Lessons in passive treatment: Towards efficient operation of a sulphate reduction – sulphide oxidation system

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Abstract Passive or managed passive treatment systems are an attractive alternative to conventional systems for AMD treatment, particularly for persistent, low-volume flows. The integrated managed passive (IMPI) process was developed in South Africa and has been implemented at demonstration scale. The paper describes parallel laboratory studies aimed at improved understanding of particularly the sulphide oxidation component. The data illustrate the importance of hydrodynamics, maintaining the correct balance between sulphide and oxygen and the importance of organic carbon in floating sulphur biofilm formation. Lessons from this study will improve performance of the demonstration plant.

Key Words acid mine drainage, passive treatment, sulphate reduction, sulphide oxidation

Introduction

Acid rock drainage (ARD) is currently a significant problem within the mining and minerals processing sector of South Africa. The biological treatment of mine waters has received increased attention due to its potential as a sustainable and economically attractive alternative to chemical treatment. Moreover, the development of a long-term sustainable technology would be a highly preferable alternative to the current treatment methods. This is due to the fact that many mines have been abandoned and therefore a cheap alternative to process the wastewater is required.

The Integrated managed passive (IMPI) system was developed by Pulles Howard and de Lange, in association with Rhodes University. The process relies on a combination of biological sulphate reduction, utilising complex organic carbon sources, and sulphide oxidation to remediate partially treated mine water. As most ARD is derived from pyrite (FeS_2), the resulting sulphate load is typically higher than the metal load, so even if metal sulphide precipitation is engineered into the process a sulphide mediation step will be required. Studies conducted by the Environmental Biotechnology Research Unit (EBRU) at Rhodes University and Golder Associates Africa (GAA) suggested that biological sulphide oxidation in a floating sulphur biofilm was a potential technology that could be utilised in the passive treatment system to achieve this. This led to the development of the linear flow channel reactor (LFCR) and the application of the IMPI technology at demonstration scale at an operational coal mine in the Mpumalanga region of South Africa. However, the demonstration plant, particularly the sulphide oxidation component, has not yet operated efficiently at its full potential. A lack of fundamental kinetic and mass balance information has contributed to this.

Therefore, a key aim of the current work was to conduct a laboratory scale study on the integrated system in order to generate the necessary kinetic and mass balance information. In addition the hydraulic regime within the LFCR was characterised to assess whether the original assumption of perfectly mixed plug flow within the system was justified. Finally, the effect of operation under a range of different conditions on the microbial population was investigated by studying the microbial ecology. These data are imperative for the characterisation and efficient operation of the demonstration plant.

Methods

Experimental system

The experimental setup consists of two sulphidogenic degrading packed bed reactor (DPBR) columns, packed with lignocellulosic material and three purpose-built LFCRs. The DPBRs were fed from the bottom as fully saturated reactors to minimise channelling and promote reactor stability. The columns were fed a synthetic solution, simulating partially treated AMD (2 000 mg/L sulphate), at a rate of 4 L/day. The synthetic AMD was supplemented with molasses (1.5 g/L) to promote sulphate reduction and mimic the operation of the demonstration plant. The column effluent was sampled every second day to determine pH, redox potential, sulphate, sulphide and occasionally to determine volatile fatty acid (VFA) concentration.

The LFCRs were operated with a liquid volume of 25 L. The reactors were closed to the surroundings (gasket sealed lid) leaving a headspace of 12.5 L. The headspace was flushed with air at a rate of 2.08 L/hour, with the exit gas passing through an alkaline scrubber to recover any in H_2S in the gas phase. The reactors were fed via the uppermost inlet port and the effluent exited via the upper-

most exit port (based on the outcome of the hydrodynamic study). The residence time (2—5 days) was determined by the feed rate. The LFCRs were designed with 15 sampling ports, across three levels, on the front wall of the reactor. Samples (5 mL) were collected from 10 of the 15 sample ports on a daily basis, along with influent and effluent samples, and analysed for pH, redox potential and the full range of sulphur compounds.

