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Effect of Ni^{2+}, V^{4+} and Mo^{6+} concentration on iron oxidation by *Acidithiobacillus ferrooxidans*

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Abstract−The ferrous oxidation ability of *Acidithiobacillus ferrooxidans* was studied in the presence of Ni^{2+}, V^{4+} and Mo^{6+} in 9 K media in order to implement the culture in the bioleaching of spent catalyst. The rate of iron oxidation decreased with increasing concentration of metal ions, but the rate of inhibition was metal-ion dependent. The tolerance limit was critical at a concentration of 25 g/L Ni^{2+}, 5 g/L V^{4+} and 0.03 g/L Mo^{6+}. The growth rate of microorganisms was negligible at concentrations of 6 g/L V^{4+} and 0.04 g/L Mo^{6+}. Levels and degree of toxicity of these ions have been quantified in terms of a toxicity index (TI). The toxicity order of metal ions was found to be $Mo^{6+}>V^{4+}>Ni^{2+}$. The significance and relevance of multi-metal ion tolerance in *Acidithiobacillus ferrooxidans* has been highlighted with respect to bioleaching of spent refinery catalyst.

Key words: Iron Oxidation Rate, *Acidithiobacillus ferrooxidans*, Adaptation, Tolerance, Toxicity Index, Bioleaching

INTRODUCTION

Microbial bioleaching is based on the natural ability of microorganisms to transform solid compounds into a soluble and extractable form. This involves enzymatic oxidation or reduction of the solid compounds, or an attack on the solid compounds by metabolic products [1,2]. Researchers are employing this technique to remove heavy metals from materials such as low-grade ores, industrial wastes, spent batteries, electronic scraps, and spent petroleum catalysts. In this regard the acidophilic, chemolithotrophic bacteria *Acidithiobacillus ferrooxidans* has been exploited world wide for various metallurgical application, the most common being bioleaching [3-6].

These autotrophs commonly inhabit the earth's most metal-rich environments, and in these habitats they develop an indigenous metal-tolerance capability. Therefore these bacteria are considered to be ideal media to study microbial metal-tolerance phenomenon [7,8].

Some toxic cations and anions can affect the ferrous iron oxidizing ability of *Acidithiobacillus ferrooxidans*. This has been studied using redox potential measurement and other spectroscopic analysis. The results suggest that the presence of Hg^{2+}, Ag^{+}, Pb^{2+} and Cd^{2+} in solution at a concentration level of more than 10 ppm inhibits the bio-oxidation of ferrous [9]. No inhibitory effect on the oxidation of ferrous ions is observed in the presence of other heavy metal cations such as As^{3+}, Mn^{2+}, Sn^{2+}, Co^{2+}, Cu^{2+} and Zn^{2+} or with anions such as Cl^{-} and NO_3^{-} up to concentrations of 10 ppm. It is suggested that the iron oxidation is inhibited due to formation of an enzyme-Fe^{2+} complex and at the higher concentrations; the mechanism is thought to be diffusion of Fe^{2+} in to the periplasmic space of the *Acidithiobacillus ferrooxidans* cells.

To design a suitable engineering process to tackle the imbalance in ecosystem, it is crucial to understand the inhibitory effect of dissolved heavy metals [10]. The work here was conducted to study of the tolerance limits of the acidophilic bacterium, *Acidithiobacillus ferrooxidans*, in the presence of a range of heavy metals at various concentrations [11].

Biofilm formation is a strategy that microorganisms might use to survive in a toxic flux of inorganic solvents containing heavy metals. Evidence in the literature suggests that biofilm populations are protected from toxic metals by the combined action of chemical, physical and physiological phenomena, and in some instances, linked to phenotypic variation among the constituent biofilm cells [12]. This is termed as "biphasic population killing," where most of the growing population is rapidly killed by a low concentration of the antimicrobial [13,14].

Since the *Acidithiobacillus ferrooxidans* can grow at higher concentrations of iron in the nutrient media, it is convenient to study the kinetics of the iron oxidation rate by analyzing Fe^{2+} over time by using a volumetric method. In this note, the iron oxidation rate of the said bacteria has been monitored out in presence of heavy metals such as Ni, V and Mo. These heavy metals form part of the major constituents in spent petroleum refinery catalysts. One other hand adaptation of the heavy metals was carried out in order to apply in bioleaching of the spent refinery catalyst. Also, several toxicity indices have been calculated to allow for a better understanding. Bioleaching of spent refinery catalyst was carried out by using both the unadapted and adapted microorganisms by varying the solid concentrations.

