rs"[1] *International Mining Congress and Exhibition of Turkey-IMCET 2003, iç) 2003, ISBN 975-395-605-3*

Bioleaching of a Complex Sulphide Ore by Mesophilic and Extremely Thermophilic Bacteria: Statistical Analysis of Data Using Ergun's Test

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ABSTRACT: This study investigates the effects of bacterial strain, salinity and pH on the bioleaching of a complex ore using mesophilic and extremely thermophilic bacteria with the statistical analysis of the results using Ergun's test. The results showed that the ore was readily amenable to the selective extraction of zinc and the extreme thermophiles as confirmed by the statistical analysis displayed .superior kinetics of dissolution of zinc compared with the mesophiles. The bioleaching performance of the extreme thermophiles was found to improve in response to the increase in acidity (pH 2.0 to 1.0) while the activity of the mesophiles was adversely affected by decreasing the pH. The statistical analysis indicated that the effect of pH was insignificant in the range of pH 1.0-1.6 for the extreme thermophiles and pH I.6-2.0 for the mesophiles. The salinity was shown to have a suppressing effect on the mesophiles. However, the extreme thermophiles appear to be halophilic in character as they could operate efficiently under the saline conditions (1-4% CI").

I INTRODUCTION

Bioleaching processes have gained importance in recent years for the extraction of metals particularly from the difficult-to-treat and low grade ores or concentrates (Rawlings et al. 2003). Bioleaching is essentially a dissolution process with the involvement of acidophilic bacteria which have the ability to oxidise ferrous iron (I) and/or elemental (2) or reduced sulphur compounds to derive the energy required for their growth and other metabolic functions (Ingledew 1982. Suzuki 2001). The reaction products, ferric iron and/or acid attack the sulphide minerals such as sphalerite (3 & 4) resulting in their dissolution (Sandetal. 2001).

$$2Fe^{2+} + 1/2O_2 + 2H^+ \xrightarrow{bacteria} 2Fe^{3+} + H_2O \quad (1)$$

$$S^0 + 3/2O_2 + H_2O \xrightarrow{bacteria} H_2SO_4 \quad (2)$$

$$ZnS + 2Fe^{3+} \rightarrow Zn^{2+} + 2Fe^{2+} + S^0 \quad (3)$$

$$ZnS + 2H^+ \rightarrow Zn^{2+} + H_2S \quad (4)$$

Mesophilic bacteria e.g. *T. ferrooxidans, L. ferrooxidans and T. thiooxidans* operating at <40°C are the most commonly used microorganisms for the bioleaching of sulphide minerals (Bosecker 1997). Thermophilic bacteria with their ability to thrive at high temperatures up to 85°C have great potential for use in bioleaching processes probably due to the improvement expected in the kinetics of metal dissolution particularly from the recalcitrant minerals such as chalcopyrite (Norris ct al. 2000, d~Hugues et al. 2002, Rawlings et al. 2003).

The rate and extent of oxidation of sulphide minerals within bioleaching processes are closely controlled by the oxidative activity (growth) of bacteria. There are many factors including pH and the toxicity of anions (e.g. CI") and cations that may affect the growth and hence the optimum leaching performance of a bacterial culture (Bosecker 1997, Dew et al. 1997, Deveci 2002). The level of salinity of process water to be used may be of great importance for the application of bioleaching processes in the areas where the availability of chloride-free process water may he limited (Budden & Spencer 1991, Weston et al. 1994).

The accurate interpretation of batch leaching data on which the development of a process is based is of vital significance for the determination or optimisation of process conditions. Despite the general availability of a variety of statistical techniques, the time-dependent nature of biolcaching data (i.e. metal concentration varying with time) restricts the use of many conventional statistical methods for the analysis of experimental data (Jordan el al. 1997). Powell & Jordan (1997) demonstrated a corrective technique based on Ergun's test (Ergun 1956) for the eradication of time dependency of leaching data.

