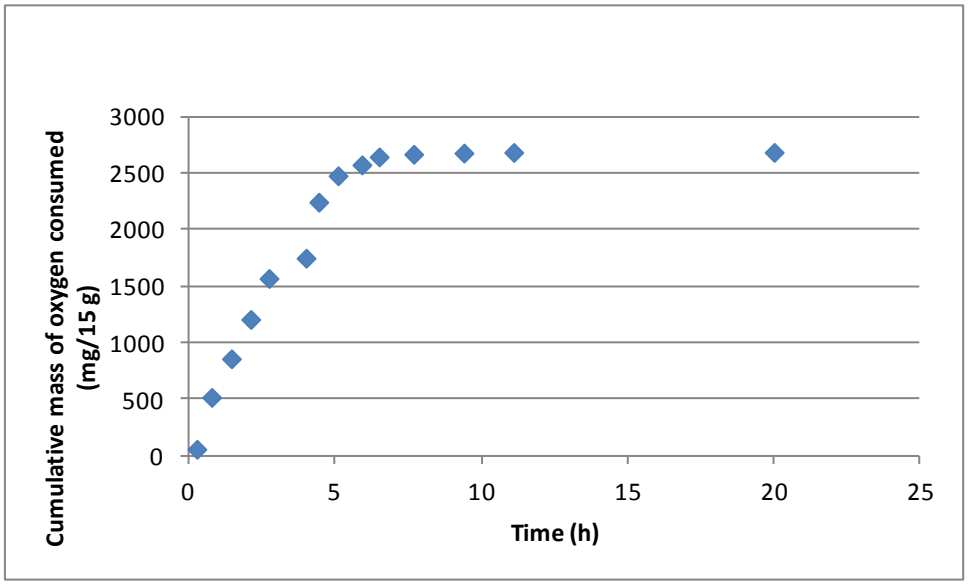


Figure 15 Cumulative mass of oxygen consumed

### 4.2.5 Biological iron oxidation

A reactor was constructed for the biological oxidation of FeS and ferrous ion (Figure 16). It was filled with plastic rosettes to support the growth of a fixed film. The liquid inside the reactor was recycled from the bottom and evenly distributed on the top of the growth media, as indicated in Figure 17. Air was sucked from the bottom upwards using a suction fan. The

reactor was loaded with a ferrous sulphate solution with a 10 g Fe/L concentration. It was seeded with a sample from another similar pilot reactor that had been in operation for a few years. Nutrients were added to support the growth of the bacteria.

Figure 16 Biological iron oxidation reactor

Figure 17 Distribution of recycled ferrous sulphate solution on top of the rosettes

### 4.2.6 Dissolution of iron sulphide in an acidic ferrous/ferric sulphate solution

The optimum pH of the process is 2. When iron sulphide is mixed with the acidic ferric sulphate solution, it is dissolved. It was observed that a fraction of the iron sulphide does not dissolve. Qualitative tests suggest that elemental sulphur is separated from the iron sulphide,

supporting the earlier comment that a mixture of FeS and $S_8$ is produced when ferric hydroxide is added to the BSR reactor effluent.

### 4.2.7 Precipitation of iron hydroxide from the acidic ferrous/ferric sulphate solution with magnesium hydroxide

When the ferric sulphate produced in the biological iron oxidation reactor is treated with magnesium hydroxide, ferric hydroxide precipitates. Magnesium sulphate with a high solubility is produced.

$$Fe_2(SO_4)_3+3Mg(OH)_2(s)\rightarrow 2Fe(OH)_3(s)+3MgSO_4 \qquad 25$$

It is important that the dosage of magnesium hydroxide should be controlled to ensure that it is completely dissolved. Several tests were done to determine the optimum pH for complete removal of iron hydroxide.

### 4.2.8 Separation of calcium carbonate from magnesium hydroxide

The experiments were carried out in a fluidized bed reactor, the dimensions of the Perspex column being 0.42 mm dia. and 1 m in height. The experimental set up is illustrated in Figure 18. A T-piece was glued to the bottom of the column to allow for the recycle and feed lines. A fine mesh was placed at the bottom of the column to prevent the carrier media from entering the feed and recycle lines. A T-piece glued at the top guided the overflow into a bucket. Fine sand (0.51 mm) was used as the carrier media and the bed depth was set at a 50% fill ratio. Two Synchron 360/166 peristaltic pumps (recycle and feed) continuously fed the reactor with the same flow rate of 32 L/h. The pH, conductivity and temperature were measured at the top of the column. A Metrohm 713 pH meter was used for pH and temperature measurements, and an Orion 4 Star meter was used to measure conductivity. Reagents, such as hydrated lime, supplied by Protea chemicals, or sodium hydroxide, supplied by Minema, were dosed with a third, adjustable speed Aqua PER-R peristaltic pump within the column at the bottom 3 cm above the mesh; this was done to prevent excessive scaling on the mesh. Effluent from the 1 $m^3$/day reactor was used as feed. The rates of calcium and magnesium precipitation were determined by frequent sampling. The method to determine the precipitation kinetics described by Benjamin et al. (1977) was followed. Since the pilot reactor was not operated at steady state, the results were not conclusive due to the large variation in the concentration of the carbonic species and the organic substances in the feed water. The tests will be continued once the performance of the biological reactor has improved.

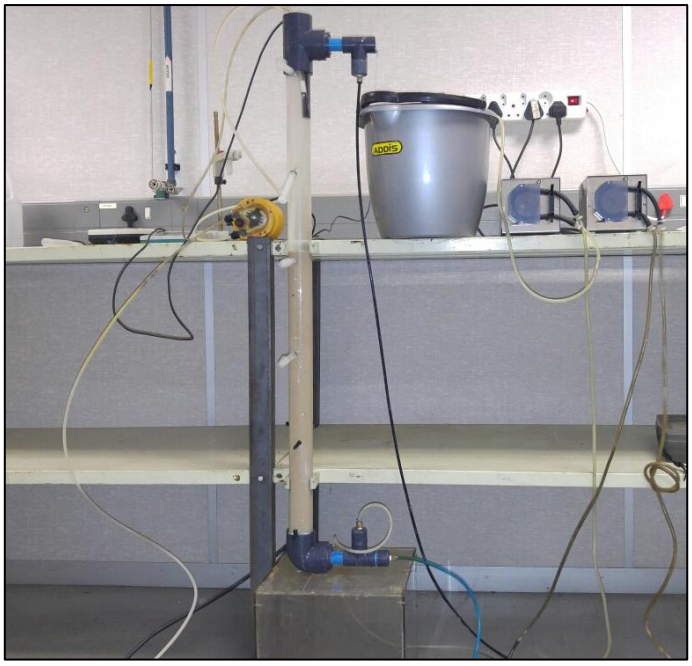

Figure 18 Fluidised bed reactor for precipitation of calcium carbonate pellets

# 5 Results

The results of the batch reactors that received silage only are shown in the figures below. The different batch reactors were operated at 10-, 20- and 30-day sludge ages, at 15°C, 25°C and 30°C, with a total of nine reactors. The objective of the experiment was to operate the reactors until steady state was reached and to determine the stoichiometric and kinetic parameters. Due to the large number of analyses required, it was necessary to abandon the initial plan to simultaneously run similar reactors with a 1:1 mixture of PSS and silage, based on mass of COD and PSS only.

## 5.1 Batch reactors with silage as carbon source

### 5.1.1 Batch reactors operated at 15°C

Sulphate was reduced to below 350 mg/L in the reactor at 10-day sludge age, but remained between 600 to 800 mg/L in the reactors operating at 20- and 30-day sludge ages. The results indicated that steady-state operation was not achieved (Figure 19) before approximately 250 days. Given regular power failures due to load shedding, as well as problems with power supply to the premises where the tests were conducted, the temperature could not be controlled accurately which resulted in a significant scatter of the results. The performance of the reactor operated at 30-days sludge age was generally poorer than expected.

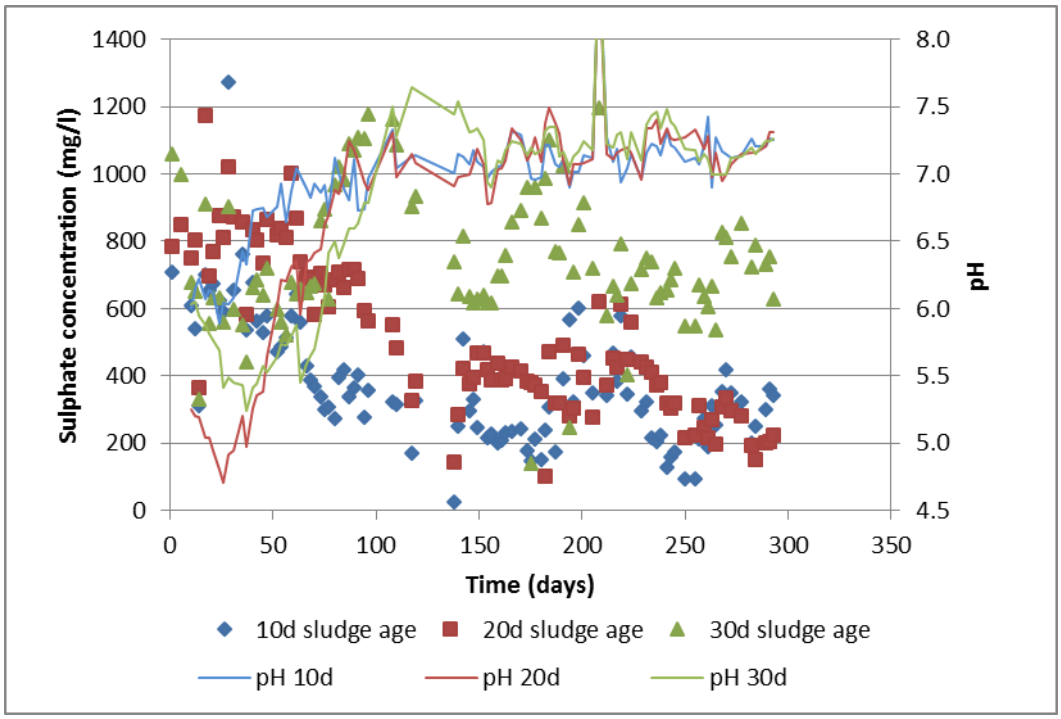


Figure 19 Batch reactor results of sulphate reduction at 15°C

There was a steady increase in sulphide concentration at 10-days sludge age. At 20-days sludge age, an increase in the sulphide concentration was only observed after more than 60

days of operation. Initially, the sulphide production at 30-days sludge age remained very low but increased rapidly after 70 days (Figure 20). Judged by the increase in sulphide concentration, the process performed better at a 30-days sludge age compared with the 10- and 20-days sludge ages. This is contrary to observation with respect to the reduction of sulphate. The production of alkalinity followed the same trend as that of sulphide production, as expected since alkalinity is produced when sulphate is removed (Figure 21).

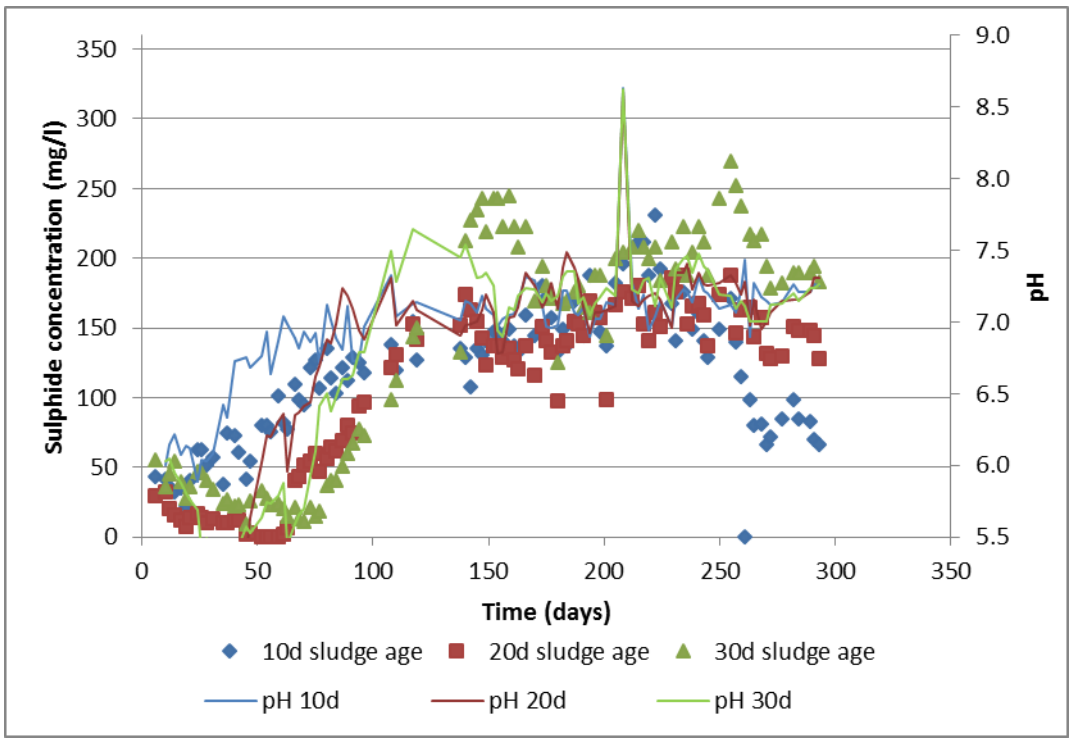


Figure 20 Batch reactor results of sulphide production at 15°C

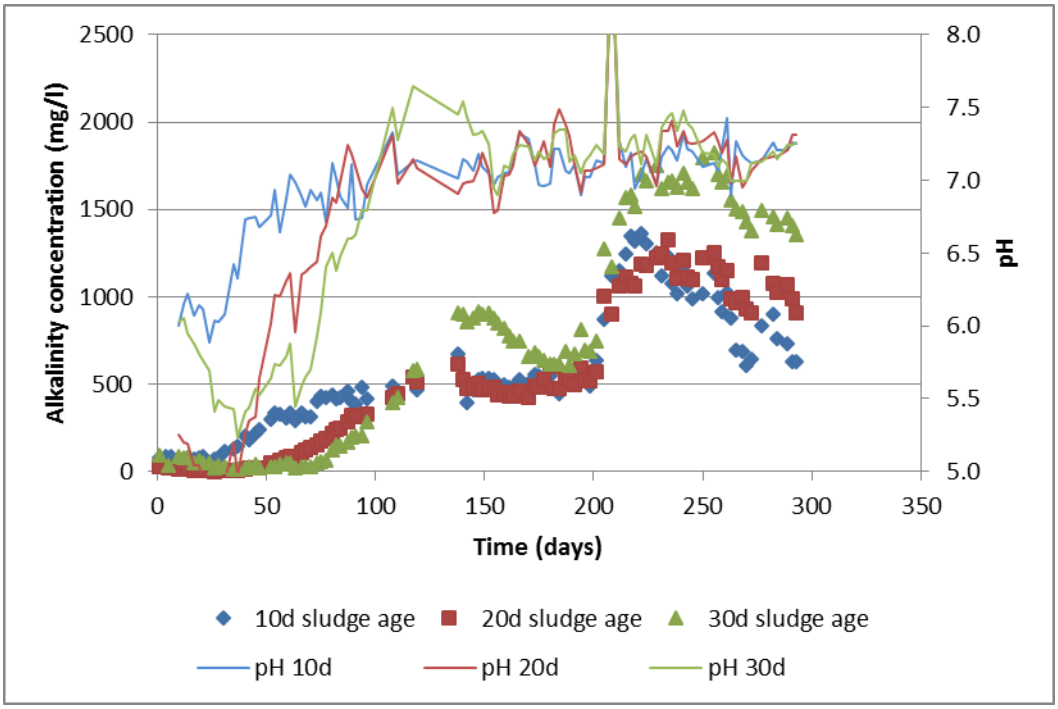


Figure 21 Batch reactor results of alkalinity production at 15°C

Fatty acids initially accumulated at a high rate at a sludge age of 30 days with a steady decline in the rate observed after 30 days of operation. The same trend was observed in the reactors operated at a 20-day sludge age, although fatty acid concentrations were lower with 20-day sludge age than with the 30-day sludge age. Much less fatty acid were produced at a sludge age of 10 days (Figure 22). After 105 days, the fatty acid concentration in all the reactors reached the same concentration. The concentration of fatty acids generally remained below 200 mg/L, with a significant scatter, as a result of unreliable power supply.

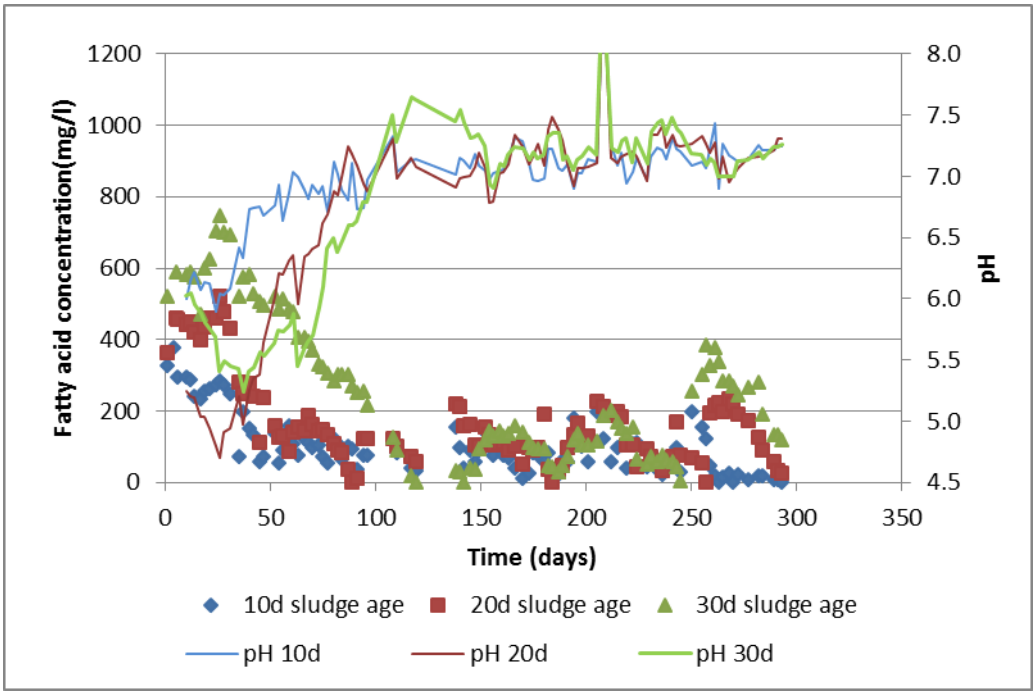


Figure 22 Batch reactor results of VFA at 15°C

The pH in the 10-day sludge age reactor was initially higher than 6 and increased steadily to pH 7. It appears to stabilize at pH 7. The pH in the 20-day sludge age reactor remained less than 5 until after the 20$^{th}$ day of operation and increased gradually. It reached a pH of 6 after 49 days and approached a pH 7 after 80 days of operation. The 30-day sludge age remained below pH 6 until the 72$^{nd}$ day and increased steadily to a pH of 6.5. Eventually, after 100 days, the pH in all the reactors was similar and varied between 6.9 and 7.5 (Figure 23), as expected for a carbon-limited sulphate-reducing process.

