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The effect of elemental sulphur and pyrite on the leaching of nickel laterites using chemolithotrophic bacteria

G.S. SIMATE* and S. NDLOVU* and M. GERICKE†

**School of Chemical and Metallurgical Engineering,*
University of the Witwatersrand, South Africa
†Biotechnology Division, Mintek, Randburg, South Africa

In this study the efficiency of using either elemental sulphur or pyrite in the bacterial leaching of nickel laterites was investigated. Mixed cultures of *Acidithiobacillus ferrooxidans*, *Acidithiobacillus caldus* and *Leptospirillum ferrooxidans* were used. By measuring released nickel and pH changes, it was found that elemental sulphur yielded considerably better results than pyrite over a duration of one month. In the initial leaching the nickel recovered in the presence of the pyrite substrate was somewhat higher than that produced in the presence of the sulphur substrate. It should be noted, however, that although there was a definite observed trend for all the pHs studied, the difference was not that very significant in terms of statistically quantification. These observed results are attributed to the fact that bacteria are not very active in the initial leaching stages. At this stage of the process, abiotic oxidation of the substrates is the dominating factor. Ferrous ions in the leach media solution abiotically oxidize to ferric ions in the presence of oxygen. At acidic pH levels, elemental sulphur is inert to abiotic oxidation although other species such as thiosulphate and tetrathionate are oxidized abiotical to sulphate. Pyrite is oxidized by ferric ions via the thiosulphate route, producing sulphuric acid. It is thus expected that pyrite will result in slightly higher pH drops and thus the observed slight differences in nickel recoveries.

Keywords: nickel laterites; chemolithotrophic microorganisms; leaching

Introduction

With an ever increasing demand for nickel and daily depletion of high grade nickel sulphide ore reserves, research on how to process the more abundant nickel laterite ore reserves is necessary. Nickel laterite ores have complex mineralogy, low nickel content, and are difficult to treat by conventional methods. However, new processes such as biotechnological leaching play an ever increasingly important role in the extraction of metals from low grade ores (Nemati *et al*., 2000). These processes offer potential economic, environmental and technical advantages over conventional methods (Nemati and Harrison, 2000; Acevedo, 2000).

Although bacterial leaching has found widespread application in the minerals industry, the use of chemolithotrophic bacteria in recovery of nickel from lateritic ores is relatively unexplored. The chemolithotrophs, which use sulphur and sulphide minerals such as pyrite as their energy sources in the bioleaching, ultimately produce sulphuric acid, which is subsequently used for the leaching of the metal values. Although nickel laterite contains metal values, it is not capable of participating in the primary chemolithotrophic bacterial oxidation process, probably because of the lack of the sulphidic content of the ore needed to produce the required sulphuric acid. Previous studies have shown that the metal value in the nickel laterites can be recovered by allowing the primary bio-oxidation of substrates such as pyrite or elemental sulphur to provide sulphuric acid solutions, which solubilize the metal content (Simate and Ndlovu, 2007; Simate and Ndlovu, 2008). These substrates are biogenically oxidized by acidophilic chemolithotrophic microorganisms for their energy supply, producing sulphuric acid (Ross, 1990), as shown in Equations [1] and [2]. The rate of recovery of nickel laterites by bacterial leaching is, however, affected by the type of substrate used to generate the acidic leaching reagent.

$$2FeS_2 + 7O_2 + 2H_2O \xrightarrow{bacteria} 2Fe^{2+} + 4SO_4^{\ 2-} + 4H^+ \qquad [1]$$

