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Full Length Research Paper

The effect of initial pH on the kinetics of ferrous-iron biooxidation at low temperature

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The general understanding in bioleaching of sulphide minerals is to keep pH low. A number of published articles have reported the effect of pH on biooxidation rates of ferrous-iron and/or sulphur by bioleaching microbes, although most of these studies were conducted at optimum or near optimum temperature for microbial performance. Consequently, a series of experiments were conducted in this study to investigate the effect of pH on biooxidation of ferrous-iron at low temperature condition (22°C) by a culture that was predominantly *Leptospirillum ferriphilum*. The maximum specific microbial activity (2.13 x10^{-3} h^{-1}) obtained at pH 1.37 was more than 10 times lower than the corresponding activity at optimum temperature. The specific rates decreased as pH increased from 1.37 to 1.88. However, the jarosite precipitation under these conditions was not significant to deplete the available iron. The result of this study, if extended to other microbes would have implications on strain selection and management of heap bioleach processes operating in cold conditions.

Keywords: Bioleaching, ferrous-iron biooxidation, *Leptospirillum ferriphilum,* low temperature.

INTRODUCTION

Bioleaching of sulphide minerals is an established technique for the recovery of copper from their sulphide ores (Plumb et al., 2008; Ojumu, 2008). It occurs via three sub-processes viz: the leaching of the sulfide mineral by ferric iron and/or acid (Equation 1), the microbial oxidation of the reduced ferrous iron back to ferric iron (Equation 2), and the microbial oxidation of the sulfur species to sulphate (Equation 3). These steps have been discussed widely in the literature (Breed and Hansford, 1999; Ojumu et al., 2006). The application of bioleaching for recovery of other metals has also been explored with some success (van Aswegen et al., 2007).

$$CuS + 2Fe^{3+} \rightarrow Cu^{2+} + 2Fe^{2+} + S^0 \quad (1)$$

$$4Fe^{2+} + O_2 + 4H^+ \xrightarrow{\text{Microorganisms}} 4Fe^{3+} + 2H \quad (2)$$

$$2S^0 + 3O_2 + 2H_2O \xrightarrow{\text{Microorganisms}} 2SO_4^{2-} + 4H^+ \quad (3)$$

The role of microbial ferrous-iron oxidation is critical to bioleaching of sulphide minerals. The microbial metabolism forms the pathway to generate the ferric-iron (Equation 1) as ferrous-iron serves as the electron donor in the microbial respiratory chain (Ingledew, 1982).

There are many studies on microbial oxidation of ferrous-iron, and some authors have used different methods with the aim of understanding the kinetics of this sub-process of bioleaching (Nemati et al., 1998; Ojumu et al., 2006). This has led to the success of tank bioleaching whereby operating conditions can be manipulated and controlled to suit the microorganisms, and hence facilitate optimal performance for efficient metal recovery. On the other hand, and very recently, studies on ferrous-iron biooxidation have been focusing on non-optimum conditions, such as those that usually occur in heap leach operation (Dopson et al., 2007; Kupka et al., 2007). In heap bioleaching, operating conditions could be erratic and cannot be controlled to optimum. Of particular importance is the operating temperature, it can be as low as 10°C in some sections within a heap bed, especially in some locations where ambient weather temperature can be

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freezing (Dopson et al., 2007). Although it has been reported that ferrous-iron biooxidation does occur at lower temperatures, microbial activities are considerably low (Franzmann et al., 2005; Dopson et al., 2007). Also of importance is the presence of protons (Equation 2) in the microbial respiratory chain (Dopson et al., 2007). An acidic condition is desirable to keep ferric-iron in solution, thus preventing loss of iron due to ferric-iron precipitation (e.g. jarosite and or schwertmannite) (Dopson et al., 2007). Breed and Hansford (1999) reported that pH range of 1.1 to 1.7 did not significantly affect specific microbial ferrous-iron oxidation rate at 37°C in a continuous stirred-tank reactor (CSTR), a high pH (pH > 2.0) will not only inhibit microbial activity (Daoud and Karamanev, 2005; van Aswegen et al., 2007; Plumb et al., 2008), but may lead to blockage of heap bed due to ferric precipitation.

There are limited data on low temperature studies on microbial ferrous-iron oxidation, and the effect of pH under this condition has not been investigated. Although ferrous-iron biooxidation cannot be sustained in a continuous flow system at temperatures below 20°C (Ojumu et al., 2009), investigating the effect of pH on the kinetics of this sub-process at low temperature conditions is critical to the understanding of leaching and metal recovery rates in heap bioleach operations, especially in locations where ambient temperature is below 20°C.