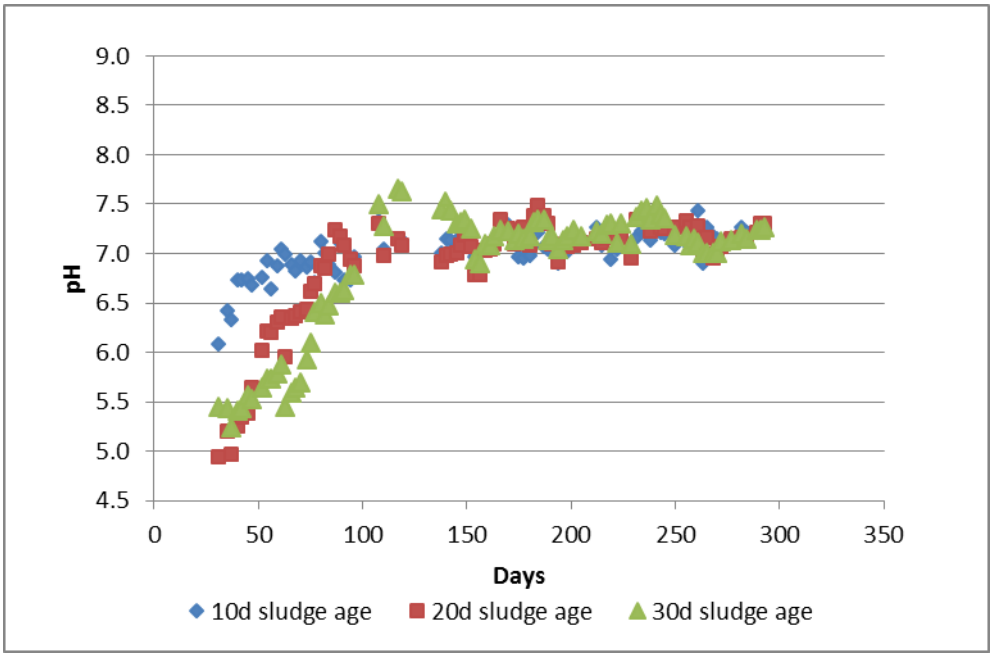


Figure 23 Batch reactor pH at 15°C

### 5.1.2 Batch reactors operated at 25°C

At 25°C, the sulphate was reduced to below 100 mg/L within 20 days of operation in the reactor operated at a 30-day sludge age. After 36 days of operation, the sulphate concentration gradually increased and continued to do so after 74 days of operation when it reached a concentration of more than 800 mg/L. The sulphate concentration of the reactor operated at a 20-day sludge age was initially very erratic but it appeared to stabilize at between 136 and 202 mg/L after 60 days of operation. There was a gradual decrease in the sulphate concentration in the reactor operated at a 10-day sludge age, approaching a value of between 220 and 260 mg/L at 74 days of operation. The results suggest that steady state operation was approached after a period of 60 to 75 day for the reactors operated at 10- and 20-days sludge age (Figure 24), achieving a final sulphate concentration generally below 400 mg/L. These reactors performed better than the reactor operated at 30-day sludge age, but unexpectedly, worse than the reactors operated at 15 °C.

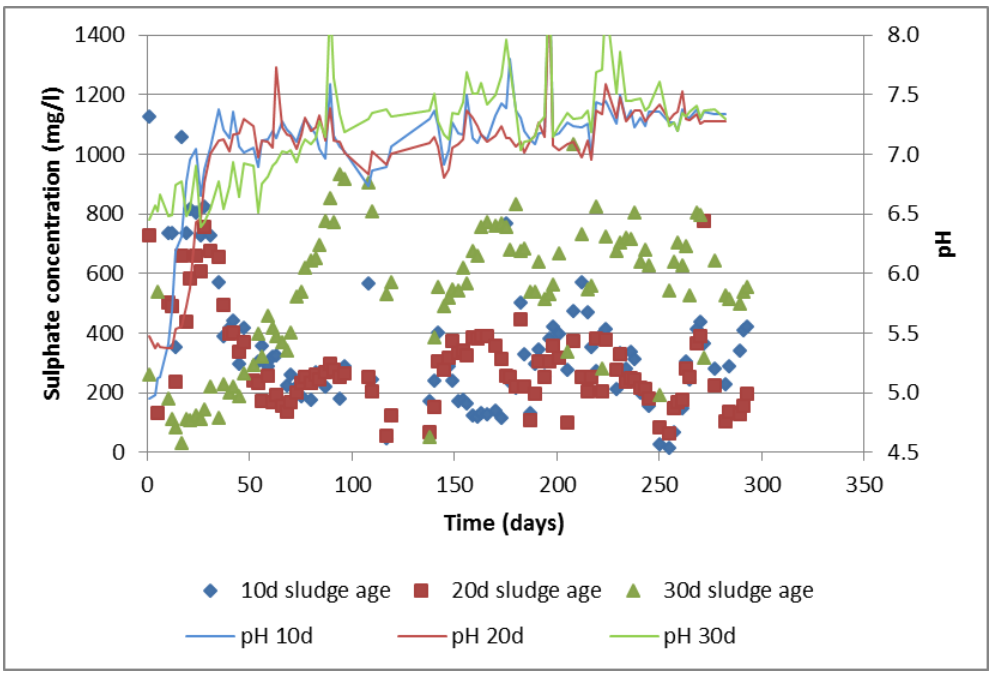


Figure 24 Batch reactor results of sulphate reduction at 25°C

The sulphide concentration in the reactor operated at a 30-day sludge age was initially high but gradually started decreasing after the 25$^{th}$ day. The sulphide concentration increased rapidly after 100 days and remained at approximately 250 mg/L for the remainder of the period. At a 20-day sludge age, there was a sharp increase in the sulphide concentration after 32 days of operation and sulphide reached a concentration of 160 mg/L by 74 days of operation. Similarly, for the reactor operated at a 10-day sludge age, there was a sharp increase in the sulphide concentration after the 15$^{th}$ day of operation. Sulphide concentration continued to increase until 145 days of operation (Figure 25). After that, the results were very erratic. The measured increase in the sulphide concentration was less than the decrease in

the sulphate concentration, indicating that not all the sulphate was accounted for. Again, the production of alkalinity followed a similar trend to that of sulphide production (Figure 26).

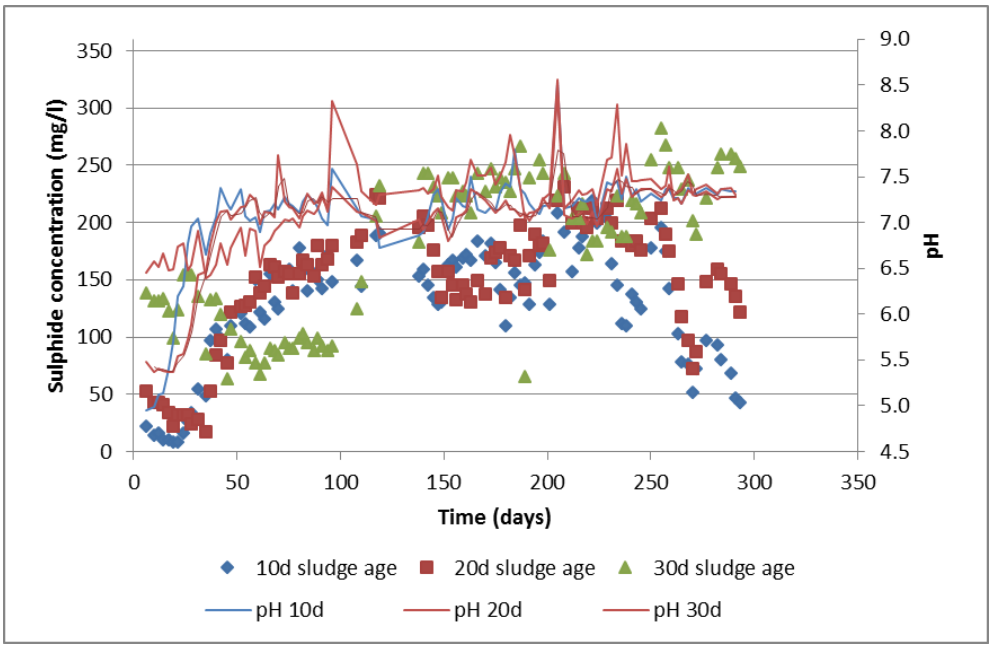


Figure 25 Batch reactor results of sulphide production at 25°C

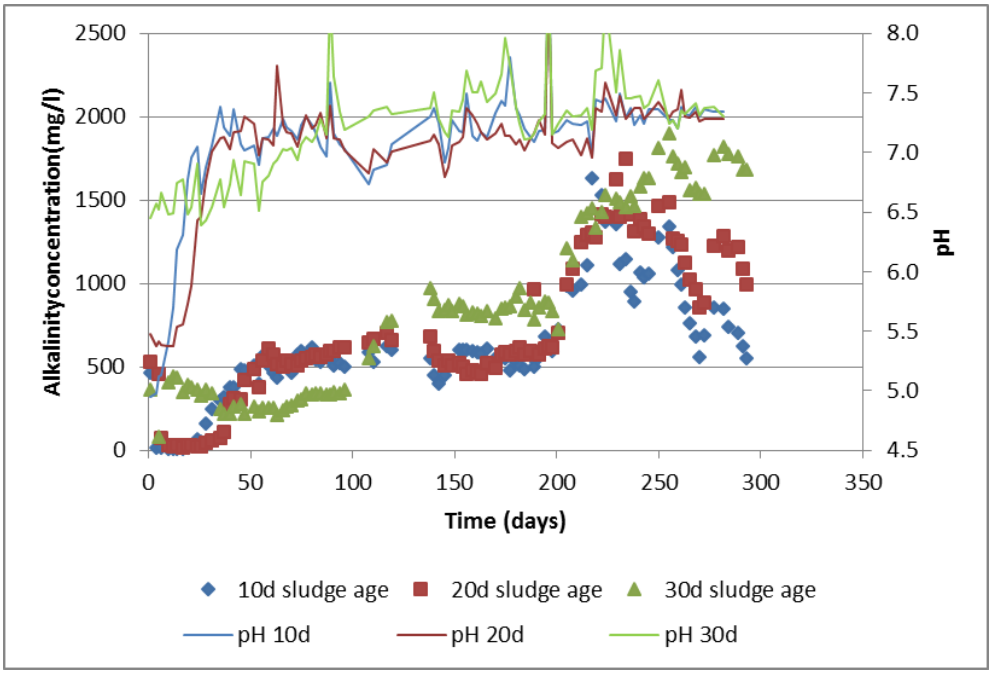


Figure 26 Batch reactor results of alkalinity production at 25°C

Fatty acids initially accumulated in all three reactors, although the concentration was slightly lower in the 10-day sludge age reactor. The fatty acids started decreasing after 16 days in the 10-day sludge age reactor and after 18 days in the 20-day sludge age reactor. Consumption of fatty acids was only observed after 32 days at a sludge-age of 30 days. The rate of decline

was steady for the first 50 days in the 10- and 20-day sludge age reactors, and remained steady for the remainder of the period, except for short periods when it increased significantly. The rate of decline in the 30-day sludge age reactor was slower and only reached a minimum concentration after 110 days. All three reactors behaved very similarly for the remaining period (Figure 27).

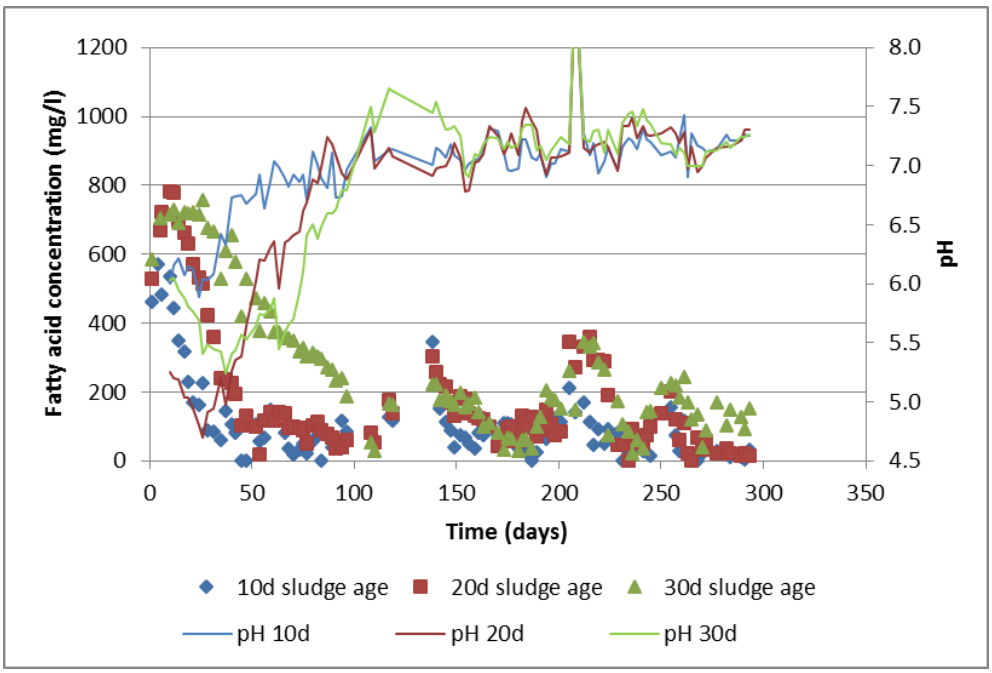


Figure 27 Batch reactor results of VFA at 25°C

The pH in the 10- and 20-day sludge age reactors was initially less than 5.5. The pH rapidly increased to pH values higher than 7 after 32 days and remained fairly constant thereafter. The 20-day sludge age reactor reached a pH of 7 after 39 days and also remained stable afterwards. The pH in the 30-day sludge age reactor was initially higher and gradually increased during the first 100 days of operation until it reached a pH between 7.0 and 7.8 for most of the time (Figure 28).

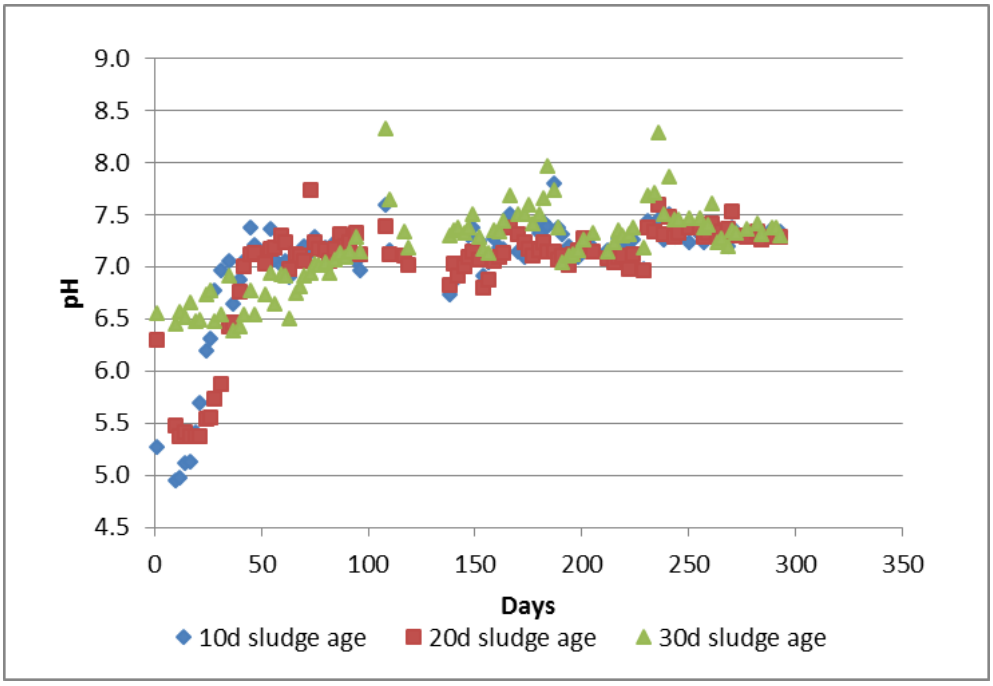


Figure 28 Batch reactor pH at 25°C

### 5.1.3 Batch reactors operated at 30°C

At 30°C, the sulphate concentration in all three reactors was initially reduced to below 600 mg/L and was further reduced to approximately 100 mg/L after 17 days of operation. There was an increase in the sulphate concentration until the 29$^{th}$ day after which the sulphate concentrations again dropped to approximately 100 mg/L on the 57$^{th}$ day of operation, in the 10- and 20-day sludge age reactors. Thereafter it varied in a cyclical manner and remained below 400 mg/L for most of the time. The sulphate concentration in these two reactors followed very similar patterns. The sulphate concentration in the 30-day sludge age reactor varied significantly more than in the 10- and 20-day sludge age reactors, with values regularly above 800 mg/L (Figure 29).