Within this study, the potential amenability of a complex sulphide ore to the selective extraction of zinc using mesophilic and extremely thermophilic bacteria was evaluated. The effects of pH and salinity (Cf ions) on the bioleaching performance of the selected cultures were investigated. Statistical assessment of the experimental results using Ergun\s test to (i) eradicate the time-dependency and to (ii) examine the differences between the varying experimental conditions (i.e. pH, bacterial strain and chloride concentration) for significance was undertaken.

2 EXPERIMENTAL

2. / *Mineral Sample*

A complex sulphide ore sample originated from the McArthur River deposit. Northern Territory of Australia was used in this study. The ore sample were found to contain sphalerite (ZnS), galena (PbS) and pyrite (FeS_2) as the major sulphide phases with a chemical composition of 16.2% Zn, 5.6% Pb and 7.95% Fe. The crushed ore sample as received was ground to -300 (jm (d_{NO}=-25() pin) prior to use in the experiments.

2.2 *Bacteria and growth media*

The mesophilic cultures, SJ2 *(T. ferrooxidans)* and the mixed culture designated WJM (Jordan 1993), and the extremely thermophilic cultures, *Sulfolobus (S. metallicus)* and DSM 1651 *(Acidiantts brierleyi)* were used in the bioleaching experiments. The growth of mesophiles (30°C) and extreme thermophiles (70°C) on the ore (1% w/v) were carried out on orbital shakers using an enriched salt solution containing $MgS0_4$.7rLO (0.4 g/1), (NH_4),S04 (0.2 g/1), $K_2HP0_4.3H_20$ (0.1 g/1) and KCl (0.1 g/1) as the growth media.

2.3 Bioleaching experiments

Bioleaching experiments were carried out in 250 ml Erlenmeyer flasks with a working volume of 100 ml. A \% w/v ore in enriched salt solution (90 ml) was prepared in a flask which was then auloclavcd prior to the inoculation with a 10 ml (10% v/v) aliquot of an appropriate culture previously grown. In the chloride experiments the calculated amounts of chloride (as NaCl) were added to the flasks to produce 1%. 2% and 4% CI' in the final volume of solution.

The oxidation of the ore was monitored by the daily removal of 1 ml samples. These samples were used to determine the metal concentration (Zn, Fe and Pb) by AAS and the pH. If exceeded, the pH was adjusted to the predetermined level by the addition of 18 M H_2S0_4. The mean values of replicate (duplicate or triplicate) experiments were presented in the results.

2.4 Statistical methodology for the analysis of data

Ergun's test in conjunction with Moving Regression Analysis was adopted as the statistical methodology for the analysis of the bioleaching data. The maximum rate of metal dissolution (max. gradient) was initially determined from the bioleaching profiles using Moving Regression Analysis (Deveci 1997) in which the linear regression lines were fitted to the selected number of data points (e.g. five data points) starting from the first data set (i.e. 1—»5) and then the second (i.e. 2—»6) and so forth. The largest gradient with a statistically acceptable correlation coefficient was assigned as an estimate of the maximum rate of metal dissolution.

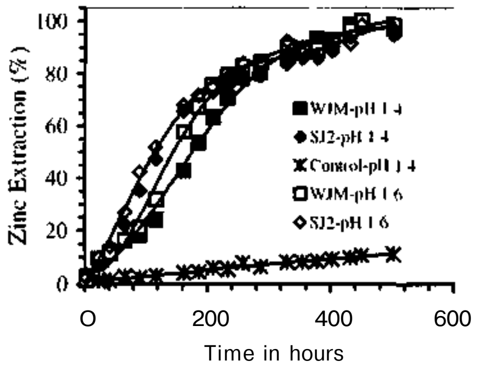
Having determined the maximum gradients for each bioleaching profile, Ergun's test was applied to eradicate the time-dependent nature of the rates of metal extraction (gradients) and to test for the differences between these for the varying pH values, the type/strain of bacteria and the chloride concentrations. The details of statistical analysis procedure used herein and the outlines of the mathematical methodology for Ergun's lest can be found elsewhere (Powell & Jordan 1997).