MATERIALS AND METHODS

1. Microorganism and Medium

The bacteria used in this experiment were a mixed culture isolated from the effluent pond water of Dalsung Tungsten and Copper Mines, South Korea. This culture was chemolithotrophic *Acidithio-*

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bacillus ferrooxidans grown in 9 K medium [15]. The nutrient media constituents were $(NH_4)_2SO_4$: 3.0 g/L, KCl: 0.1 g/L, K_2HPO_4: 0.5 g/L, $MgSO_4 \cdot 7H_2O$: 0.5 g/L, and $FeSO_4 \cdot 7H_2O$: 44.2 g/L. The required amount of oxygen and carbon dioxides was fulfilled from dissolved stoichiometric air by agitating the media in Erlenmeyer flasks at 150 rpm. The fully grown media was filtered and the filtrate was centrifuged for 20 minutes at 10,000 rpm and 20 °C using a Mega21R, refrigerated centrifuge. The pelletized biomass at the bottom of centrifuge tube was dissolved in distilled water after the supernatant liquor was decanted. The cell suspension was kept in an incubator for the next set of experiments.

2. Iron Oxidation and Adaptation

The chemolithotrophic *Acidithiobacillus ferrooxidans* is able to oxidize ferrous sulfate to ferric sulfate under the aerobic acidic conditions required for their growth and metabolism, according to the following reaction [16]:

$$4FeSO_4 + 2H_2SO_4 + O_2 \xrightarrow{At.\ ferrooxidans} 2Fe_2(SO_4)_3 + 2H_2O \quad (1)$$

It is essential to carry out the adaptation study prior to the growth study as the bacteria are very sensitive to the culture medium. Hence, adaptation was conducted by sub-culturing the bacteria in the particular culture medium several times until the growth rate of the bacteria attained a stationary phase. The focus of this study was to curb the lengthy lag phase so that the overall kinetics of the process would increase. An iron oxidation study without bacteria was conducted as a microbial control.

3. Metal Tolerance Study

All chemicals used in this experiment were analytical grade reagents (AR) unless otherwise stated, and all aqueous solutions were prepared by using de-ionized water. For Ni^{2+} and V^{4+}, 1 L of stock solution was prepared by dissolving $NiSO_4 \cdot 6H_2O$ and 97% $VOSO_4 \cdot xH_2O$, respectively, in distilled water in volumetric flasks. A stock solution of molybdenum was taken from an AAS standard molybdenum solution (elemental molybdenum powder dissolved in aqua regia).

Different concentrations of heavy metal solutions (100 mL lots) were prepared from the stock solution in the 9 K media. Experiments used low concentration initially which were gradually increased after the bacteria adapted to the previous concentration by way of serial adaptation. The concentrations of the heavy metals were varied as follows: Ni^{2+} from 0.1 g/L to 25 g/L, V^{4+} from 0.1 g/L to 7 g/L and Mo^{6+} from 0.01 g/L to 0.05 g/L. The iron oxidation ability of the isolated culture in the presence of the heavy metal solutions was compared to that in the absence of the metals. From the comparison, the toxicity indices of the heavy metals at different concentrations were determined. The data at low concentrations of Ni^{2+} and V^{4+} were not derived since this had no effect on these conditions.

4. Bioleaching

Bacterial leaching of spent refinery catalyst was tested in shake flasks at different solid concentrations by using both the adapted and unadapted strains. For the unadapted strains, solid concentrations were 5 g/L, 10 g/L, and 20 g/L, and for the adapted strains, solid concentrations varied from 5 g/L to 50 g/L. The total volume of slurry in each flask was 100 mL. The conditions of bioleaching were an initial pH of 2.0, an initial ferrous concentration of 9 g/L, a temperature of 30 °C and a shaking speed of 150 rpm. All the experiments were run over a two-weeks interval and liquid samples were taken periodically for ICP analysis.

5. Analysis

The concentrations of ferrous and total iron in the medium were analyzed by a titration method using 0.1 N potassium dichromate as titrant and barium diphenylamine-4-sulfonate (BDAS) as a redox indicator [17]. The pH of the solutions was measured with an Orion-720+ pH meter. Metal values in leach liquor were analyzed by ICP-AES, Spectro Analytical Instruments (CIROS).

All the experiments, including tolerance tests and bioleaching tests, were performed in duplicate. The average deviation between the replicates was found to be within ±5%.

