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Development of *Thiobacillus ferrooxidans* ATCC 19859 strains tolerant to copper and zinc

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Abstract. A study was carried out to develop strains of *Thiobacillus ferrooxidans* ATCC 19859 tolerant to higher levels of heavy metal ions. Strains of *T. ferrooxidans* capable of growing in Cu^{2+} (30 g/L) and Zn^{2+} (60 g/L) have been obtained. The ability of strains tolerant to either copper or zinc to grow in medium containing both the metals has been examined. The copper-tolerant strain (25 g/L) grows better in the medium containing both metals (Cu^{2+} 25 g/L and Zn^{2+} 40 g/L) compared to the zinc-tolerant strain (40 g/L).

Keywords. *Thiobacillus ferrooxidans*; tolerance to copper; tolerance to zinc; iron oxidation.

1. Introduction

Thiobacillus ferrooxidans is the most widely studied micro-organism in biohydrometallurgical processes because of its ability to dissolve metals, mainly from sulphide minerals. Microbiological leaching of sulphide-bearing concentrates has also been reported (Torma *et al* 1972; Torma and Subramanian 1974; Sakaguchi *et al* 1976). During leaching of concentrates the level of the metal ions in solution increases markedly. For an organism to play an effective role in leaching process, it has to be tolerant to high levels of metal ions. For optimum recovery of metals from mineral concentrates, strains tolerant to higher concentrations of heavy metal ions singly or in combination, need to be developed (Olson and Kelly 1986). The present study was undertaken to develop strains of *T. ferrooxidans* tolerant to higher levels of Cu^{2+} and Zn^{2+}. The ability of these strains to grow in medium containing both Cu^{2+} and Zn^{2+} has also been investigated.

2. Materials and methods

T. ferrooxidans ATCC 19859 was obtained from Prof. H L Ehrlich, Rensselaer Polytechnic Institute, Troy, New York, USA.

The organism was maintained in 9K medium at pH 2·3 (Silverman and Lundgren 1959). Ferrous sulphate solution (44·8 g of $FeSO_4 \cdot 7H_2O$ in 100 ml of medium) was sterilized by passing through a Millipore membrane filter (pore size: 0·45 μm). To 80 ml of medium in a 250 ml Erlenmeyer flask, sterilized by autoclaving, 10 ml of ferrous sulphate solution was added. This was then inoculated with 10 ml of a 72 h old culture of *T. ferrooxidans*. The flask was kept on a rotary shaker (240 rpm) at $30 \pm 2°C$. The growth was monitored by quantitating the ferrous-iron oxidized at 24 h intervals.

The concentration of ferrous-iron in the culture was determined as follows: 2 ml of 10 M H_2SO_4 was added to 1 ml of the culture in 100 ml conical flask and diluted with 25 ml of distilled water. To this 0·1 ml of *o*-phosphoric acid was added. The

solution was titrated with 0·025 N potassium dichromate using 0·2 ml of barium diphenyl amine sulphonate as the indicator. Under the conditions of assay 1 ml of potassium dichromate solution used corresponds to 1·4 mg of ferrous-iron.

Strains of *T. ferrooxidans* tolerant to high levels of metals were obtained by repeated culturing of the organism in 9K medium supplemented with indicated levels of Cu^{2+} and Zn^{2+}. The sulphate salts of the metals were used in the study and were of analar grade quality. Redox potential and pH were monitored with a platinum-saturated calomel electrode couple and combined electrode respectively.

Strains of *T. ferrooxidans* made tolerant to either Cu^{2+} (25 g/L) or Zn^{2+} (40 g/L) were used to examine their ability to grow in media containing both the metals at the levels indicated. For this 9K medium supplemented with Cu^{2+} (25 g/L) and Zn^{2+} (40 g/L) was inoculated with the culture (10% v/v) of the tolerant strains. The growth was monitored by following the extent of ferrous iron oxidized by the method described above.

3. Results and discussion

The growth pattern of *T. ferrooxidans* ATCC 19859 in 9 K medium containing different levels of Cu^{2+} is shown in figure 1A. The growth of the organism was slightly inhibited upto 48 h at 5 g/L of Cu^{2+}. However, this inhibition was not evident at later stages of growth (96 h). A marked inhibition was observed at 10 g/L or more of Cu^{2+}. However, on prolonged incubation (192 h) the organism showed recovery in growth at 10 g/L of Cu^{2+} but not at 20 g/L of Cu^{2+} (figure 1A). The growth profile of *T. ferrooxidans* tolerant to 10 g/L of Cu^{2+} when subcultured into medium containing higher levels of Cu^{2+} is presented in figure 1B. It is evident that

