The European Journal of Mineral Processing and Environmental Protection
Vol.2, No.3, 1303-0868, 2002, pp. 141-150

ejmp&ep

Effect of Salinity on the Oxidative Activity of Acidophilic Bacteria during Bioleaching of a Complex Zn/Pb Sulphide Ore

H. Deveci*

Karadeniz Technical University, Mining Engineering Department, 61080 Trabzon, Turkey

Received 3 April 2002 accepted 10 June 2002

ABSTRACT

The presence of some anions and cations at certain levels in the bioleaching environment may exert an inhibitory effect on the growth and hence leaching activity of a bacterial culture. In this respect, the quality of process water available with particular reference to salinity can be of prime importance for the application or development of a bioleaching process for a particular feed at an operation site.

The current study investigates the extent to which salinity up to 8% Cl^- (~80g/l) affects the bioleaching activity of mesophilic, moderately and extremely thermophilic strains of bacteria during the bioleaching of a complex Zn/Pb sulphide ore. The results indicated that salinity can adversely influence the "optimum" bioleaching activity of mesophiles and moderate thermophiles; the extent being dependent upon the strain (and type) of bacteria and the concentration of chloride. The mesophilic WJM strain was found to oxidise the complex ore at concentrations of up to 0.8% Cl^- (~8g/l) without any significant effect on the extraction of zinc while the limited extraction of zinc by DSM 583 strain occurred at 0.2% Cl^-. It was noted that mesophiles can be adapted to tolerate 0.8-1% Cl^- (~8-10g/l) in solution. The bioleaching ability of the strains of moderate themophiles was adversely influenced even at 0.2% Cl^- (~2g/l). On the other hand, the extreme thermophiles were shown to perform well under saline conditions up to 5% Cl^- (~50g/l). This probably indicates the halophilic peculiarity of the extreme thermophiles compared with the mesophiles and the moderate thermophiles.

Keywords: Acidophilic bacteria; Bioleaching; Chloride

1. INTRODUCTION

Biooxidation of gold bearing arsenopyrite/pyrite concentrates using mesophilic and moderately thermophilic bacteria as a pre-treatment step prior to cyanidation has already been proved as an economically viable and competitive alternative to conventional roasting and pressure oxidation (Dew *et al.*, 1997; Miller, 1997). This has stimulated the extension of the technology for the extraction of other metals such as Cu, Zn, Co and Ni (Brierley and Brierley, 1999; Miller *et al.*, 1999; Gilbertson, 2000).

Iron and/or sulphur oxidising strains of mesophilic bacteria such as *Thiobacillus ferrooxidans, Leptosprillum ferrooxidans* and *Thiobacillus thiooxidans* operating at 40°C are the most extensively studied microorganisms for the oxidation sulphide minerals with commercial interest (Dew *et al.*, 1997; Jordan *et al.*, 1996). Since the kinetics of metal dissolution tend to increase with temperature, the commercial application of thermophilic bacteria which can operate at temperatures exceeding 45°C has great potential for improving the rates of metal extraction from sulphide minerals. Accordingly, in recent years several commercial bioleaching processes using thermophilic bacteria have been developed for the extraction of base metals (Cu, Ni and Zn) and successfully operated at pilot scale with the reports of imminent commercial realisation (Miller *et al.*, 1999; Staden, 1998; Gilbertson, 2000).

* Corresponding author. E-mail: hdeveci@ktu.edu.tr

Bioleaching of the sulphide minerals is based on the exploitation of the ability of the acidophilic bacteria to oxidise ferrous iron (Eq. 1) and/or reduced sulphur compounds (Eq. 2 for elemental sulphur) as substrates whereby the bacteria derive the energy required for the growth and other metabolic functions (Ingledew, 1982). The oxidation of ferrous iron and sulphur compounds by bacteria results in the production of ferric iron and/or acid which attack sulphide minerals (Eqs 3-4 for ZnS) (Sand *et al.*, 2001). This leads to the enhanced dissolution of metals from sulphides in the presence of bacteria.

$$2FeSO_4 + 1/2O_2 + H_2SO_4 \xrightarrow{\text{bacteria}} Fe_2(SO_4)_3 + H_2O \quad (1)$$

