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Bioleaching of Low-Grade Copper Ores Using *Thiobacillus ferrooxidans*

Scientific Note

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INTRODUCTION

Microbial leaching of copper ores using *T. ferrooxidans* is becoming increasingly important, and it is estimated to account for more than 10% of copper output throughout the world. However, although extensive studies are reported on bioleaching of metal sulfides using *T. ferrooxidans,* there is still limited understanding about the exact mechanism of the electron transfer process involved.

T. ferrooxidans, a gram-negative chemolithotropic bacterium, uses Fe^{2+} as the sole source of energy and assimilates CO_2 at the expense of energy derived from oxidative phosphorylation (*1*). Electron transport chain components involved in the oxidation of Fe^{2+} reportedly include rusticyanin and several cytochromes of a and c types (*2*). Rusticyanin is a soluble type 1 blue copper protein present in the periplasmic space of the bacterium, consisting of a single polypeptide chain with one copper atom as a cofactor. It has a mol wt of 16,300 Da and has an absorption maxima at 597 (*3*).

Diethyl dithiocarbamate [DEDC] reportedly shows antimicrobial activity against some organisms, such as *E. coli, Klebsiella pneumoniae,*

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Proteus vulgaris, Salmonella, Serratia, Cadida and so on (4). Since its action on *T. ferrooxidans* has not been observed so far, DEDC has been used in our present study as a tool for understanding the electron transfer mechanism involving rusticyanin in the bioleaching process.

METHODS

A sample of chalcopyrite obtained from M/s. Hindustan Copper Limited, Ghatshila showed a copper concentration of 0.755% using 372-Perkin Elmer Atomic Absorptions Spectrophotometer. *T. ferrooxidans* strain 3C was obtained from Indian Institute of Chemical Biology, Calcutta. The liquid medium most frequently used for the growth of *T. ferrooxidans* is designated as 9K medium (5). Composition of this medium is given below:

Components	Amounts, g
$(NH_4)_2SO_4$	3.00
KCl	0.10
K_2HPO_4	0.50
$MgSO_4$, $7H_2O$	0.50
$Ca(NO_3)_2$	0.01
Distilled water	700 mL
$FeSO_4$ (energy source)	300 mL of a 14.77% w/v solution
pH adjusted to 2 with dilute H_2SO_4	

The strain 3C was adapted to the copper ore with higher leaching efficiency by serial passages in 9K basal salt medium containing a higher concentration of copper ore. The process was continued until reaching a steady state of leaching by the strain.

Effect of DEDC on the Growth of the Organism

Actively grown cells of *T. ferrooxidans* were inoculated in 9K medium, pH 2.0, in flasks containing different amounts of DEDC to find out the effect of DEDC on the growth of the organism. One flask free of DEDC was used as control. All the flasks were maintained at 30°C and were stirred at 200 rpm for 72 h.

Determination of the Biomass

Biomass was determined by following the method of Visca et al. (6). Samples of the culture were taken at different time intervals, filtered through a Whatman no. 1 filter paper to remove insoluble material, and the cell mass, collected from the filtrate by centrifugation, was washed with acid water. The pelleted bacteria were suspended in 1.0*N* NaOH and

hydrolyzed for 10 min at 100°C. The protein content was estimated by the procedure of Lowry et al. (7) using bovine serum albumin as the standard.

Effect of DEDC on Copper Extraction

Shake flask leaching experiments were carried out in 500-mL Erlenmeyer flasks containing 180 mL of iron-free 9K medium, pH 2.0, 10 g mineral substrate (ore), and 20 mL of active microbial culture, previously adapted to the ore concentrate being leached. In the ore slurry, 1.5×10^{-3} *M* DEDC was added directly to one flask. In a separate flask, the inoculum to be used (20 mL) was pretreated with the same concentration of DEDC (1.5×10^{-3}) for 12 h. The cells were washed and allowed to grow on ore slurry. Another flask free of DEDC was inoculated as a control experiment. The cell cultures in all the flasks were cultivated on a rotary shaker at 200 rpm at 30°C for 30 d. Samples were removed from the leach solution periodically and analyzed for copper extraction. The removed sample was replaced with 9K iron-free nutrient medium.