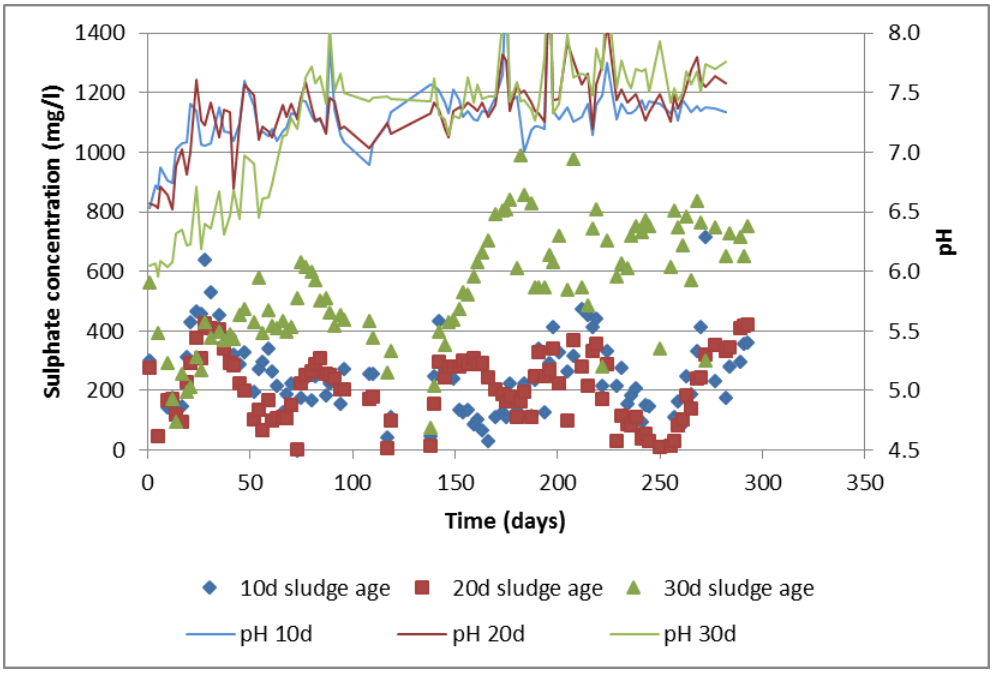


Figure 29 Batch reactor results of sulphate reduction at 30°C

Initially, the sulphide concentrations in all three reactors were very similar, with an average value of approximately 90 mg/L. It remained constant for the first 28 days. The sulphide concentration in the 10- and 20-day sludge age reactors gradually increased until it reached a concentration slightly above 200 mg/L after 125 days. The two reactors performed in a similar way. Clearly, it was not possible to account for all the sulphate that was reduced during this period. The sulphide concentration in the 30-day sludge age reactor, however, gradually decreased until the 57$^{th}$ day; thereafter, it started to increase and reached a maximum concentration of 260 mg/L after 125 days of operation (Figure 30). After approximately 240 days, the sulphide concentration in all three reactors dropped significantly. The production of alkalinity followed a similar trend as that of sulphide production in the 10- and 20-day sludge age reactors (Figure 31). The alkalinity in the 30-day sludge age reactor remained constant at a value slightly higher than 100 mg/L for the first 70 days; thereafter, it increased to more than 1000 mg/L after 130 days. It was reduced temporarily and increased to 1900 mg/L on the 220$^{th}$ day, it steadily decreased, thereafter, for the remainder of the period. The alkalinity in the 10- and 20-day sludge age reactors followed a similar pattern.

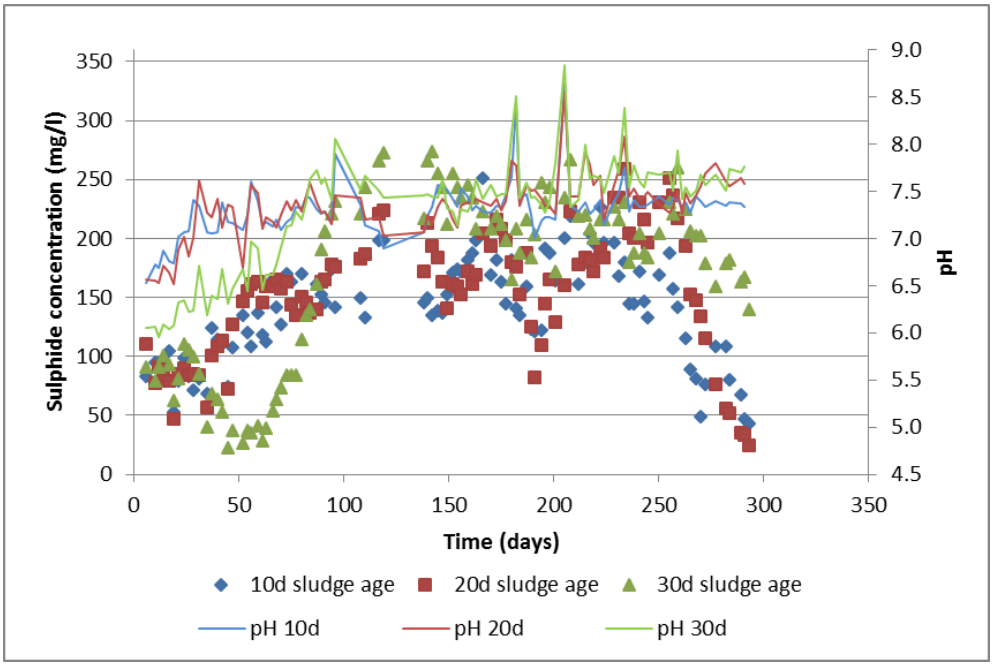


Figure 30 Batch reactor results of sulphide production at 30°C

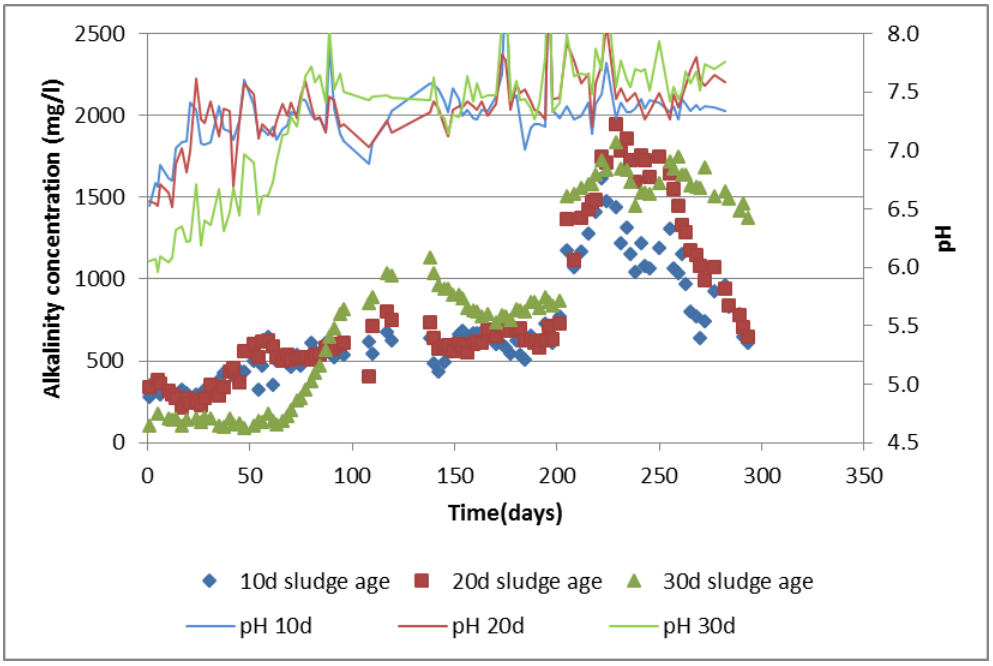


Figure 31 Batch reactor results of alkalinity production at 30°C

The trend of all three reactors with respect to VFA production was very similar to the trend in the three reactors at 25°C, except that higher concentrations were measured initially and consumption of the fatty acids began earlier. Fatty acids initially accumulated in all three reactors, although it was slightly lower in the 10-day sludge age reactor. The fatty acids started decreasing after 10 days in the 10-day sludge age reactor, and after 16 days in the 20-day sludge age reactor. Consumption of fatty acids was only observed after 18 days at a sludge

age of 30 days. The VFA concentration reduced to less than 200 mg/L after 50 days in the 10- and 20-day sludge age reactors (Figure 32). The VFA concentration in the 30-day sludge age reactor reached a minimum after 100 days and remained below 200 mg/L for most of the time.

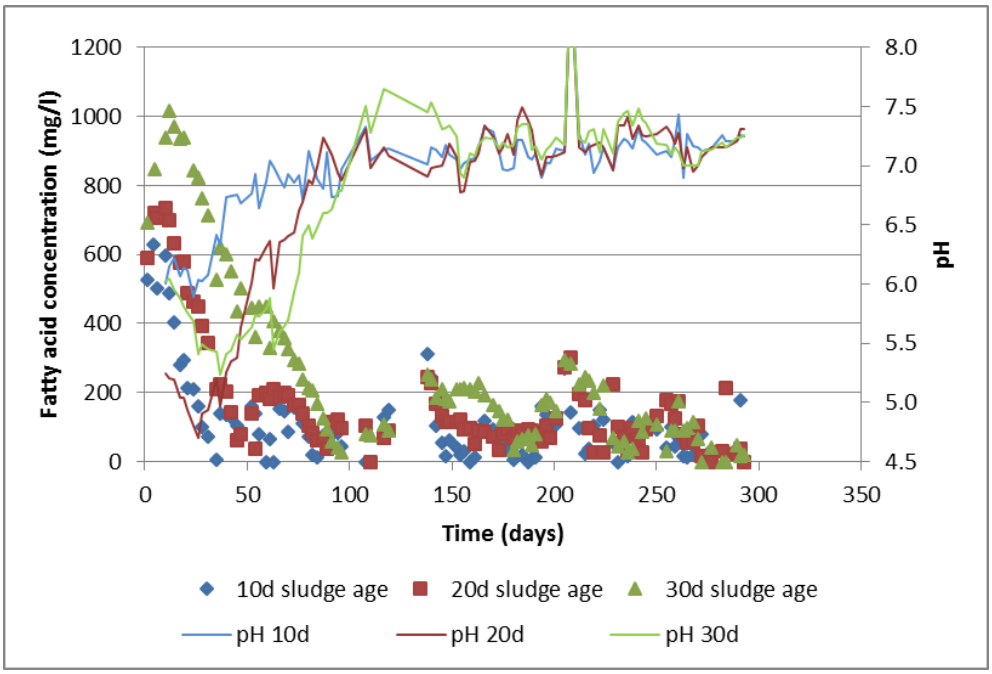


Figure 32 Batch reactor results of VFA (volatile fatty acids) at 30°C

The pH in the 10- and 20-day sludge age reactors was initially slightly more than 6.5. The pH increased to values higher than 7 after 20–23 days and remained fairly constant thereafter. The pH in the 30-day sludge age reactor was initially lower than that of the other two reactors, but it gradually increased until it reached a pH of approximately 7.5 after 90 days of operation, and remained fairly constant for the remainder of the period (Figure 33).

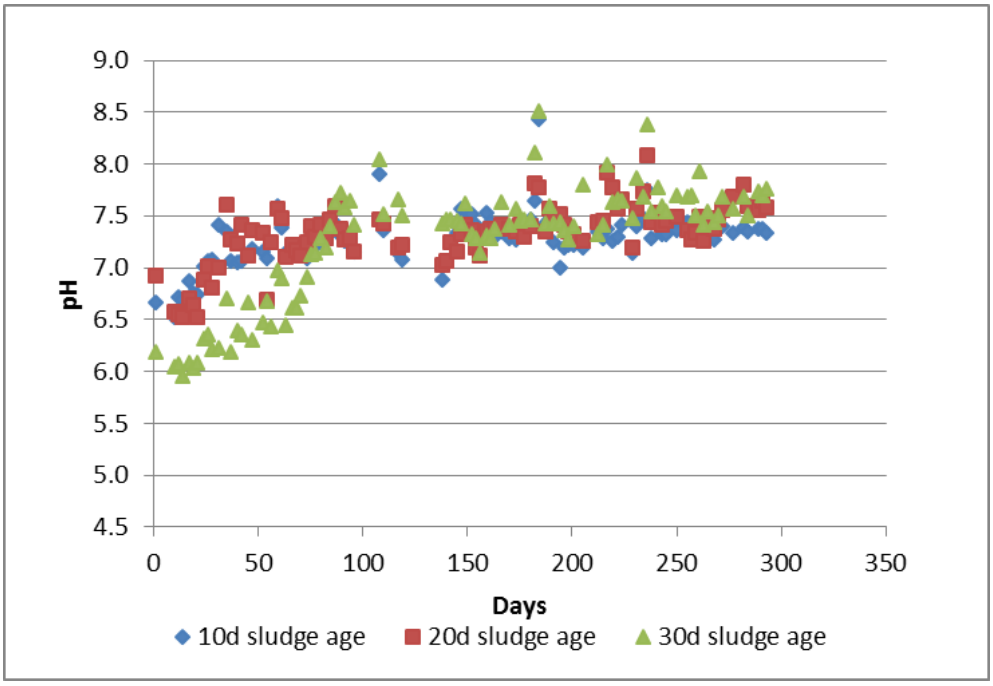


Figure 33 Batch reactor pH at 30°C

## 5.2 Discussion of the results

Sulphate reduction was observed in all the reactors at the different temperatures and different sludge ages, where silage was used as a carbon source. The results suggest that the reactors generally performed better initially with respect to sulphate reduction at the shorter 10-day sludge age, at all the temperatures. Although no attempt was made to determine the reason for this observation, it is speculated that other groups of organisms, such as acidogens were growing more slowly compared to sulphidogens, and were washed out at a short sludge age. Considering that the success of silage production and preservation is dependent on the selective cultivation of acidogens, it can be assumed that the biomass of viable acidogens initially exceeded that of the sulphidogens by orders of magnitude. Operating the reactors at a longer sludge age gives the acidogens a competitive advantage. Controlling the sludge age appears to be the most important operating parameter to select for sulphidogens during start-up and operation.

An unexpected outcome of the results was the poor removal of sulphate in the reactors operated at 30-day sludge age at all the temperatures. Unfortunately, due to the interrupted power supply during the test period, there is no convincing explanation for this observation. However, it may be postulated that inhibitory compounds may be formed when the more recalcitrant lignocellulose is decomposed at a longer sludge age. With the more reliable power supply, the reactors are now operated for an extended period of time in order to ensure steady operation and to obtain more reliable results. As explained earlier, it was observed that the fibrous silage particles caused blockages at the sampling points which made the accurate control of sludge age difficult. It was also not possible to prevent air from

entering the reactors during the sampling. The reactors were modified to overcome these potential problems without discontinuing the current performance of the reactors.

Accumulation of fatty acids, which results in a reduction of pH, inhibits the growth of sulphidogens. The sulphidogens performed particularly poorly at a low temperature (15°C) and a 30-day sludge age. There was, however, an indication that the pH gradually increased as the fatty acids were consumed, which may eventually favour the growth of sulphidogens. It appears that the fatty acids introduced with the silage during feeding are diluted to sufficiently low concentrations at a short sludge age so that the inhibitory effect is reduced. Once the reactors approached the apparent steady state, the VFAs were generally reduced to less than 200 mg/L.

The initial feed rate of silage and control of sludge age are therefore important aspects that need to be considered during the start-up of biological sulphate reactors. The ratio of alkalinity to hydrogen sulphide measured was higher than the expected value of 2.9 mg $CaCO_3$/mg $H_2S$ produced, and suggests that the measurement of hydrogen sulphide was not accurate, probably due to volatilization of the hydrogen sulphide gas from the samples after sampling and during analysis. Zinc sulphate was added to the samples in order to prevent the loss of hydrogen sulphide from solution.

# 6 Design of the 1 $m^3$/d pilot plant and performance of the unit processes

The design and construction of each unit process is discussed in this section, as well as the preliminary pilot results.

A schematic layout of the existing pilot plant, excluding the acid neutralization step, is illustrated in Figure 34 and in Figure 35. As mentioned above, it is the intention that AMD from the Western Basin will be treated, at which point acid neutralization will be included. This will involve the recycling of a portion of the effluent from the BSR reactor, whereupon the bicarbonate alkalinity in the recycled effluent will neutralise the feed water, and the sulphide in solution will affect the precipitation of metal sulphide, specifically iron sulphide.

The start-up procedure of the reactor with a low pH feed will be particularly important. pH neutralization of the first batch will be required and the feed rate of the carbon source should be controlled to prevent accumulation of VFAs. Ideally, the reactor should be inoculated with active biomass in order to accelerate the hydrolysis of the complex organic material.

This is discussed further in section 6.1.2.