$$2S^0 + 3O_2 + 2H_2O \xrightarrow{bacteria} 2SO_4^{\ 2-} + 4H^+ \qquad [2]$$

$$NiO + 2H^+ \rightarrow Ni^{2+} + H_2O \qquad [3]$$

The sulphuric acid produced lowers the pH of the reaction media (Equations [1] and [2]). The pH or hydrogen ion concentration, [H^+], is an important parameter that can be utilized to determine the extent to which nickel laterites dissolve (Equation [3]). It also determines the diversity of the microorganisms in a colony, e.g., obligate acidophiles such as those used in this study require low pH for growth since their membranes dissolve and cells lyse at neutrality (Todar, 2000). The solution pH in a given bioleaching operation is determined by the balance between the acid-producing and acid-consuming reactions. In the construction and use of culture media, one must always consider the optimum pH for growth. Therefore, the primary objective of this particular work was to investigate the pH requirements for the bacterial leaching of nickel laterites using a mixed culture of chemolithotrophic microorganisms in the presence of externally added substrates of elemental sulphur and pyrite.

Materials and methods

Ore samples and preparation

The nickel ore was crushed and was classified into -75+63 μm size fractions using standard sieve plates. This size range was used because it was the size class in which most of the nickel laterite material fell in terms of mass. In addition, previous studies have shown that this particular size range gave higher nickel recoveries (Valix *et al*., 2001; Tang and Valix, 2006) not only due to the large percentage of nickel in the ore but also due to the higher survival of the microorganisms during the bioleaching process at this particular size range. The chemical composition of nickel was determined prior to chemical leaching experiments. The typical chemical composition of various oxides in the laterite ore used is given in Table I.

Table I
Chemical composition of nickel laterite ore sample

Mineral	SiO_2	Fe_2O_3	Cr_2O_3	Al_2O_3	MgO	NiO	CoO	CaO	MnO	CuO	C	S	P
Mass %	52.79	21.86	0.99	2.5	7.46	1.87	0.25	0.68	1.28	0.01	0.119	0.034	0.064

Microbes

A mixed culture of chemolithotrophic microorganisms (*Acidithiobacillus Ferrooxidans*, *Acidithiobacillus caldus* and *Leptospirillum Ferrooxidans*) used in the experiments was provided by Mintek, South Africa. The bacteria were cultured in standard 9K nutrient medium (Silverman and Lundgren, 1959).

Experimentation

Bioleaching experiments were carried out in 250 ml Erlenmeyer flasks with 100 ml of slurry. The slurry comprised a mixture of 5 g nickel laterite ore and 100 ml of medium together with an appropriate amount of sterilized energy sources (30% w/w elemental sulphur and 56% w/w pyrite to that of nickel laterite). These quantities of energy sources were chosen so as to have the same sulphur content in both elemental sulphur and pyrite. The slurries were inoculated with 10% (v/v) mixed bacterial culture. The pH of the mixtures was adjusted to 1.0, 1.5, 2.0 and 2.5, respectively. These pH ranges (i.e. pH <3) were chosen because they are known to optimize the growth of acidophilic microorganisms (Norris and Johnson, 1998). Some experiments were also run at initial pH of 1.5 using fresh liquid medium with an appropriate amount of sterilized energy source but without the inoculation of bacteria for comparison with inoculated experiments. Uninoculated distilled water and nutrient media were run as sterile at an initial pH of 1.5.

The flasks were covered with pieces of aluminium foil to reduce evaporation and prevent contamination, but allow free supply of air, and then incubated in a platform shaking incubator at 30°C and 190 rpm. The pH profiles and redox potentials of the leach solution were measured but not controlled throughout the leach period using 744 pH meter Metrohm. The redox potentials readings were obtained using the Ag/AgCl, 3M KCl reference electrode and subsequently converted to the standard hydrogen electrode (SHE) (Friis *et al*., 1998). Samples (1.5-ml) were drawn from flasks every three days to determine the concentration of nickel dissolved using the Varian SpectrAA-55B atomic absorption spectrophotometer. Solution loss through sampling was compensated by the addition of distilled water or 9K medium. The nickel recovery during the leaching of nickel laterites at any sampling time was calculated as a percentage of nickel in the liquid phase (concentration) to that in the original nickel laterite ore.