$$S^o + 3/2O_2 + H_2O \xrightarrow{\text{bacteria}} H_2SO_4 \quad (2)$$

$$ZnS + 1/2O_2 + H_2SO_4 \rightarrow ZnSO_4 + H_2O + S^o \quad (3)$$

$$ZnS + Fe_2(SO_4)_3 \rightarrow ZnSO_4 + 2FeSO_4 + S^o \quad (4)$$

The degree of enhancement in the metal dissolution is intimately controlled by the oxidising activity (growth) of bacteria. These microorganisms in general obtain their cellular material from inorganic sources such as CO_2, NH_4^+, K^+, Mg^{2+}, Ca^{2+}, SO_4^{2-} and PO_4^{3-} present in the growth media (Tuovinen and Bhatti, 1999). However, the presence of some anions such as Cl^- and cations at certain levels in the bioleaching environment may exert an inhibitory effect on the growth and hence the leaching performance of a bacterial culture. In this regard, the quality of process water available with particular reference to salinity can be of prime importance for the application of the bioleaching processes since chloride-free process water may not be available at an operation site. Budden and Spencer (1991) and Weston *et al.* (1994) have reported the analyses of some borehole waters with a Cl^- content of 12.7-25.7g/l and 110g/l respectively. Depending on the concentration of chloride that a bacterial culture can tolerate the treatment of poor quality water may be required, which would in turn lead to the increase in both operating and capital costs (Budden and Spencer, 1991).

Leong *et al.* (1993) reported that the dissolution of copper from a high grade ore by a mesophilic mixed culture of *Thiobacilli* was adversely affected in the presence of up to 8g/l Cl^-. Weston *et al.* (1994) observed a reduction in the oxidation rate of pyrite with an increase in the concentration of Cl^- above 2g/l during the pilot scale test work on a pyritic gold concentrate using the BIOX® culture. Budden and Spencer (1991) claimed that moderately thermophilic bacteria (BacTech®'s culture) can operate well under saline conditions (0-5% NaCl).

The current study reports the results of an investigation undertaken to evaluate the Cl^- tolerance limits of all three groups of bacteria; mesophiles, moderate and extreme thermophiles during the bioleaching of a complex ore.

2. EXPERIMENTAL

2.1. Mineralogy and Chemical Composition of the Ore

Samples of the McArthur River complex ore were obtained from the mine site in the Northern Territory of Australia (kindly via MIM Holdings, owners of the deposit). The chemical and mineralogical analyses of the ore are shown in Table 1. Mineralogical analysis of the ore using the XRD and SEM revealed that the ore consisted predominantly of sphalerite (ZnS), galena (PbS) and pyrite (FeS_2) as the important sulphide phases. The sphalerite, galena and pyrite were found to be very fine grained and intimately associated with each other to such a degree that even fine grinding (<10µm) would not have produced complete liberation. A particle size of <300µm (d_{80}= ~250µm) were used in the experiments.

Table 1
Chemical and mineralogical composition of the McArthur River complex ore used in this study

Metal content						Mineralogical content
Zn (%)	Fe (%)	Pb (%)	Cu (%)	S (%)	Ag (g/t)	Sphalerite, galena, pyrite, chalcopyrite, quartz, dolomite and various silicate phases such as ortochlase
16.2	7.95	5.60	0.27	15.2	59	

2.2. Bacterial Cultures

Two strains of mesophiles, moderate and extreme thermophiles were used in this study. The mesophiles were *Thiobacillus ferrooxidans* DSM 583 (from Deutsche Sammlung von Mikroorganismen (DSM) culture collection) and the mixed enrichment culture designated WJM (an isolate from the acid mine drainage waters of Wheal Jane mine, Cornwall, UK) composed of iron and sulphur oxidisers such as *Thiobacillus ferrooxidans*, and heterotrophic bacteria. The strains of moderate thermophiles were YTF1 and THWX (*Sulfobacillus acidophilus*) which were kindly provided by Dr D.B. Johnson (University of Wales, Bangor, UK). The extreme thermophiles, DSM 1651 (*Acidianus Brierleyi*) and *Sulfolobus* were available in the culture collection of Camborne School of Mines and had previously been obtained from the DSM culture collection and kindly from Dr. P.R. Norris (Department of Biological Sciences, Warwick University, UK) respectively. All cultures were grown (and maintained) on the ore sample (1% w/v) prior to the experimental work. The growth of the cultures was carried out in an enriched salt solution containing $MgSO_4.7H_2O$ (0.4g/l), $(NH_4)_2SO_4$ (0.2g/l), $K_2HPO_4.3H_2O$ (0.1g/l) and KCl (0.1g/l). Yeast extract (0.02% w/v) was also added to support the growth of moderate thermophiles.

2.3. Bioleaching experiments

Bioleaching experiments (in duplicate) were carried out in 250ml Erlenmeyer flasks. Enriched salt solution (90ml) was transferred into each flask to which 1g of the ore sample (1% w/v pulp density) was added. The calculated (stoichiometric) amount of chloride (as NaCl) was added to each flask to produce the required chloride concentration in the final volume of solution. The flasks were then autoclaved under the pressure of 1atm at 121°C for 20 minutes. Following autoclaving each flask was inoculated under aseptic conditions with a 10ml aliquot of the selected culture producing a final volume of slurry of approximately 100ml. The control flasks were prepared using the same procedure with the exception that, instead of being inoculated with a bacterial culture, 10ml of 5% thymol in methanol was transferred to prevent any bacterial activity for the duration of experiments. For the experiments using the mesophiles an orbital shaker operating at 100rpm within a warm room controlled to a temperature of 30°C was used to shake the flasks. The moderate and extreme thermophile experiments were performed using a separate incubator rotating at 160rpm, thermostated at the appropriate temperature such as 50°C for moderate thermophiles and 70°C for extreme thermophiles.