3 RESULTS AND DISCUSSION

3.1 Bioleaching tests using mesophiles

Figure 1 illustrates the bioleaching of the ore using WJM and SJ2 cultures at pH 1.4 and 1.6. Compared with the acid leach as indicated by the control, the contribution of the mesophilic bacteria to the dissolution of zinc was substantial with an enhancement of > 12-fold in the rate and of >8-tbld in the recovery. Over 95% of zinc was dissolved in the presence of both cultures at the both pH levels.

An increase in the pH from 1.4 to 1.6 had a positive effect on the bioleaching performance of both cultures with an increase in the dissolution rate of zinc observed for WJM culture in particular (Fig. 1). These findings suggested that the optimum pH for both cultures may well be above pH 1.6 as, in fact, further tests using WJM culture showed that the dissolution of zinc further improved with increasing the pH to 2.0 (Fig. 2).



Fιgme 1 Exrraclinn üt zinc lιoιn the ore using me.sophilic bacteι ια at 3()°C & pH 1.4 & 1.6

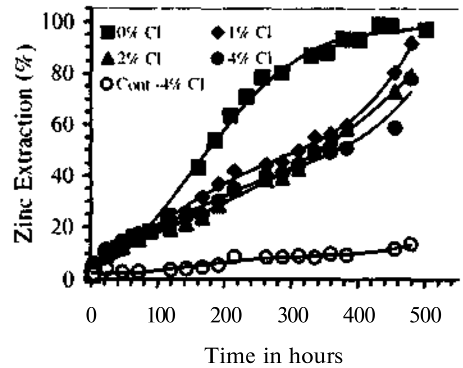


Figure 3. Effect of added chlonde (0-4% CD on the extraction of zinc fiom the oie (1% w/v) usin« WJM cultuie at 30°C & pH 1 4

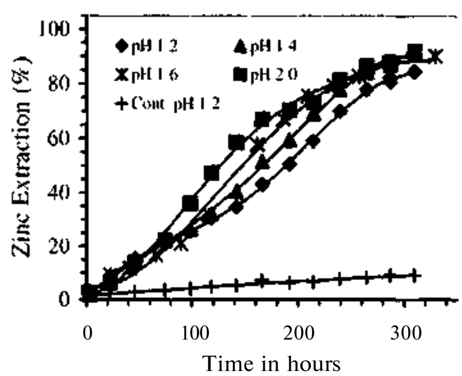


Figure 2. Hftect of pH (1.2-2 0) on the extt action of zinc fiom the ore (1% w/v) using WJM culture at WC

This was consistent with the optimum pH range of 2.0-2.5 for me.sophilic bacteria (Bosecker 1997). However, the potential for the precipitation of iron would increase with decreasing acidity and could become significant at high pHs as the pulp density increases leading to the increase in the availability of precipitate forming ions such as ferric iron. Considering the extensive dissolution of zinc occurred at pH 1.2 and 1.4 (Figs. 1-2) both cultures can be adapted to operate efficiently at low pHs as demonstrated by Porro et al. (1989).