The bacterial population was determined by measuring turbidity or optical density of the bacterial suspension using a UV-Visible double beam spectrophotometer (Model 4802). Since turbidity is directly proportional to the number of cells, this property was used as an indicator for bacterial concentration. The cells suspended in the suspension interrupt the passage of light, allowing less light to reach the photoelectric cell, and the amount of light transmitted through the suspension was measured as percentage transmission (or %T). The turbidity for cell suspension was measured at 550 nm (Plumb *et al*., 2008) against sterile 9K media as a reference. The wavelength of 550 nm is chosen because at this wavelength changes in both size of the cells as well as changes in the total nucleotide concentrations are reflected (Alupoaei and García-Rubio, 2004).

Note that the relationships among absorbance (A), transmittance (T) and optical density (OD) are as follows: $T = \frac{I}{I_0}$, where I is the light passing through the sample and I_0 is the light hitting the sample. A = $\log_{10}T$. Optical density is a measure of absorbance and is related to transmittance by the following expression; OD = 2 – log (%T).

Other available methods based upon measurement of nitrogen or protein content or ammonia consumption, and that of using the phase contrast microscope were less convenient and slower than the UV-Visible double beam spectrophotometer. On the other hand, the UV-Visible spectrophotometer is a versatile, quantitative, rapid, and reliable analytical tool (Alupoaei and García-Rubio, 2004). It must be noted, however, that this method measures both the dead and active bacteria as it does not differentiate between the two states. However, the results obtained used in conjunction with pH and redox potential changes would reasonably outweigh the disadvantages of counting the dead bacteria. Prior to reading, the samples were filtered through Whatman filter number 1 to remove any solid particles.

Results and discussion

Effect of substrate type on nickel recovery

In the first two weeks there were no significant differences in nickel recoveries, though slightly higher recoveries of nickel were observed in the presence of the pyrite substrate than sulphur substrate (Figure 1a). Initially ferrous ions in the 9K media oxidize to ferric ions according to reaction [4] below.

$$4Fe^{2+} + O_2 + 4H^+ \rightarrow 4Fe^{3+} + 2H_2O \qquad [4]$$

The ferric ions in turn attack pyrite producing ferrous ions and sulphuric acid through the thiosulphate mechanism (Schippers *et al*., 1996; Schippers and Sand, 1999). In the case of elemental sulphur under acidic conditions, oxidation is exclusively carried out by bacteria (Friedrich *et al*., 2001; Rohwerder *et al*., 2003; Rohwerder and Sand, 2007) although other sulphur species such as thiosulphate and tetrathionate are oxidized abiotically to sulphate (Schippers and Sand, 1999; Rohwerder *et al*., 2003). It is also expected, in the initial stages, that the bacteria are not very active; so abiotic oxidation of the substrates is supposedly dominant. However, higher recoveries were observed for the sulphur substrate than for the pyrite substrate at all pH levels after two weeks of leaching (Figure 1b). This showed that there was more acid produced by the leaching bacteria from the sulphur substrate than from pyrite substrate after two weeks.

Effect of initial pH on nickel recovery

Figures 2 and 3 show that the dissolution of nickel laterites is largely dependent on the initial solution pH. Although there was a general increase in the rate of nickel dissolution with time for all pH levels, higher recoveries of nickel were observed at lower pH with about 50% nickel being extracted within the first two weeks at initial pH level of 1.0. The higher recoveries at lower pHs indicate the possible dominance of abiotic leaching during this dissolution stage due to the high levels of acid in solution.

Figures 2 and 3 also show that a rise in the nickel recovery was more for the pH of 2.5 after two weeks. This was attributed to the higher rate of acidification at this pH level (Figure 4 and 5). Previous studies have shown that higher rates of acidification imply that there were higher microbial activities (Rossi 1990; Hanford and Vargas, 2001; Schippers and Sand, 1999). Acidification in this context means production of H^+ ions. The production of H^+ ions is depicted by the pH drop, and that pH is a measure of concentration of H^+ ions (i.e. pH = -log10[H^+]).