Sampling of each flask was carried out daily by removing a 1ml aliquot of the leach solution which was then used for analysis of metals (Zn, Fe and Pb) by Atomic Absorption Spectrometry (AAS) and for monitoring of pH. Adjustment of the pH was undertaken only if the pH value exceeded the initial pre-set values of pH 1.6 for mesophiles and moderate thermophiles, and pH 1.4 for extreme thermophiles.

3. RESULTS AND DISCUSSION

3.1. Effect of Chloride on Mesophiles

Figure 1-4 illustrate the dissolution of zinc and iron from the complex ore using mesophilic strains WJM and DSM 583 in a range of 0-5% Cl^- added. In the presence of strains (0% Cl^-) there was a significant enhancement in the extraction of zinc and iron in comparison with the chemical acid leaching in the absence of strains as indicated by the control. The results also indicated that the addition of chloride exerted an adverse effect on the oxidative activity of the bacterial cultures with the extent being dependent upon the strain and concentration of chloride added.

As shown in Figure 1-2 for WJM strain, the addition of chloride led to a lag period in the dissolution of zinc and iron with the extent of the lag period having increased with increasing the concentration of chloride. This increase in the lag period was possibly the indication of the adverse effect of chloride on the bioleaching activity of the strain. During these initial periods, the adaptation of the strain or selection of the more resilient cells within the bacterial population may be presumed to have occurred since a substantial increase in the dissolution of zinc and iron occurred particularly at Cl^- concentrations of ≤0.8% following these initial periods.

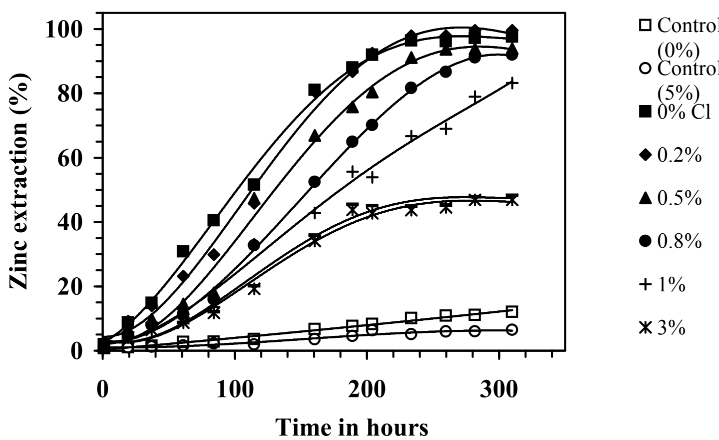

Figure 1. Effect of chloride on the extraction of zinc from the ore (1% w/v) by WJM strain at pH 1.6 and 30°C

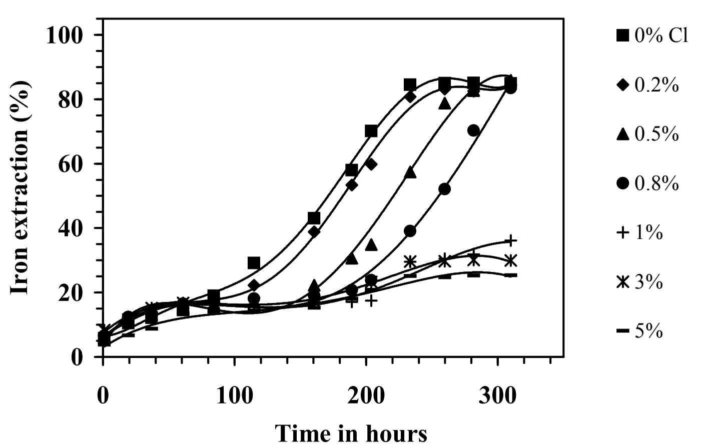

Figure 2. Effect of chloride on the extraction of iron from the ore (1% w/v) by WJM strain at pH 1.6 and 30°C

The extraction of zinc was comparable at ≤0.8% Cl^- with a slight decrease at 0.5-0.8% Cl^- over the bioleaching period (Figure 1). Further increase in the Cl^- level resulted in significant reduction in the rate and extent of metal dissolution. The extraction of zinc at 3-5% Cl^- appeared to be prematurely levelled off after 189h. This could be attributed to the formation/encrustation of a passivating layer such as elemental sulphur and/or ferric iron precipitates on the surfaces of sphalerite particles. In this respect, the presence of relatively high concentrations of Na^+ such as ~20 g/l at 3% Cl^- (introduced in the form of NaCl) could have promoted the precipitation of sodium jarosite (Eq. 5) providing that the bacteria were able to generate ferric iron under these conditions.