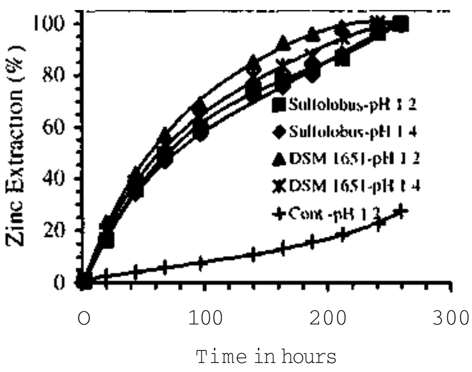
The addition of chloride (1-4% CI") appeared to severely inhibit the activity of WJM culture (at pH 1.4 and 30°C) with a marked decrease in the dissolution rate and extent of zinc as shown in Figure 3. Leong et al. (1993) also noted the detrimental effect of chloride (up to 8 g/1 CD on the bioleaching of a copper ore using mixed cultures of *Thiobacilli.* These investigators also reported that adaptation of bacteria to chloride levels of up lo 5 g/1 was possible ameliorating the deleterious effect of chloride on the dissolution process. In a recent study Deveci (2002) observed that the extent of the adverse effect of chlonde depended on the bacterial strain and the concentrations of chloride with the possibility of adaptation of mesophiles up to -8-10 g/1 CI". Weston et al. (1994) argued thai the deleterious effect of salt was not due to the chloride present but rather due lo the coexisting ions such as sodium leading to the formation of jarosite precipitates since they observed no apparent effect of salinity at 1-2 g/1 CI" *on* the bacterial oxidation rate when operating at lower pHs (1.1-1.3). Dew et al. (1997) suggested that high concentrations of chloride in solution could damage the membrane of the bacteria.

3.2 Bioleaching lests using extreme thermophiles

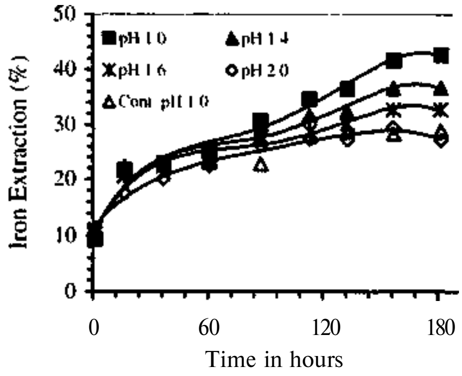
Figure 4 shows the bioleaching of the ore using the extremely thermophilic strains *Sulfolohus* and DSM 1651 *(Acidianus Brierleyi)* at pH 1.2 and 1.4. Compared with the mesophiles (Figs. 1-2) the extreme thermophiles were able to dissolve >99% of the zinc-present in the ore over a relatively short incubation period of 260 hours.

Although the zinc extraction was practically complete at the end of incubation period, both the extreme thermophiles consistently exhibited a better performance for the dissolution of zinc at a pH of 1.2'. This was consistent with the data presented in Figure 5 showing the effect of pH (1.0 to 2.0) on the bioleaching activity of DSM 1651 culture.

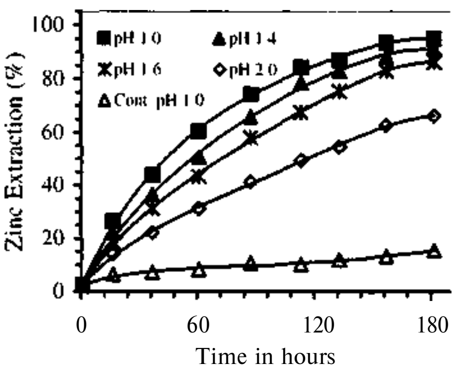
It was observed (Fig.5) that the rate and extent of dissolution of zinc increased with decreasing the pH in contrast to the mesophiles which did not illustrate



Figuie 4 Extinction of zinc from the oie using extiemely theimoplulic bactena at 70°C & pH I 2 & I 4



Figuie 6. Effect of pH (I 0-2.0) on the exliaction of iron from ihe ore (I 'h w/v) using DSM 1651 culture at 70°C



Figuie 5. Effect of pH (1.0-2 0) on the extiaction of zinc from the o i e d * w/v) using DSM 1651 culture at70°C

such a trend in response to the decrease in pH (Fig. 2). A limited zinc extraction of only 66% at a dissolution rate of 6.8 mg/l/h occurred at pH 2.0 compared with 95% Zn recovery (at 12.7 mg/l/h) recorded at pH 1.0. Jordan (1993) had shown that the dissolution rate of zinc and copper from a complex concentrate by the extreme thermophile *Sulfolobus* increased with decreasing the pH in the range of 2.0-1.2 in agreement with the current findings.