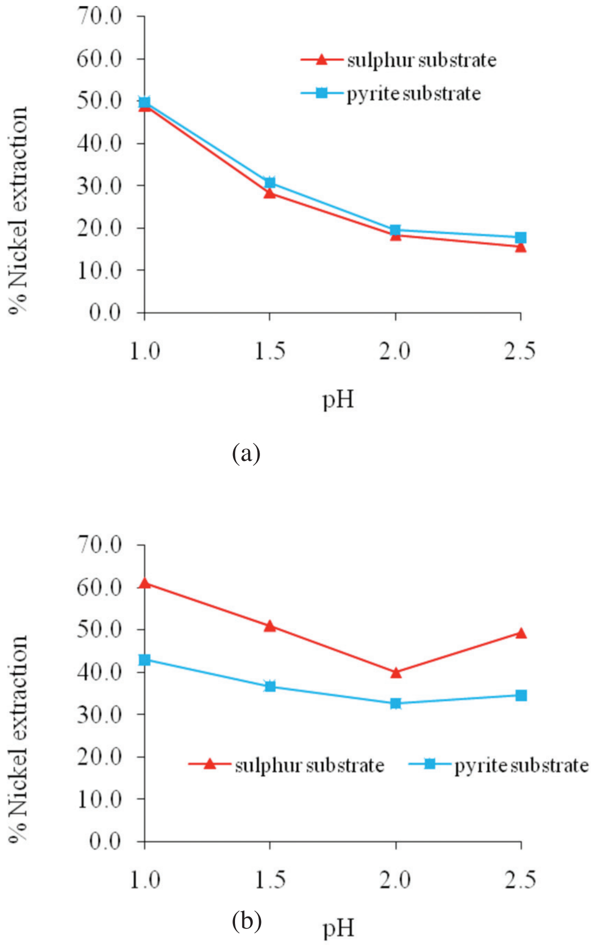

Figure 1. Dissolution rates of nickel laterites as a function of substrate type at different pH. (a) is recoveries within two weeks; (b) is recoveries in a month

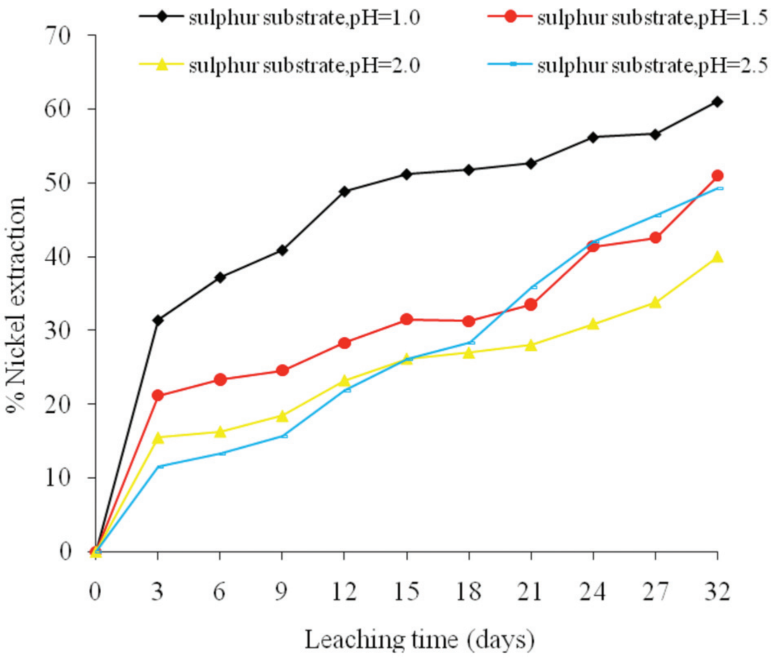

Figure 2. Effect of pH on the leachability of nickel laterite with sulphur substrate