In the low temperature study by Dopson et al. (2007), the effect of pH was not investigated, but where investigated by Plumb et al. (2008); the optimum temperatures, T_{OPT} (T_{OPT}, > 30°C in all cases) of the corresponding microbes were used (Franzmann et al., 2005). Previous studies investi-gating the effect of initial pH on the biooxidation process in batch cultures used the biooxidation rate constant as a measure of the efficiency of the biooxidation process, with the largest biooxidation rate constant yielding the most efficient biooxidation (Nowaczyk et al., 1999; Mousavi et al., 2006). It is important to note that those studies were performed with *Acidothiobacillus ferrooxidans* at the microbial optimum temperature. Thus, there is no infor-mation on the effect of initial pH on the biooxidation process at low temperatures.

Rate constant calculation

The kinetics of microbial ferrous-iron oxidation has been reported to follow a first order rate law with respect to the substrate concentration (Nowaczyk et al., 1999, Daoud and Karamanev, 2005, Franzmann et al., 2005, Plumb et al., 2008) as shown in Equation 4.

$$-r_{Fe^{2+}} = kC_{Fe^{2+}} \quad (4)$$

By substituting the rate law into the performance equation for a constant-volume batch system (Fogler, 2006), and upon integration, Equation 5 is derived.

$$\ln\left(\frac{C_{Fe^{2+}}}{C_{Fe^{2+}0}}\right) = -kt \quad (5)$$

Where, $C_{Fe^{2+}0}$ and $C_{Fe^{2+}}$ are initial and final substrate concentrations (g L^{-1}), respectively, and *k* is the specific reaction constant (h^{-1}). Therefore, by plotting $\ln(C_{Fe^{2+}} / C_{Fe^{2+}0})$ versus time during the growth phase, the slope of the curve would give a slope equal to the negative of the rate constant (Fogler, 2006).

Thus, the present investigation focus on the effect of initial solution pH on the kinetics of microbial ferrous-iron oxidation by *Leptospirillum ferriphilum* at a low temperature, with the view to understanding the contribution of these non-optimal conditions on the sub-process with respect to metal recovery in a typical heap bioleach operation.

MATERIALS AND METHODS

Bacterial culture

The bacterial culture used was obtained from the Centre for Bioprocess Engineering Research (CeBER) at the University of Cape Town, South Africa, where it was maintained on ferrous-iron in a chemostat. This culture consisted predominantly of *L. ferriphilum*, and originally from tank bioleaching operation in Gamsberg, South Africa (Ojumu et al., 2008).

Growth medium

The ferrous-iron growth medium consisted of 5 g/l of Fe^{2+} (added as $FeSO_4$. $7H_2O$), 1.11 g/l K_2SO_4, 0.53 g/l $(NH_4)_2HPO_4$, 1.83 g/l $(NH_4)_2SO_4$ and 10 ml/l of Vishniac solution – a trace element solution which has been described elsewhere (Ojumu, 2008). The growth medium was adjusted to the desired pH using concentrated H_2SO_4.

Experimental procedure

The initial pH was varied in order to investigate its effect on the kinetics of microbial ferrous-iron oxidation. Five experiments were performed in 500 ml stirred conical flasks. Fifty millilitres (50 ml) of the bacterial culture (from a chemostat running at 30°C and 48 h residence time) was added to 200 ml of growth medium in each flask. The flasks were then loosely covered with cotton wool to allow aeration. Finally, the flasks were placed on the rotary shaker with a rotation speed of 200 rpm and the temperature was maintained at 22°C in an air conditioned room/incubator. The ferric-iron and total iron concentrations in the flasks were measured initially. The pH in each flask was adjusted to 0.8, 1.37, 1.55, 1.88 and 2.06

