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The Effect of pH and Temperature on Biological Sulphate Reduction

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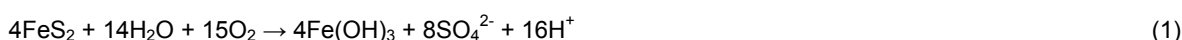
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Acid mine drainage (AMD) is a worldwide problem caused mainly by mining activities and is known for its adverse impacts on the environment due to low pH, high metal content and high sulphate concentrations. Lime neutralisation is a conventional way of treating AMD, however, this method is costly and produces sludge that is difficult to dispose of. Biological treatment of AMD using sulphate reducing bacteria has been identified as a cost-effective alternative treatment method due to its advantages that include simultaneously precipitating metals, reducing sulphate concentration and increasing pH. In this study, the effect of temperature and pH using complex organic compounds as substrates in a downflow packed bed reactor was investigated. Temperature was found to have a major impact on sulphate reduction. Sulphate reduction dropped from about 88 % to about 67 % when the temperature was slowly decreased from 30 °C to 10 °C. The decrease in pH from pH 6 to pH 5 was observed to have minimal effect on sulphate reduction.

1. Introduction

Acid mine drainage (AMD) is a worldwide problem known for its adverse impacts on the environment and is characterised by low pH, high metal content and sulphate concentrations. The main cause of AMD is current and past mining activities (Akcil and Koldas, 2006). AMD is formed when sulphide-bearing minerals, such as pyrite, chalcopyrite, chalcocite and arsenopyrite are exposed to oxygen and water (Othman et al., 2017). From the above-mentioned sulphide minerals, pyrite is the most abundant mineral on the planet and it can be associated with the formation of AMD (Johnson and Hallberg, 2005). The overall pyrite oxidation equation is shown in Eq(1) (Kaksonen and Puhakka, 2007):



In South Africa, sulphate concentrations in AMD are typically above 2000 mg/L and the required sulphate concentration for discharge is 200 – 600 mg/L. Similarly, the World Health Organisation recommends 250 mg/L for drinking water (Arnold et al., 2016).

A more conventional way of treating AMD is the application of a chemical neutralising agent such as lime, calcium carbonate and sodium hydroxide, with lime neutralisation being the most commonly used chemical treatment in South Africa. Although this method has been successful in neutralising AMD and precipitating metals, it has not been efficient in reducing sulphate concentrations (Arnold et al., 2016). Moreover, this method has proven to be costly and produces large sludge volumes that are difficult to dispose of. In addition to chemical treatment, physical treatment of AMD has also been used which includes ion exchange and membrane filtration. Although these methods are efficient, they require pre-treatment of AMD (Luptakova et al., 2013).

Biological sulphate reduction (BSR) has been identified as an alternative, sustainable and cost-effective method for the treatment of AMD (Neale et al., 2017). BSR is a semi-passive remediation technology that uses sulphate reducing bacteria (SRB) to metabolically reduce sulphate to sulphide. This treatment process has advantages over other treatment processes because it removes sulphates while simultaneously removing metals as sulphide precipitates and increasing pH (Luptakova et al., 2013). The successfulness of this

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process solely depends on the activity of the microorganisms which is governed by operating conditions, of which the most critical ones are temperature, pH and the availability of carbon source as an electron donor. Sheoran et al. (2010) explicitly review most of these factors.

SRB utilises sulphate present in AMD as the terminal electron acceptor to metabolise organic matter resulting in sulphate being reduced to sulphide under anaerobic conditions (Macingova and Luptakova, 2012). SRB uses a wide range of substrates such as simple organic compounds (acetate, lactate, molasses, butyrate, propionate, hydrogen), alcohols (methanol, ethanol) (Liamleam and Annachatre, 2007) and complex organic sources (cow manure, goat manure, buffalo manure, sawdust) as electron donors

Eq(2) shows how SRB oxidises an organic substrate (CH_2O) while reducing sulphate, produce alkalinity which raises the pH and hydrogen sulphide which precipitates the metals as metal sulphides Eq(3)(Dvorak et al., 1992):



where M represents metals.