$$Na^+ + 3Fe^{3+} + 2SO_4^{2-} + 6H_2O \rightarrow NaFe_3(SO_4)_2(OH)_6 + 6H^+ \quad (5)$$

The onset of a substantial dissolution of iron was coincident with the occurrence of a significant extraction of zinc, ~45% Zn at 0% Cl^- (Figure 1-2). This was probably a reflection of the electrochemical aspects of the dissolution process peculiar to the complex sulphide systems with "preferential or selective" oxidation of sphalerite over pyrite (Natarajan, 1990). The continual oxidation of ferrous iron by bacteria was presumed to occur within the bioleaching environment. This would probably lead to the gradual build-up of ferric iron as a consequence of depletion of more active sulphides such as sphalerite and, more importantly, proliferation of the bacterial population with increasing oxidation capacity. Had the redox potential of the system approached/surmounted the rest potential of the pyrite, the dissolution of iron would have been promoted. This was consistent with the observed pattern for the dissolution of iron (Figure 2).

Figure 3-4 illustrate the effect of added Cl^- on the bioleaching performance of DSM 583 strain. Compared with WJM strain, DSM 583 strain appeared to be more sensitive to Cl^- ions in that severe inhibition of the oxidising activity of the strain was discernible even at 0.2% Cl^- with a 17% decrease in the final extraction of zinc. This highlights the different sensitivity of both strains to tolerate Cl^- ions. An apparent decrease in the concentration of iron in solution at 0.2% Cl^- after 260h was observed for DSM 583 strain. This could be attributed to the formation of presumably jarosite type precipitates at the operating pH of 1.6. In this regard, the limited zinc extraction at 0.2% Cl^- may have been due to the encrustation of precipitates on the surfaces of sphalerite particles possibly leading to the impediment of the further zinc release.

The dissolution of lead within the experiments where no chloride was added was minimal; over 98% of lead reported to the residues most likely in the form of either "insoluble" lead sulphate or undissolved galena irrespective of the strain (and type) of bacteria used (the solubility of $PbSO_4$ is 0.045g/l at 25°C (Forward and Peters, 1985)). However, the solubilisation of lead was promoted with increasing the concentration of Cl^- added such as 5% Pb at 1% Cl^- and 100% Pb (~0.44g/l) at 5% Cl^-. It should be noted that lead chloride is reasonably soluble (the solubility of $PbCl_2$ is 10.7g/l at 25°C (Forward and Peters, 1985)) and the solubility of lead increases with increasing Cl^- concentration and temperature probably due to the formation of soluble lead-chloro complexes, $PbCl_i^{2-i}$ where i=1-4 (Holdich and Lawson, 1987).

Following the termination of the experiments, the cultures from the flasks at each Cl^- level were then subcultured on ferrous iron (100mM, pH 1.7) and the oxidation of ferrous iron was observed to occur in the flasks which had received inoculum from the flasks containing up to 0.5% Cl^- for DSM 583 strain and up to 1% Cl^- for WJM strain. It was noted that a prolonged period for the oxidation of ferrous iron was necessary for the strains which had been exposed to the increasing concentrations of Cl^-. Adaptation of both strains to Cl^- was also carried out on ferrous iron using serial subculturing procedures. Although the oxidation of ferrous iron was relatively slow, both strains were developed to tolerate 0.8% and 1% Cl^- (~8 and 10g/l) respectively beyond which there was little success.

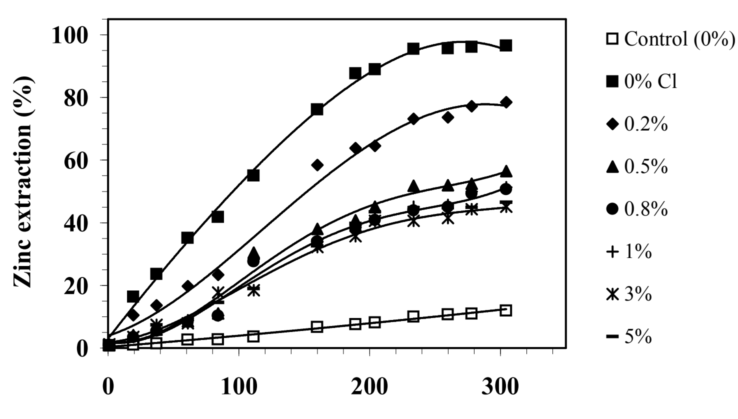

Figure 3. Effect of chloride on the extraction of zinc from the ore (1% w/v) by DSM 583 strain at pH 1.6 and 30°C