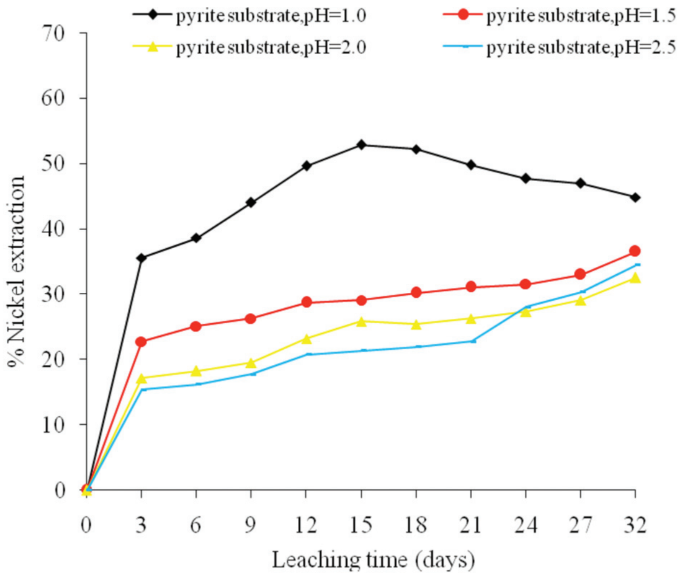

Figure 3. Effect of pH on the leachability of nickel laterites with pyrite substrate