Sample Analysis

During the course of leaching experiments, 5-mL samples of ore slurry were collected from the shake flasks and immediately frozen in order to halt bacterial activity. The samples were thawed and filtered through a Whatman no. 1 filter paper to remove ore particles, and then centrifuged at 10,000*g* for 10 min in order to remove any bacterial cells. The resulting clear samples were then analyzed for copper by Atomic Absorption Spectrophotometry.

Effect of DEDC on Rusticyanin

Rusticyanin purified by following the method of Cox and Boxer (3) was made to react with 1 m*M* DEDC in 10 m*M* Na acetate buffer, pH 5.5. Absorption maxima of this complex were compared with the absolute spectrum of isolated rusticyanin.

RESULTS AND DISCUSSION

Figure 1 indicates that growth continued up to 72 h, in the absence of DEDC, in 9K medium. However, with an increase in concentration of DEDC in the medium, growth was retarded (b,c), and it was almost negligible with a DEDC concentration of $3\times10^{-3}M$ (d).

It was also found that, in the absence of DEDC (Fig. 2 a), leaching continued for up to 30 d. There is only a small lag phase, and copper concentration obtained was approx 150 ppm maximum in approx 30 d. In the presence of DEDC, however, the lag phase continued up to 12 d, and

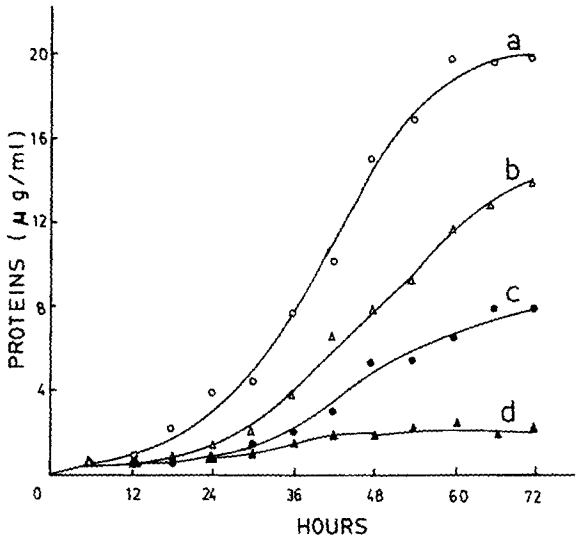

Fig. 1. Inhibitory effect of DEDC on the growth of *T. ferrooxidans*. Curve a, control; curves b, c, and d containing $10^{-3}M$, $2\times10^{-3}M$, $3\times10^{-3}M$ of DEDC, respectively.

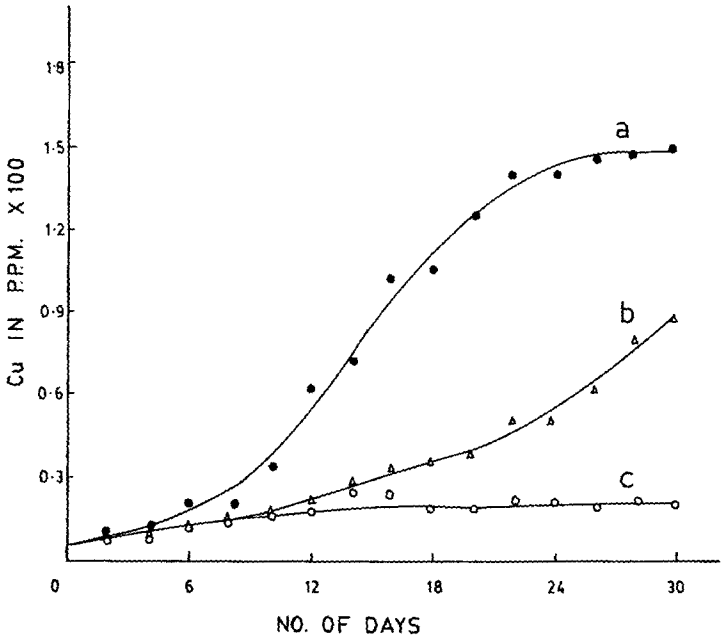

Fig. 2. Effect of DEDC on the extraction of copper. Curve a, control; curve b containing $1.5\times10^{-3}M$ DEDC in the medium; curve c, inoculum pretreated with $1.5\times10^{-3}M$ DEDC.