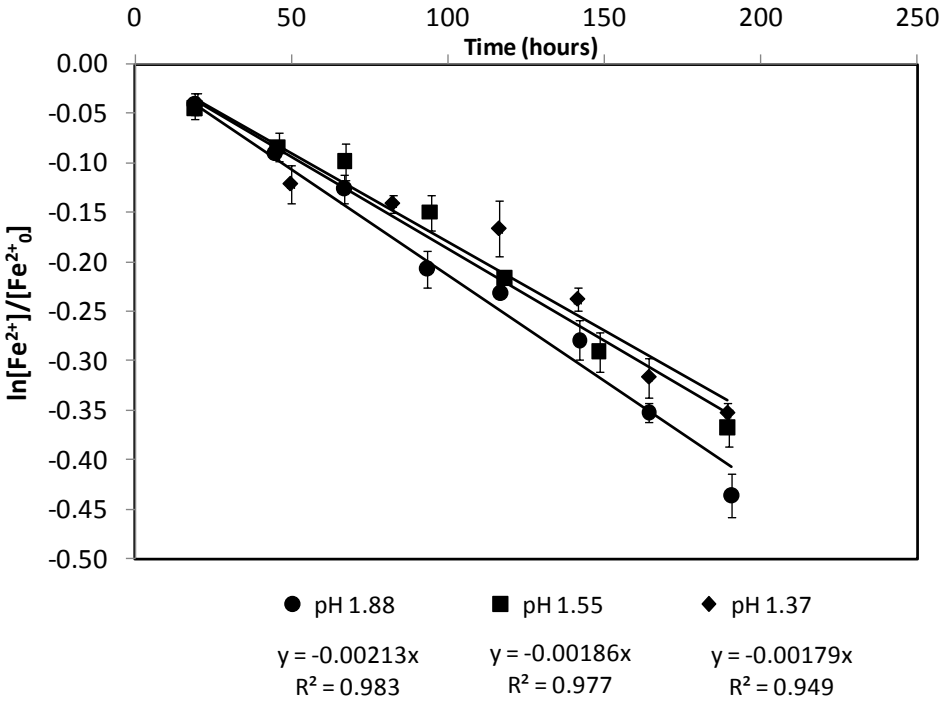

Figure 1. The first order rate plot showing the plot of ln (Fe^{2+}/Fe_0^{2+}) versus time at various pHs.

using 98% sulphuric acid. Each experiment was carried out in triplicate. The ferrous iron concentration was monitored at specific time intervals, and by subtracting the obtained ferrous iron concentration from the total iron concentration, the ferric iron concentration was obtained at the specified time intervals.

Analysis

Iron analysis

The ferrous iron and total iron concentrations were determined at the start of the experiments by titrating with potassium dichromate using barium diphenylamine sulphonate (BDS) as an indicator (Vogel, 1989). The ferric and ferrous-iron concentrations were also determined via solution potential measurement using Pt–Ag/AgCl electrode. The total iron concentration was assumed to be constant throughout the investigation. Ferric precipitation was used as a proxy measure of the iron loss during the process, and this was determined at the end of each run; the liquid in each flask was filtered using a vacuum flask and filter paper (25 µm pore size). The solids on the filter paper were then returned back to the corresponding flask by washing them with concentrated hydrochloric acid. HCl readily dissolves the filtered jarosite and that attached to the wall of the flask. Finally, the precipitate was measured as total iron concentration by dissolving samples in concentrated HCl prior to measurement by using an atomic absorption spectrophotometer (Perkin Elmer AA 3300).

RESULTS AND DISCUSSION

Several experimental runs were performed in triplicates, testing the effect of the various pHs ranged from 0.8 to 2.06. All experiments were left to run for approximately 190 h before termination. The temperature was kept constant at 22°C.

There was no reasonable biooxidation observed at pH 0.8 and 2.06 after about 80 h before the experiments were terminated. Although the immediate reason for the lack of biooxidation at pH 0.8 could be attributed to acid inhibition (Nemati et al., 1998; Plumb et al., 2008), the inhibition observed in this study was aggravated by the lower temperature condition unlike the previous study by Plumb et al. (2008) which was conducted at near optimum temperature condition of 38.6°C. Lack of oxidation at pH 2.06 shows that microbial activity is hampered, as reported by van Aswegen et al. (2007). On the contrary, the recent study by Ojumu (2008) showed a reduced, but significant oxidation at pH 0.8 and 2.0; however, the study was conducted at an elevated temperature of 42°C, and in a chemostat operation.

Figure 1 shows the plot of the first order rate equation, for pH measured at 1.37, 1.55 and 1.88. The results show that the biooxidation rates decreased with increasing order of the pH as revealed by the calculated specific rate constants. Although Plumb et al. (2008) observed a rapid oxidation trend between pH 1.0 to 2.0, reporting an optimum pH of 2.0 for *L. ferriphilum* at 38.6°C, a similar study with *A. ferrooxidans* also showed an increasing trend in oxidation rates between pH 1.6 to 2.0 at 35 and 40°C,

