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A Column Bioleaching Model for Chalcocite: An Investigation of Oxygen Limitation and Bacterial Inoculation on Leaching

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Abstract

A model for column bioleaching is investigated to identify and understand aspects of bacteria in bioleaching applications, which have implications in heap bioleaching operations. The model is used to simulate scenarios that would otherwise be time consuming to perform experimentally.

This study uses a model of bacterial transport and attachment/detachment to ore particles, with a bioleaching model for the depletion of a copper-sulphide, also accounting for liquid and gas flow and gas/liquid oxygen mass transfer. The model includes aspects such as oxygen and ferrous ion consumption, coupled with leaching of a copper-sulphide via the shrinking core model.

The model is used to investigate how the rate of leaching is affected by the bacterial concentration in the columns, by the bacterial regeneration of the leaching oxidant ferric ions. The model is also used to assess the impact of oxygen limitation and inoculation method on the copper leaching.

Some comparison of the model with experimental data will be shown.

INTRODUCTION

Heap bioleaching is a hydrometallurgical process by which large heaped piles of lowgrade ore (eg copper, zinc) are leached with acidic solution for long time periods. The process involves the application of acid in water based solution and availability of bacteria within the heap, which may occur either naturally or seeded in solution. The injection of air into the heap (sparging) is of upmost importance to keep the aerobic bacteria alive, for optimal leaching. The solution soaks into the ore and leaches the metal into solution and is then processed. The copper sulfide chalcocite (Cu_2S) is considered in this work in combination with pyrite (FeS_2). Ferric ions are used in intra-particle leaching of copper, to produce ferrous and copper ions in solution (equation (1)) for the leaching of chalcocite, and in the dissolution of pyrite (equation (2)).

$$Cu_2S + 4Fe^{3+} \longrightarrow 2Cu^{2+} + 4Fe^{2+} + S$$
(1)

$$\text{FeS}_2 + 8\text{H}_2\text{O} + 14\text{Fe}^{3+} \rightarrow 15\text{Fe}^{2+} + 2\text{SO}_4^{2-} + 16\text{H}^+$$
 (2)

$$2Fe^{2+} + 0.5O_2 + 2H^+ \xrightarrow{\text{bacteria}} 2Fe^{3+} + H_2O$$
(3)

Sulfur and iron oxidizing acidophilic bacteria such as *Acidithiobacillus ferrooxidans* are involved when ferrous ions are catalyzed to ferric ions (equation (3)), which increases the overall reaction rate significantly (Meruane and Vargas, 2003). The optimal growth of bacteria is strongly coupled with iron, sulfur, oxygen, temperature and pH levels. Bacteria are known to attach to ore surfaces (Escobar *et al*, 1996), and this can be beneficial to leaching by maintaining a high bacterial concentration in the heap.

To gain an understanding of the fundamental processes occurring in a heap, experiments are often performed with a column of ore, and one can sample effluent liquid data and record quantities such as iron levels, copper, bacteria (free) and the solution (electric) potential. Consequently recent modelling efforts have been directed towards the simulation of column bioleaching (Dixon and Petersen, 2003; Neuburg et al, 1991). Indeed the only researchers to account for bacterial transport, growth and attachment/detachment in the heap bioleaching model are Dixon and Petersen (2003) and Neuburg et al (1991). Unfortunately these authors have not discussed particular aspects of the model, including the effect of inoculation on the process, and the effect that poor aeration can have on leaching. These researchers used a high sparging rate, in an attempt to eliminate the oxygen limitation on bioleaching, however there are circumstances in heap bioleaching whereby there may not be sufficient airflow and hence oxygen. An understanding of oxygen limitation is of importance for a real heap bioleaching operation, which can have lateral as well as vertical air flow (Leahy, Schwarz and Davidson, 2003)), and regions of low air flow and hence low oxygen levels (Bartlett, 1998). These may be due to variations in bed permeability (pore space clogging due to jarosite precipitation or localized liquid saturation), or a large sparger spacing and associated oxygen depleted regions in between spargers (Sidborn and

Moreno, 2003). Relying on natural convection has also been shown to result in oxygen limitation (Casas *et al*, 1998). Consequently there is a need for an investigation on how reduced aeration affects the bioleaching in a column.