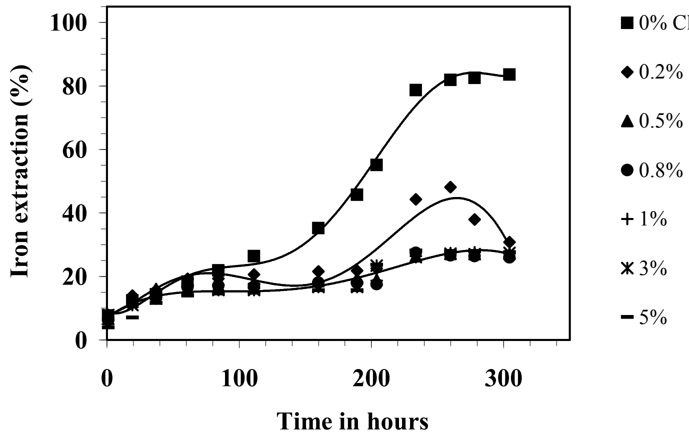

Figure 4. Effect of chloride on the extraction of iron from the ore (1% w/v) by DSM 583 strain at pH 1.6 and 30°C

Leong *et al.* (1993) observed the detrimental effect of chloride (0-8g/l Cl^-) on the dissolution of copper using mixed cultures of Thiobacilli. These investigators also showed the adaptation of bacteria to chloride levels of 5g/l ameliorating the deleterious effect of chloride on the dissolution process. Vorreiter and Madgwick (1982) demonstrated that the average release of copper decreased (by 80%) in the presence of ~21g/l Cl^- (as NaCl) during the bioleaching of a low grade copper ore using *T. ferrooxidans* in a percolation column. They also noted a decrease in the number of viable cells (*T. ferrooxidans*) from 10^3 to <10 cells/ml on average.

Weston *et al.* (1994) reported the results of pilot plant and bench scale test work carried out using the BIOX® process for the biooxidation of a refractory gold concentrate with no apparent effect of salinity at 1-2g/l Cl^- on the bacterial oxidation rate when operating at lower pHs (1.1-1.3). They argued that the deleterious effect of salt was not due to the chloride present but rather due to the coexisting ions such as sodium leading to the formation of jarosite precipitates. They also reported a significant reduction in the subsequent extraction of gold by cyanidation at high Cl^- levels which was attributed to the increasing amount of jarosite precipitates under these conditions. Dew *et al.* (1997) suggested that high concentrations of chloride in solution could damage the membrane of the bacteria. They also reported that the negative effect of chloride on the oxidation of ferrous iron by the BIOX® culture was negligible in a range of 0-5g/l Cl^-, thereafter the oxidation rate of ferrous iron significantly decreased by 25% at 7g/l Cl^- with complete inhibition of the activity of bacteria at Cl^- concentrations of ≥19g/l.

It can be inferred that although the salinity exerts an adverse effect on the oxidising activity of mesophiles depending on the concentration of Cl^- and the strain of bacteria, the adaptation of the mesophilic bacteria up to 0.8-1% Cl^- (~8-10g/l) appears to be possible. However, the presence of the counter ions such as sodium may present further problems partaking in the formation of potentially deleterious jarosites.

3.2. Effect of Chloride on Moderate Thermophiles

The addition of chloride severely impeded the oxidative activity of YTF1 strain even at the lowest Cl^- level tested (0.2%) as illustrated in Figure 5. The strain completely solubilised the zinc available in the ore in the absence of added Cl^- (0% Cl^-). However, a severe decrease in the rate and extent of zinc extraction was observed in response to the addition of chloride at 0.2-8% Cl^- where a limited extraction of zinc occurred over the bioleaching period.

On the contrary, previous studies by the author (Deveci, 1997) had demonstrated that the addition of chloride (1-4% Cl^-) had no apparent effect on the dissolution of zinc by YTF1 strain from the ore but all be at a slower rate (at 0% Cl ~9.5mg Zn/l/h compared with 16.5mg Zn/l/h in the current study). These findings were later confirmed using the same strain at 0-3% Cl^- (Deveci, 2001). These earlier tests (Deveci, 1997) had been performed in the absence of yeast extract. The limited ability of the strain for the oxidation of ferrous iron had been noted and attributed to the maintenance of the strain in the absence of yeast extract. It should be noted that many pure strains of moderate thermophiles including those used in this study require yeast extract to support the growth of these microorganisms (Deveci, 2001; Ghauri and Johnson, 1991). The results of the current tests (at 0% Cl^-) showed that the dissolution rate of zinc was enhanced 1.7-fold in the presence of 0.02% w/v yeast extract compared with the earlier studies. The rationale behind this behaviour of YTF1 strain maintained in the presence and absence of yeast extract under saline conditions was not understood.