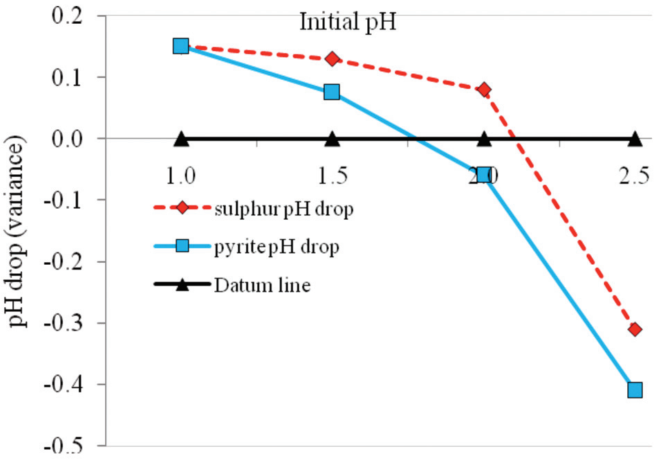

Figure 4. pH drop for sulphur and pyrite substrates within two weeks

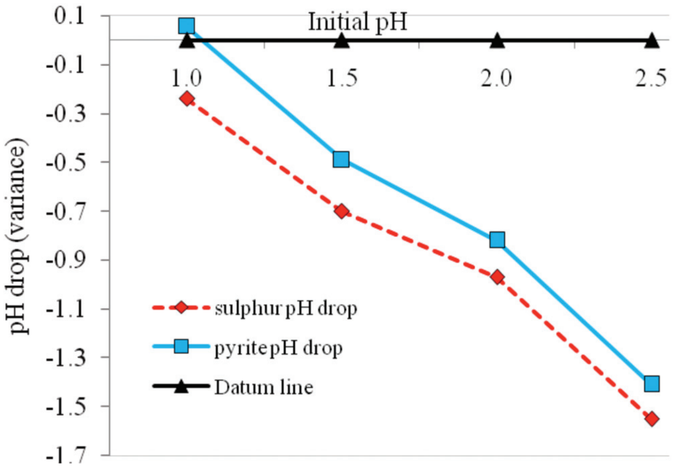

Figure 5. pH drop for sulphur and pyrite substrates in a month

Effect of substrate on pH drop

The pH rise for all the pH levels tested (i.e. pH 1.0, 1.5, 2.0, 2.5) was initially higher for sulphur than for pyrite within two weeks (Figure 4). Bacteria are expected not to be very active in the early stages of leaching. Initially, the pH rise was due to the consumption of the added acid since there was low production of acid through either abiotic oxidation or biotic oxidation of the substrates. However, in the case of the pyrite substrate, the abiotic oxidation of ferrous ions in the 9K medium to ferric ions leads to the subsequent oxidation of pyrite to produce ferrous ions and sulphuric acid. As a result there is a slight balance between the acid consuming and producing reactions and thus the pH rise is less than that for the sulphur substrate. In the subsequent leaching period pH drop was higher for the sulphur substrate than for pyrite at the same initial pH, implying that there was higher rate of acidification with the sulphur substrate as a result of bacterial activity (Figure 5). The results in Figures 4 and 5 also show that the pH decreased rapidly at higher initial pH, 2.5>2.0>1.5>1.0. This can be attributed to either; (1) the bacteria were naturally inactive at the low pH level or, (2) metal toxicity due to higher metal recoveries (due to initial rapid chemical leaching) at low pH. Furthermore, at higher pH both elemental sulphur and ferrous iron are oxidized abiotically at significant rates (Rohwerder *et al.*, 2003). The trend in pH changes, arising from the substrates, was also observed during the identification of influential factors in the previous studies (Simate and Ndlovu, 2008). The results of the differences in pH changes can also be seen in the recoveries, being slightly higher in the presence of the pyrite substrate in the early stages, and subsequently higher with the sulphur substrates (Figures 1).

Effect of substrate on bacterial growth

Figure 6 shows the bacterial growth curve that was taken during the leaching period. The figure shows that bacterial concentration was higher for sulphur substrate than pyrite throughout the leaching period. The higher microbial growth with sulphur is as result of more energy gained from the oxidation of sulphur compared to the oxidation of ferrous ions (Rawlings *et al.*, 1999; Yu *et al.*, 2001). At the solution pH of 2, the Gibbs free energy released from the oxidation of ferrous iron is -9.49 kcal/mol, and that released from elemental sulphur is -185.9 kcal/mol (Yu *et al.*, 2001).

The trends in the nickel recoveries (Figure 1a) and pH drops (Figures 4) compared to bacterial concentration in the first two weeks (Figure 6) indicate that the rate of abiotic oxidation of pyrite was higher than the biotic oxidation of sulphur in the beginning. The higher amount of nickel recovered in the presence of the sulphur substrate after the two weeks tally with the peak of bacteria population as seen in Figure 6. These observations, therefore, show that a period of more than two weeks is an effective duration for the bacterial leaching of nickel laterites. After two weeks the high acid levels from the biotic oxidation of the sulphur substrates result in the higher nickel recoveries in the presence of the sulphur substrate than pyrite substrate. It seems, therefore, that the effect of bacteria on the nickel laterite dissolution depends mostly on the relationship between the bacteria and the substrate added to the media.

Effect of media composition on pH and oxidation reduction potential

Figures 7 and 8 show the effects of different media composition on pH and oxidation reduction potential (ORP), respectively. It is observed in Figure 7 that for inoculated experiments, there was a slight increase of pH initially and a subsequent decrease of pH thereafter. This was most likely due to the predominance of abiotic oxidation process initially.