RESULTS AND DISCUSSIONS

1. Determination of Iron Oxidation Rate

At the initial stage of inoculation, it took 120 hours to oxidize 9 g/L ferrous in medium with almost 60 hours of bacterial lag phase time after two lots of subculturing; the reaction time was decreased to 30 hours with reduction in the lag period to 0 hours. The reaction time was decreased from 30 hours to 10 hours in order to oxidize 80% of the ferrous in media. The bacterial concentration was 4×10^8 cells/mL. No flat curve was observed through the iron oxidation path since the active bacteria followed the bacterial logarithmic phase. The residual ferrous concentration in solution was decreased as a function of time, and the plot of ferrous concentration versus time shows the kinetics of the iron oxidation. The graph, shown in Fig. 1, illustrates the iron oxidation of the stated strain at its fully active stage. The iron oxidation rate was estimated from the slope as $(\partial [Fe(II)])/\partial t$, and it was found to be 0.779 $g \cdot L^{-1} \cdot hr^{-1}$.

2. Effect of Single Metal Ions

The growth responses of *Acidithiobacillus ferrooxidans* in terms of iron oxidation rate at different concentrations of heavy metal ions in liquid cultures were studied. In this investigation, the concentration of nickel ion in the nutrient medium was varied from 1 g/L to 25 g/L, and the iron oxidation rate (IOR) was evaluated by measuring the residual ferrous concentration over time. The overall data are shown in Table 1. From the table, it can be seen that the iron

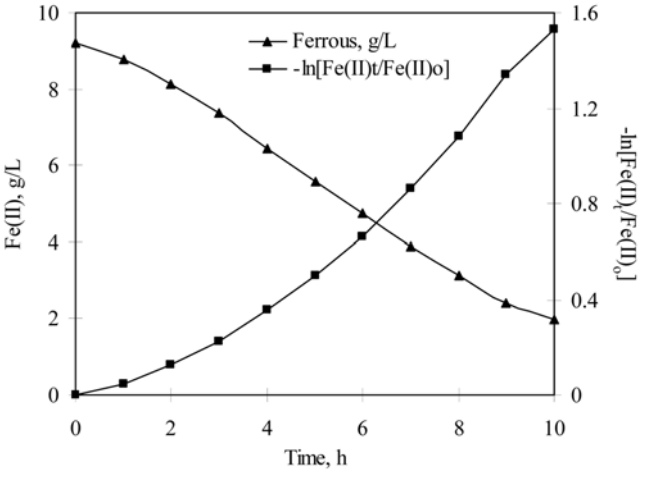

Fig. 1. Iron Oxidation by *Acidithiobacillus ferrooxidans* in logarithmic phase (Conditions: initial pH 2.0, 30 °C inoculum volume 10 mL, stirring speed 150 rpm).

Table 1. Iron oxidation study for adapted strain of *Acidithiobacillus ferrooxidans* in the presence of single and mixed heavy metal ions (Conditions: initial pH 2.0, 30 °C, inoculum volume 10 mL, stirring speed 150 rpm)

Metals	Concentration of metals, g/L	Iron oxidation rate, $g \cdot L^{-1} \cdot hr^{-1}$	Toxicity index	Correlation coefficient
-	0	0.779	-	0.9998
Ni^{2+}	1	0.777	1.0	0.9996
	5	0.691	1.2	0.9981
	10	0.619	1.25	0.9975
	15	0.612	1.3	0.9996
	20	0.577	1.5	0.9974
	25	0.526	1.6	0.9926
V^{4+}	0.5	0.683	1.2	0.9918
	1	0.616	1.3	0.9944
	2	0.609	1.3	0.9898
	3	0.558	1.5	0.9924
	4	0.516	1.7	0.9921
	5	0.434	2.0	0.9909
	6	0.233	4.5	0.9821
	7	0.069	-	0.9685
Mo^{6+}	0.01	0.522	1.7	0.9912
	0.02	0.506	1.7	0.9875
	0.03	0.472	1.8	09884
	0.04	0.092	9.8	0.9827
	0.05	0.013	-	0.9258
$Ni^{2+}+V^{4+}+Mo^{6+}$	10+2+0.02	0.448	1.7	0.9858
	10+2+0.03	0.409	1.8	0.9876
	10+5+0.02	0.393	2.0	0.9879
	10+5+0.03	0.372	2.2	0.9783