In this work we aim to investigate the application of a model for the column bioleaching process and use it to investigate the effects of oxygen limitation and inoculation on the leaching process.

COLUMN BIOLEACHING MODEL

Problem Definition

There are many coupled processes occurring in the column, with the interaction of bacteria, oxygen, ferric and ferrous ions, along with the copper-sulfide.

The process is summarized as follows, and is assumed to involve 8 components:

those in the solid phase (assumed to be stationary)

- the un-leached copper remaining in the ore, described by a shrinking core model
- bacteria attached to the ore from the liquid phase, which detach proportionally to the number attached, have Monod growth kinetics (dependent on oxygen and ferrous ions), and also have a given death rate.

those species in the gas phase

• oxygen which exchanges with the liquid phase,

and those species in the (flowing) liquid phase

• oxygen which exchanges with the gas phase

- ferrous and ferric ions which are converted back and forth; ferric ions regenerated from bacteria, and ferric ions converted to ferrous ions in leaching
- free bacteria which exchange with the attached bacteria, have Monod growth kinetics (dependent on oxygen and ferrous ions), and also have a given death rate.

The transport of these components is governed by an advection-diffusion equation with source terms for each moving phase to describe the processes just mentioned. The gas and liquid velocities are assumed to be constant and given by their respective application flow rate divided by the area of application.

Model Formulation

The scalar equation for the gas oxygen concentration C_g at time *t* (seconds) is given by the well known advection diffusion equation in unsaturated porous media for a gas occupying a volume fraction ε_g

$$\frac{\partial(\varepsilon_{g}C_{g})}{\partial t} = D_{g}\frac{\partial^{2}(\varepsilon_{g}C_{g})}{\partial z^{2}} - v_{g}\frac{\partial(\varepsilon_{g}C_{g})}{\partial z} + S_{g}$$
(4)

where D_g is the diffusion (dispersion) coefficient for oxygen in gas, with z the distance from the bottom of the column, and S_g is the source term for the gas phase, representing the oxygen mass transfer to and from the liquid phase.

Similarly, the transport equation for the liquid species C_i is given by the advectiondiffusion equation for the liquid species: dissolved oxygen, free bacteria, ferrous ions and ferric ions as

$$\frac{\partial(\varepsilon_L C_i)}{\partial t} = D_g \frac{\partial^2(\varepsilon_L C_i)}{\partial z^2} + v_L \frac{\partial(\varepsilon_L C_i)}{\partial z} + S_{L,i}$$
(5)

where ε_L is the volume fraction of liquid, D_L is the diffusion (dispersion) coefficient for oxygen in liquid, and S_i is the source/sink term for the i_{th} species. This term represents the source/sink for each species and represents attachment/detachment, bacterial growth and death, oxygen and ferrous ion consumption, and ferric ion regeneration for the respective species as outlined above.

The attached population of bacteria ψ (to the stationary solid phase) have the form

$$\frac{\partial(\varepsilon_{ore}\psi)}{\partial t} = S_{\psi} \tag{6}$$

where S_{ψ} the source term for the attached bacteria population representing bacterial growth and death, attachment and detachment. In (6) ε_{ore} is the ore bed density defined as $\varepsilon_{ore} = \rho_b(1-\gamma)$, ρ_b is the ore density and γ the porosity of the bed given by $\gamma = \varepsilon_L + \varepsilon_g$.