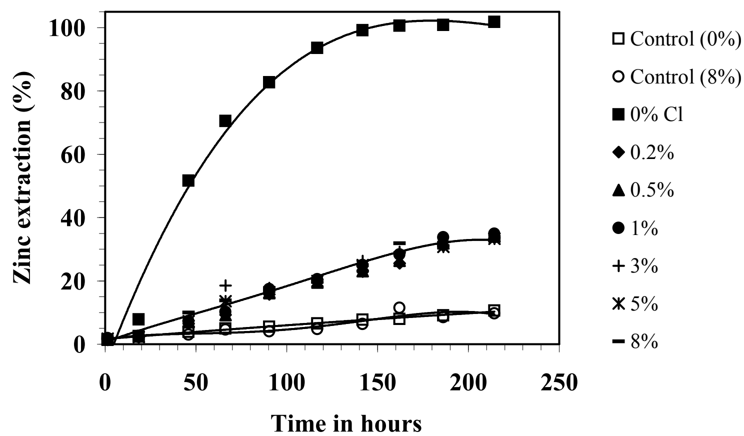

Figure 5. Effect of chloride on the extraction of zinc from the ore (1% w/v) by YTF1 strain at pH 1.6, 50°C and 0.02% w/v yeast extract

The effect of chloride on the bioleaching activity of THWX strain was also investigated as shown in Figure 6. The dissolution of zinc at 0.2% Cl^- was significantly faster (3.8 times) than that produced by YTF1 strain (Figure 5) at the same level of chloride. This probably indicates the varying sensitivity of both strains to chloride ions. The extraction of zinc by THWX strain though was severely suppressed at 0.5-3% Cl^- (Figure 6).

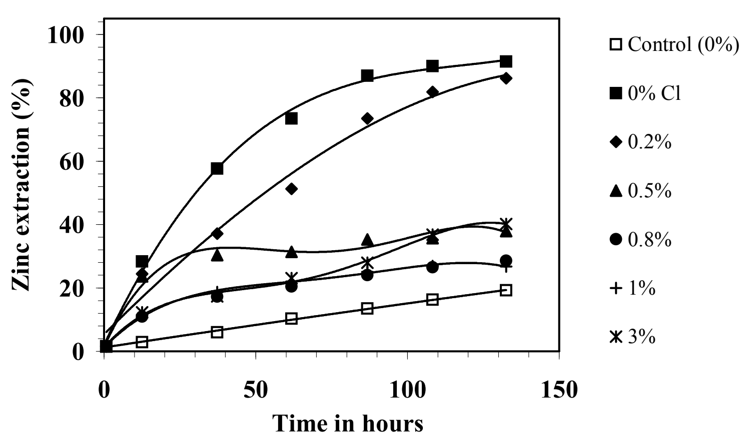

Figure 6. Effect of chloride on the extraction of zinc from the ore (1% w/v) by THWX strain at pH 1.6, 50oC and 0.02% w/v yeast extract

Although there appeared a significant impact on the bioleaching ability of the strains used in this study even at 0.2% Cl^-, Budden and Spencer (1991) reported that the BacTech®'s moderately thermophilic culture was able to oxidise a pyrite concentrate at a rate comparable (25% decrease) to the control (0% Cl^-) in a range of 0.6-3% Cl^-. They attributed the observed difference in the oxidation rate to the precipitation of iron rather than the inhibition of the bacterial activity since their microscopic examination of the culture revealed no apparent difference between the control and the chloride tests. No change in the number of bacteria or the morphology of the cells or the motility of the bacteria was noted in the presence of salt (NaCl). In a separate study, Budden and Spencer (1993) also demonstrated that the extraction rate of copper from a chalcopyrite concentrate decreased by 20% in a range of ~0.3-1% Cl^- (added as NaCl) and by 50% at ~1.2% Cl^-. These investigators noted an initial lag period; the extent being proportional to the amount of chloride present. However, in the subsequent cycles of bioleaching process, they no longer observed the lag time that was attributed to the adaptation of the culture to the saline conditions.

3.3. Effect of Chloride on Extreme Thermophiles

Complete dissolution of zinc was achieved by *Sulfolobus* at all the chloride concentrations tested (0-5% Cl^-) over the bioleaching period (Figure 7). The addition of chloride (0.2-5% Cl^-) appeared to have a slightly positive effect on the dissolution of zinc compared with no addition of chloride. A similar pattern for metal dissolution under the saline conditions was also produced by DSM 1651 strain as shown in Figure 8. These findings are consistent with the earlier findings using both strains at 1-4% Cl^- (Deveci, 1997).

The addition of chloride in the range of 0-3% Cl^- also appeared to improve the dissolution of zinc by DSM 1651 strain particularly after the initial 63h (Figure 8). The extraction of zinc at 5% Cl^- was similar to that with no chloride addition during the initial period of 139h despite the relatively low zinc recovery over the bioleaching period. Further increase in the concentration of chloride (at 8% Cl^-) adversely affected the bioleaching process and the final extraction of zinc was limited to only 77%.