Hydrodynamic study

A theoretical calculation of Reynolds number for the LFCR suggested very little turbulence. A laminar flow profile would have significant implications for operation in terms of residence time and sulphide mass transport to the biofilm. It was suspected that challenging and short-circuiting could have contributed to sub-optimal performance of the demonstration plant. In order to test this experimentally a number of different tracer studies were performed in order to characterise the fluid flow within the LFCR. All experiments were conducted at ambient temperature and pressure using a feed rate of 5 L/h (120 L/d) which is similar to the highest flow rate (132 L/d) used on the pilot reactors. Phenolphthalein is an indicator that turns solutions bright pink in a pH range from pH 8.2—12 and colourless below pH 8.2. The reactor was filled with 25 L of sodium hydroxide (1.68 mM) and indicator added to turn the entire volume pink. A number of experiments were performed using different acid concentrations and cylindrical baffles (to induce turbulence). The experiments were repeated for each of the three feed points and in the presence and absence of cylindrical baffles.

Scanning electron microscopy

Samples of the planktonic and biofilm associated populations were prepared for SEM. The solution samples were processed by filtering the appropriate volume through a 0.22 µm nylon membrane filter. The A portion of the biofilm was carefully removed and placed on a membrane filter. The samples were fixed in 1% gluteraldehyde for 48 hours at 4 °C, followed by dehydration using ethanol (10 min at 30, 50, 70, 90, 95 and 100% ethanol). The planktonic samples were subjected to critical point drying prior to carbon coating. The biofilm samples were transferred to a 2 mL Eppendorf tube and impregnated with expoxy resin. Once set, the resin encased sample was removed from the tube and ground and polished until the biofilm was exposed at the surface. The electron dispersive X-ray analysis (EDX) technique was utilised to determine the elemental composition of specific areas with the field of view.

Microbial ecology study

The microbial ecology within the sulphide oxidising system was characterised using molecular biology techniques. DNA was extracted from both planktonic and biofilm samples using the Roche High Pure Template Preparation kit, according to the manufacturer's instructions. The extracted DNA was amplified by polymerase chain reaction (PCR) using primer sets targeting the small ribosomal subunit RNA gene (16S for prokaryotes and 18S for eukaryotes). Amplification products were separated using agarose gel electrophoresis.

PCR amplified DNA bands were excised from the gels and purified using the QIAquick Gel Extraction Kit (QIAGEN ™), according to the manufacturer's instructions. The recovered DNA amplicon (3 µL) was ligated into pGEM-T Easy™ (Promega). The ligation mixture contained the following: 2X ligase buffer, 1 µL T4 ligase, 1 µL pGEM-T Easy™ and 3 µL DNA. The ligation mix was incubated at 4^{0}C overnight. The ligation mixture was used to transform competent E. coli cells, which were plated (100 µL) onto LB plates containing 0.5 mM ampicillin, 0.5 mM IPTG (Isopropyl-Beta-D-thiogalactoside) and 80 µg/mL X-gal (5-Bromo-4-chloro-3-indolyl-β-D-galactoside). The plates were incubated at 37 °C overnight and plasmid DNA was extracted from positive tranformants using the GenElute™ hp Plasmid Miniprep Kit (SIGMA- ALDRICH). Purified samples were sent for sequencing. Microbial identification was performed by comparing the resulting sequences against known sequences using the National Centre for Biotechnology Information's (NCBI) basic local sequence alignment tool (BLAST).

Results and discussion

DPBR performance

The DPBRs are packed with layers of lignocellulosic material (wood chips, straw and cow manure) and were originally inoculated with rumen fluid. The columns used in the current study had been operated at a collaborating organisation for 18 months, prior to being shipped to UCT, where they have been running for over a year. Previous work has shown that carbon liberation is initially high and sustains efficient sulphate reduction. However, the sulphate reduction efficiency steadily decreases until a "steady state" is reached. This is most likely associated with the kinetics of lignocelluloses decomposition. In the current study the sulphide concentration in the DPBR effluent was typically around 200 mg/L, which represents about 30% reduction efficiency. In addition, the level of sulphate reduction is not significantly higher than that which could be sustained by the addition of 1.5 g/L molasses. This points to slow or inefficient lignocellulose degradation or the selection of a microbial population

that has become dependent on molasses. Analysis of the DPBR effluent showed no residual sugars and acetate concentrations below 5 mg/L. The sulphide oxidation reactors were therefore severely carbon limited.