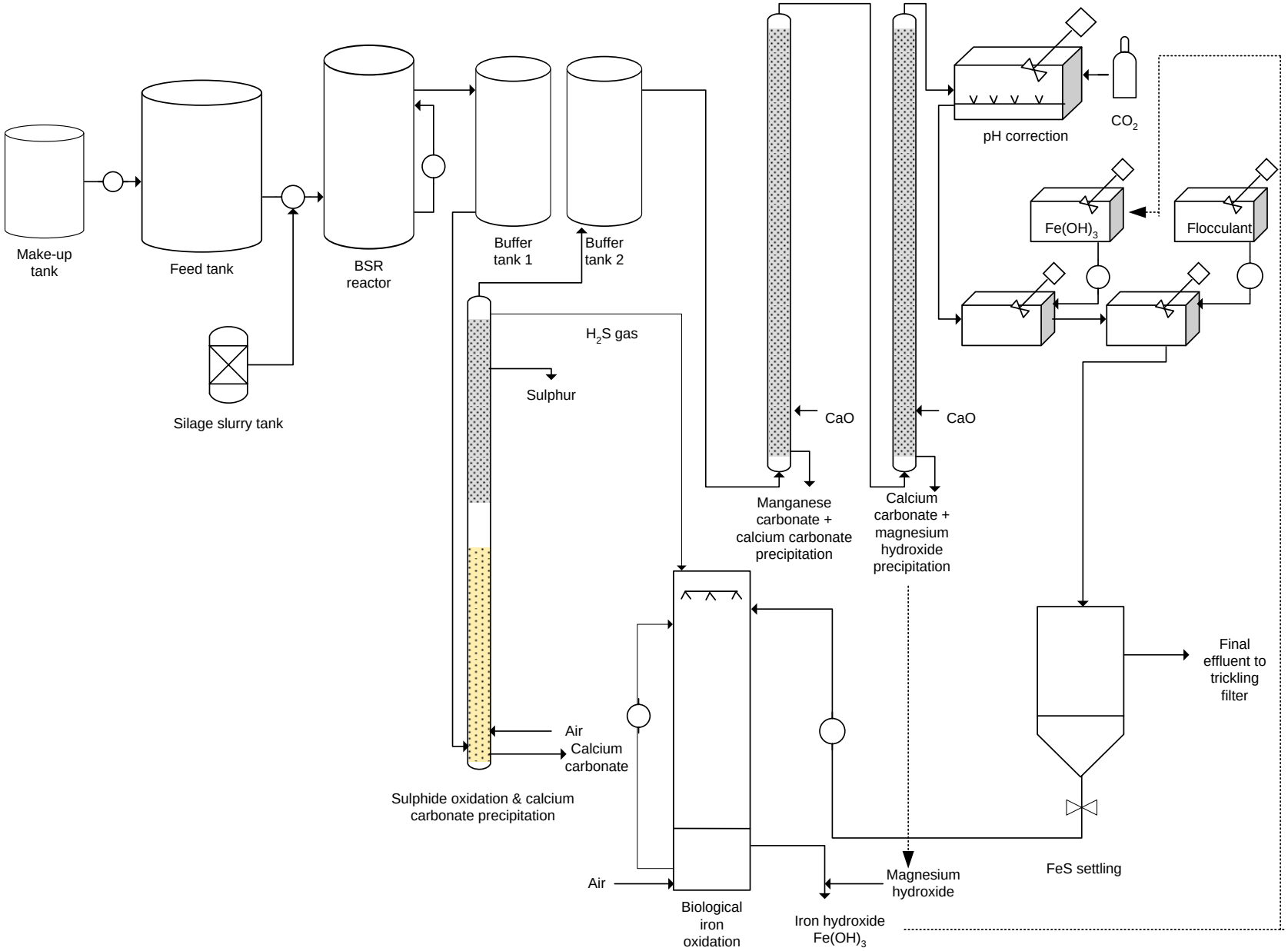


Figure 34 Schematic layout of the pilot reactor, excluding acid neutralization

Figure 35 Pilot plant with 1 $m^3$ capacity

## 6.1 Biological sulphate reduction

### 6.1.1 Maize silage as carbon source

It is worth noting that a mixture of other sources of organic material, such as carbohydrates, proteins or fats may change the relative ratios of C, H, O and N. The reader is referred to the paper by Poinapen and Ekama (2010) for a thorough discussion. Using the information in

Table **3** as a guide, it can be concluded that the VS of typical silage consists of 9.8% crude protein, 3.6% fat, oil and grease (FOG) and 86.6% carbohydrates. Using this information and following the approach as described by Grady et al. (1999), the composition of silage is $C_{0.246}H_{0.481}O_{0.225}N_{0.006}$. The redox half-reaction for silage as electron donor ($R_d$) is as follows:

$$C_{0.246}H_{0.481}O_{0.225}N_{0.006}+0.268H_2O \rightarrow 0.246CO_2+0.006NH_4^+ +0.994H^+ +e^- \qquad 26$$

This result implies that the formula of silage closely approaches that of carbohydrates ($CH_2O$). The protein and FOG content of the silage does not change the formula significantly. Based on equation 2.9, the COD of silage is 1.12 g COD/g VS compared with 1.07 g COD/g VSS for carbohydrates. To simplify the calculations, silage will be modelled as a carbohydrate.

Following the same procedure as above, assuming a yield coefficient, $Y_H$, of approximately 0.113 g COD biomass/g COD organics hydrolyzed as previously, the overall stoichiometric reaction (R) for silage is:

$$2.255CH_2O + SO_4^{2-} + 0.051NH_4^+ + 2H^+ \rightarrow 0.051C_5H_7O_2N + H_2S + 2CO_2 + 0.153H_2O \qquad 27$$

The mass ratio between silage and sulphate is 0.705 g VSS/g $SO_4^{2-}$. Assuming the lowest expected value for silage biodegradability of 70% and 95% VS of total dry mass, 1.06 g dry silage/g $SO_4^{2-}$ is required. Assuming 70% moisture content, 3.53 g moist silage/g $SO_4^{2-}$ is required. Expressed in terms of COD, the ratio is 0.754 g COD/g $SO_4^{2-}$. Therefore, an estimated 1.077 g silage COD/g $SO_4^{2-}$ is required. The estimated available COD of moist silage is 0.21 g COD/g silage.

The nitrogen content is equivalent to 0.012 mg N/mg VSS and compares well with other reported values of 0.014 to 0.015 mg N/mg VSS (Browne et al., 2005; Marten et al., 2008). The stoichiometric requirement for nitrogen to sustain the growth of the SRP, is 0.012 mg N/mg VSS, an exact match of the supply. This has several important implications:

- Nitrogen is probably the growth-limiting factor, especially when more mature plants are harvested;
- Assuming Monod kinetics, the half saturation constant, $K_s$, must be determined to determine the minimum effluent ammonia-nitrogen concentration required to sustain the growth of biomass at a reasonable rate;
- Contrary to the use of PSS as energy and carbon source, the effluent ammonia-nitrogen concentration can be accurately controlled at concentrations below the standard discharge limits when silage is used as substrate. There is therefore no need for additional effort to remove the ammonia-nitrogen from the effluent. This reduces the investment in terms of capital costs and energy consumption to remove nitrogen from the effluent;
- A total of four biological processes are included in the suite of unit processes, including the BSR reactor. The other processes are the sulphide to sulphur oxidation process, the biological iron oxidation process and the final effluent treatment process for removal of residual COD in the final effluent. Each of these processes will require a minimum nitrogen concentration for assimilation. It will therefore be possible to operate the BSR process at an effluent nitrogen concentration slightly higher than the discharge standard limit without a concern of non-compliance as it will be consumed in the downstream processes.

Alkalinity and saline ammonia are produced in sufficient quantities to buffer the effluent when proteinaceous material is degraded in methanogenic systems. In sulphate-reducing processes, alkalinity is produced as a result of the reduction of sulphate to sulphide, while the hydrogen sulphide and bisulphide weak acid/weak base pair plays a significant role in buffering the effluent (Poinapen & Ekama, 2010). Consequently, no additional alkalinity is required when carbohydrates are used as raw material. Based on the stoichiometric relationship in equation 34, acidity is consumed (or alkalinity is produced) at a rate of 1.042 g $CaCO_3$/g $SO_4^{2-}$ consumed. This is equivalent to 2.942 mg $CaCO_3$/g $H_2S$ produced.

The practical aspects of using silage as the carbon source for the BSR are discussed further in section 7.1.

### 6.1.2 Acid neutralization and metal sulphide precipitation

These results suggest that a fraction of the BSR effluent can be recycled to a process upstream of the BSR in order to precipitate iron sulphide and to partially neutralise the AMD. The results also suggest that iron can be selectively precipitated and recovered as slurry, while manganese remains in solution. A schematic diagram to illustrate the acid neutralization and FeS precipitation process is presented in Figure 36. The process will consist of a mixed reactor receiving AMD feed and a fraction of the BSR effluent. Following the mixed reactor is a reactor-clarifier with a dual function. It is fitted with a flash mixer and dosed with an organic flocculent. The flocculated suspended solids will then flow into a settling zone for clarification and thickening. The thickened FeS slurry will then be introduced into an aeration basin for biological iron oxidation. A fraction of the thickened sludge may be introduced to the neutralization reactor as an aid to enhance the precipitation and clarification process.

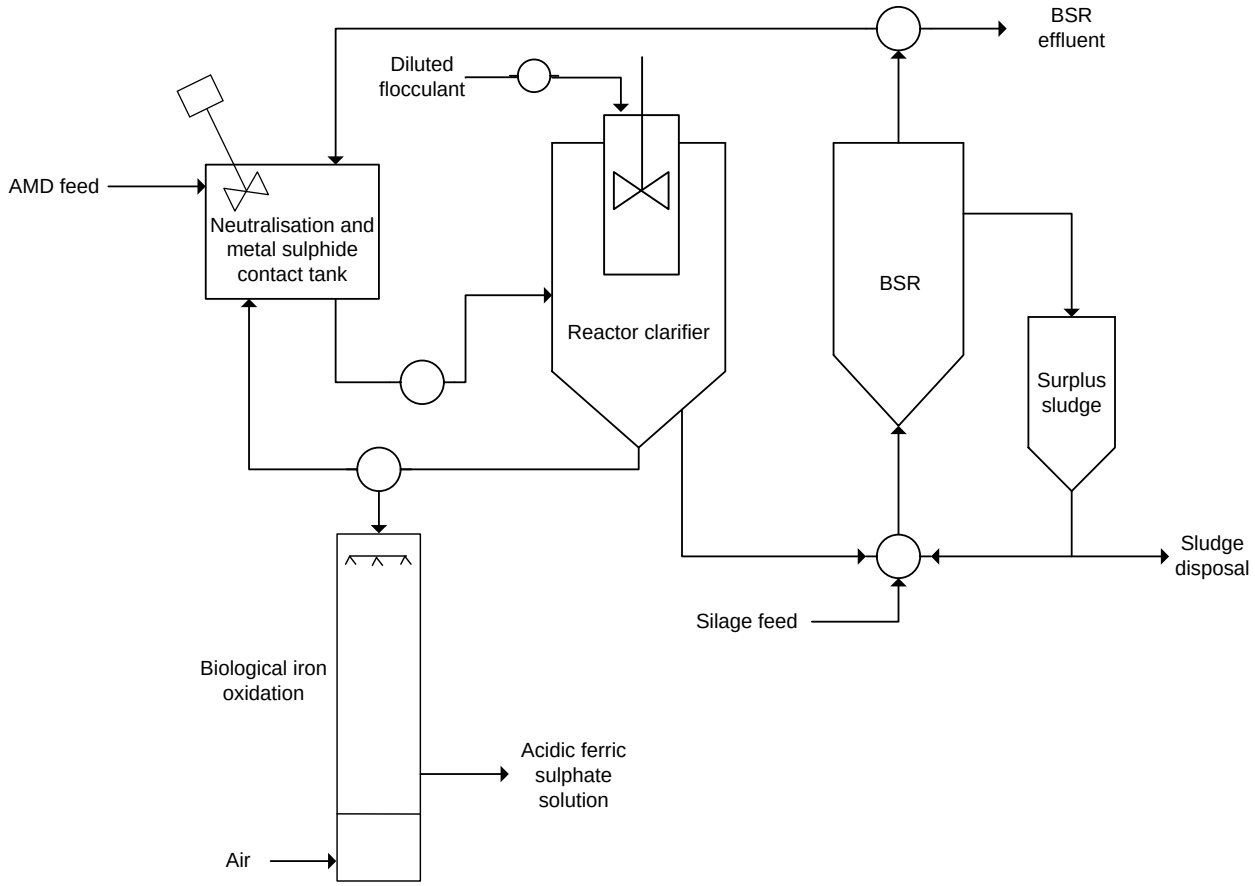


Figure 36 Schematic diagram to illustrate the acid neutralization and FeS precipitation process

A mass balance of sulphate around the mixed reactor can be used to calculate the recycle rate.

Mass of sulphate in = mass of sulphate out 28

$$Q_{in}[SO_4^{2-}]_{in}+Q_R[SO_4^{2-}]_{out}=(Q_{in}+Q_R)[SO_4^{2-}]_{mix} \quad 29$$

where:

| | |
|---|---|
| $Q_{in}$ | = AMD feed flow rate |
| $Q_R$ | = recycle flow rate |
| $[SO_4^{2-}]_{in}$ | = sulphate concentration in untreated AMD |
| $[SO_4^{2-}]_{out}$ | = sulphate concentration in BSR reactor effluent |
| $[SO_4^{2-}]_{mix}$ | = sulphate concentration in mixed reactor |

Dividing equation 29 by $Q_{in}$ and let $\alpha = Q_R/Q_{in}$, the recycle ratio, α, can be calculated by the following relationship:

$$\alpha = \frac{[SO_4^{2-}]_{mix}-[SO_4^{2-}]_{in}}{[SO_4^{2-}]_{out}-[SO_4^{2-}]_{mix}} \quad 30$$

Assuming a sulphate concentration of 3314 mg/L in the AMD feed and 250 mg/L in the BSR effluent or recycle, the effect of different recycle ratios on the precipitation of FeS and acid neutralisation was simulated using Visual Minteq. The results are illustrated in Figure 37.

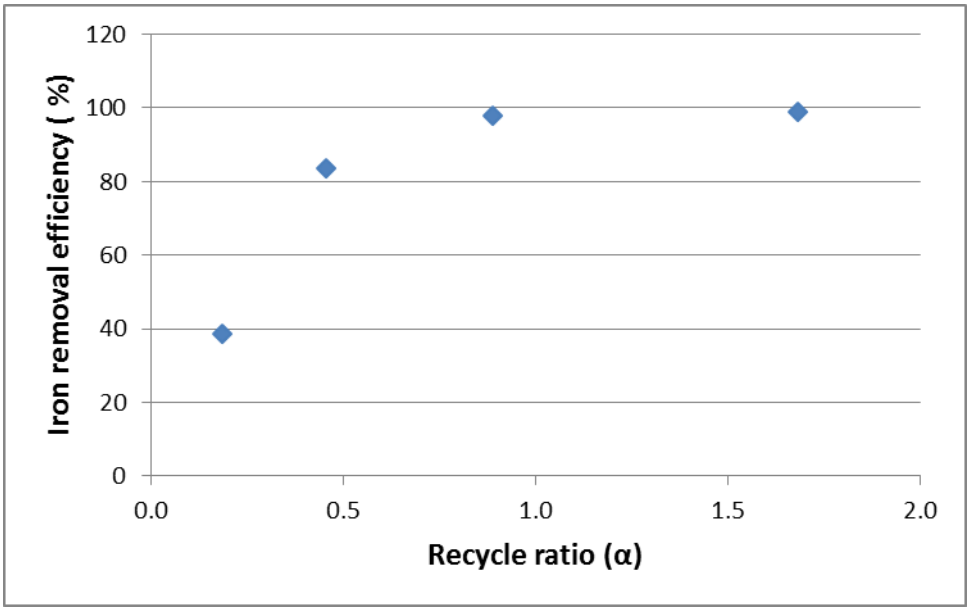


Figure 37 Effect of recycle rate on iron removal

Introducing recycling had a significant impact on the pH. As the recycle ratio increased, there was a rapid increase in the pH until the dissolved iron precipitated. When most of the FeS was precipitated, the pH increased further but approached a pH slightly higher than 6, which is close to the pKa value for the carbonic acid/bicarbonate weak acid and conjugated base pair (see Figure 38).

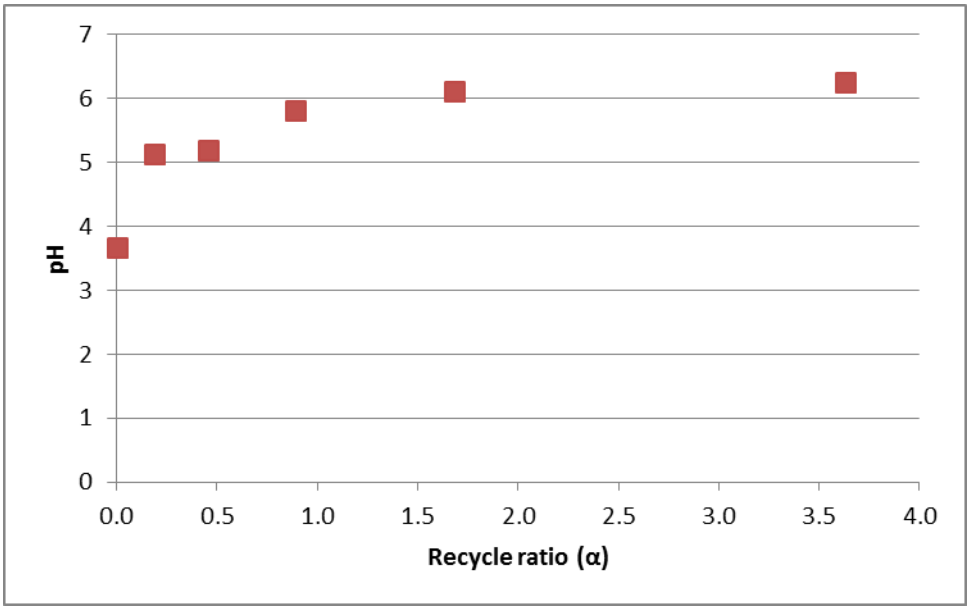


Figure 38 Effect of the recycle ratio on pH

It is interesting to simulate the partial pressures of carbon dioxide and hydrogen sulphide as well. There was initially only a slight increase in the partial pressure of hydrogen sulphide, due to the reaction with iron. At high recycle ratios, $\alpha \geq 2$, the total partial pressure approaches that of the ambient pressure. One can therefore conclude that the optimum recycle ratio is approximately 1. Incomplete iron removal will be observed at a recycle ratio <1, while hydrogen sulphide will be lost to the atmosphere at higher recycle ratios.

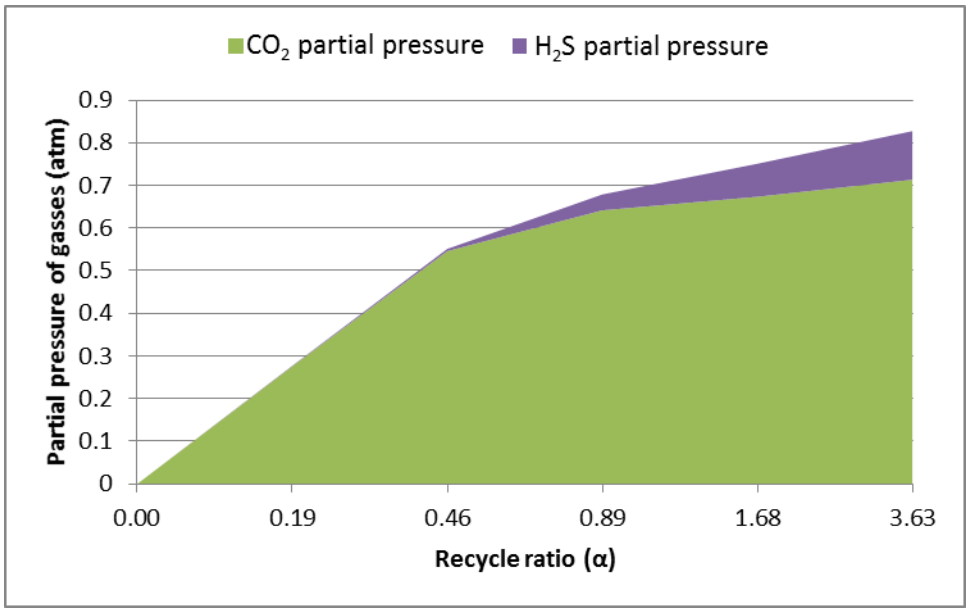


Figure 39 $CO_2$ and $H_2S$ partial pressures as a function of the recycle ratio

The composition of the water after partial acid neutralization and iron removal is summarized in Table 9.