The enhancement in the bioleaching performance of DSM 1651 culture with increasing acidity was also evident from the bioleaching profiles obtained for iron (Fig. 6). The limited extraction of iron (i.e. 43% at pH 1.0) compared with extensive extraction of zinc (i.e. 95%) may be an indication of preferential/selective oxidation of sphalerite over pyrite. This is consistent with the electrochemical properties of both minerals with sphalerite having a lower rest potential and hence being electrochemically more active than pyrite under the oxidising conditions (Natarajan 1990).

Figures 7-8 show the effect of salinity (0-4% CI") on the extraction of zinc by both the extreme thermophiles at pH 1.2. The response of both cultures to the saline environment was similar in character with an identical pattern for metal extraction under the same experimental conditions. In contrast to the suppressing effect on the mesophiles, the addition of chloride appeared to produce a positive effect on the extraction of zinc by the extreme thermophiles particularly following an initial period of -50 h. To illustrate, over 98%) of zinc was extracted by DSM 1651 culture at 1-4% CI' compared with 85% Zn recovery in the absence of added chloride.

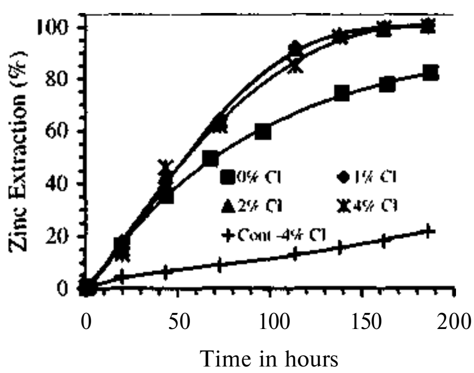
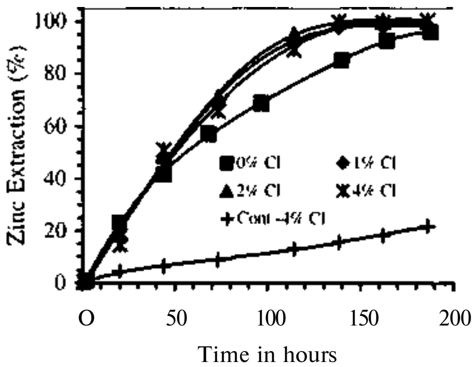


Figure 7. Effect of added chloride (0-4% CD on the extraction of zinc fiom the ore (1% w/v) using *Sitlfbliihits* cultuie at 70°C & pH I 2



Figuie 8 Effect of added chloride (0-4% CI") on the extinction of zinc from the ore (\'/i w/v) using DSM I65I culture at 70°C&pH 1.2

These findings were consistent with the published data for the extreme thermophiles (Deveci 2002) where it was reported that the addition of chloride up to -30-50 g/l Cl" improved the metal extraction by the extreme thermophiles with the apparent adverse effect at -80 g/l CI". It may be inferred that the extreme thermophiles are halophilic in nature with their ability to tolerate extremely saline environments.

It is of prime importance to note that the formation of jarosite-type precipitates in particular could present problems in the extremely thermophilic systems operating at high temperatures (70°C) presumably due to the availability of counter ions such as Na^+ present in saline environments. Such precipitates could adversely affect the metal extraction via the formation of a passivating layer on the un reacted minerals surface (Weston et al. 1994). In this respect, the ability of the extreme thermophiles to operate at low pHs (Fig. 5) could become important from the overall process point of view to minimise the formation of potentially deleterious precipitates. It should be noted that the decrease in pH would probably alleviate the formation of jarosite-type precipitates (Dutrizac 1983).