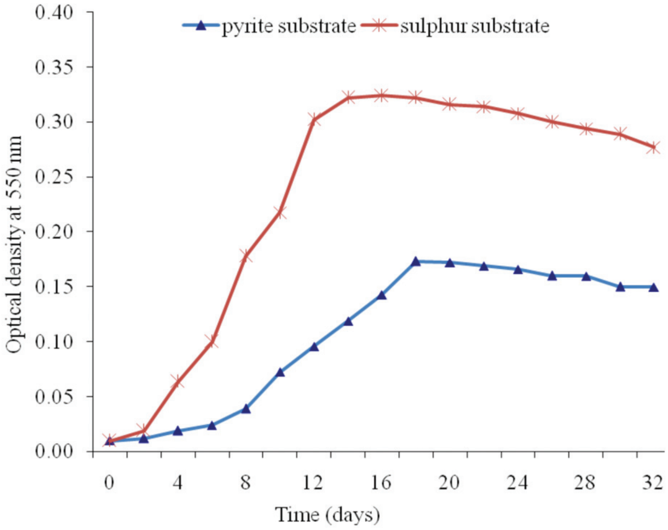

Figure 6. Optical density at 550 nm as a function of time at pH of 1.5

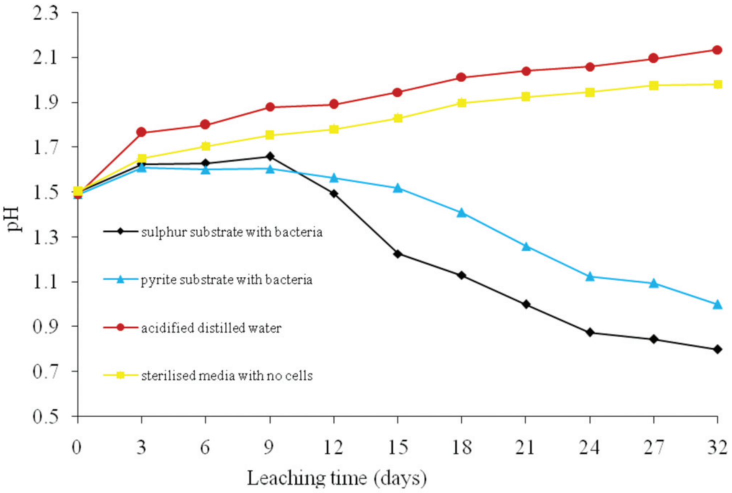

Figure 7. Evolution of pH with time at initial pH of 1.5 for different media compositions

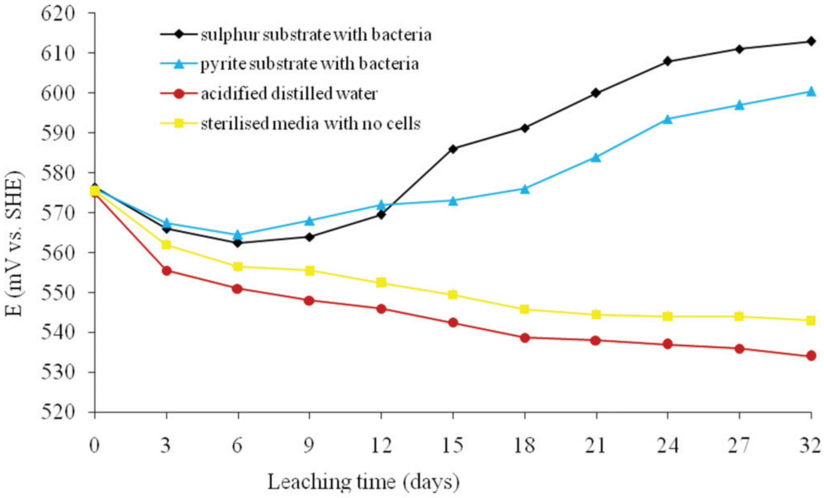

Figure 8. Evolution of ORP with time at initial pH of 1.5 for different media composition

Even though acid production took place in the long-term through the subsequent bioxidation of sulphur or pyrite, the initial net leaching process was likely acid consuming and hence the initially pH rise. In summary, the overall decrease in pH observed in the inoculated media is attributed to the oxidation of elemental or reduced sulphur to sulphuric acid by the leaching bacteria (see Equations [1] and [2]).

In sterile controls (acidified distilled water, and sterilized media with no cells), the pH decrease was not observed. An initial rise and final decrease in pH was observed in experiments where uninoculated sulphur and pyrite media were used (results not shown here). However, the pH decrease for these uninoculated media was still lower than the inoculated media. This shows the efficacy of the presence of bacteria in the production of acid leading to pH reduction. The pH drop observed with uninoculated pyrite media can be attributed to the slow pyrite oxidation by oxygen in the presence of water forming ferrous iron and sulphate (Larsson *et al*., 1990) according to Equation [5] below. Sulphur was assumed to be slowly oxidized by oxygen in the presence of water according to Reaction [6] below (Hanford and Vargas, 2001).

$$2FeS_2 + 7O_2 + 2H_2O \rightarrow 2Fe^{2+} + 4SO_4^{2-} + 4H^+ \qquad [5]$$

$$0.125S_8 + 1.5O_2 + H_2O \rightarrow SO_4^{2-} + 2H^+ \qquad [6]$$

Figure 8 shows that higher ORPs were obtained with inoculated media than sterile media. The metal ion recoveries are higher with inoculated media than sterile media, thus implying higher ionic activities in the inoculated media. The ORP, which in the framework of this study, is a measure of the activities or strength of metal ions in relation to their concentration, is thus higher in the inoculated media.