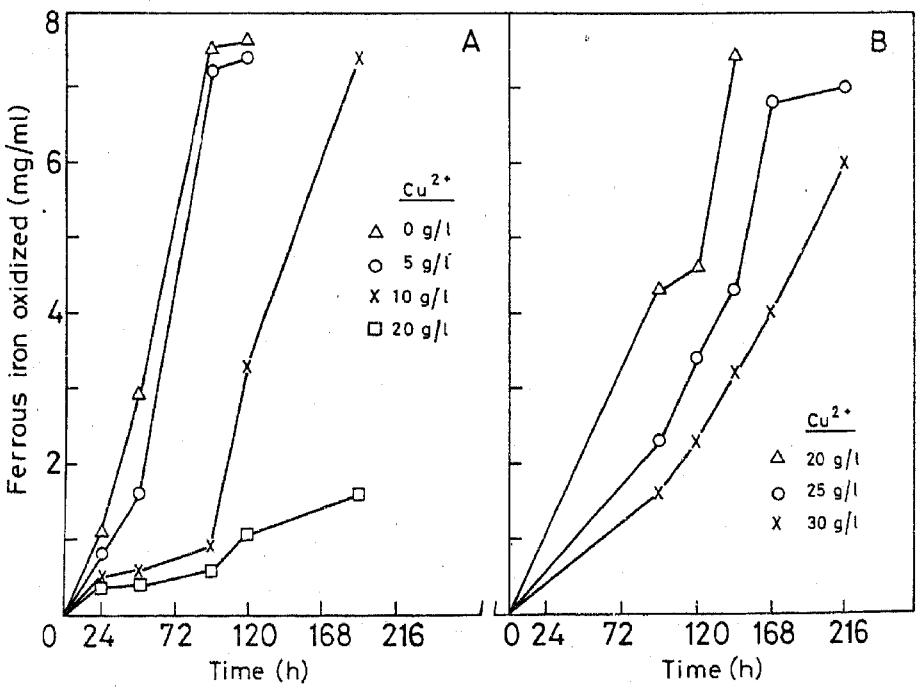

Figure 1. Development of tolerance of *T. ferrooxidans* to increasing levels of Cu^{2+}. Tolerance of (**A**) unadapted and (**B**) adapted (10 g/L) strains to different concentrations of Cu^{2+}.

the strain tolerant to 10 g/L of Cu^{2+} was able to grow at higher concentrations of Cu^{2+} (20 g/L and above) while an unadapted culture was totally inhibited.

The redox potential values were found to increase rapidly when a tolerant strain was cultured in a medium containing metal ions. At the same time there was no significant changes in pH values during growth. Earlier reports have shown inhibitory concentrations of Cu^{2+} to be more than 1 g/L (Tuovinen *et al* 1971) whereas *T. ferrooxidans* ATCC 19859 is inhibited only at 5 g/L and above.

Figure 2A represents the growth of *T. ferrooxidans* in medium containing different levels of Zn^{2+}. The growth of the organism was inhibited by Zn^{2+} (20 g/L) only during initial stages (48 h). After 48 h the growth in the presence of Zn^{2+} was comparable with the control. The extent of inhibition observed during the initial 48 h was proportional to the concentration of Zn^{2+} in the medium. The ability of the strain made tolerant to Zn^{2+} (20 g/L) to grow at higher levels of Zn^{2+} is presented in figure 2B. The adapted/tolerant strain shows a better growth rate than the unadapted strain. However, at high concentrations of Zn^{2+} an inhibition was observed. The tolerance to Zn^{2+} could however be developed by repeated subculturing in the presence of the metal ion. Other reports have shown that *T. ferrooxidans* was inhibited by Zn^{2+} at more than 10 g/L (Tuovinen *et al* 1971). In this study we have developed *T. ferrooxidans* ATCC 19859 strains tolerant to Cu^{2+} (25–30 g/L) and Zn^{2+} (60 g/L).

The strain tolerant to Zn^{2+} (40 g/L) when cultured in medium containing Cu^{2+} (row 1) shows poor growth upto 12 days (table 1). Under similar conditions Cu^{2+}-tolerant strain (row 2) grows very well in the presence of Zn^{2+} (40 g/L) and reaches the maximal growth much earlier (12 days). It showed a consistently higher rate of growth compared to the Zn^{2+}-tolerant one.