With respect to the behaviour of lead within the experiments where no chloride was added, the solubilisation of lead was minimal with over 98% of lead having reported to the residues most likely in the form of either insoluble lead sulphate or undissolved galena irrespective of the type (and strain) of bacteria and pH. This rellects the selective extraction of zinc from the complex ore within the bioleaching process. However, the dissolution of lead, most likely via lead-chloro complexes was promoted with the addition and/or increasing the concentration of chloride in solution. To illustrate, the extraction of lead increased from 4 to 69% with increasing the level of CI" from 1 to 4% during the bioleaching of the ore using *Siilfolohus* culture at 70°C.

3.3 Statistical Analysis of the Results

Statistical tests for the significance of the differences in the maximum dissolution rates of zinc under the experimental conditions were carried out in respect of the methodology previously outlined i.e. Ergun's test-essentially One-way Analysis of Variance (ANOVA) for gradients. This postulates the equality of the maximum gradients (dissolution rates) as a Null Hypothesis. The outcomes of the statistical analysis ot the various tests are summarised in Table 1 where the significance of the differences is indicated either at 5% ("significant") or 1% ("highly significant") or 0.1% ("extremely significant") levels.

Initially the contribution of the mesophilic and extremely thermophilic cultures of bacteria to the dissolution of zinc was tested as indicated by the "control versus bacteria"" in Table I. It was assumed that the (maximum) dissolution rate of zinc occurred in the control was equal to that in the bioleaching (i.e. Null Hypothesis). This hypothesis was rejected in that the differences in the rates were clearly "extremely significant"'. In other words, the dissolution rate of zinc was substantially enhanced in the presence of all the cultures. Since this initial assumption was rejected, subsequent tests were performed for differences between the dissolution rates produced at different conditions in an attempt to compare the performances of strains (and groups) of bacteria, to determine the optimum pH (i.e. the pH effect) for the cultures concerned, and to examine the effect of chloride on the bacterial cultures.

Ergun's lest did not detect any significant difference between the maximum rates of dissolution of zinc produced by the mesophiles (WJM and SJ2) at pH 1.6 and the extreme thermophiles *(Sulfolobus* and DSM 1651) al pH 1.2 and 1.4. However, the differences in the performance of WJM and SJ2 cultures at pH 1.4 appeared to be statistically significant probably as an indication of the better acid tolerance of SJ2 culture.

Statistical analysis of the pH data for WJM and DSM 1651 cultures suggests that the pH in the range of 1.0-2.0 is an important parameter affecting the dissolulion process. Notwithstanding this, the differences recorded in the dissolution rates of zinc were found to be statistically insignificant in the pH ranges of 1.6-2.0 and 1.0-1.6 for WJM and DSM 1651 cultures respectively (Table 1). These may be regarded as the optimum pH range for the process using these cultures.

In a similar manner, Ergun's test was applied to evaluate the effect of chloride on the dissolution of zinc by the mesophilc WJM and the extreme thermophile *Sulfolobus* and DSM I65I cultures. These tests affirmed the inhibitory effect of chloride on the

Table 1 The summary of the statistical analysis of the experimental icsults using hrgun's test ("a" represents the level of significanee and the test results are presented as "significant" at 5%. "highly significant" ât l%> and "extremely significant" at 0.1% levels)