oxidation rates varied from 0.777 to 0.526 $g \cdot L^{-1} \cdot hr^{-1}$ with increasing nickel ion concentration from 1 g/L to 25 g/L. There was no significant variation observed in iron oxidation rate at different concentrations, indicating Ni^{2+} is harmless in form of the growth of *Acidithiobacillus ferrooxidans*. Also, Ni^{2+} may have a positive affinity to form the biofilms in a lid to overcome the changes in permeability of the cell membrane due to the high concentration of heavy metal ions in the liquid medium [12]. However, there was a general decrease in the IOR with increasing metal concentration from 5 g/L to 25 g/L, though the growth patterns were not particularly affected at lower concentrations of the heavy metals. Although large parts of the biofilms were able to protect their cells, a smaller fraction of growing microorganisms was incapable of surviving due to the weaker biofilms. Thus, there was a smooth decrease in IOR with heavy metal ions concentration. This result was established by plotting the specific iron oxidation with time at different concentration of nickel ions. The data are shown in Fig. 2(a). From the figure, the slopes of the curves decrease with increasing nickel ion concentration, but there are no sharp decreases in the slopes. This may be due to inadequate molecular oxygen in the growth medium. In the presence of higher concentrations of metal ion at constant temperature and pressure, the solubility of oxygen is hindered because a part of total water interacts with the metal ion [18,19]. The lack of oxygen in the growth medium was severely affected on the iron oxidation because molecular oxygen is the basic reagent of reaction (1) and the toxicity effect is minimal. Hence the concentration of nickel ion has little effect on the growth of *Acidithiobacillus ferrooxidans* in 9 K media, but higher concentration of metal ion reduced the levels of dissolved oxygen in the growth medium.

Bacterial growth in the presence of different vanadium ion concentrations as a form of specific iron oxidation is shown in Fig. 2(b). Bacterial ferrous oxidation ability in the presence of V^{4+} shows a marked effect even at lower concentrations. The path of the specific oxidation curve in the presence of 0.5 g/L vanadium is quite disparate compared to that of in the absence of metal. This is due to the effect of selected heavy metal ions on the rate of iron oxidation [19]. From Table 1, the iron oxidation rate decreased to 0.683 $g \cdot L^{-1} \cdot hr^{-1}$ in the presence of 0.5 g/L V^{4+} and gradually decreased with increasing concentration; increasing vanadium has a more toxic effect on the growth and metabolism of *Acidithiobacillus ferrooxidans*. The iron oxidation rate was around halved at 5 g/L vanadium compared with blank metal ion and became almost flat at 7 g/L. The iron oxidation rate was 0.516 $g \cdot L^{-1} \cdot hr^{-1}$ in the presence of 4 g/L vanadium, and this value was comparable to that with nickel at 25 g/L. This shows vanadium is more toxic than nickel. The heavy metal toxicity may have accounted for the observed decrease in the microbial oxidation rate, as heavy metals have been reported to be toxic to microorganisms at sufficiently high concentrations [20]. The presence of excess heavy metal ion in the bacterial liquid growth medium hinders the production of proteins and enzymes. Hence,

vanadium concentration above 5 g/L impacts adversely on the binary growth of the bacterial cell. It is clear that the presence of heavy metals decreases the oxidative capacity of the bacterial species studied. Furthermore, cellular growth is inhibited as the metal concentration in the medium increases, leading to a progressive decrease in the oxidation rate [11].

A specific iron oxidation study of *Acidithiobacillus ferrooxidans* with respect to molybdenum was carried out in cultures supplemented with 0.01, 0.02, 0.03, 0.04 and 0.05 g/L. Data are shown in Fig. 2(c).