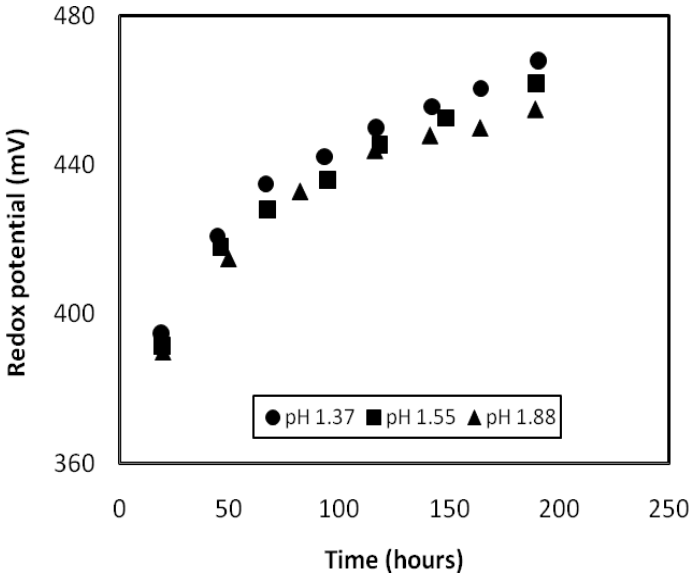

Figure 2. Plot of redox potential as a proxy measure of ferric-to ferrous ration with time.

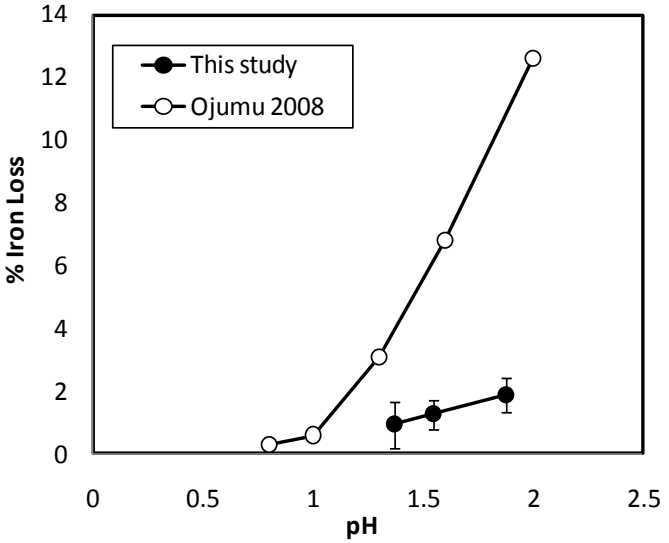

Figure 3. Plot of percentage of total iron loss versus reactor pH compared to previous study by Ojumu 2008.

however, the trend was reversed at lower temperatures, 25 and 30°C (Daoud and Karamanev, 2005). The maximum specific rate obtained in this study, 2.13 x10^{-3} (h^{-1}), at pH 1.37 is about 10 times smaller compared to the value reported by Plumb et al. (2008) for *L. ferriphilum* at an optimum temperature of 38.6°C. This pH is comparable to the previous study by Ojumu (2008) where maximum microbial activity was reported at pH 1.3. However, the calculated mean rate of oxidation at 190 h is also 10 times smaller than the reported values by Daoud and Karamanev (2005) at 46 h.

Although the biooxidation reaction was about half completed as shown by the final redox potential measurements (Figure 2), the possibility of ferric precipitation and/or biofilm was checked due to the extended periods of experimentation. However, the analysis of the ferric precipitation as a proxy measure of jarosite shows that it was not significant, especially when compared to the previous study (Figure 3). About 3% of total iron was lost due to ferric precipitation after two residence time, in a chemostat of microbial ferrous-iron oxidation that was maintained at pH 1.3, 40°C temperature and 16 h residence time (Ojumu, 2008). Although pH 1.37 gave minimum precipitation, the percentage iron lost due to precipitation increases insignificantly over the range of pHs investigated, as indicated by the standard deviation values.

Jarosite precipitation has been reported to be significant at about pH 2.0 in previous studies, which often makes analysis difficult (Ojumu, 2008; Plumb et al., 2008). However, ferric-iron precipitation at higher pHs is minimised at lower operating temperature (Pogliani and Donati, 2000; Daoud and Karamanev, 2005; Plumb et al., 2008; Dutrizac and Chen, 2010). Sustaining low temperature leaching may not be achievable across the heap bed because of the non-uniform temperatures that exist in various sections of a typical heap; this condition may be desirable at least at the region close to the surface (that is, low temperature region), to keep the critical reagent, iron, in solution.

Conclusion

Relationship between microbial activities (measured as specific rates) and pH has been described. This study showed that under cold conditions below the normal optimum temperature for microbial activity, the microbial activities of cultures, predominantly *L. ferriphilum* decreased with increasing pH within the range 1.37 to 1.88. However, the activity was significantly inhibited at 0.8 and 2.06. Jarosite precipitation was insignificant as almost all the iron was in solution. Thus, extension of this study to cover other bioleaching microbes and development of psychrotolerant bacteria for biohydrometallurgical application may have implication for selection of suitable strain at different pH for bioleach heap operations in cold locations.

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