Most research that has been done so far has been focussed on metal removal in AMD. Those that focus on sulphate reduction are mostly conducted at optimum conditions and they mostly use simple compounds such as methanol, ethanol, lactate and acetate as substrates, which are costly. Mintek has conducted bench-scale tests on biological sulphate reduction that led to the commissioning of a pilot plant at a coal mine in Mpumalanga province, South Africa (Neale et al., 2017). It was observed that the amount of lime needed to adjust AMD to a comfortable pH ($\text{pH} > 5$), is considerable and results in an excessive amount of sludge. It was also observed that during winter seasons the performance of the plant decreased. For this reason, the purpose of this study was to determine the effect of decreasing pH with the intention of minimising the amount of lime used in the pH adjustment step as well as to understand the process' robustness with changing temperature. This study sheds new light to the BSR process and how it is likely to be impacted by environmental conditions while using complex organic compounds as substrates which are less expensive (Zagury et al., 2007).

2. Materials and methods

2.1 Acid Mine Drainage

The experiments were carried out using raw AMD from a coal mine in Mpumalanga province, South Africa. Table 1 shows the typical composition of the AMD discharged from the coal mine and the allowable discharge limits into a water source in South Africa (DWAf, 2013).

Table 1: Typical AMD composition from coal mine and allowable discharge limits

Parameter	pH	SO_4^{2-} mg/L	Mg mg/L	Al mg/L	Mn mg/L	Cu mg/L	Zn mg/L
Value	3	3000	204.62	61.79	28.84	3.24	2.21
Value ¹	5.5-9.5	200-600	100	0.1	0.1	0.01	0.1

¹Allowable discharge limits into a water source in South Africa.

2.2 Inoculum

The anaerobic mixed sulphate reducing culture used was collected from one of the reactors at the pilot plant that has been running in a coal mine in Mpumalanga province, South Africa. To inoculate the reactors, the inoculum was mixed with mine water that was adjusted to pH approximately 6.5. The reactors were inoculated using 30% inoculum and 70% neutralised mine water.

2.3 Analytical methods

The turbidimetric method was used to measure sulphate concentrations using a Merck Spectroquant[®] Prove 300. All sulphate samples were filtered using 0.22 μm membrane syringe filters to prevent suspended solids from interfering with the results. The pH was measured using a Metrohm pH sensor and a Hamilton redox sensor was used for redox potential measurements. Metal concentrations were analysed using a Thermo Icap Q ICP-MS equipped with a Perfluoroalkoxy (PFA) nebuliser, a quartz spray chamber and a 2.5 mm internal diameter quartz injector.

2.4 Experimental setup and operation.

Three water jacketed packed bed reactors were operated in parallel in a continuous downflow mode. Mine water was fed at the top of the reactors at a speed of 5.6 rpm. A PolyScience Whipspercool heater/chiller was used to control the reactor temperatures. The working volume of each reactor was 8 L. Initially, each reactor was packed with woodchips, wood shavings, hay, lucerne straw and cow manure as support for the SRB biofilm. Complex organic substrates, namely cow manure and lucerne pellets were used as main substrates for these experiments and they were replenished once every week. During startup, the reactors were operated in a batch mode at 30 °C for 10 days. Recirculation was introduced for a week after the 10th day. Due to the depletion of sulphate (which was proof that SRB were active) recirculation was stopped and neutralised AMD (at pH 6) was fed slowly while simultaneously getting the reactors to the desired operating conditions shown in Table 2. This was achieved after 60 days from the day the reactors started operating.

Table 2: Operating conditions for each reactor

	Reactor 1	Reactor 2	Reactor 3
Temperature (°C)	30	30	10
pH	6	5	5
Hydraulic residence time (days)	7	7	7

3. Results and discussion

3.1 The effect of temperature

Temperature is one of the factors that affect the efficiency of biological sulphate reduction. Bacterial growth and activity are highly affected by temperature with low temperature known for slowing down their metabolic activity (Doshi, 2006). Redox potential is an important indicator of the overall reducing capacity in biological sulphate reduction. For biological sulphate reduction to occur, a minimum of at least -150 mV is required with -300 mV being a good indicator of a strong reducing environment (Salo et al., 2017). Figure 1 shows how the effluent pH and effluent redox potential varied while operating at 30 °C and 10 °C with the feed AMD at pH 5.