Conclusions

This study investigated the bacterial leaching of nickel laterites using a mixed culture of chemolithotrophic microorganisms in the presence of externally added sulphur containing material (elemental sulphur and pyrite). The bacterial oxidation of sulphur and pyrite produces sulphuric acid, which dissolves nickel laterite to yield the required nickel metal.

The results presented showed that the dissolution rates of nickel laterite were high in low pH media and high ORP, and in the presence of bacteria. However, the study showed that microbial activities, depicted by acidification, were lower at lower initial pH levels. Although uninoculated media with energy sources appeared to induce acidification, this was less than that in active bacterial cultures. This shows that under similar conditions, an inoculated media is more effective than an uninoculated media.

This study has also shown the relationships between bacterial activities, depicted by acidification and the type of substrate. The recoveries were slightly higher for pyrite in the early stages of leaching and subsequently significantly higher for sulphur at all the initial pH levels studied. The test results and the high nickel recovery yield demonstrated that sulphur was more effective as a substrate than pyrite. It seems, therefore, that the effect of bacteria on the dissolution of minerals that are not capable of taking part in the primary chemolithotrophic bacterial oxidation process depends mostly on the relationship between the bacteria and the substrate energy source added to the media.

Previous studies have shown that mixed cultures tend to be superior to those containing only iron- or sulphur oxidizing bacteria. However, little is known about how microorganisms interacted in this study, and how such interactions may have benefited or detracted the mineral dissolution process. It is, therefore, recommended that further studies should detect and differentiate the various species used during the bioleaching experiments.

Overall, this study has opened up a new era for the potential application of chemolithotrophic microorganisms for the commercial processing of the difficult-to-process low grade nickel laterite ores. In regions where the nickel laterite orebody exists the sustainability of the process will depend on the supply of sulphur-containing material (commercial sulphur or metal sulphide) for energy requirements of the bacteria. This is likely to form the dominant operating cost component of the bacterial leaching of nickel laterite ores using chemolithotrophic microorganisms. In addition, this process is promising because sulphuric acid is produced *in situ* whereas in processes such as high pressure acid leaching, sulphuric acid is produced in external facilities. With the addition of sulphur-containing material, the use of chemolithotrophic bacteria can be extended to the leaching of other low grade non-sulphide containing ores. Some examples where this process may be applied include silicate ores, oxidic converter furnace slags and refractory oxides.

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Geoffrey S. Simate
Masters Student, University of Witwatersrand, South Africa

Three years experience in metallurgical operations for Zambia's major mining conglomerate (Zambia Consolidated Copper Mines Ltd). Worked for one year with Konkola Copper Mines Plc after privatisation of Zambia Consolidated Copper Mines Ltd. Worked for one year with Anglovaal Mining Zambia Ltd Research Laboratory as a Process Engineer. Worked for four years with Chambishi Metals Plc as a Process Controller. Worked for one year with Maamba Collieries Ltd as a Plants Manager with the responsibility for ensuring efficient, safe and cost effective operations of the entire metallurgical operations. In my career I have been involved with plant commissioning, trouble shooting, pilot plant tests, plant efficiency tests and have provided technical expertise to the plant processes in order to achieve maximum efficiency. Have experience with leaching, solvent extraction, ion exchange, thickening, and electrowinning commercial plant operations. Fully qualified Quality Assurance Internal Auditor (BSI and RT&A). Scheduled to graduate with an MSc (Eng) in Metallurgy and Materials Engineering from the University of Witwatersrand in May, 2009.