Statistical Test	F value	F critic (ıı)	Significance
Mesophiles			
Control vs bacteria (WJM and S.I2) at pH 1.4	32.27	16.39(0.1%)	Extremely significant
pH U vs pH 1.6 (SJ2)	0.76	5.99 (5%)	Not significant
WJM vs SJ2 at pH 1.6	0.06	5.99 (5%)	Not significant
WIMvsSJ2atpH 1.4	6.38	5.99(5%)	Significant
pH effect on WJM (pH 1.2-2.0)	9.71	5.95(1%)	Highly significant
pH 1.2 vs pH 2.0 (WJM)	20.98	13.75(1%.)	Highly significant
pH U vs pH 2.0 (WJM)	14.00	13.75(1%)	Highly significant
pH 1.6vspH2.()(WJM)	0.35	5.99 (5%)	Not significant
pH U vs pH 16 (WJM)	8.95	5.99(5%)	Significant
CI clfect on WJM (0% vs 1% CI")	13.39	5.99(5%)	Significant
IV, CI" vs 23-CI" vs 4% CI"	1.68	5.99 (5%)	Not significant
Extreme thermophiles			
Control vs bacteria (Sulfolobus and DSM 1651) at pH 1.4	30.15	16.39(0.1%)	Extremely significant
pH 1 2vspH 1.4 (Sulfolobus)	0.51	5.99 (5%)	Not significant
Sulfolobus vs DSM 1651 at pH 1.2	0.23	5.99(5%)	Not significant
pH effect on DSM 1651 (pH 1.0-2.0)	5.33	4.89(1%)	Highly significant
pH 1.0 vs pH 1.2 (DSM 1651)	0.04	5.99 (5%)	Not significant
pH I.OvspH 14 (DSM 1651)	0.78	5.99(5%)	Not significant
pH I.OvspH 1.6(DSM 1651)	3.13	5.99 (5%)	Not significant
pH 1 0vspH2.0(DSM 1651)	13.65	5.99 (5%)	Significant
pH 1.6 vs pH 2.0 (DSM 1651)	10.81	5.99 (5%)	Significant
CI" effect on Sulfolobus (0% vs 4% CD	1.06	5.99(5%)	Not significant
CI" effect on DSM 1651 (0% vs 4% CD	0.51	5.99(5%.)	Not significant
Comparison of mesophiles with extreme thermophiles			
WJM (at pH 2.0) vs DSM 1651 (at pH 1.0)	9.46	5.99(5%)	Significant

dissolution of zinc by WJM culture and the ability of both the extreme thermophiles to oxidise the ore efficiently under the saline conditions. Finally, the "best" performed cultures of mesophiles (WJM at pH 2.0) and extreme thermophiles (DSM 1651 at pH 1.0) were compared with the results indicating that the extreme thermophiles have superior capacity for the bioleaching of the ore.

4 CONCLUSIONS

The bioleaching tests performed within the current study have shown that the complex sulphide ore is readily amenable to the selective bacterial extraction of zinc with lead present in the ore remaining in the residues during the bioleaching process.

The pH appears to exert a significant effect on the dissolution process controlling the oxidative activity of the mesophiles and extreme thermophiles. The bioleaching performance of the mesophiles tends to decrease with increasing acidity from pH 2.0 to 1.2. The extreme thermophiles however have greater tolerance for acidity than the mesophiles as the extraction of zinc was observed to improve with decreasing the pH in the range of 1.0-2.0. Statistical analysis of the pH data allowed the determination of the optimum pH range to be pH 1.0-1.6 for the extreme thermophiles and pH 1.6-2.0 for the mesophiles since the differences in the dissolution rates of zinc produced by the respective cultures within these ranges of pH were found to be statistically insignificant.

The quality of process water with particular reference to salinity appears to be of practical importance for the mesophiles since the addition of chloride (1-4% CI') was shown to have a detrimental effect on the bioleaching activity of the mesophilic WJM culture. These findings were consistent with the results of the statistical analysis of the data. However, the extreme thermophiles were found to consistently perform well under the saline conditions tested indicating the halophilic nature of these microorganisms.

The statistical analysis of the experimental result also affirmed that the extreme thermophiles exhibit superior kinetics for the dissolution of zinc from the ore compared with the mesophiles. The statistical methodology adopted herein has proved a useful tool for the accurate interpretation of the batch experimental results and it can be used for the optimisation (or estimation) of process parameters.

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