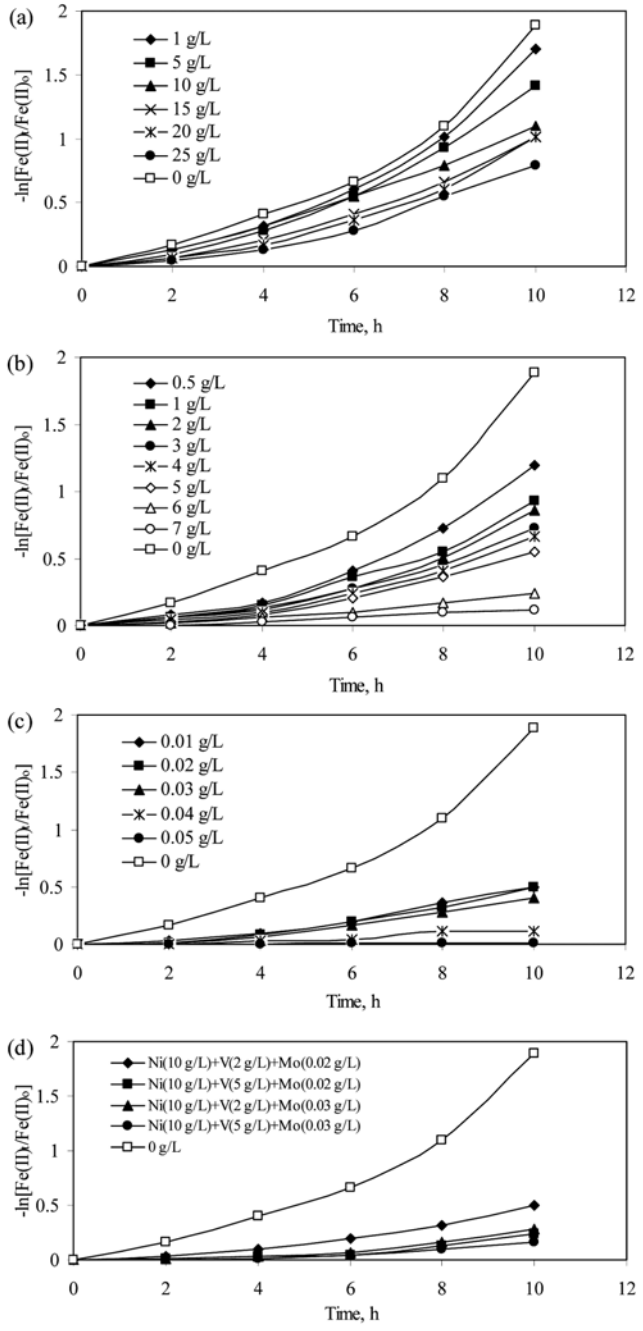

Fig. 2. Specific iron oxidation rate in the presence of different concentration (a) Ni^{2+}, (b) V^{4+}, (c) Mo^{6+}, (d) Ni^{2+}+V^{4+}+Mo^{6+} (Conditions: initial pH 2.0, 30 °C inoculum volume 10 mL, stirring speed 150 rpm).

The rate of reaction was hindered at a much lower concentration of 0.01 g/L and it was calculated to be 0.522 $g \cdot L^{-1} \cdot hr^{-1}$ shown in Table 1. The maximum molybdenum concentration that the bacteria were able to tolerate was 0.03 g/L. At concentrations of 0.04 g/L and 0.05 g/L of molybdenum in the media, the iron oxidation rate was quite low compared to that of without metal, showing a high toxicity level of molybdenum on bacterial growth. This effect was accompanied by a significant decrease in the bacterial production per gram of ferrous oxidation. This may be due to the formation of the enzyme-Fe^{2+}-Mo^{6+} complex as a intermediate product, which resulted in no electron transfer between the enzymes and Fe^{2+} and thus no oxidation in the growth medium [9]. Since the strain was a mixed culture of iron-oxidizing bacteria, the presence of heterotrophic microorganisms cannot be ignored. In some references in the literature, it was observed that microorganisms of activated sludge showed a reduction in bacterial viable counts and fungal mycelia development after contamination by heavy metals [21].

3. Effect of Mixed Ni^{2+}, V^{4+} and Mo^{6+}

A growth study in the presence of collective Ni^{2+}, V^{4+} and Mo^{6+} was under taken with varying concentration of three mixed metal ions in solution as shown in Fig. 2(d). In this experiment, four sets of ternary metal solutions were used: 10 : 2 : 0.02 g/L, 10 : 5 : 0.02 g/L, 10 : 2 : 0.03 g/L and 10 : 5 : 0.03 g/L of Ni^{2+}, V^{4+} and Mo^{6+} respectively. It was envisaged that if the concentration of any of the metal ions in the mixture attained a significant inhibition level, when present alone, the bacterial oxidation would be totally impeded even if the other metal ions in the ternary mixture were present in lower concentrations. For the concentrations of different metal ions lower than the respective critical levels, the inhibitory effect on bacterial ferrous ion oxidation was seen to be partial and cumulative with respect to their type and concentrations. In a high concentration ternary solution, i.e., Ni^{2+} with 10 g/L, the iron oxidation rate was 0.372 $g \cdot L^{-1} \cdot hr^{-1}$ whereas in individual 10 g/L Ni^{2+}, the iron oxidation rate was 0.619 $g \cdot L^{-1} \cdot hr^{-1}$. This is due to the inhibitory effect of combined metals present in the ternary solution. Also, it indicates that the toxicity of vanadium and molybdenum predominates over nickel even if its concentration is higher than the former. At a constant concentration of Ni^{2+}, with varying V^{4+} and Mo^{6+}, the toxicity of molybdenum increased with increasing concentration to only 0.01 g/L, which is equivalent to 3 g/L V^{4+}. When the rates are compared, it is evident that the collective toxicity of three metals prevails, since the iron oxidation rate was 0.372 $g \cdot L^{-1} \cdot hr^{-1}$ at a concentration ratio 10 : 5 : 0.03 g/L of Ni^{2+}, V^{4+} and Mo^{6+} respectively, whereas the reaction rates were higher in the individual metal solutions. Therefore, it can be stated that the effect of ternary metal ion combinations is more detrimental to bacterial ferrous ion oxidation than similar levels of the metal ions in a single metal solution. Furthermore, the relative order of toxicity observed in single metal ion studies remains in the ternary combinations.