**Table 9 Composition of mixed reactor**

| Component | Total dissolved (mg/L) |
|---|---|
| pH | 5.87 |
| $Ca^{2+}$ | 548 |
| $Cl^{-}$ | 71 |
| $CO_3^{2-}$ | 1,906 |
| $Fe^{2+}$ | 10 |
| $HS^{-}$ | 201 |
| $K^{+}$ | 17 |
| $Mg^{2+}$ | 193 |
| $Mn^{2+}$ | 56 |
| $Na^{+}$ | 110 |
| $SO_4^{2-}$ | 1788 |
| FeS(ppt) | 432 |

Based on a combined flow rate (feed and recycle) of 2 $m^3$/d, and assuming a hydraulic rate of 0.7 m/h in the reactor-clarifier, the diameter of the settling tank should be 390 mm. Based on previous experience, the polymer dosing rate was 0.75 mg/L or equivalent to 1.5 g/d. In order to achieve a concentration of 5 g Fe/L in the biological iron oxidation reactor, the FeS in the reactor-clarifier underflow should be thickened by maintaining an underflow rate of 7.2 L/h. The design parameters of the mixed tank and reactor-clarifier combined are summarized in **Table 10**. Variable speed pumps should be used to allow for process flexibility and optimization. It will not be practical to operate these units continuously with the small flow rates without the risk of blockages. The pilot plant was therefore operated in a sequencing batch mode and operated at preset time intervals, allowing higher instantaneous flow rates. The mixed tank and reactor-clarifier were combined in a single reactor with sufficient capacity to store the FeS between the sequencing stages. The biological iron oxidation reactor received FeS from this neutralization step as well as from the precipitation of sulphide from iron hydroxide, described further in section 6.1.4.5.

**Table 10 Design parameters of the mixed tank and reactor-clarifier combined in a sequencing batch reactor**

| Parameter | Unit | Design value |
|---|---|---|
| $Q_{in}$ | $m^3/d$ | 1 |
| α | (-) | 1 |
| $Q_{in}(1+\alpha)$ | $m^3/d$ | 2 |
| Residence time in mixed reactor | min | 30 |
| Mixed reactor volume (operated intermittently for 50% of the time) | $m^3$ | 0.084 |
| FeS in suspension | g/d | 863 as FeS or 548 (as Fe) |
| Underflow rate (for continuous operation) | L/h | 7.2 |

### 6.1.3 Biological sulphate reducing reactor: Pilot plant design and performance

The combined feed and recycle flow rate into the BSR is 1.83 $m^3/d$ when the underflow rate of 7 L/h is taken into consideration. It was originally intended that two vertical reactors would be used, to be operated in series or in parallel, each with a diameter of 0.76 m, equivalent to an area of 0.454 $m^2$, and a height of 1.84 m. However, a single reactor was used and the results indicated that a second reactor was not necessary.

The sludge blanket can be maintained at varying heights to determine the optimal hydraulic loading rate. An internal recycle from the top of the sludge bed to the bottom has been implemented and can be varied to determine the optimum recycle rate.

Approximately 10 kg of silage was dosed to the reactor daily. It was initially intended that silage would be fed to the reactor using a manually operated auger feeder. During the laboratory batch reactor trials, it became clear that when silage is placed in water with a low sulphate concentration, such as tap water, the pH remains at approximately 4 and there is very little deterioration in the VFA concentration of the silage over time, indicating that it remains self-preserved. From a practical point of view, this has made the storage and handling of the silage much easier. It can be dosed as a slurry into the BSR reactor, eliminating the difficulties associated with feeding of dry solids. It was also observed that when the silage particles were not wetted properly before being dosed, the captured air made them buoyant, causing them to migrate to the surface of the reactor.

The BSR reactor is currently operating at a sludge age of 20 days, based on the results obtained from the batch reactor tests (see section 5.1), but is still reaching steady state. Once steady state is achieved, this will be modified to optimize the process, if necessary.

A concern of the BSR process is the risk of releasing toxic hydrogen sulphide gas from the reactor. Based on the fact that acidity is consumed (or alkalinity is produced) at a rate of

1.042 g $CaCO_3$/g $SO_4^{2-}$ consumed, and using the AMD feed water quality in Table 8, the effect of the BSR process, using silage as carbon source, was simulated with the aim of reducing the sulphate concentration to 250 mg/L (see Table 11) using Visual Minteq version 3.0 (Gustavsson, 2013). Note that the results are reported in moles/L. All the simulations were done at 25°C. The results suggest that virtually all the iron precipitates as FeS. The calculated pH of the effluent is 6.38. The results suggest that 31% of the sulphide produced in the process is consumed when FeS precipitates. Another important consideration is the partial pressure of carbon dioxide at 0.78 bar and that of hydrogen sulphide at 0.16 bar. The water vapour pressure is 0.03 bar. The total expected vapour pressure is therefore 0.97 bar. Randfontein in the Western Basin is situated at an altitude of 1715 m above sea level. This corresponds to an atmospheric pressure of 0.82 bar, which is lower than the sum of the partial pressures. Closure of the reactors and containment of the gas is therefore an important consideration.

**Table 11 Composition of BSR effluent**

| Component | Total dissolved (mol/L) | % dissolved | Total precipitated (mol/L) | % precipitated |
|---|---|---|---|---|
| $Ca^{2+}$ | 0.01370 | 100.00 | 0.00000 | 0.00 |
| $Cl^-$ | 0.00200 | 100.00 | 0.00000 | 0.00 |
| $CO_3^{2-}$ | 0.06380 | 100.00 | 0.00000 | 0.00 |
| $Fe^{2+}$ | 0.00005 | 0.55 | 0.00995 | 99.45 |
| $H^+$ | 0.10612 | 100.00 | 0.00000 | 0.00 |
| $HS^-$ | 0.02196 | 68.82 | 0.00995 | 31.18 |
| $K^+$ | 0.00043 | 100.00 | 0.00000 | 0.00 |
| $Mg^{2+}$ | 0.00794 | 100.00 | 0.00000 | 0.00 |
| $Mn^{2+}$ | 0.00102 | 100.00 | 0.00000 | 0.00 |
| $Na^+$ | 0.00478 | 100.00 | 0.00000 | 0.00 |
| $SO_4^{2-}$ | 0.00260 | 100.00 | 0.00000 | 0.00 |

The water is scale-forming only with respect to the carbonates of calcium and magnesium. All the oversaturated minerals that may precipitate are highlighted in red (Table 12).

**Table 12 Possible solid species with the oversaturated species highlighted**

| Mineral | log IAP | Sat. index |
|---|---|---|
| Aragonite | -7.842 | 0.494 |
| Artinite | 2.132 | -7.468 |
| Brucite | 10.195 | -6.905 |
| $CaCO_3xH_2O(s)$ | -7.843 | -0.698 |
| Calcite | -7.842 | 0.637 |
| CaS(s) | 1.613 | -9.567 |
| Dolomite (disordered) | -15.904 | 0.636 |
| Dolomite (ordered) | -15.904 | 1.186 |
| Epsomite | -5.735 | -3.609 |
| $Fe(OH)_2$ (am) | 5.202 | -8.288 |
| $Fe(OH)_2$ (c) | 5.202 | -7.688 |
| FeS (ppt) | -3.6 | -0.65 |
| Gypsum | -5.514 | -0.904 |
| Halite | -5.222 | -6.772 |
| Huntite | -32.028 | -2.06 |
| Hydromagnesite | -22.054 | -13.288 |
| Mackinawite | -3.6 | 0 |
| Magnesite | -8.062 | -0.602 |
| Melanterite | -10.729 | -8.52 |
| $Mg(OH)_2$ (active) | 10.195 | -8.599 |
| $Mg_2(OH)_3Cl:4H_2O(s)$ | 11.213 | -14.787 |
| $MgCO_3:5H_2O(s)$ | -8.064 | -3.524 |
| MgS(s) | 1.393 | -16.287 |
| Mirabilite | -8.023 | -6.909 |
| $MnCl_2:4H_2O(s)$ | -11.405 | -14.12 |
| $MnCO_3$ (am) | -11.308 | -0.808 |
| MnS (grn) | -1.852 | -1.832 |
| MnS (pnk) | -1.852 | -4.832 |
| $MnSO_4(s)$ | -8.979 | -11.562 |
| Natron | -10.351 | -9.04 |
| Nesquehonite | -8.063 | -3.393 |
| Periclase | 10.196 | -11.388 |
| Portlandite | 10.415 | -12.289 |
| Pyrochroite | 6.95 | -8.244 |
| Rhodochrosite | -11.308 | -0.308 |
| Siderite | -13.055 | -2.465 |
| Thenardite | -8.019 | -8.341 |
| Thermonatrite | -10.348 | -10.985 |
| Vaterite | -7.842 | 0.071 |

### 6.1.4 Removal of sulphide from the effluent of the biological reactor

#### 6.1.4.1 Biological sulphide oxidation for sulphur production

It was initially proposed that all the sulphide in the effluent of the biological sulphate-reducing reactor be precipitated as iron sulphide, using iron hydroxide. There was, however, one important drawback to the process. The amount of magnesium in the AMD is not sufficient, and additional magnesium oxide or hydroxide will be required to recover all the sulphate in the feed, which will contribute significantly to the treatment cost. Biological sulphide oxidation is an attractive alternative for sulphur recovery.

It was intended that autotrophic denitrification with the SANI process would be applied for the removal of ammonia from the effluent of the biological sulphate-reducing reactor, and the laboratory-scale reactors were set up accordingly. As described in section 6.1.1 above, the nitrogen content of silage was low which resulted in very little ammonia in the effluent. As a result, the SANI process was unnecessary, but continued to run, and the conditions in the anoxic reactor were correct for the biological oxidation of sulphide to elemental sulphur. These requirements are described in more detail below.

#### 6.1.4.2 Biological sulphide oxidation process design

One of the key aspects of the oxidation of sulphide to sulphur is the production of hydroxyl alkalinity. It is not desirable to operate the reactor at high pH values (pH >8). Using the information of the BSR effluent quality in Table 11, the effect of sulphide oxidation was simulated. Based on the findings of the laboratory-scale tests, where elemental sulphur was produced in the anoxic SANI reactor, it was intended that two fluidised bed reactors would follow the BSR, with pH correction and calcium carbonate removal occurring in the first reactor through the introduction of air to strip off carbon dioxide, and sulphide oxidation occurring in the second reactor. The alkalinity produced in the BSR process effects calcium carbonate precipitation on the seed material. In the second reactor, hydroxyl alkalinity is produced in the process of sulphide oxidation (Figure 40), which is then recycled back to the first reactor. In this manner, there will be sufficient alkalinity to ensure that all the calcium (99.7%) is removed in the process while 63% of the carbonates are removed as a result of calcite precipitation. The results show that most of the calcite precipitates soon after commencing with aeration, with 94% of the calcium precipitated after 22% removal of the sulphide. This can be explained in terms of the stripping of carbon dioxide and the poor buffering capacity of the water at pH values close to 8. The advantage is that no additional chemicals are required for the softening of the water with respect to calcium hardness.

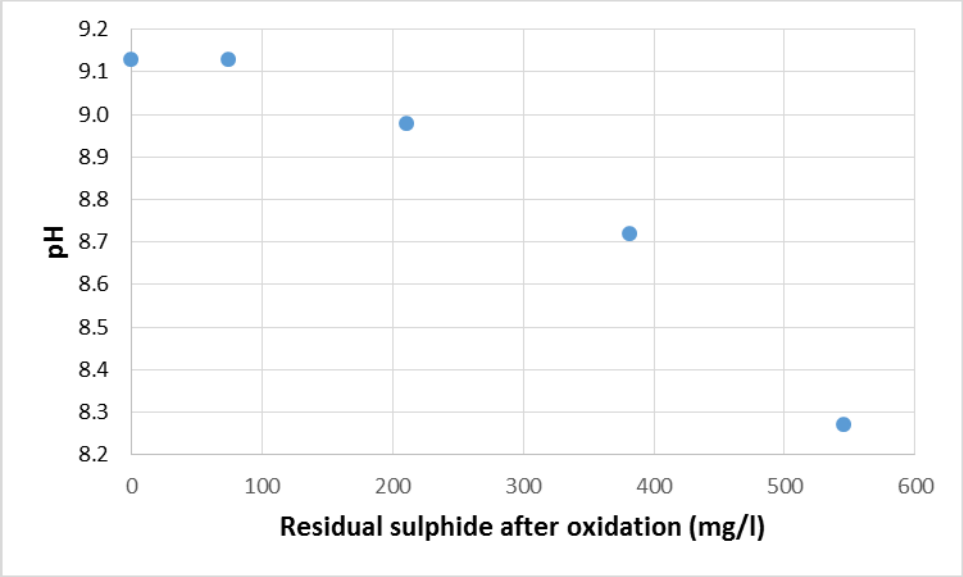

Figure 40 Increase of pH as a result of alkalinity produced during sulphide oxidation

The results of the laboratory-scale reactors suggested that fluidized reactors with sand as a support medium had the potential to support the growth of sulphide oxidizing bacteria (SOB) in the second reactor, as well as to act as seed material for calcium carbonate precipitation in the first reactor. As the calcium carbonate precipitates on the sand particles, they grow in size and density, and can be recovered from the bottom of the reactor as calcium carbonate pellets. In the laboratory-scale reactors, the sulphur formed in the sulphide oxidation reactor was removed from the sand as a result of abrasion and accumulated on top of the bed (Figure 41). In this manner, the sulphur concentration in close proximity to the biofilm was low and the reaction between bisulphide and sulphur was limited. The sulphide concentration at the top of the reactor, where the sulphur collected, was too low for significant side reactions. Due to the rapid growth of calcite on the sand particles in the first reactor, a biofilm was not established on the particle surfaces, thereby limiting the biological oxidation process to the second reactor. Based on these findings, it was planned that two 6 m-high transparent PVC pipes, each with a diameter of 65 mm, would be used in series in the pilot plant. Sand with a mean diameter of 510 µm was initially used, with the intention of maintaining the upflow rate at 21 m/h to fluidize the reactors. The second reactor was aerated to oxidize the sulphide, while the high pH effluent from the second reactor was recycled to the first reactor to effect calcite precipitation. Some operational problems were experienced during the start-up of the process due to the weight of the sand in the column and the distribution of air which resulted in poor carbon dioxide stripping.

Figure 41 Picture illustrating accumulation of biosulphur particles on top of fluidised bed reactor

In order to overcome the disadvantages associated with sand as a support media, plastic beads with a density just below that of water (approximately 0.98 g/cm$^3$) were used instead for the sulphide oxidation reactor. This ensured that the beads remained floating. Limestone powder was used for the calcium carbonate reactor to act as seed material for the precipitation of calcium carbonate. The particle size distribution of commercially available limestone particles is presented in Table 13. The settling rates of the particles were calculated, using the procedure as outlined by Richardson et al. (2002), and are presented in Figure 42.

**Table 13 Sieve analysis of commercially available limestone used for neutralization of AMD (Maree, 2014)**

| % of mass passed through the sieve | Metlime | LimeChem (-4.75) | Limedust |
|---|---|---|---|
| | Size (µm) | Size (µm) | Size (µm) |
| 10 | 4.27 | 18.05 | 3.91 |
| 20 | 8.07 | 31.8 | 5.56 |
| 30 | 13.56 | 46.32 | 11.9 |
| 40 | 17.47 | 58.08 | 21.39 |
| 50 | 20.89 | 67.36 | 30.67 |
| 60 | 24.64 | 75.89 | 43.06 |
| 70 | 29.58 | 84.77 | 52.88 |
| 80 | 37.7 | 95.45 | 61.21 |
| 90 | 52.86 | 100.6 | 71.08 |
| 95 | 66.11 | 105.6 | 79.39 |

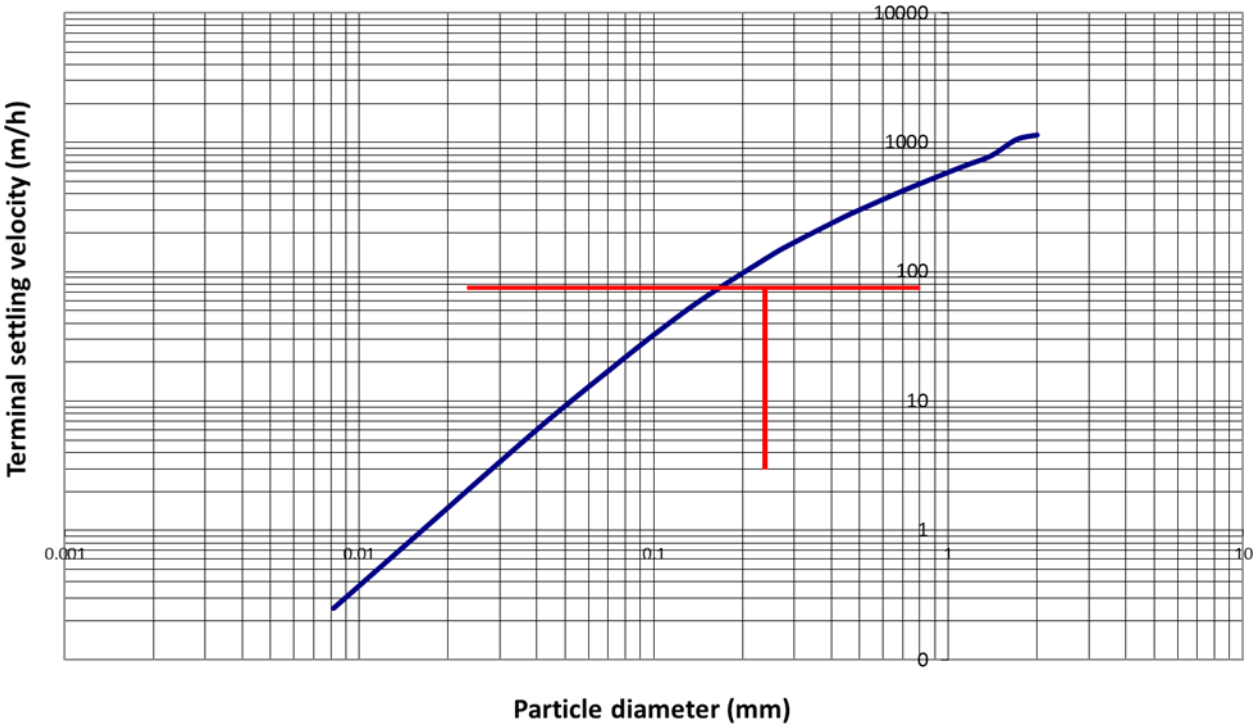


Figure 42 Terminal settling velocity of limestone particles in water as a function of particle size

The sparging of hydrogen sulphide gas from the effluent as a result of aeration was an issue with the first reactor. In order to minimize the loss of $H_2S$ and to streamline the process, a combined sulphide oxidation-calcium carbonate precipitation reactor was suggested. This also allowed for the internal recycling of hydroxyl alkalinity. A single 6 m column was then employed that contained both the plastic beads for sulphide oxidation and the limestone powder for calcium carbonate precipitation. The relative densities of the two materials ensured that they remained spatially separated in the column. The headspace gas was recycled to the biological iron oxidation reactor. Using limestone powder dispersed in the fluidized reactor instead of sand offers the advantages of lower minimum fluidization velocities and consequently less energy required for pumping and aeration, and a higher surface area per mass of material used. As the sulphur accumulates on top of the fluidized calcite bed and below the floating plastic media, harvesting of the sulphur can take place just above the liquid/calcite interface. Air is supplied at the bottom of the reactor to strip carbon dioxide and ascends through the plastic media with the sulphur-oxidizing bacteria attached to supply oxygen (Figure 43).