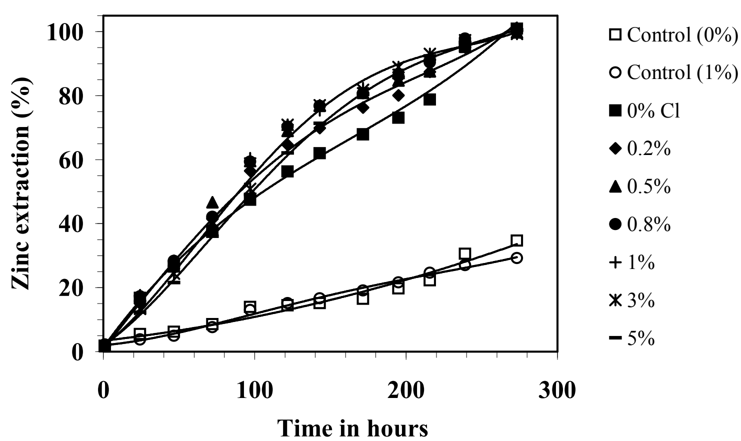

Figure 7. Effect of chloride on the extraction of zinc from the ore (1% w/v) by *Sulfolobus* strain at pH 1.4 and 70°C

It should be noted that there was no evidence for the precipitation of iron (presumably due to the relatively low operating pH of 1.4) in the current experiments using the extreme thermophiles. Notwithstanding this, the precipitation of ferric iron as jarosites would probably be promoted by the increase in temperature but this may be partially alleviated by decreasing pH (Dutrizac, 1983). The extreme thermophiles can efficiently operate at high levels of acidity as low as pH 1 (Deveci, 2001).

These findings indicate that the extreme thermophiles are halophilic in character as these microorganisms are able to operate under extremely saline environments at chloride concentrations up to 5% Cl^- (~50g/l).

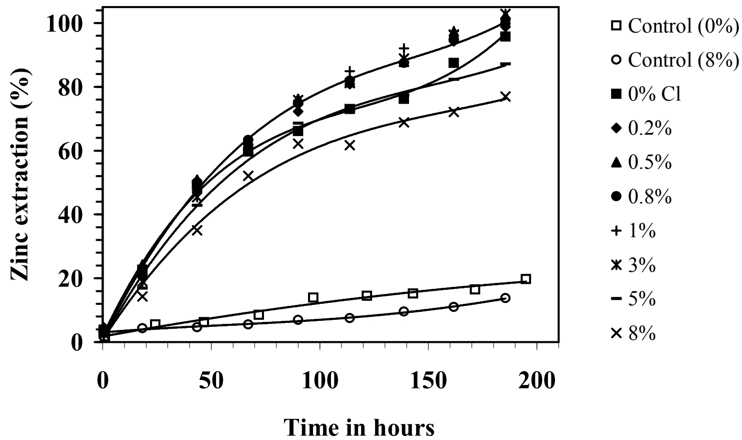

Figure 8. Effect of chloride on the extraction of zinc from the ore (1% w/v) by DSM 1651 strain at pH 1.4 and 70°C

4. CONCLUSIONS

Investigations into the chloride tolerance of the selected strains of mesophiles, moderate and extreme thermophiles showed that the quality of process water with respect to salinity is of prime importance for the bioleaching processes since it affects the activity of the bacteria and the chemistry of the dissolution process. The following conclusions can be drawn from this study:

i) The salinity exerts an adverse effect on the bioleaching activity of the mesophiles and moderate thermophiles resulting in a decrease in the rate and extent of zinc extraction from the ore. The extent of the adverse effect depends on the concentration of chloride and the strain of bacteria used.
ii) The adaptation of mesophilic bacteria to 0.8-1% Cl^- (~8-10g/l) appears to be possible. Chloride tolerance of moderate thermophiles appears to be limited to a Cl^- concentration of 0.2% (~2g/l).
iii) The extreme thermophiles are halophilic in character with their ability to perform under extremely saline environments at Cl^- concentrations up to 5% (~50g/l).
iv) The presence of counter ions such as Na^+ may present further problems as they could promote the formation of potentially deleterious ferric precipitates.
v) The solubilisation of lead increases with increasing the concentration of Cl^- probably due to the formation of lead-chloro complexes. In the absence of added Cl^- >98% of the lead reports to the residues.

This work highlights the vital need to consider the quality of process water available at the early stages of the development of bioleaching proposals and the potential benefit of using extreme thermophiles rather than mesophiles and moderate thermophiles for the bioleaching of ores/concentrates in saline environments.

ACKNOWLEDGEMENTS

The author would like to express his sincere thanks and appreciation to the late Dr. C.V. Phillips for his support, assistance and guidance, and to the Turkish Ministry of Education for financial support during his studies at Camborne School of Mines, University of Exeter, UK.