At this juncture, the bacteria-catalyzed iron oxidation reaction is assumed to be zero order to factor because the initial concentration of ferrous, acid and molecular oxygen in all experiments was carried out with constant initial ferrous sulfate concentration, initial pH, temperature and agitation. Hence the reaction rate differed only because of the heavy metal ion content in the reactor. Neglecting the effect of dissolved oxygen, due to the higher concentration of metals in solution, a kinetic model can be disclosed with this reaction based

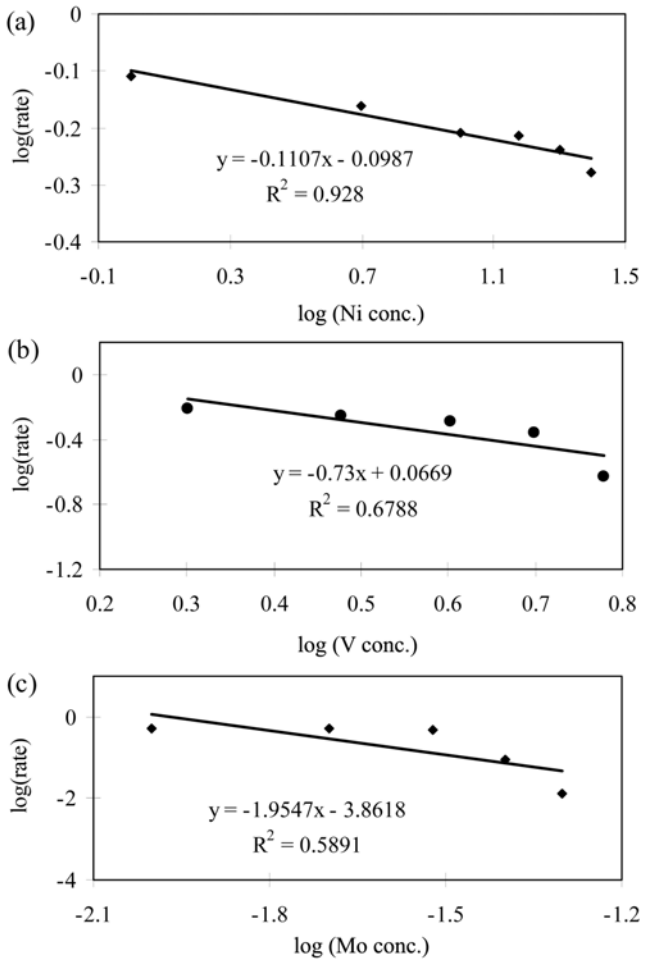

Fig. 3. Dependence of iron oxidation rate with respect to the concentrations of metal ions (a) Ni^{2+}, (b) V^{4+}, (c) Mo^{6+}.

on the concentration of heavy metals, keeping all other parameters constant. The slope of the plot in Fig. 3, log(Rate) vs log(heavy metal concentration) demonstrates the iron oxidation kinetics with metal ion concentrations. Fig. 3(a), (b) and (c) relate to the individual specific rate plot for nickel, vanadium and molybdenum, respectively. The rate can be written as follows:

$$R=K\times[Ni^{2+}]^{-0.1}\times[V^{4+}]^{-0.7}\times[Mo^{6+}]^{-1.9} \quad (2)$$

where, R is rate of iron oxidation, K is the empirical constant that depends on dissolved oxygen, initial ferrous concentration, initial acid concentration, temperature and the specific rate constant of iron oxidation in the absence metal ions.

4. Toxicity Index Determination

The toxicity index is defined as the ratio of the time required to oxidize 80% of the total ferrous in media by a strain in the presence of a dissolved metal ion at a particular concentration, to that required by the unadapted strain in the absence of any dissolved metal ion [22]. In this study the toxicity indices of all the three metal ions and multi-metal ions have been determined and data are given in Table 1. No significant bacterial growth was observed above the concentration mentioned in Table 1; hence we have removed this data.