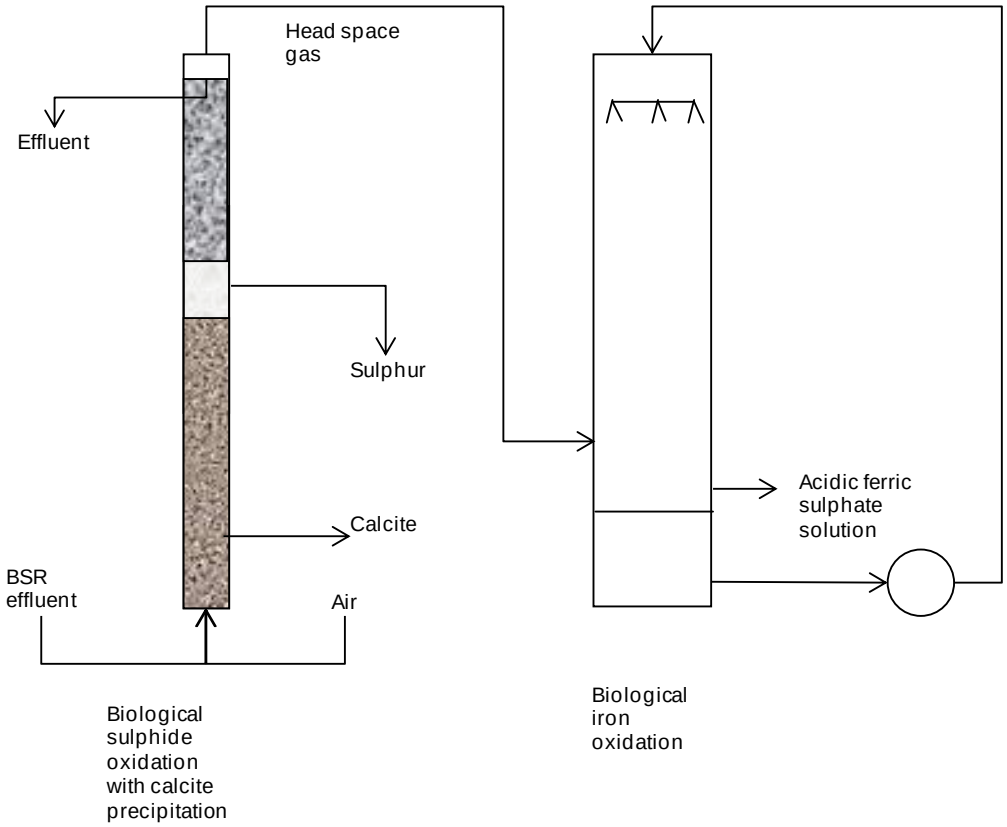


Figure 43 Biological sulphide oxidizing reactor combined with calcite precipitation and integrated with the biological iron-oxidizing reactor

#### 6.1.4.3 Removal of manganese and magnesium

Following the combined biological sulphide oxidation-calcium carbonate precipitation reactor, lime will need to be dosed in order to increase the pH to just below 10.73 in order to remove the manganese as manganese carbonate, but avoid the precipitation of magnesium hydroxide. Since 37% of the carbonic species remain in solution after the sulphide removal step, calcite will be precipitated simultaneously. This requires 148 mg/L hydrated lime. A further 370 mg/L lime is required to precipitate the magnesium as magnesium hydroxide, together with further calcium carbonate precipitation.

Two 6 m columns of clear PVC with a diameter of 65 mm have been included in the pilot plant for the manganese carbonate and magnesium hydroxide removal. As the synthetic solution did not contain heavy metals, there was no need to removed manganese. Once AMD is used, this process will be fully tested. Lime slurry was dosed to effect magnesium carbonate and calcium carbonate precipitation as mixed slurry.

The magnesium hydroxide can be recycled for the precipitation of iron hydroxide for sulphide removal. It will be of great advantage if the calcite can be separated from the magnesium hydroxide. Laboratory-scale trials using a fluidized bed reactor in an attempt to recover calcite as pellets in the presence of the flocculated magnesium hydroxide suspension are currently

underway. There is no information on this process, and a design of a pilot reactor, if the process can be demonstrated successfully, can only be offered later.

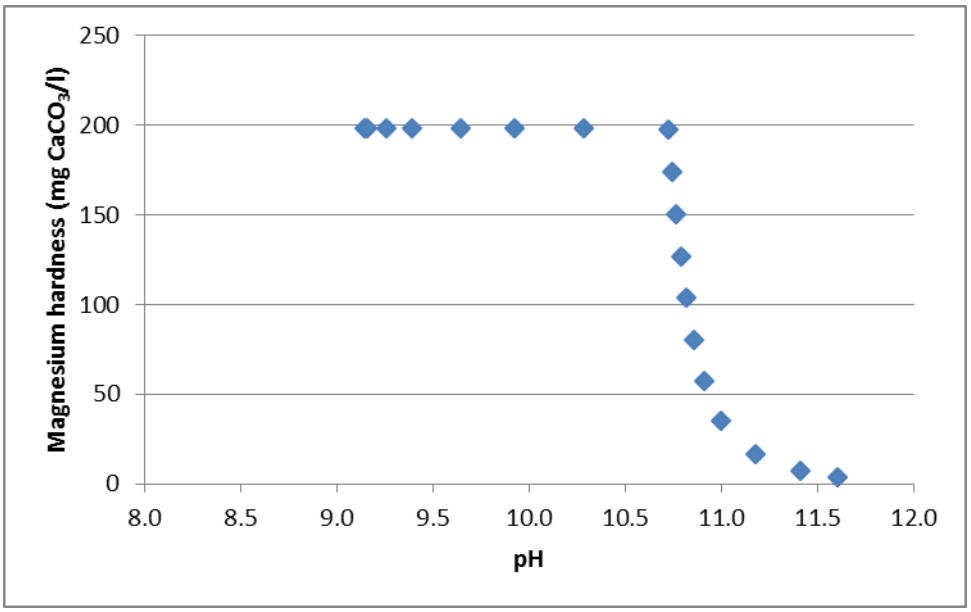


Figure 44 Magnesium hydroxide precipitation as a function of pH

#### 6.1.4.4 Stabilization of the effluent

After magnesium removal, the effluent had a pH higher than 11. Carbon dioxide is preferred for the pH adjustment since no ions will be introduced in the process and a surplus will be available from the process, for example, the stripping of carbon dioxide from the sulphide oxidation reactor and the calcining of calcite. The results of the simulation indicated that 66 mg/L carbon dioxide was required for pH adjustment. This was necessary to reduce the pH prior to the removal of residual sulphide as iron sulphide with the addition of iron hydroxide.

#### 6.1.4.5 Precipitation of sulphide with iron hydroxide

Because of the requirement that the ratio of sulphide to oxygen should exceed 2:1 to ensure the production of sulphur, only approximately 75% of the total sulphide in the effluent of the biological sulphate-reducing reactor can be converted to sulphur. There is therefore still a requirement for iron hydroxide precipitation and regeneration of the iron hydroxide.

Bench-scale studies were conducted with promising results demonstrating that iron hydroxide can be recovered and recycled, and that magnesium sulphate can be recovered as a by-product. Successful demonstration of this unit process indicated that an external supply of iron hydroxide may not be critical. The process works on the principle that biological iron sulphide oxidation at pH 2 will produce ferric sulphate. Bio-leaching of iron sulphide is an established process. The acid ferric sulphate solution is then neutralized with magnesium hydroxide or magnesium to produce soluble magnesium sulphate and insoluble ferric hydroxide. The iron hydroxide is recovered in a filter press and washed to produce a clean iron hydroxide cake. The iron hydroxide is re-suspended and dosed into the sulphide-rich

effluent from the BSR reactors to precipitate iron sulphide. In this manner, the iron is regenerated continuously. The magnesium sulphate can be recovered through evaporation in ponds or evaporators, or through crystallization using the principle of eutectic freezing or freeze crystallization. Since the feed AMD contains a high concentration of magnesium, magnesium hydroxide can be recovered using lime softening as a source of magnesium.

The proposed process is illustrated in Figure 45 below, followed by a more detailed description of each step of the overall process.

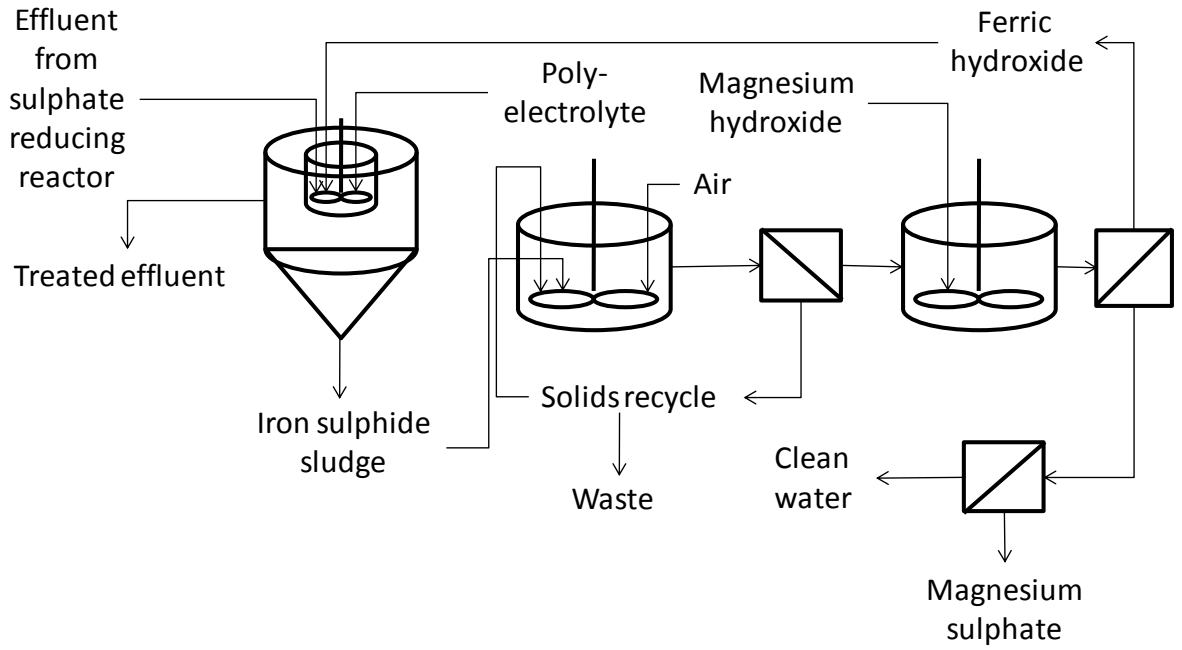


Figure 45 Proposed sulphide removal process

The process for sulphide removal may be described step-wise in more detail as follows:

**Step 1:** Precipitate sulphide with $Fe(OH)_3$ as $Fe_2S_3$

$$2Fe(OH)_3(s)+3H_2S \rightarrow Fe_2S_3(s)+6H_2O \quad 31$$

There is evidence that the $Fe_2S_3$ formed in the process is a mixture of FeS and elemental sulphur in a molar ratio of 2:1. Poulton et al. (2004) proposed the following reaction scheme:

In a surface complex formation, hydrogen sulphide diffuses to the surface of the iron hydroxide particle:

$$\equiv Fe^{III}OH+HS^- \leftrightarrow \equiv Fe^{III}S^-+H_2O \quad 32$$

This complex undergoes electron transfer:

$$\equiv Fe^{III}S^{-} \leftrightarrow \equiv Fe^{II}S \qquad 33$$

The oxidized product is then released from the particle surface:

$$\equiv Fe^{II}S + H_2O \leftrightarrow Fe^{II}OH_2^{+} + S^{\cdot\cdot} \qquad 34$$

Iron is then detached from the surface:

$$\equiv Fe^{II}OH_2^{+} \leftrightarrow \text{new surface site} + Fe^{2+} \qquad 35$$

Elemental sulphur is predominantly formed:

$$8\equiv FeOH + 8S^{\cdot\cdot} \rightarrow S_8^0 + 8Fe^{2+} \qquad 36$$

The Fe(II) formed may then react with additional hydrogen sulphide in the bulk solution to produce $FeS_s$.

$$Fe^{2+} + HS^{-} \leftrightarrow FeS_s + H^{+} \qquad 37$$

This mechanism suggests two very important aspects:

- alkalinity is consumed in the process
- elemental sulphur is produced.

The latter observation has a very important implication. While amorphous FeS is readily soluble in acidic solutions; elemental sulphur is not. It may therefore be possible to separate elemental sulphur by dissolving it in an acidic solution.

**Step 2:** Separate $Fe_2S_3$ from liquid using flocculation and gravity settling

Steps 1 and 2 (Figure 46) were practiced at the 10 Ml/day BioSURE plant operated in Springs, Ekurhuleni, treating extraneous water from Pamodzi Gold's mines. Iron hydroxide produced in the high-density settling process at the mine was used to precipitate sulphide. This process was attractive, since dissolved sulphide could be reduced reliably to concentrations below 10 mg/L in a simple process.

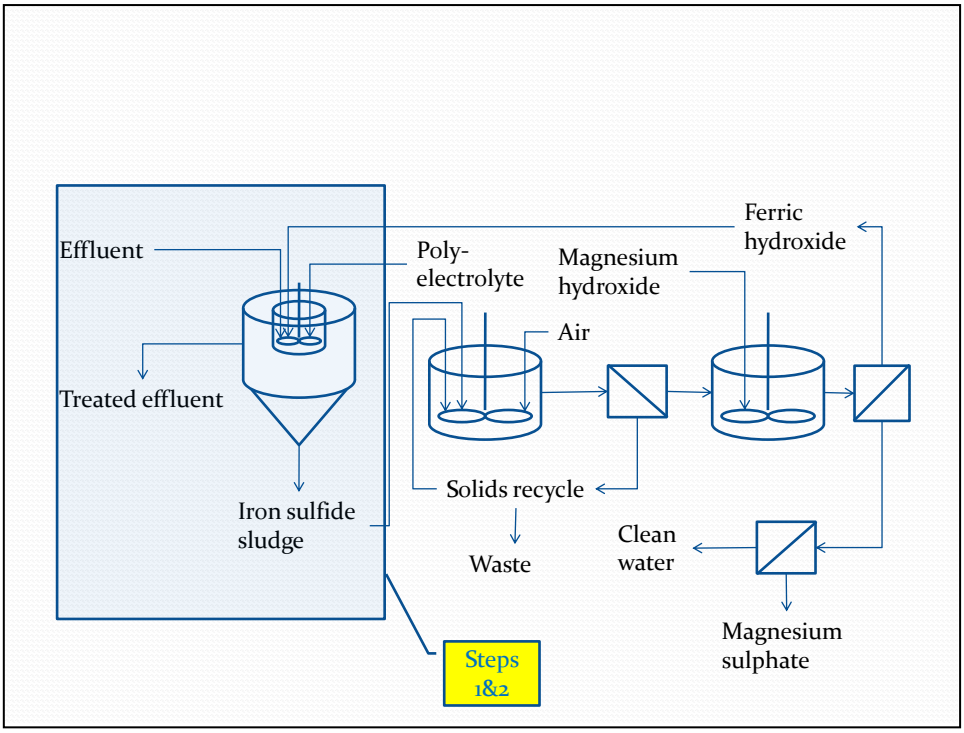


Figure 46 Steps 1 and 2 of the proposed sulphide removal process

Lime was used at the mine to precipitate iron hydroxide. It contained large quantities of particulate calcium carbonate. The particulate material contaminated the iron sulphide, making any further processing impossible. It also contained dissolved sulphate that contaminated the final effluent with a high risk of non-compliance. Final disposal of the solid material was costly and not without environmental risks. The experience gained when operating this process prompted further studies that led to the development of a process to regenerate iron hydroxide from precipitated iron sulphide and to recover magnesium sulphate as a by-product. Further processing of iron sulphide and recovery of useful by-products will improve the affordability of any BSR process.

The following treatment steps were investigated as a means of processing the iron sulphide in order to recover ferric hydroxide and valuable by-products.