The rate of copper sulfide leaching given by

$$R_{cps} = -\varepsilon_{ore} G \frac{\partial \alpha}{\partial t}$$
⁽⁷⁾

where α is the copper fraction remaining in the ore given by the shrinking core equation (Neuburg *et al*, 1991)

$$\frac{\partial \alpha}{\partial t} = \frac{3\alpha^{2/3} F e^{3+}}{\tau_c + 6\tau_d \alpha^{1/3} (1 - \alpha^{1/3})}$$
(8)

where τ_c , τ_d are given by

$$\tau_c = \frac{\delta \varphi \rho_b M_{Fe}}{\beta M_{ore}} \tag{9}$$

and

$$\tau_d = \frac{\delta^2 \sigma \, G \varphi \rho_b M_{Fe}}{D_{eff} M_{ore}} \tag{10}$$

where G is the grade of the ore, σ is the stoichiometric coefficient, φ is the particle shape factor, β is the intrinsic rate of oxidation and D_{eff} is the effective diffusion coefficient of the oxidant (Fe^{3+}) through the particle.

Model Boundary Conditions

The system of partial differential equations was solved in *CFX4* with the following assumptions: initial levels of oxygen in the gas and liquid phase are atmospheric, initially no bacteria either attached or in solution, and small levels of ferric and ferrous ions. The boundary conditions are: atmospheric levels of oxygen at the top and bottom of the column in the liquid and gas phases respectively, with a pulse of bacteria injected at a known concentration, and dissolved iron inflow of: 0.7g/L ferrous and 0.7g/L ferric ions.

NUMERICAL RESULTS AND DISCUSSION

Model Validation

An experimental data set from the literature is used for the validation of the model (Dixon and Petersen, 2003), a column bioleaching operation of low grade chalcocite ore mixed with pyrite, with the operation in a 5 metre column of 10 cm in diameter lasting 120 days. It is assumed the column was operated on a once through basis but it is uncertain whether the columns were inoculated and what the ferric and ferrous ions and acid concentrations were in the leaching solution. In this work we assume the column was inoculated for a small amount of time with bacteria at a fixed concentration, and that the solution consisted of certain amounts of ferric and ferrous ion as described above.

The ore contained chalcocite and pyrite with weight grades 0.9% and 3.5% respectively, as well as acid soluble copper sulphide (presumably copper oxide), with 0.5% grade. It is assumed the acid soluble copper sulfide would have been flushed out very quickly with the acid in solution and is not included in the simulation. To compare the simulation with the copper leaching data, which includes the leaching of the acid soluble copper sulfide, in Figure 1(a) the copper extracted α is plotted with the value

$$\alpha' = \alpha \frac{0.9}{1.4} + \frac{0.5}{1.4} \tag{11}$$

The model comparison to the effluent data of bacterial concentrations, solution potential (*Eh*), iron levels and the overall copper extracted is shown in Figures 1(a)-(f) (see Table 1 for simulation parameters). We see that the model successfully predicts the variables involved in the copper extraction. The attached bacteria have a constant distribution throughout the column (Figure 1(f)) with the concentration range in latter stages of the simulation around 5×10^{11} cells/kg ore, and this is a typical value found in practice (Bouffard and Dixon, 2003).

The initial stage of the ferric ion comparison does not match the data, and there are several reasons for this discrepancy. The actual operating conditions of the column are unknown, and it is not even known whether the column was operated on a once through basis, or whether the column was inoculated. It is possible also that some ferric ions could also have been precipitated to jarosite, a hardening of ferric ion from solution to the solid surface. Jarosite precipitation is complex process, but has been reported (Readett et al, 2003) to be dominant (so that little ferric ions will remain in solution) when the pH is above 3, and others have reported a pH over of 2.5 (Roman and Benner, 1973). In this experiment Dixon and Petersen (2003) report a pH of up to 2.8 in the early stages where the ferric ions are low. However the timing of the fraction of ferric ions to total iron is comparable to the data (Figure 1(e)) and this model obtains a qualitative and quantative fit of bacteria to the data, whereas Dixon and Petersen (2003) model this data and do not achieve such close comparison. The model is able to predict the bioleaching of chalcocite, and associated phenomena and this suggests the model can be used to investigate sub-processes within the column such as oxygen limitation and inoculation method.