It can be observed that the presence of nickel does not render toxicity up to 1 g/L, since the time taken by the active bacterial strain to oxidize 9 g/L of Fe(II) in presence of 1 g/L nickel in solution is exactly equal to that in the absence of the metal. But the toxicity index is almost unity up to 15 g/L nickel in solution for an adapted strain. Beyond this concentration, the toxicity index is higher, which illustrates that the adapted strain can grow easily in nickel concentration up to 15 g/L. In the presence of vanadium, the toxicity index is increased with increasing concentration of V^{4+} in solution. At 6 g/L V^{4+} ion, the toxicity index is a maximum at 4.5, as it took a maximum of 54 hours to oxidize 80% of total ferrous sulphate in solution by using the active adapted bacterial strains. No significant bacterial growth was observed above 6 g/L vanadium in the bacterial growth medium. With increasing toxicity index of the metal, the iron oxidation rate of the bacteria decreased. Molybdenum was found to be the most toxic metal, as in the presence of 0.01 g/L, the toxicity index was 1.7, which was significantly different from Ni^{2+} and V^{4+}. In the presence of 0.04 g/L of Mo^{6+} in solution, the reaction time was 118 hours to oxidize the maximum fraction of ferrous, and it was almost diminished above this concentration of molybdenum. In the multi-metal ion solution, the toxicity was partial and cumulative according to the behavior and character of the cations. Hence, the relative toxicities of the above metals were: $Mo^{6+}>V^{4+}>Ni^{2+}$.

5. Bioleaching of Vanadium Rich Spent Catalyst

Performance of the adapted and unadapted culture was tested for leaching Ni, V and Mo from spent refinery catalyst. Since the major interest in this study was to assess the performance of the strains when metal ions accumulate to toxic levels, the experiments were conducted at higher solid-liquid ratios. In the adapted strain, the solid concentrations were varied from 5 g/L to 50 g/L, whereas these were varied from 5 g/L to 20 g/L in the unadapted strain. The efficiency of leaching, as well as metals concentrations with the unadapted and adapted strains for different heavy metals, are shown in Figs. 4 and 5, respectively. As expected, the efficiency of leaching was significantly better in the adapted cells than in the unadapted strain. After 2 weeks of leaching, the amount of vanadium and molybdenum leached was almost doubled in the adapted strain related to the unadapted one, whereas not much difference was found in the nickel leaching rate. The heavy metal concentrations in leach liquor were well matched with the tolerance capacity. Also, the effect

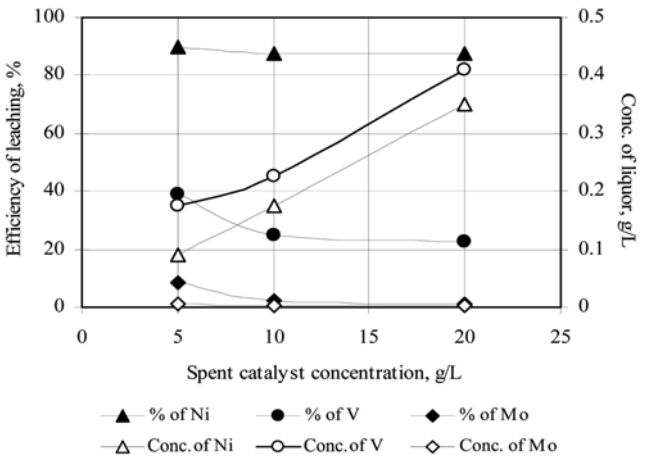

Fig. 4. Bioleaching of spent catalyst with unadapted culture at different solid concentrations (Conditions: initial pH 2.0, 30 °C initial ferrous conc. 9 g/L, stirring speed 180 rpm).

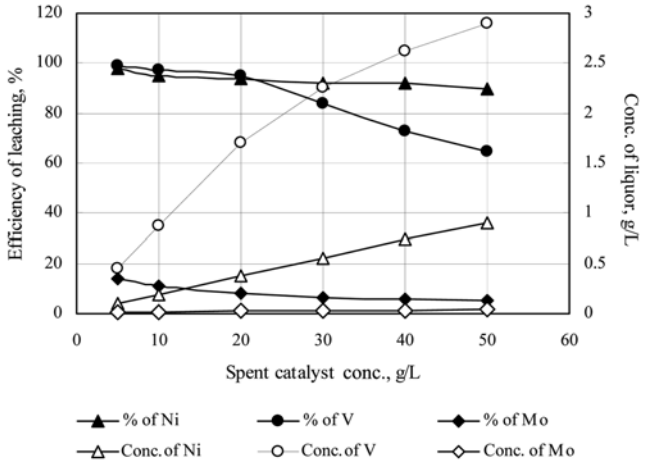

Fig. 5. Bioleaching of spent catalyst with adapted culture at different solid concentrations (Conditions: initial pH 2.0, 30 °C initial ferrous conc. 9 g/L, stirring speed 180 rpm).

of the solids concentration was examined up to 50 g/L in the adapted strain and the leaching rate was unaffected. In the unadapted strain, the V^{4+} and Mo^{6+} leaching was drastically decreased with increasing solids concentrations even at 20 g/L levels. The nickel leaching rate was not affected in both strains, as the leach Ni concentration in leach liquor was less than the maximum concentration of adaptability. The overall leaching trend was $Ni^{2+}>V^{4+}>Mo^{6+}$, whereas a reverse trend was observed in case of toxicity.