**Step 3:** Oxidize $Fe_2S_3$ to $Fe_2(SO_4)_3$ and neutralize the acidity

This step (Figure 47) involves a biological ferrous oxidation process; its mechanism and kinetics have been studied extensively. The optimum pH for the reaction is approximately 2 and the reaction proceeds as follows:

$$Fe_2S_3(s)+6O_2 \rightarrow Fe_2(SO_4)_3 \qquad 38$$

It is important to note that carry-over of suspended organic material from the biological sulphide removal reactors may interfere with the C-autotrophic biological iron oxidation

process. Iron-oxidizing bacteria derive their energy from the oxidation of ferrous iron to ferric iron and their source of carbon from carbon dioxide. In the presence of high concentrations of organic material, aerobic heterotrophic bacteria will compete with and suppress the growth of the iron-oxidizing bacteria. Consequently, the presence of organic material may interfere with the biological iron oxidation process and must be removed first. Care must be taken to remove the suspended solids by gravity settling before dosing with $Fe(OH)_3$. The clarified effluent will be used as feed into this ferrous oxidation process.

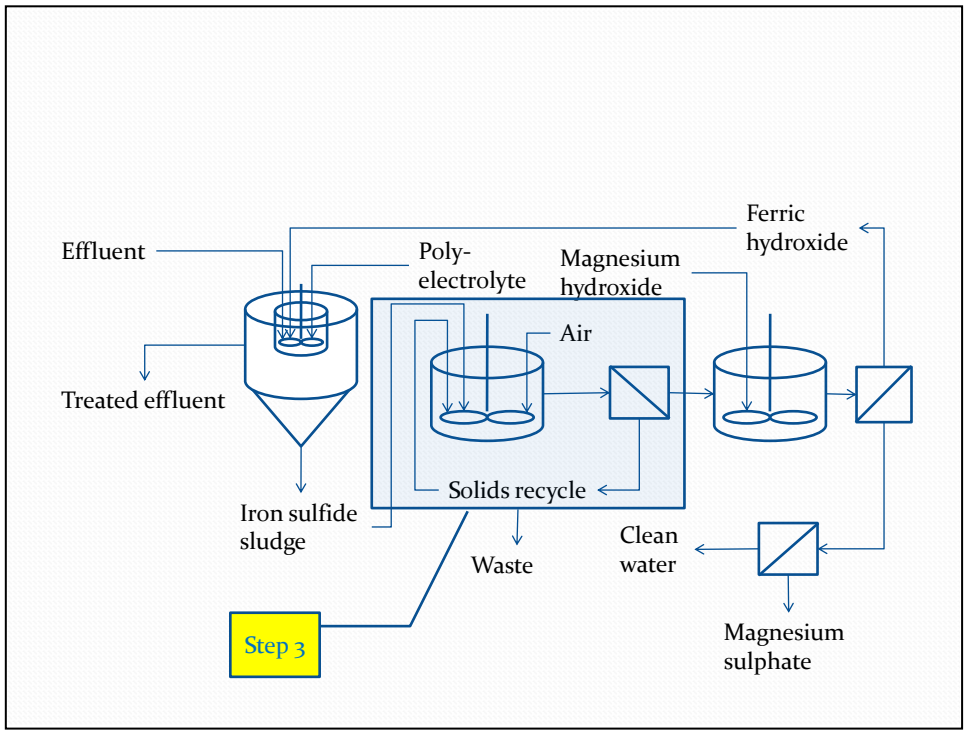


Figure 47 Step 3 of the proposed sulphide removal process

Raising the pH will cause iron hydroxide to precipitate. Alkalis such as $Mg(OH)_2$ or $MgCO_3$ can be used. The neutralisation and precipitation reaction with magnesium hydroxide is shown below:

$$Fe_2(SO_4)_3+3Mg(OH)_2(s)\rightarrow 2Fe(OH)_3(s)+3MgSO_4 \qquad 39$$

Precipitation of $Fe(OH)_3$ is complete at a relatively low pH so that all the $Mg(OH)_2$ will be dissolved. The $MgSO_4$ produced in the process has a high solubility. Precipitated $Fe(OH)_3$ will be the only solids in suspension and selective recovery of $Fe(OH)_3$ from the solution is possible.

Sodium-based alkalis such as $Na_2CO_3$ or NaOH are not considered to avoid the addition of sodium in the final effluent. $Ca(OH)_2$ or $CaCO_3$ will react with sulphate and precipitate as gypsum to produce a mixture of ferric hydroxide and gypsum, with no option to separate the iron hydroxide for reuse, and introduction of sulphate in the treated effluent.

Commercially available magnesium oxide will be used for this study to avoid dependence on magnesium hydroxide recovered from the proposed softening process.

**Step 4:** Separate $Fe(OH)_3$ from liquid and recycle to precipitate sulphide (step 1)

Gravity settling followed by a filter press or belt filter will be required to limit the mass of sulphate recycled (Figure 48). Recycling of iron hydroxide will ensure that the process is largely independent from an external source.

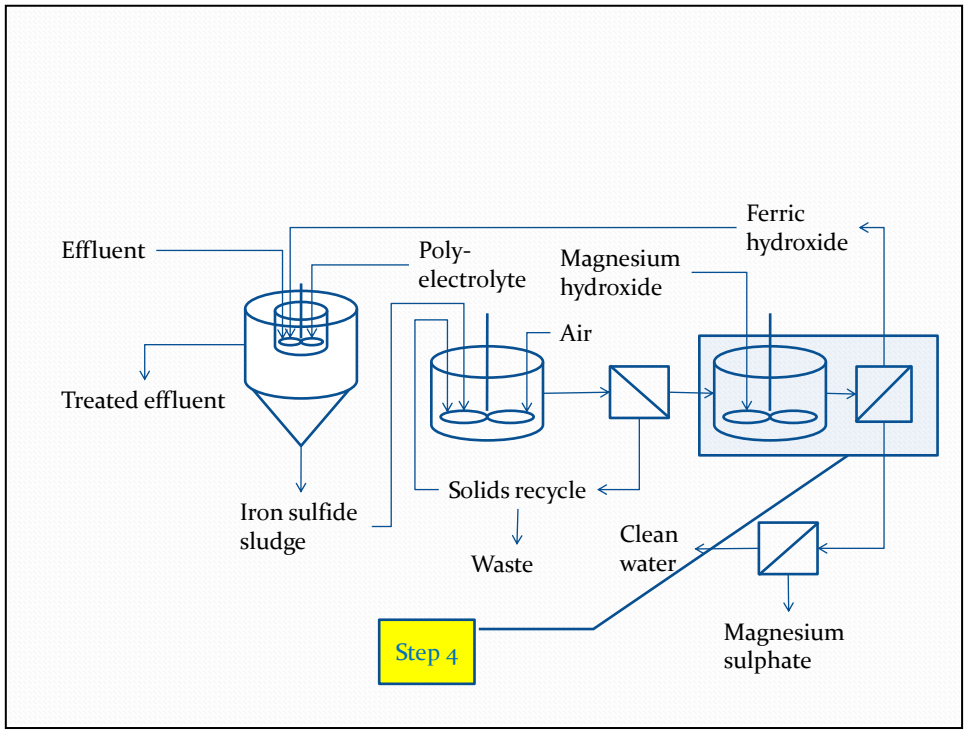


Figure 48 Step 4 of the sulphide removal process

**Step 5:** Concentrate $MgSO_4$ through evaporation or membrane technology, and crystallize

Evaporation, freeze crystallization or eutectic freeze crystallization (EFC) can be used to recover magnesium sulphate (Figure 49).

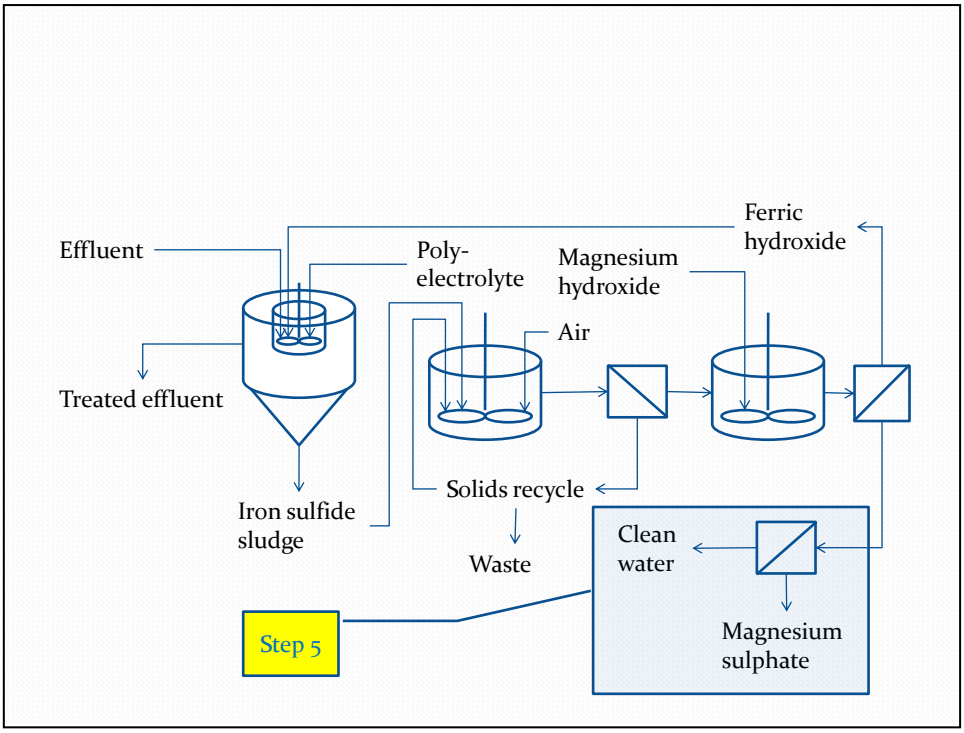


Figure 49 Step 5 of the sulphide removal process

This process illustrates that sulphide can be effectively precipitated from the effluent of BSR processes using recycled ferric hydroxide. It also demonstrates that valuable by-products may be recovered from the process, thereby reducing the disposal of waste products. The proposed process configuration consists of five steps. The individual steps make use of known technology and are integrated with each other. The purpose of the demonstration plant is to monitor each step under different operating conditions, with the emphasis on upstream disturbances and downstream impacts. This will allow for determination of reliable scale-up factors and identification of important critical operational issues.

### 6.1.5 Precipitation of sulphide with iron hydroxide: Pilot plant design and preliminary results

The same arguments apply for using a sequencing batch process for the precipitation of sulphide with iron hydroxide as for the acid neutralization and FeS precipitation reactor. As it is expected that only FeS will precipitate in the latter process, a single biological iron-oxidizing reactor will be used for both the FeS initially precipitated and that precipitated in the final sulphide removal step. The reactor is filled with rosettes, as illustrated in Figure 50. Magnesium hydroxide will be added to the acidic ferric sulphate solution and ferric hydroxide will be precipitated. The dewatered ferric hydroxide will then be dosed as a slurry to react with the surplus sulphide.

Figure 50 Biological iron oxidation reactor showing the distribution of recycled ferrous sulphate solution on top of the rosettes (right)

# 7 Design of demonstration plant

A schematic diagram of the proposed process for the demonstration plant, which is the next step in the scale-up of the technology, is presented in Figure 51. It is the intention that this diagram will be the basis for the demonstration plant engineering design, and as more information becomes available from the pilot plant this diagram will become more detailed, until the final process and instrumentation diagram (P&ID) can be compiled.

## 7.1 Silage logistics and dosing requirements

One of the main considerations for the feasibility of the process on a large scale is the availability and accessibility of a reliable carbon source. While silage may be applied as a supplementary source when there is a locally available source of COD that is easily available such as waste organic material or PSS, in some cases silage may be the sole carbon source for the process such as would be the case in more remote areas.

Maize has a high yield, in the order of 50 t/ha for dry land production under normal climactic conditions and 100 t/ha when irrigated. It is the intention that a portion of the treated effluent would be used for irrigation so as to ensure maximum yield. If a conservative biodegradability of 70% is assumed, approximately 9.2 t of silage are required for every Ml treated per day, based on a sulphate concentration of 3000 mg/L and a discharge limit of 250 mg/L. Therefore, for a 10 Ml/d plant, 92 t of silage per day will be required. If an average yield of 50 t/ha is assumed, this will require 672 ha of land. At R500/t, the cost for silage is R4-60 per kl treated. This is equivalent to R1750/t sulphate treated.

Ideally, the silage should be cultivated near to the intended plant site. This will minimise transport requirements. Practically, a farmer in the area should be contracted to produce the maize and silage on a dedicated basis. Close proximity of farmlands to the plant will allow for irrigation of crops with the treated water, resulting in a greater yield and smaller cultivated area.

Where a treatment plant is to be located in close proximity to a waste water treatment works, PSS may be a logical choice of carbon source, although maize silage could be cultivated on the sludge lands as an alternative. This offers the advantages of reducing the nutrient load to the land which may ultimately affect the quality of the groundwater, and will mean that a primary sewage conveyance pipeline will not be necessary.

The choice of carbon source will ultimately be site-specific, and will in most cases involve a combination of sources, with silage acting as a supplementary source. Where biodegradable industrial waste is available, disposal agreements should be put in place with those industries to offer a mutually beneficial relationship, potentially providing a source of income to the plant where those industries would otherwise pay for disposal in landfill.

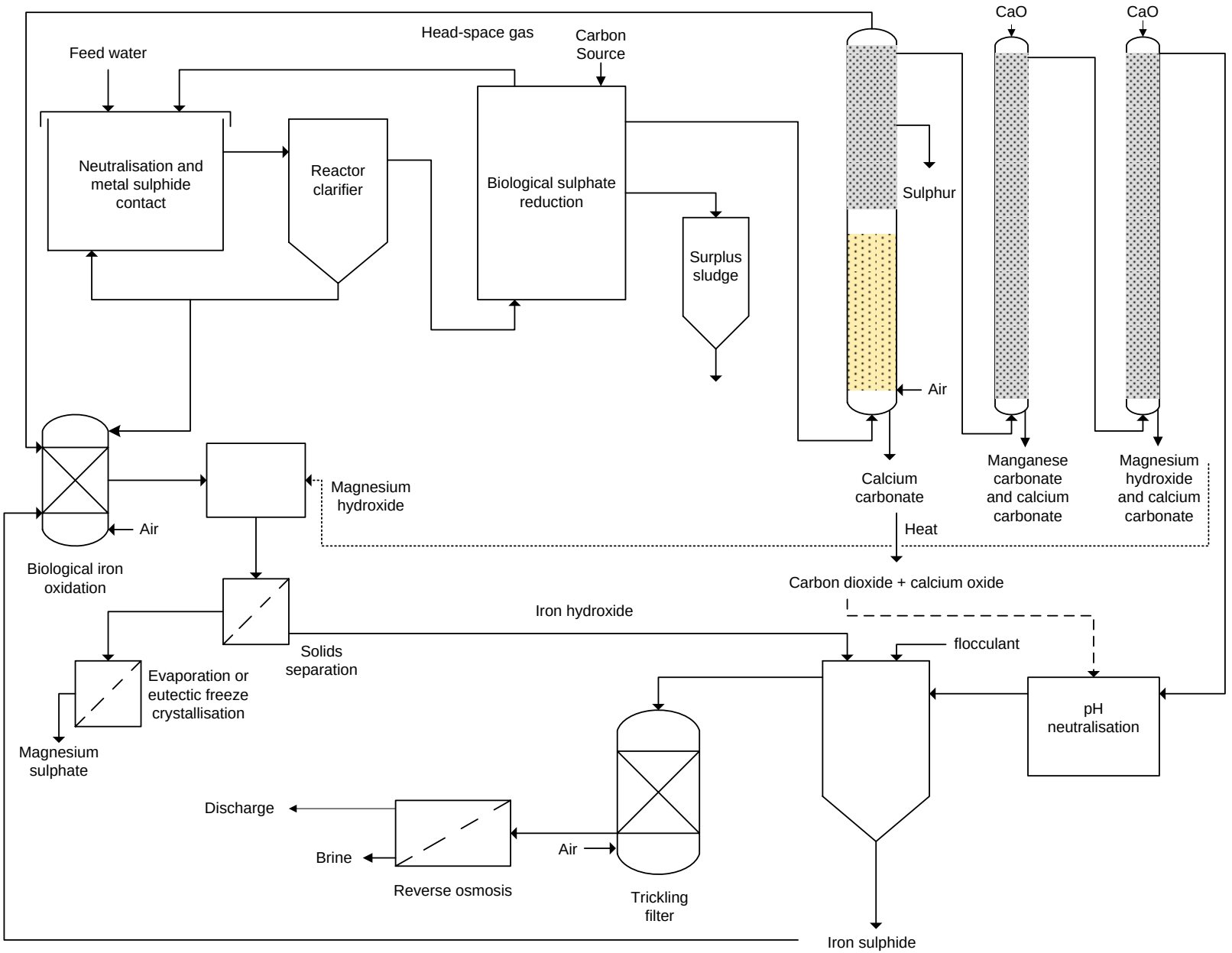


Figure 51 Schematic process layout of proposed demonstration plant

# 8 Novelty and advantages over the prior art; motivation for a new patent

## 8.1 Novelty over prior art

It is our opinion that, although some of the components of the above process are similar to those claimed in relevant patents, the process as a whole presents a unique solution to the treatment of acidic, sulphate- and metal-containing waste water, such as that which is typical of AMD. This is mainly due to the manner in which sulphide is removed from the solution, and the fact that the feed water can be neutralized and all the calcium can be removed using only biogenically produced alkalinity in the form of bicarbonate alkalinity and hydroxyl ions, without the need to introduce additional lime from an external source.

### 8.1.1 Rose patents (US6, 197196 and US6,203,700)

In the first US patent (US6, 197196, 2001), Rose and Hart proposed an "accelerated hydrolysis reactor" for the hydrolysis of complex carbonaceous material, with at least three settling "valleys" where material collects and is recycled, and where some sulphate reduction to sulphide takes place, followed by a separate sulphide-reducing reactor that received only readily biodegradable COD. In the EU patent (EP 1 124 763), this was reduced to a single reactor, but with the same design as the "accelerated hydrolysis reactor", where both hydrolysis and the biological sulphide reduction occur in the same reactor. This was renamed the "falling sludge blanket" reactor in this patent. The initial US patent specified the design of the BSR reactor as being a "UASB reactor, an expanded bed granular reactor, a stirred reactor, or the like", as it was separated from the accelerated hydrolysis reactor; but in the EU patent the design is very specific with respect to the BSR, defined as a falling sludge blanket reactor with the reaction occurring as the sludge falls through the solution. The BSR reactor in our process differs from this falling sludge blanket reactor, and, depending on the COD source, is likely to be either a UASB reactor or a fixed media reactor.