Oxygen Limitation in Column

The aeration rate was varied below the base case $v_g = 1.1 \times 10^{-3} m/s$ to observe the effect on copper extraction. It is expected that lower oxygen causes a lower bacterial concentration in the column causing a decreased ferric ion concentration and hence lower copper extraction rate. In Figure 2(a) we see that for the sparging rate ($v_g/10$) the copper extraction is slower initially compared to the base case, and the bacterial concentration is not greatly different (Figure 2(b)) in the effluent initially. In the long run the copper extraction for the middle aeration rate catches up to the highest sparging rate so that although the middle air velocity slows the copper extraction, it will not ultimately inhibit the copper extraction. Another decrease in the sparging rate $(v_g/100)$ sees a much larger decrease in the copper extraction, which suggests air velocities below 10^{-4} m/s should be avoided.

We need to look inside the column to understand why the bacterial concentrations for the lower sparging rates are eventually highest. At 37 days (Figure 3(a)-(d)) the total bacteria is higher throughout the column for the highest sparging rate, and so the ferric ions concentration and associated copper extraction is higher. In Figure 4(a)-(d) after 93 days the bacterial concentration is greater for the middle sparging rate ($v_g/10$), and as time increases (Figure 2(b)) the bacterial concentration start to decrease and drop below those of the lower sparging rates. This is simply because the higher sparging rate achieves faster copper extraction (high ferric ion and low ferrous ion levels) with the ferrous ions becoming limiting to bacterial growth sooner.

In Figures 3, 4 and 5 we see that bacteria become growth limited when the oxygen concentration drops below 15% (or 1.1 mg/L), which is similar to the experimentally determined value range 1-1.2mg/L (Witne and Phillips 2001, Neuburg et al, 1991). After 116 days (Figure 5(c)) the oxygen levels for the middle sparging rate have increased through-out the column to be above 20%, and do not limit the bacteria, which are now evenly distributed. This is only possible if the bacterial concentration lower down in the column have decreased and less oxygen is used on the way up, and this is the case, as can be seen on comparing the bacterial and oxygen levels at 93 days and 116 days in Figures 4(c),(d) and 5(c),(d) respectively. The decrease in bacterial concentration further down the column is due to the significant decrease in total ferrous ions in the column after 50 days (Figure 2(b)), causing a limitation to the bacterial growth. This decrease in ferrous ions occurs gradually as the copper is leached from the lower section of the column (where bacteria had earlier not been limited by oxygen), and the reaction rate decreases, causing the source term for ferrous ion to drop. Consequently the ferric ions concentration is high enough everywhere to produce good leaching thereafter, and explains why the copper extraction catches up the highest sparging rate (Figure 2(a)).

Effect of Inoculation

The effect of the inoculation method is discussed here, whereby the duration and concentration of the bacterial injection is varied to observe the associated affect on the bacteria in effluent and copper extraction.

Length of Inoculation

The length of time that the bacteria are injected in the column is investigated in this section, to compare the resultant bacterial behaviour and copper extraction. In Figure 6(a)-(b), we see the effect of variation of the time period of inoculation, varying from 100 seconds to 80000 seconds. The results show that the bacteria in effluent are essentially the same regardless of the length of inoculation and are shifted later in time as the time period for inoculation decreases, so that the overall copper extraction is also shifted later. For each case, eventually the same behaviour is evident, so that the copper extraction is no different in the long term. This behaviour can be explained by the fact that bacteria initially grow exponentially, and since the injection concentration is the same, eventually the bacterial concentration all reach the same peak (and drop due to ferrous ion limitation) regardless of the length of inoculation.

Effect of Concentration of Inoculation

The concentration of bacteria injected in the column is investigated in this section, to observe the bacterial behaviour and associated copper extraction. In Figure 6(c)-(d) we see a very similar effect to the proceeding section, whereby the inoculation concentration does not significantly change the overall copper extraction. Again this is because the growth is exponential and although the bacteria take longer to grow to the peak concentration, the same behaviour is observed, regardless of the initial bacterial concentration.