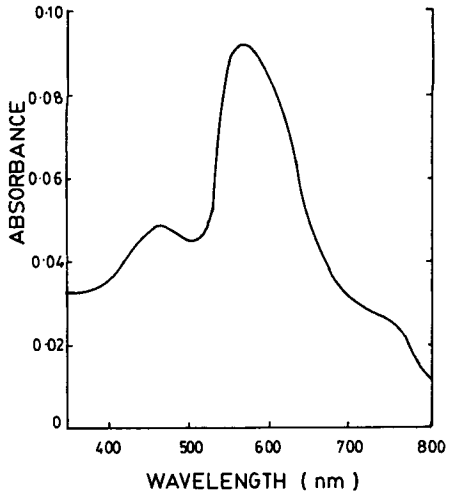

Fig. 3. Optical spectra of purified rusticyanin (1 mg protein/mL).

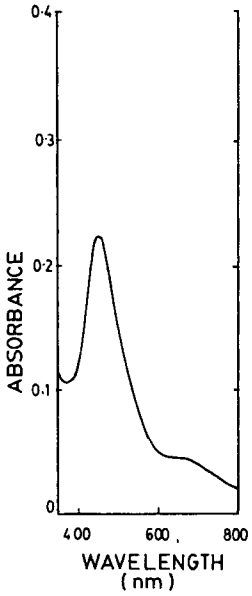

Fig. 4. Spectrum of rusticyanin (0.01 mg/mL) DEDC (1 m*M* in 10 m*M* Na acetate buffer, pH 5.5) complex.

maximum copper concentration reached only 90 ppm during the same period (Fig. 2 b). It is interesting to note that with overnight DEDC pretreated inoculum leaching was almost completely inhibited.

Figure 3 shows the spectrum of isolated rusticyanin. The protein has an absorption maximum at 597 nm. When a concentrated solution of purified rusticyanin was made to react with DEDC, the absorption maximum of rusticyanin disappeared, and instead, a new absorption maximum at 450 nm appeared (Fig. 4). The spectrum of this rusticyanin—DEDC com-

plex was essentially identical to that of copper—DEDC complex (*8*) in solution. Hence, it is evident that copper of rusticyanin forms a stable complex with DEDC. This indicates that DEDC is a metal complexing agent and has a strong affinity for copper. Copper is an essential and integral part of rusticyanin. During electron transport, copper of rusticyanin undergoes Cu^{2+}/Cu^{1+} transition (*9*). Hence, inhibition of growth may be owing to complexation of copper of rusticyanin by DEDC, which in turn disrupts the biochemical pathway of electron transport.

Rusticyanin has been postulated as the initial acceptor of electron from Fe^{2+}, but kinetic studies have indicated that the electron transfer is slow to account for the iron-dependent reduction of cytochromes in intact organism (*9*). Recently, an acid stable cytochrome c has been suggested as the primary acceptor of electron (*9,10*) from Fe^{2+} at pH 2.0 (pH of the growth medium) transferring the electron to rusticyanin, which effects electron transfer to iron cytochrome c reductase at pH 5.5 (the pH at which cytochromes are active) and ultimately to oxygen.

On the basis of above discussion, Hazra and coworkers have suggested an electron transport sequence (*11*). Results of bioleaching experiments in the presence and absence of DEDC further confirm the role of rusticyanin in the electron transport process.

ACKNOWLEDGMENTS

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