In our process, although biological sulphide production forms the core, the effluent is handled in a completely different manner to that described in the Rose et al. patents. The carbon source is specified as "sewage, settled sewage, settled sewage solids, tannery wastewater, brewery wastewater, starch manufacture wastewater and paper pulp wastewater". The carbon source in our proposed invention does include PSS, but also includes other industrial waste carbon sources such as abattoir waste, and most importantly, maize silage. No mention is made in the Rose patent applications of any fermented plant products as a potential carbon source.

In the Rose process, $H_2S$ gas is "seeped" from the reactor headspaces using an inert gas such as nitrogen. This is transferred to a gas separator to recover the hydrogen sulphide gas, a portion of which is brought into contact with the feed to precipitate metal sulphides. In our process, the hydrogen sulphide gas is not purged from the headspace of the reactor to recycle back to

precipitate the metal sulphides. The sulphides in solution are used for this purpose. The effluent is polished in two steps in the Rose patent, one is an elongated trench reactor for settling of solids and further biological suphate reduction, and the other a high-rate algal pond for polishing the effluent by removal of nitrates and phosphates. Our process makes use of an aerated trickling filter to biologically polish any remaining COD and ammonia from the effluent. It is expected that the ammonia concentration will be very low if maize silage is used as the carbon source.

In the Rose et al. patents, no mention is made of the removal of sulphides that remain in solution. It is expected that these would be oxidized back to sulphates in the high-rate algal pond (HRAP), thus defeating the objective of sulphate removal. US6, 197196, 2001 mentions the option to convert hydrogen sulphide gas to elemental sulphur in a sulphide oxidation stage, once it is purged from the headspace of the reactor and separated from the inert carrier gas, but no mention is made of whether this occurs biologically. It is stated that this step occurs in the gas phase. Our process deals with the sulphides in solution by removing them in a step-wise manner that results in little waste. That is, approximately 75% of the sulphide generated in the BSR reactor that is not used for metal sulphide precipitation in the first step will be converted to elemental sulphur biologically in a fluidised biological sulphide oxidation reactor. This reaction occurs in the liquid phase and not the gaseous phase as implied by the Rose patent. The remaining portion of sulphides is removed by blending the stream with iron hydroxide to effect the precipitation of iron sulphide.

After the Rose HRAP, algal biomass is harvested for use in a second HRAP, referred to as a “stress reactor” which is termed the “alkalization stage”. It is claimed that the algal biomass generates alkalinity which can be used for the neutralization of the feed water before it enters the accelerated hydrolysis reactor/BSR process. No mention is made of using alkalinity produced in the BSR process by the sulphate-reducing bacteria for the neutralization of the feed water and to effect metal sulphide precipitation.

This “alkalization reactor” is the subject of the second US patent by Rose et al. (US6,203,700, 2001), and the “biological alkalinity” that is referred to in this patent is the alkalinity generated in theory by the algae, not the sulphate-reducing or oxidizing bacteria as in the case of our process. It can be argued that although the algae may increase the pH of the water through photosynthethesis and the uptake of carbon dioxide, this is a temporary situation that is diurnal due to the cyclical nature of photosynthesis and respiration, where oxygen is taken up and carbon dioxide released in the dark. Algae themselves do not generate bicarbonate alkalinity or hydroxyl ions.

### 8.1.2 Rowley patent (US 5,587,079)

A US patent filed by Rowley et al. in 1996 ("Process for treating solutions containing sulphate and metal ions") was cited as prior art during the Rose patent application process. According to the claims of the Rowley patent, the BSR reactor is fed by the products of a partial oxidation burner (CO and $H_2$) that is fed with hydrocarbon fuel, therefore the BSR process is considered autotrophic. Mention is made of the possibility to include a "carbon nutrient" with the purpose of generating carbonate ions. No mention is made of specific carbon sources such as sewage sludge, organic industrial waste or maize silage. It is claimed that the effluent of the BSR process can then be aerated to precipitate carbonates in series, specifically magnesium carbonate and calcium carbonate. A batch-wise settling process is proposed, which differs from the fluidised pellet bed that is proposed in our process. It must be argued that it will not be possible to precipitate magnesium carbonate, rather that magnesium can only be removed as magnesium hydroxide by the addition of lime to increase the pH to above 10.73. No mention is made of the addition of lime. In our process, lime is added to effect the precipitation of magnesium hydroxide. This lime is a product of the process through the hydration of the calcined calcium oxide from the first calcium carbonate precipitation step.

In the Rowley process, $H_2S$ is stripped from BSR solution, not just seeped from the headspace as in the Rose patent. The carrier gas is nitrogen from the partial oxidation burner. The $H_2S$ gas is added to the feed to precipitate metal sulphides. This is the same as in the Rose patent; in our process, the sulphides remain in solution.

The Rowley process refers to the precipitation of the remaining sulphides as sulphide salts; alkaline sodium or calcium salts are added to the stream to create calcium or sodium sulphide salts. As described above, our process proposes that the sulphides are removed by biological sulphide oxidation to form elemental sulphur, followed by precipitation of the remaining sulphides as iron sulphide by contacting the stream with ferric iron hydroxide.

### 8.1.3 Advantages over the prior art

The modified BioSURE Process has various advantages over the prior art described in the relevant patents above:

1. The use of maize silage as a carbon source has an advantage over other carbon sources mentioned in the prior art in that it can be stored for long periods of time without loss of integrity. It can therefore be used to supplement inconsistent supply sources, such as organic industrial waste, without risk to the process. Maize silage is also much lower in protein than other carbon sources such as sewage sludge or industrial organic wastes, and therefore the ammonia concentration in the effluent of the BSR will be much lower

than when these carbon sources are used. The requirement for a polishing step is therefore minimal.

2. It is possible to selectively precipitate only iron as iron sulphide in the neutralization/metal precipitation step. Prior art claims that sequential metal sulphide precipitation can be effected in this step by recycling the alkalinity produced in the BSR process, but it is not possible to remove any other metal in this stage using only biogenically generated carbonate alkalinity from the BSR.
3. It is possible to selectively precipitate calcium carbonate as calcium carbonate pellets after the BSR process, without the need to add an external source of lime. The biogenic bicarbonate alkalinity from the BSR process and the recycled hydroxyl ions from the biological sulphide oxidation process are sufficient for this purpose.
4. It is possible to sequentially separate manganese carbonate and magnesium hydroxide by the addition of hydrated lime from the calcining process. An external supply of lime is, therefore, again not necessary. Both the manganese carbonate and magnesium hydroxide will be unavoidably contaminated with precipitated calcium carbonate. The manganese carbonate will be a stable waste product, but the magnesium hydroxide/calcium carbonate stream can be utilized in the process for the neutralization of the effluent of the biological iron-oxidizing reactor.
5. The sulphide that is not removed by biological sulphide oxidation is precipitated as iron sulphide by the addition of iron hydroxide. This, together with the iron sulphide precipitated in the first step, can be oxidized in the biological iron-oxidizing reactor so as to continuously regenerate and recycle the iron hydroxide, and the process is therefore not dependant on a continuous external supply source.
6. The biological sulphur that is produced differs from that which is produced chemically in that it is hydrophilic. This makes it highly sought after as it can easily be wetted, such as required for soil conditioning applications.

## 9 Renaming of the BioSURE Process

During the reference group meeting held for the project on the 3rd of December, 2014, it was agreed by all reference group members that a name change from the current “Rhodes BioSURE Process” should be implemented for the following reasons:

1. The current name does not have a significant market presence, and the image that it has is not necessarily positive
2. There are perceived problems with the BioSURE Process such as the production of large amounts of iron sulphide sludge and the availability of a reliable carbon source. These have now been solved through the regeneration and recycling of iron hydroxide and the employment of maize silage as a reliable and stable carbon source.

3. The process has changed significantly and materially from the original process developed and patented by Rose et al., upon which the BioSURE Process was named. The changes include both significant modification to the existing unit processes, deletion of others, and the addition of new unit processes, as discussed in Section 8 above.
4. A provisional patent application was filed for the new process in July 2014, followed by a PCT patent application and full patent application for Argentina in July 2015, which effectively separates it completely from the original patents.
5. The CSIR are also referring to their biological process as “BioSURE”, and this has caused confusion.
6. A new name is an opportunity to re-launch the technology.

It was decided that there is no need to keep any portion of the original name. It was suggested that the four biological processes that are integrated to form the full process be used as the basis for the new name. These include:

1. Biological sulphate reduction (SuRe)
2. Biological sulphide oxidation (SuOx)
3. Biological iron oxidation (Ferrox)
4. Nutrient removal in a trickling filter (Trickling/Tertiary)

In this light, VitaOne8 has proposed that the name be changed from BioSURE to VitaSOFT, with “Vita” meaning living, and the acronym SOFT referring to the four biological processes above. SOFT also refers to the non-biological chemical softening processes that are applied, including the precipitation of calcium carbonate using only biogenically produced alkalinity, and the addition of lime to remove manganese as manganese carbonate and magnesium as magnesium hydroxide. The VitaSOFT logo is presented in Figure 52 below.


Figure 52 VitaSOFT logo

# 10 Opportunities for technology demonstration

## 10.1 200 $m^3$/d demonstration plant in the Western Basin

A the short-term intervention on the Witwatersrand involves treating mine water in HDS (high-density sludge) processes to a level that it can be discharged to the environment. In the medium term, it is planned to implement reverse osmosis to remove salts from the water. These treatment options are costly, with high ongoing operational costs and the production of large amounts of sludge and brine.

The VitaSOFT technology has the following advantages when compared with other technologies for AMD treatment:

- It has the potential to eliminate the need for a HDS neutralization step, as sufficient alkalinity is produced biogenically in the BSR process to neutralize the AMD and to precipitate the dissolved metals as metal sulphides and carbonates. This will reduce the volume of solid waste, with the potential to recover valuable by-products.
- It is possible to selectively precipitate calcium carbonate as calcium carbonate pellets after the BSR process, without the need to add an external source of lime. The biogenic bicarbonate alkalinity from the BSR process and the recycled hydroxyl ions from the biological sulphide oxidation process are sufficient for this purpose.
- There is the potential for co-disposal of industrial biodegradable organic waste such as dairy and abattoir waste, which offers an opportunity to recover some of the operating costs by charging industries for disposal of solids that may otherwise require costly and risky disposal in landfill. The use of maize silage as a carbon source has an advantage over other carbon sources in that it can be stored for long periods of time without loss of integrity. It can therefore be used to supplement inconsistent supply sources, such as organic industrial waste, without risk to the process. Maize silage is also much lower in protein than other carbon sources such as sewage sludge or industrial organic wastes, and therefore the ammonia concentration in the effluent of the BSR will be much lower than when these carbon sources are used. The requirement for a polishing step is therefore minimal.
- The process serves as an effective pre-treatment process for a reverse osmosis membrane system, with the fouling potential greatly reduced due to the removal of sulphates, calcium, magnesium, manganese and alkalinity. Because there is only a requirement to remove mono-valent ions in the reverse osmosis system, lower pressures can be applied, thus saving energy.

The anticipated CapEx and OpEx costs of the VitaSOFT technology compare very favourably with other popular technologies, especially when viewed over an expected life of more than five years.

The estimated CapEx of the plant is comparable to that of a biological nutrient removal WWTP, at R7 million to R10 million per 1000 $m^3$ treated, depending on site-specific conditions. The OpEx can be estimated at approximately R7.50/$m^3$, with minimal waste disposal requirements. The capital cost is comparable to that of an HDS/RO process, with the OpEx of the HDS/RO estimated at approximately R9.00/$m^3$. This excludes waste disposal and limestone costs, which are the highest contributors. This is based on information from the long-term feasibility study conducted by the Department of Water and Sanitation (formerly the Department of Water Affairs) (DWA, 2013).

The goal of the project is to demonstrate the VitaSOFT Process on a scale of 200 $m^3$/d in the Western Basin of the Witwatersrand, to prove the suitability of the technology for AMD treatment in the long term. The technology is aimed not only at the effective treatment of toxic AMD water, but also to create a potential industrial water resource, to reduce the load of organic matter requiring disposal into the environment, and to produce valuable by-products. The project deliverables include the plant design and construction and its successful operation, with the objective of gathering sufficient data on the newly developed unit processes to ensure successful commercialisation.

At the start of this project, the water quality in the Western Basin was viewed as the 'worst-case scenario', as the water had the highest acidity concentration, the lowest pH, the highest concentration of sulphates and the highest concentration of dissolved heavy metals of the three basins. As such, the water in this basin is of an ideal quality on which to demonstrate the acid neutralising and metal precipitating abilities of the process. There are obvious advantages to proving the technology on a "worst-case" water source, which will lend credibility to the process. The latest water quality reports for the Western Basin do, however, indicate that the water quality is improving with time with respect to pH and heavy metals concentration, most likely due to the current pumping and treatment activities.

Various by-products may be generated in the process. These include magnesium sulphate, elemental sulphur and calcium carbonate. As yet, there are no off-take agreements in place for any of these products, and a study should be undertaken to determine the market potential. The sale of by-products, together with the revenue from waste disposal, present an opportunity to recover some of the process operating costs on full scale, and there would be value in demonstrating a market for these products at the demonstration stage.

The treated water may be viewed as a resource, and the Department of Water and Sanitation (DWS) will need to explore the potential for the reuse of this water.

According to the mine water treatment agreement, DWS will only consider a 3 Ml/d plant producing effluent compliant with the intended reuse as a successful AMD treatment technology

demonstrator. The period of operation should be sufficient to take diurnal, weekly, monthly, seasonal, and annual patterns in weather, influent, and operating conditions into account. As it will not be possible to scale the plant from its current 1 $m^3$/d size to 3 Ml/d, it is planned to design the plant in such a way as to have a scalable modular unit from which a full-scale design can be extrapolated, while complying with the monitoring requirements set by DWS. It is intended to demonstrate that the VitaSOFT technology can produce water of either industrial water quality, quality suitable for discharge to the environment, or potable water, so all of the relevant parameters will need to be measured.

The demonstration phase of the project will consist of eight work packages, each comprising specific milestones:

- WP1: Identification of a suitable site, and evaluation of site-specific water quality, including conceptual process design
- WP2: Full engineering design, planning and preparation, including compilation of P&IDs
- WP3: Procurement of materials and allocation of human resources
- WP4: Plant manufacture and on-site installation
- WP5: Plant commissioning and on-site laboratory establishment, and operations handover
- WP6: 18-month plant operation
- WP7: Compilation of draft operations and maintenance manual for full-scale plant
- WP8: Drafting of commercialisation strategy and business plan

## 11 Conclusions

Maize silage was identified as an alternative carbon source to PSS (primary sewage sludge), with various advantages over PSS, such as the ability to store it for long periods of time without loss of quality, a higher percentage biodegradability when compared with PSS, and a lower nitrogen content. It was hypothesized that sufficient alkalinity could be generated in the BSR process to neutralize AMD and to precipitate contaminating metals as metal sulphides without the need for an upstream HDS process. Biological iron oxidation was suggested as a means to regenerate the iron hydroxide required for sulphide removal, so that a constant supply would not be required. Finally, it was suggested that the effluent of the BSR process could be softened and stabilized by removal of calcium carbonate as magnesium hydroxide, to reduce the salinity of the water in order to meet the final effluent standards for discharge.

These hypotheses were tested first on bench scale and then in a 1 $m^3$/d pilot plant. The pilot plant was constructed and operated at VitaOne8's research and development facilities in Pretoria, using synthetic water resembling that of the Western Basin of the Witwatersrand.

It was demonstrated that maize silage is a valid alternative to PSS as a carbon source for BSR, which can be applied either as a supplementary carbon source where PSS is available, or as a primary source where there are no alternatives. The lower nitrogen content of silage when compared with PSS resulted in a lower ammonia concentration in the BSR effluent. The implication of this is that there is no requirement for integration of the process with a WWTP as was required for the BioSURE Process at Grootvlei mine, or for an alternative nitrification/denitrification step. It was planned to test the SANI process (sulphate reduction, autotrophic denitrification and nitrification integrated process) as an option for ammonia removal, which was ultimately unnecessary. However, the reactor configuration for the SANI process enabled the fortuitous and unintended occurrence of biological sulphide oxidation, the observation of which led to the integration of a biological sulphide oxidation reactor into the process. Results have shown that a significant portion of the sulphide can be removed biologically as elemental sulphur, further reducing the requirement for iron hydroxide.

It was demonstrated that sufficient alkalinity is generated biogenically between the biological sulphate-reducing and sulphide-oxidizing processes to not only neutralize the incoming AMD, but also to precipitate all the calcium in the water as calcium carbonate without the need for lime addition. Lime was demonstrated to only be required for the removal of manganese and magnesium in a two-stage process. The cost saving of lime and limestone is therefore two-fold; there is no requirement for an upstream HDS process, and less lime is required for desalination than would typically be required without the contribution of the biogenic alkalinity.

Biological iron oxidation was successfully demonstrated as a viable means to regenerate iron hydroxide from iron sulphide.

Sufficient information was therefore generated to confirm the validity of the process. It differs significantly from the original patents by Rose et al., and the BioSURE Process as applied at the Ancor site for Grootvlei mine. In this light, a provisional patent was filed in July, 2014, where the integrated process was described. The novelty and advantages over the prior art are described in more detail in this document. A PCT patent application was filed for the process in July, 2015, as well as a full patent application in Argentina, which is not a PCT member country.

Because the newly developed process differs sufficiently from the initial patents and original BioSURE Process, the decision was taken to change the name of the process to VitaSOFT, an acronym that refers to the four integrated biological processes.

Based on the results of the pilot study, a preliminary process design for a 200 $m^3$/d demonstration plant has been developed. The technology presents a viable long-term treatment solution for the treatment of mine impacted water.

The operation of the bench-scale reactors needs to be continued until steady state is achieved, to determine the kinetic values and for comparison with the established results where only PSS was used. The operation of the pilot reactor will also be continued in order to produce sufficient effluent to determine the operating parameters of the downstream processes more accurately.

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