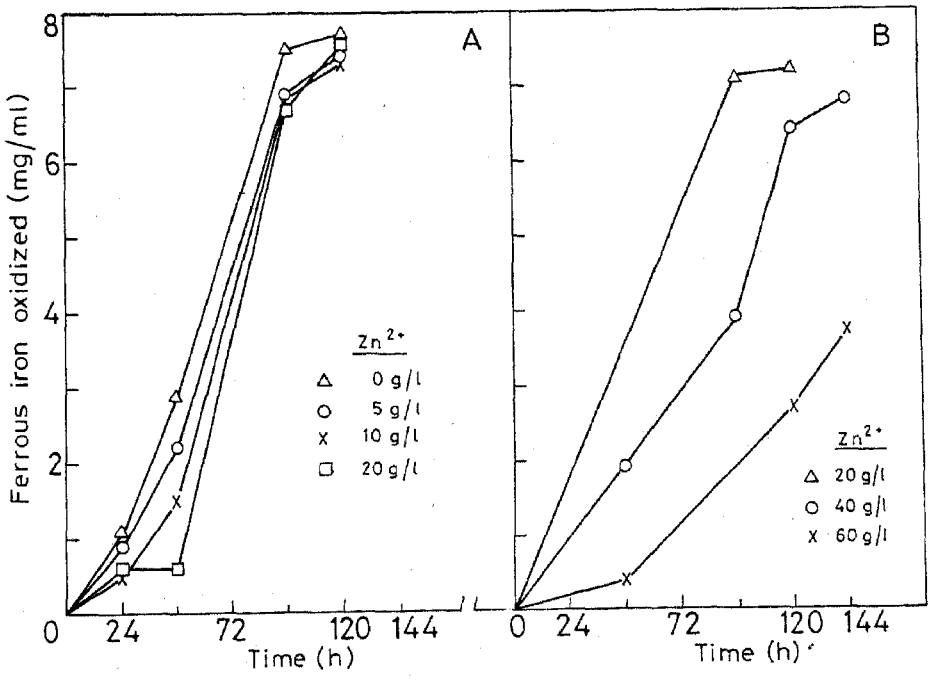

Figure 2. Development of tolerance of *T. ferrooxidans* to increasing levels of Zn^{2+}. Tolerance of (**A**) unadapted and (**B**) adapted (20 g/L) strains to different concentrations of Zn^{2+}.

Table 1. Growth of *T. ferrooxidans* tolerant to Cu^{2+} and Zn^{2+}, in 9K medium containing both metals.

Culture	Cultured in 9K medium containing	Incubation in days				
		2	6	9	12	25
Zinc tolerant strain†	Cu^{2+} 25 g/L	1·6#	1·9	2·2	2·5	6·1
Copper tolerant strain*	Zn^{2+} 40 g/L	1·8	3·3	6·5	8·4	—
Copper tolerant strain	Cu^{2+} 25 g/L + Zn^{2+} 40 g/L	2·5	2·6	2·8	3·2	6·8
Zinc tolerant strain	Cu^{2+} 25 g/L + Zn^{2+} 40 g/L	2·1	2·5	2·6	2·8	3·5
Copper tolerant strain + zinc tolerant strain@	Cu^{2+} 25 g/L + Zn^{2+} 40 g/L	2·5	2·8	2·8	3·3	6·8
Unadapted strain	Cu^{2+} 25 g/L + Zn^{2+} 40 g/L	0·1	2·1	2·3	2·6	3·6

#Figures are (mg/ml) Fe^{2+} oxidized; †40 g/L of Zn^{2+}, full growth occurs in 96 h; *25 g/L of Cu^{2+}, full growth occurs in 96 h; @Inoculum consisted of 5 ml Cu^{2+} and Zn^{2+} tolerant cultures each.

The Cu^{2+}-tolerant strain (row 3) can well tolerate the presence of both metals and reaches maximal growth by 25 days. In contrast the Zn^{2+}-tolerant strain (row 4) grew only 50% as efficient and is comparable with the growth shown by the unadapted strain. The 9 K medium containing both the metals was inoculated with equal volumes of cultures tolerant to Cu^{2+} and Zn^{2+}. The growth pattern was similar to the one shown by Cu^{2+}-tolerant strain. The results from the present study reveal that a Cu^{2+}-tolerant strain is better equipped to overcome the inhibition caused by Zn^{2+}. Tuovinen *et al* (1971) have shown that *T. ferrooxidans* adapted to zinc or nickel are more tolerant to copper compared to unadapted cultures.

The mechanism of metal tolerance is supposed to be due to the ability of the cells to exclude metal ions from their internal structures (Kuznetsov *et al* 1963). Ingledew and Cobley (1980) and Mjoli and Kulpa (personal communication) have shown that Fe^{2+} oxidation enzymes are membrane bound in *T. ferrooxidans*. The lag phase that was observed on exposure to metal ions indicates the possibility of an adaptation process taking place for the protection of membrane-bound Fe^{2+} oxidation enzymes. Also the prolonged lag phase may represent the time when natural selection of metal-tolerant cells takes place (Groudeva *et al* 1981) while the other susceptible cells in the population succumb to the toxicity of the heavy metals. Thus, development of strains tolerant to Cu^{2+} and Zn^{2+} could be the result of adaptation and/or selection of tolerant strains.

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