CONCLUSIONS

The iron oxidation rate of the isolated iron oxidizing bacteria was found to be 0.779 $g \cdot L^{-1} \cdot hr^{-1}$. The adaptation of *Acidithiobacillus ferrooxidans* to Ni^{2+}, V^{4+} and Mo^{6+} was successfully done with poor adaptability of Mo^{6+} in 9 K media. A tolerance study at set heavy metal concentration was established by comparing the iron oxidation rate of the bacteria in the presence of heavy metals to that in the absence of the metals. Nickel proved to be significant adaptation up to 25 g/L, whereas vanadium was less so at 5 g/L. The adaptability of molybdenum was quite low, indicating toxicity in the growth media even at concentrations as low as 0.01 g/L. The tolerance levels of V^{4+} and Mo^{6+} were found to be 5 g/L and 0.03 g/L, respectively. The synergistic toxic effect due to the presence of multi-metal ions in the medium on ferrous oxidation by *Acidithiobacillus ferrooxidans* has been demonstrated in this work. The toxicity order is $Mo^{6+}>V^{4+}>Ni^{2+}$. In the presence of multi-metal ions, the inhibitory effect on bacterial activity is cumulative with respect to individual metal ions.

The bioleaching was not affected at 50 g/L solid concentration with the adapted strain, whereas the leaching rate was very low even at 20 g/L solid concentration in the unadapted strain.

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RFERENCES

1. S.-Y. Shi and Z.-H. Fang, *Int. J. Miner. Process*, **76**, 3 (2005).
2. P. R. Norris, *Microbial mineral recovery*, McGraw-Hill, New York (1990).
3. C. Solisio, A. Lodi and F. Veglio, *Waste Management*, **22**, 667 (2002).
4. O. M. Lee, J. H. Oh, M. Libert, D. S. Hwang, Y. D. Choi, J. H. Park, U. S. Chung, B. R. Jo, M. J. Kim and S. J. Lee, *Korean J. Chem. Eng.*, **25**, 787 (2008).
5. T. Das, G. Roychaudhury and S. Ayyappan, *Biometals*, **11**(2), 125 (1998).
6. K. S. Rao, A. Mishra, D. Pradhan, G. R. Chaudhary, B. K. Mohapatra, T. Das, L. B. Sukla and B. K. Mishra, *Korean J. Chem. Eng.*, **25**, 524 (2008).
7. M. Dopson, B. A. Craig, P. R. Koppineedi and P. L. Bond, *Microbiology*, **149**, 1959 (2003).
8. M. Patra, N. Bhowmik, B Bandopadhyay and A. Sharma, *Environmental and Experimental Botany*, **52**, 199 (2004).
9. G. C. De, D. J. Oliver and B. M. Pesic, *Hydrometallurgy*, **44**, 53 (1997).
10. G. Cabrera, R. Perez, J. M. Gomez, A. Abalos and D. Cantero, *Journal of Hazardous Materials*, **A135**, 40 (2006).
11. G. Cabrera, J. M. Gomez and D. Cantero, *Process Biochemistry*, **40**, 2683 (2005).
12. J. J. Harrison, H. Ceri and R. J. Turner, *Nature Reviews Microbiology*, **5**, 928 (2007).
13. A. Spoering and K. Lewis, *J. Bacteriol.*, **183**, 6746 (2001).
14. J. J. Harrison, R. J. Turner and H. Ceri, *Environ. Microbiol.*, **7**, 981 (2005).
15. M. P. Silverman and D. G. Lundgren, *J. Bacteriol.*, **77**, 642 (1959).
16. W. C. Nemati, *Biotechnol. Bioeng.*, **53**(5), 478 (1997).
17. A. I. Vogel, *A text book of inorganic analysis*, Longmans (1961).
18. D. Tromans, *Hydrometallurgy*, **50**, 279 (1998).
19. C. T. Mathews and R. G. Robin, *Aust. Inst. Min. Met.*, **242**, 47 (1997).
20. J. E. Duddridge and M. Wainwright, *Wat. Res.*, **14**, 1605 (1980).
21. G. Bitton and V. Freihoffer, *Microb. Ecol.*, **4**, 119 (1978).
22. A. Das, J. M. Modak and K. A. Natarajan, *Minerals Engineering*, **10**(7), 743 (1997).