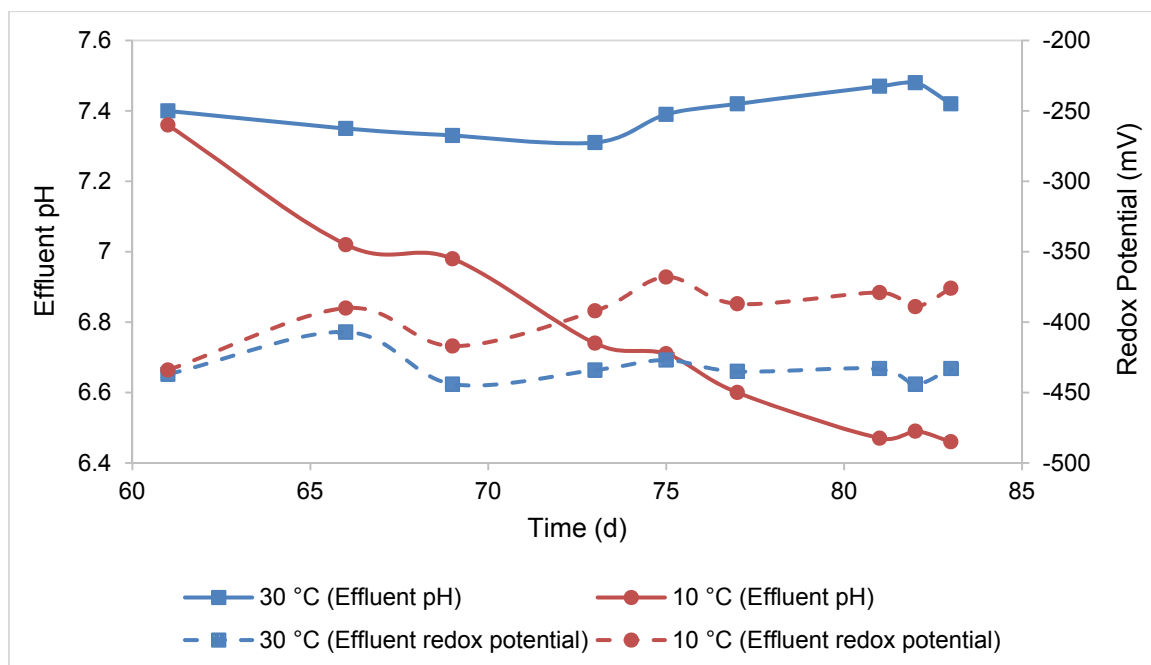


Figure 1: Changes in effluent pH and redox potential

The redox potential at 30 °C was below -400 mV throughout the experiment which demonstrates that the reducing conditions were successfully attained. This is evident from the effluent pH which steadied above 7 due to the alkalinity produced in the reactor, as shown in Eq(2). At 10 °C however, the redox potential increased over time stabilizing between -400 mV and -350 mV after day 73. Although reductive conditions

were reached, due to the low temperature and subsequent decrease in SRB metabolic activity, the biochemical cycle required for sulphate reduction was not efficient. The decrease in the metabolic activity of SRB resulted in less alkalinity being produced which explains the decrease in effluent pH at this temperature. Sulphate reduction was maintained above 90 % remaining fairly constant around 99 % after day 75 at 30 °C, as depicted in Figure 2. This can be attributed to the optimal metabolic performance of SRB at this temperature. At 10 °C, sulphate reduction also indicated a decrease in SRB activity as evident from the decline in sulphate reduction from 88 % at day 61 to 67 % at day 81. After day 81, the sulphate reduction increased from 67 % to about 74 %. This might have been due to SRB acclimatising to the low temperature and hence the increase in sulphate reduction. From the results presented, it is can be seen that the decrease in temperature has a notable impact on SRB activity.