CONCLUSIONS

A simplified model for the bioleaching of copper sulfides is presented which compares very well to experimental data of a column bioleaching operation. The model was used to investigate several aspects that had previously not been discussed in the literature, including the effect of poor oxygenation on copper extraction and the effect of inoculation method. It was found that copper leaching was slowed by a reduction in the air flow rate, but ultimately not inhibited for sparging air velocities of at least 10^{-4} m/s. Interestingly, for the middle sparging rate, bacteria were initially low at the top of the column causing poor extraction in the top. However, once the bottom of the column was leached the top was re-oxygenated and bacteria grew to high enough levels for the copper extraction to improve. For lower air velocities than 10^{-4} m/s a significant reduction in the long term copper extraction was observed due to low bacterial concentrations throughout the majority of the column. It was also found that the copper extraction is not sensitive to the inoculation method, in regard to the length and concentration of inoculation. Further work should incorporate several more aspects, including a 2D air flow model for a real heap configuration, the effect of jarosite precipitation, and the effects of thermal variations within a heap.

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References

- Bartlett, R W, 1992, Solution Mining: Leaching and Fluid Recovery of Materials, pp 24-53, Gordon and Breach Science Publishers: Amsterdam.
- Bouffard, S C and Dixon, D G, (2001), Investigative study into the hydrodynamics of heap leaching processes, *Met Mat Trans*, 32B:763-776.
- Bouffard, S C and Dixon, D G, (2003), Mathematical modelling of pyritic refractory gold ore heap biooxidation: model development and isothermal column simulations, in *Hydrometallurgy 2003 Fifth International Conference in honour of Professor Ian Ritchie Volume 1: Leaching and Solution Purification*, (Ed(s): Young, C A, Alfantazi, A W, Anderson, C G, Dreisinger, D B, Harris, B and James, A), pp 275-288 (TMS).

- Casas, J M, Martinez, J, Moreno, L, and Vargas, T, (1998), Bioleaching model of a copper-sulphide ore bed in heap and dump configurations, *Met Mat Trans*, 29B:899-909.
- Dixon, D G, (2003), Heap leach modelling-the current state of the art, in Hydrometallurgy 2003 - Fifth International Conference in honour of Professor Ian Ritchie Volume 1: Leaching and Solution Purification, (Ed(s): Young, C A, Alfantazi, A W, Anderson, C G, Dreisinger, D B, Harris, B and James, A), pp 289-314 (TMS).
- Dixon D G and Petersen, J, (2003), Comprehensive modelling study of chalcocite column and heap bioleaching, in *Copper 2003-Cobre 2003 Volume VI Hydrometallurgy of Copper (Book 2)*, (Ed(s): Riveros, P A, Dixon, D G, Dreisinger, D B and Menacho, J) pp 493-515.
- Escobar, B, Jedlicki E, Wiertz, J and Vargas, T, (1996), A method for evaluating the proportion of free and attached bacteria in the bioleaching of chalcopyrite with *Thiobacillus ferrooxidans*, *Hydrometallurgy*, 40:1-10.
- Leahy, M J, Schwarz, M P and Davidson, M R, (2003), An Air Sparging CFD model for heap bioleaching of copper-sulphide, in *Proceedings Third International Conference on CFD in the Minerals and Process Industries*, pp 581-586, (CSIRO, Melbourne, Australia)
- Meruane, G and Vargas, T, (2003), Bacterial oxidation of ferrous iron by *Acidithiobacillus ferrooxidans* in the pH range 2.5-7.0, *Hydrometallurgy*, 71:149-158.
- Neuburg, H J, Castillo, J A, Herrera, M N, Wiertz, J V, Vargas, T and Badilla-Ohlbaum, R, (1996), A model for the bacterial leaching of copper sulphide ores in pilot-scale columns, *Int J Min Processing*, 31:247-264.