REFERENCES

Brierley, J.A. and Brierley, C.L., Present and future commercial applications of biohydrometallurgy. Biohydrometallurgy and the Environment toward the Mining of the 21st Century, Part A, IBS'99, ed. R. Amils and A. Ballester. 1999, pp. 81-89, Madrid, Spain.

Budden, J.R. and Spencer, P.A., The effect of temperature and water quality on bacterial oxidation: The advantages of a moderately thermophilic culture over conventional Thiobacillus cultures. Randol Gold Forum, Cairns, 1991, pp. 271-274.

Budden, J.R. and Spencer, P.A., Tolerance to temperature and water quality for bacterial oxidation: The benefits of BacTech®`s moderately thermophilic culture. FEMS Microbiology Rev., 1993, **11**, 191-196.

Deveci, H., Investigation of the selective dissolution of metals from a complex sulphide ore using acidic ferric sulphate solutions and acidophilic bacteria. MSc Dissertation, 1997, Camborne School of Mines, University of Exeter, UK, 86 pp.

Deveci, H., Bacterial leaching of complex zinc/lead sulphides using mesophilic and thermophilic bacteria. PhD Thesis, 2001, Camborne School of Mines, University of Exeter, UK, 320 pp.

Dew, D.W., Lawson, E.N. and Broadhurst, J.L., The BIOX® process for biooxidation of gold bearing ores or concentrates. Biomining: Theory, Microbes and Industrial Processes, ed. D.E. Rawlings. Springer-Verlag, Berlin, 1997, pp. 45-79.

Dutrizac, J.E., Factors affecting alkali jarosite precipitation. Metal. Trans. B, 1983, **14B**, 531-539

Forward, F.A. and Peters, E., Leaching principles. SME Mineral Processing Handbook, ed. Weiss N.L, 1985, Vol. 2, New York, pp. 13 (6-12).

Ghauri, M.A. and Johnson, B.D., Physiological diversity amongst some moderately thermophilic iron oxidising bacteria. FEMS Microbiology Ecology, 1991, **85**, 327-334.

Gilbertson, B., Creating value through innovation: Biotechnology in mining. IMM Trans. C, 2000, **109**, 61-67.

Jordan, M.A., McGinness, S. and Phillips, C.V., Acidophilic bacteria - their potential mining and environmental applications. Minerals Engineering, 1996, **9**(2), 169-181.

Holdich, R.G. and Lawson, G.J., The solubility of aqueous lead chloride solutions. Hydrometallurgy, 1987, **19**, 199-208.

Ingledew, W.J., *Thiobacillus ferrooxidans*: The bioenergetics of an acidophilic chemolithotroph. Biochimica et Biophysica Acta, 1982, **683**, 89-117.

Leong, B.J.Y., Dreisinger, D.B., Lo, M., Branion, R.M.R., Hackl, R.P., Gormerly, L.S. and Crombie, D.R., The microbiological leaching of a sulphidic copper ore in a strongly saline medium I: Shake flask and column studies. Biohydrometallurgical Technologies Vol. 1: Proc. of Int. Biohydrometallurgy Symp., ed. A.E. Torma, J.E. Wey and V.I. Lakshmanan. 1993, pp. 117-126, USA.

Miller, P.C., The design and operating practice of bacterial oxidation plant using moderate thermophiles. Biomining: Theory, Microbes and Industrial Processes, ed. D.E. Rawlings. Springer-Verlag, Berlin, 1997, pp. 81-100.

Miller, P.C., Rhodes, M.K., Winby, R., Pinches, A. and Staden, P.J., Commercialisation of bioleaching for metal extraction. Minerals and Metallurgical Processing, 1999, **16**(4), 42-50.

Natarajan, K.A., Electrochemical aspects of bioleaching of base metal sulphides. Microbiol Mineral Recovery, ed. H.L. Ehrlich and C.L. Brierley. McGraw-Hill, 1990, pp. 79-106.

Sand, W., Gehrke, T., Jozsa, P.G. and Shippers, A., (Bio)chemistry of bacterial leaching-direct vs. indirect bioleaching. Hydrometallurgy, 2001, **59**(2-3), 159-175.

Staden, P.J., The Mintek Bactech copper bioleach process. ALTA 98: Copper Sulphides Symposium, 1998, Brisbane, Australia.

Tuovinen, O.H. and Bhatti, T.M., Microbiological leaching of uranium ores. Minerals and Metallurgical Processing, 1999, **16**(4), 51-60.

Vorreiter, A. and Madgwick, J.C., The effect of sodium chloride on bacterial leaching of low-grade copper ore. Proc. Aus. Inst. Min. Metall., 1982, No. 284, pp. 63-66.

Weston, T., Perkins, J., Ritchie, I. and Marais, H., Concentrate biooxidation in a hypersaline environment for Knowna Belle. Biomine`94, Int. Conference and Workshop Applications of Biotechnology to the Minerals Industry, Australian Mineral Foundation, 1994, Perth, W.A.