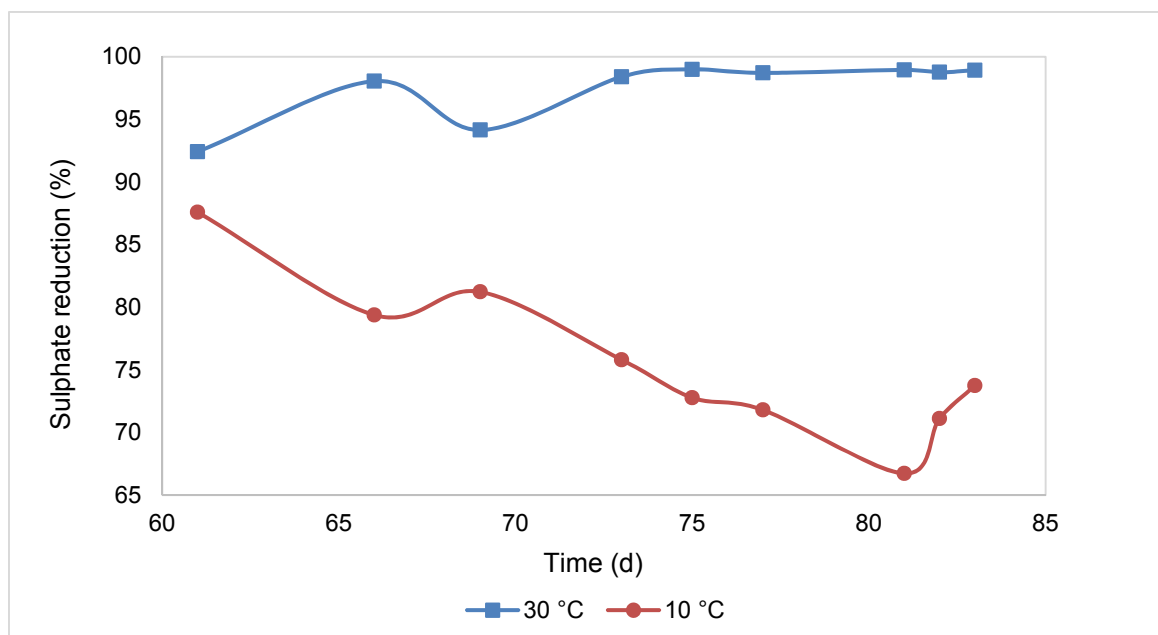


Figure 2: Temperature effect on sulphate reduction.

3.2 The effect of pH

As mentioned before, the two reactors were operated at the same conditions during startup. The pH in reactor 2 was reduced from pH 6 to pH 5 a week before day 61, the sulphate reduction before the drop was stable at 98 % (data not shown). Due to this drop in pH, sulphate reduction decreased to 92.5 % but started to increase gradually reaching maximum on day 74 as shown in Figure 3. Jong and Parry (2006) observed the same trend when they decreased the pH in their study. This was because an adaptation period was required by SRB to adjust to the new pH environment. After acclimatisation, sulphate reduction increased and stabilised between 98 % and 99 % after day 75. Sulphate reduction in reactor 1 (pH 6) remained fairly constant between 97 % and 99 % throughout the experiment. Comparing sulphate reduction at pH 5 and pH 6, it can be seen that there was little impact due to the drop in pH. Given enough time, SRB acclimatises to the new conditions sufficiently to return to similar sulphate reduction performances of higher a pH. After acclimatising to the new conditions, reactor 2 is seen to be performing marginally better than reactor 1 although the difference does not appear to be significant ($p = 0.06$, for a period of stable reduction values). From the results presented in Figure 3, it can be concluded that pH 5 and pH 6 yields similar sulphate reduction efficiency, with pH 5 yielding higher sulphate reduction. This was expected as the studied pHs falls within the optimum pH for SRB.