- Sidborn, M and Moreno, L, (2003), Model for bacterial leaching of copper sulphides by forced aeration, in *Proceedings of the 15th International Biohydrometallurgy Symposium (IBS 2003)*, paper 33, Athens, Greece.
- Readett, D, Sylwestrzak, L, Franzmann, P D, Plumb, J J, Robertson, W R, Gibson J A E and Watling, H, (2003), The life cycle of a chalcocite heap bioleach system, in *Hydrometallurgy 2003 Fifth International Conference in honour of Professor Ian Ritchie Volume 1: Leaching and Solution Purification*, (Ed(s): Young, C A, Alfantazi, A W, Anderson, C G, Dreisinger, D B, Harris, B and James, A), pp 365-374 (TMS).
- Roman, R J and Benner, B R, (1973), The dissolution of copper concentrates, *Minerals Sci Engng*, 5(1):3-24.
- Witne, J Y, Phillips, C V, (2001), Bioleaching of OK Tedi copper concentrate in oxygen and carbon dioxide-enriched air, *Min Engng*, 14(1):25-48.
- Zeballos, F, Barbosa Filho, O, and Jose de Carvalho, R, (2003), Mathematical modeling of the chemical and bacterial leaching of copper ores in stack, in *Proceedings of the 15th International Biohydrometallurgy Symposium (IBS 2003)*, paper 32, Athens, Greece.

Captions

Table 1: Parameters used in 5m-column simulation

Figure 1: Data and model copper extraction (a) data and model bacteria concentration (b) data and model Eh (mV) (c) data and model ferrous and ferric ion concentration (d) ferric ion concentration/total iron concentration (e) vs time and attached bacterial concentration after 116 days (f)

Figure 2: (a) Model bacteria concentration and copper extracted and (b) ferrous ions for very low ($v_g/100$), low ($v_g/10$) and base (v_g) vs time.

Figure 3: Model (a) copper extracted, (b) ferric ion concentration, (c) total bacterial concentration (d) oxygen normalized, for very low ($v_g/100$), low ($v_g/10$) and base (v_g) vs distance from column bottom, after 37 days.

Figure 4: Model (a) copper extracted, (b) ferric ion concentration, (c) total bacterial concentration (d) oxygen normalized, for very low ($v_g/100$), low ($v_g/10$), and base (v_g) vs distance from column bottom, after 93 days.

Figure 5: Model (a) copper extracted, (b) ferric ion concentration, (c) total bacterial concentration (d) oxygen normalized, for very low ($v_g/100$), low ($v_g/10$) and base (v_g) vs distance from column bottom, after 116 days.

Figure 6: Model (a) copper extraction and (b) bacteria concentration vs time for variation in seed time and (c) copper extraction and (d) bacteria concentration vs time for variation in inoculation concentration.

Parameter	Value	Units	Reference
\mathcal{E}_L	0.2	m^3/m^3	Bouffard and Dixon (2001)
\mathcal{E}_{g}	0.2	m^3/m^3	Bouffard and Dixon (2001)
v_g	1.1×10^{-3}	m/s	Dixon (2003)
v_L	5.29x10 ⁻⁶	m/s	Dixon (2003)
δ	0.01	т	Dixon (2003)
G	0.9%	-	Dixon (2003)
β	1.30x10 ⁻⁷	m/s	Neuburg <i>et al</i> (1991)
\mathcal{E}_{ore}	1410	g/L	Dixon (2003)
D_{eff}	9.5x10 ⁻¹¹	m^2/s	Zeballos et al (2003)
φ	0.65	-	Neuburg <i>et al</i> (1991)
σ	4	-	Neuburg et al (1991)

 Table 1: Parameters used in 5m-column simulation



Figure 1: Data and model copper extraction (a) data and model bacteria concentration (b) data and model Eh (mV) (c) data and model ferrous and ferric ion concentration (d) ferric ion concentration/total iron concentration (e) vs time and attached bacterial concentration after 116 days (f)



(a)

Figure 2: (a) Model bacteria concentration and copper extracted and (b) ferrous ions for very low ($v_g/100$), low ($v_g/10$) and base (v_g) vs time.



Figure 3: Model (a) copper extracted, (b) ferric ion concentration, (c) total bacterial concentration (d) oxygen normalized, for very low ($v_g/100$), low ($v_g/10$) and base (v_g) vs distance from column bottom, after 37 days.



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Figure 6: Model (a) copper extraction and (b) bacteria concentration vs time for variation in seed time and (c) copper extraction and (d) bacteria concentration vs time for variation in inoculation concentration.