Hydrodynamic study

The phenolphthalein tracer study clearly showed the absence of turbulent mixing and the partitioning of the solution into stagnant and motile zones. The minor density difference between the acid and base resulted in the acid sinking to the base of the reactor (Figure 1). A similar density gradient would exist for sulphide. When the lowest outflow port was used the residence time of the influent solution was less than 20 minutes, compared to five hours when perfectly mixed plug flow was assumed. The issue could be addressed by directing flow out through the uppermost outlet. In this case the incoming solution reached the end of the reactor and could not flow out. The result was displacement of fluid in a vertical direction, ultimately achieving complete mixing. This was confirmed for sulphide by analysing all 15 sampling points.

Performance of sulphide oxidation reactors

Previous work, conducted during the development phase of the IMPI process (Bowker, 2002; Molwantwa, 2007), described the floating sulphur biofilm as passing through three stages of development. The "thin" stage was characterised by an almost transparent layer on the surface of the reactor. This was rapidly followed by the "sticky" phase, where the biofilm grew in thickness and developed a light brown colour. At this point the biofilm was largely organic in nature. The final phase was termed "brittle" and was characterised by significant elemental sulphur deposition. The appearance changed to a characteristic creamy-yellow colour and the film became brittle due to the accumulated sulphur. The initial work was performed under carbon sufficient conditions.

During the current study, the biofilm did not pass through the "sticky" phase. In some of the initial tests biofilm formation was very slow and a complete biofilm did not develop. A continuous biofilm is necessary to present a sufficient barrier to oxygen diffusion such that the redox environment and reagent (HS^- and O_2) stoicheometry are conducive to partial oxidation of sulphide to sulphur. In the experiments where biofilm formation was incomplete sulphide oxidation was efficient, but the majority was oxidised completely back to sulphate.

Supplementation of the LCFR with 20 g of acetate at the start of the experiment promoted biofilm formation, although it did not pass through the "sticky" phase. The performance of the system improved significantly and the majority of the sulphur was deposited in the biofilm (Table 1). Extensive development of the methodologies for quantifying the sulphur species, particularly polysulphides, was performed. This allowed for the sulphur balance across the system to be closed for the first time. The reactors were fitted with an airtight lid and the headspace was continuously flushed into a sulphide trap. Analysis of the solution at the end of the experiment indicated an insignificant amount of hydrogen sulphide gas evolution during the experiment. This is significant in terms of scaling-up the process due to the environmental and health risks associated with hydrogen sulphide.

Optimisation of the sample preparation method for SEM by introducing the resin impreg-

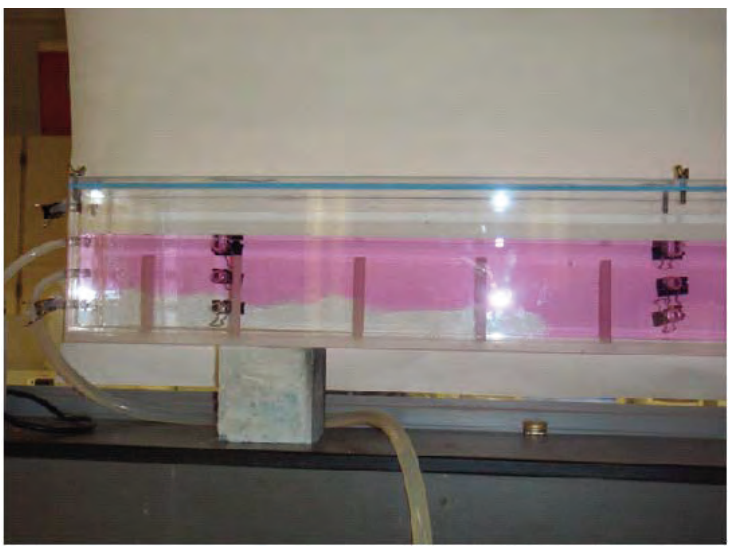

Figure 1 *Phenolphthalein tracer study showing laminar parabolic flow patter and density gradient*

Table 1 *Summary of sulphur species over the duration of the experiment. Thiosulphate was not detected in significant amounts. All values are expressed as millimoles.*

	Sulphide	Sulphur		Sulphate	Polysulphide
		Colloidal	In biofilm		
In	725.52	18.84	-	1184.1	-
Out	152.79	25.49	-	1217.1	4.0
Consumed	572.73		-	-	-
Produced	-	6.65	527	33.0	4.0

nation step allowed for excellent preservation of the biofilm structure. The microbial cells, sulphur granules, extracellular polymeric substance (EPS) matrix and voids and macropores were clearly visible (Figure 2). The EDX analysis allowed confirmation of the sulphur particles and also the composition of an inorganic crust that appeared on the upper surface of the biofilm. This was confirmed as calcium sulphate which most likely crystallised due to evaporation at the surface. The SEM images also provided confirmatory evidence for the microbial ecology work, particularly with respect to organisms not normally associated with sulphide biofilms (eg Spirochaetes).