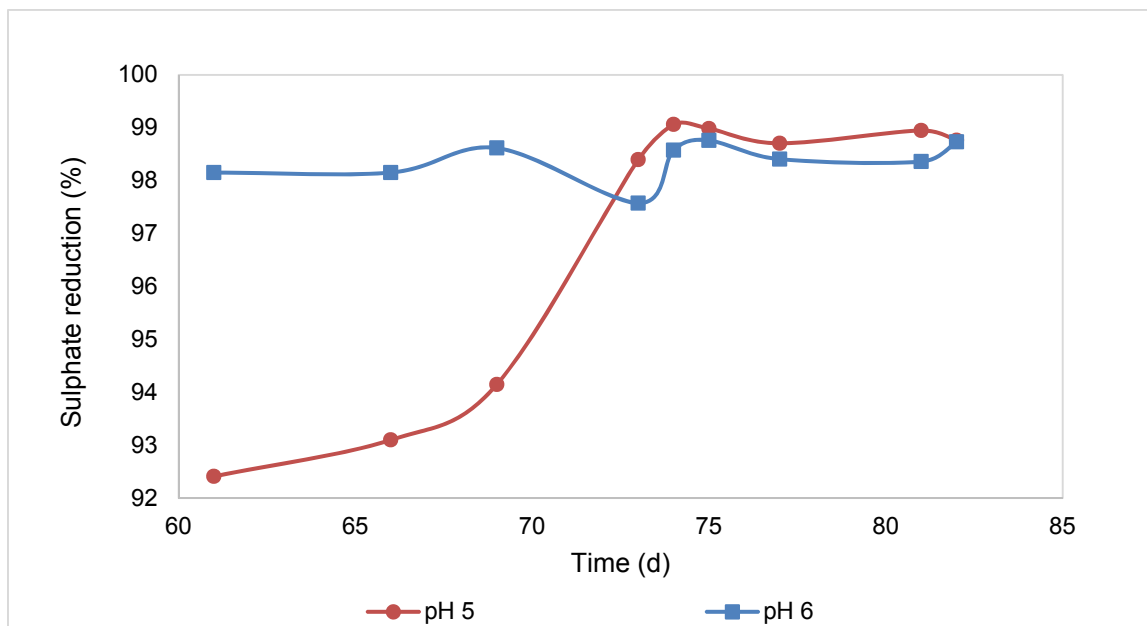


Figure 3: pH effect of sulphate reduction

3.3 Metal removal

Metal concentrations after pH adjustment and at the end of each experiment are presented in Table 3. It is evident that biological sulphate reduction process has the potential to remove metals such as Mn, Cu and Zn. Reactor 1 (30 °C, pH 6), 2 (30 °C, pH 5) and 3 (10 °C, pH 5) achieved 85.9%, 80.7% and 74.8% Mn removal respectively. Reactor 1 showed better performance due to the favourable conditions under which it was operating. Most of the Al, Cu and Zn was precipitated during the adjustment step probably as hydroxides or oxyhydroxides (Lee et al., 2002). Conversely, adjusting the AMD pH seems to increase the Mg concentration. Mg concentration before AMD pH adjustment was 204.62 mg/L but after adjusting to pH 5 and pH 6, the concentration increased to 249.06 mg/L and 225.36 mg/L respectively. The observed increase of Mg in all the reactor's effluents is presumably due to leaching from the substrates. Jong and Parry (2003) found that biological sulphate reduction process was not effective in removing Mg.

Table 3: Metal concentration before pH adjustment, after pH adjustment and in each reactor effluent

	Mg mg/L	Al mg/L	Mn mg/L	Cu mg/L	Zn mg/L
Feed before ¹	204.62	61.79	28.84	3.241	2.211
Feed after, pH 5 ²	249.06	5.46	27.40	2.388	1.437
Feed after, pH 6 ³	225.36	2.33	24.49	0.471	0.639
Reactor 1 effluent	502.72	1.97	3.46	0.099	0.301
Reactor 2 effluent	408.08	2.10	5.29	0.106	0.276
Reactor 3 effluent	502.35	0.96	6.90	0,029	0.633

¹Raw AMD

²AMD after adjusting to pH 5

³AMD after adjusting to pH 6

4. Conclusions

In this study, the effect of pH and temperature on biological sulphate reduction was investigated. A decrease in temperature showed a notable drop in sulphate reduction which was due to the decrease in SRB metabolic activity. The decrease in pH showed little effect on sulphate reduction, however decreasing pH required an adaptation period for SRB to get used to the new pH. Metals were also removed in the reactors operating at different conditions. 85.9%, 80.7% and 74.8% Mn were removed in reactor 1, 2 and 3 respectively. Some of

the metals such as Al, Cu and Zn were precipitated during the pH adjustment step, however, Mg concentrations were found to increase in the reactors' effluent which could be attributed to leaching from the substrates that were used. Based on these results, it can be seen that sulphate reduction is possible at lower temperature albeit at much-reduced efficiencies. There are currently further studies being conducted to investigate how the interaction between temperature and pH influence sulphate reduction, which provides more insight on how operating conditions can be manipulated to minimise the impact of environmental variables.

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