Microbial ecology

The microbial ecology work confirmed the identification of seven component species, based on the sequences of the 16S or 18S ribosomal RNA gene. These included three autotrophic sulphide oxidising bacteria: Thiomonas intermedia, Chlorobium limicola and Chromatium okenii. In addition a Spirochaete bacterium was detected, which was most likely Spirochaeta perfillievii, a recently described new species (Dubinina *et al.* , 2011). These results differed from those of Bowker (2002) and Molwantwa (2007) who found a greater number of heterotrophic sulphide oxidisers. This can be accounted for by the organic carbon limitation in the current system. Aside from the sulphide oxidisers, two species of sulphate reducing bacteria were detected. These would have been carried over from the DPBR. A yeast, Rhodotorula mucilaginosa, was detected in several samples. This organism has been found in sulphidic tannery effluent and was thought to metabolise organics that would otherwise be inhibitory to autotrophic sulphur oxidisers.

Conclusions

The integrated reactor system has been operated successfully, achieving over 80% sulphide oxidation, with the majority being converted to sulphur in the biofilm. However, this was achieved under conditions where the sulphide loading into the LFCR was controlled and additional organic carbon was provided.

The data presented in this report represents

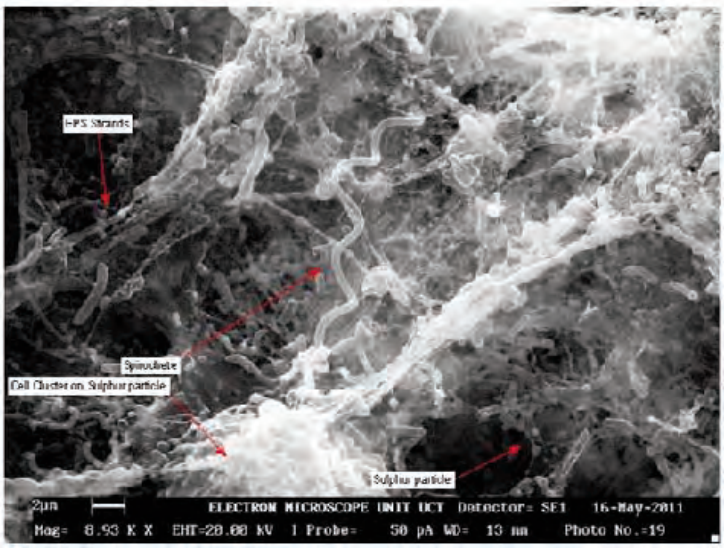

Figure 2: *SEM image of underside of floating sulphur biofilm*

the most complete characterisation of the LFCR system to date. The extensive sampling protocol and suite of analyses showed the inhomogeneity within the reactor, primarily driven by the hydrodynamics. Despite this, an almost complete sulphur species mass balance across the system was possible. The results from the experimental runs and ongoing experiments highlight a number of important issues for large scale process operation.

Liquid flow in the LFCR follows a laminar, parabolic pattern, with a stagnant upper zone and a motile lower zone. To avoid short circuiting and promote mixing the exit port needs to be at the top of the LFCR channel.

The ratio of oxygen to sulphide in the region of the biofilm is critical to ensure the desired product formation. The oxygen partial pressure remains constant implying that significant fluctuations in the sulphide concentration entering the system could have a substantial effect on system performance, particularly during the early stages of biofilm development. Once a continuous biofilm is in place the oxygen mass transfer is regulated by diffusion across the boundary.

Heterotrophic sulphide oxidising microorganisms play an important role in biofilm formation so the provision of sufficient organic carbon is critical to ensure effective biofilm formation. Insufficient organic material compromises the structural integrity of the biofilm and results in process inefficiency.

The long term performance of the DPBR should be monitored with respect to the organic carbon released. Previous work has shown that organic carbon liberation from lignocellulosic material is initially high, but drops to a stable level controlled by the breakdown of complex organic molecules. The rate of degradation is typically insufficient to sustain complete sulphate reduction. At this point the effluent is largely devoid of carbon, so carbon supplementation into the sulphide oxidation reactor will be required.

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