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Selection of *Leptospirillum ferrooxidans* SRPCBL and development for enhanced ferric regeneration in stirred tank and airlift column reactor

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Abstract

Presence of *Leptospirillum ferrooxidans* plays significant role in ferric sulphate generation during bioleaching process. Thus, an attempt was made to select *L. ferrooxidans* from the polymetallic concentrate leachate and further developed it for enhanced ferric iron regeneration from the leachate in shake flask, stirred tank and column reactor. When ferric to ferrous iron ratio in the shake flask reached to 20:1, *L. ferrooxidans* out competed *Acidithiobacillus ferrooxidans* and accounted for more than 99% of the total population. The isolate was confirmed by 16S rRNA genes sequence analysis and named as *L. ferrooxidans* SRPCBL. When the culture was exposure to UV dose and the oxidation–reduction potential of the inoculation medium was adjusted to 400 mV by ferrous:ferric iron ratio, the IOR reached to as high as 1.2 g/L/h in shake flask, even with initial ferrous iron concentration of 200 g/L. The chalcopyrite concentrate leachate containing 12.8, 15.7, and 42.0 g/L ferrous iron, ferric iron and copper, respectively was studied for ferric iron regeneration with the developed polymetallic resistant *L. ferrooxidans* SRPCBL in stirred tank and a developed biofilm airlift column, the highest IOR achieved were 2.20 g/L/h and 3.1 g/L/h, respectively, with ferrous oxidation efficiency of 98%. The ferric regeneration ability of the developed isolate from the leachate proves useful for a two-stage metal extraction process.

Keywords: *Leptospirillum ferrooxidans*; Ferric iron regeneration; Airlift column; Stirred tank reactor

1. Introduction

Metal bioextraction is highly dependent on ferrous iron oxidizing ability of the organisms as the biologically generated ferric iron and protons are responsible for carrying out the metal extraction reactions. Iron oxidation rate and oxidation–reduction potential of the medium are the key parameters for bioextraction of metals from the ores and concentrates (Mousavi et al., 2008). *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*, *Leptospirillum ferrooxidans* and *Leptospirillum ferriphilum* play a pivotal role in the oxidation of iron and sulphidic minerals. The highest, iron oxidation rate (IOR) reported for *A. ferrooxidans* and *L. ferriphilum* is 3.1 g/L/h and 8.2 g/L/h, which are with pure cultures under optimum conditions (Long et al., 2003; Kinnunen and Puhakka, 2004). Ferric iron generation ability of these organisms maintains the high Eh of the leaching system. However, the growth of *A. ferrooxidans* is inhibited above 36 mM ferric iron concentration whereas; *L. ferrooxidans* is resistant even to 500 mM ferric iron concentration (Curutcher et al., 1992). The kinetics of ferrous iron oxidation by *L. ferriphilum* was investigated at pH below 1.0 (Ozkaya et al., 2007) in the presence of 20 g and 60 g of ferrous and ferric iron, respectively. The finding showed that the ferrous iron oxidation was competitively inhibited by ferric iron and the competitive inhibition constant achieved was 830 mg/L. As bioleaching process progresses, the ferric iron concentration in the leachate becomes high as compared to ferrous iron so, *L. ferrooxidans* out competes to *A. ferrooxidans* in the

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leaching system (Boon et al., 1999; Ebrahimi et al., 2005; Rawlings et al., 1999). During continuous bioleaching process the steady state ferric iron concentration is high and under such condition *A. ferrooxidans* is less important then *L. ferrooxidans* or a combination of *L. ferrooxidans* and *A. thiooxidans* (Rawlings, 2005). In this context, the study was undertaken to isolate *L. ferrooxidans* from the bioleachate of polymetallic bulk concentrate and develop it for enhanced ferric iron generation in the presence of high ferrous iron, ferric iron and heavy metal ion in the medium.

2. Methods

2.1. Organism

A bioleachate of polymetallic bulk concentrate of Gujarat Mineral Development Corporation (GMDC) was used as source of iron oxidizing bacteria (Tipre and Dave, 2004).

2.2. Selection, isolation and identification of L. ferrooxidans

Polymetallic bulk concentrate bioleachate obtained from the bioreactor after bioleaching process was inoculated (10% v/v) in filter sterilized SR medium containing g/L: $(NH_4)_2SO_4$, 20; K_2HPO_4, 0.25; $MgSO_4{\cdot}7H_2O$, 0.25; $FeSO_4{\cdot}7H_2O$, 20 and pH 1.8. The inoculated and uninoculated (control) flasks were incubated on rotary shaker at 150 rpm and 30 ± 2 °C temperature. Once, more than 95% ferrous iron was oxidized, further 20 g/L ferrous sulphate was added in the ongoing flasks for 29 cycles. The oxidized 0.1 mL sample from 20th cycle from the ongoing biooxidation flask was inoculated on SR medium solidified by addition of 8 g/L agarose. The inoculated plates were incubated for seven days at 30 ± 2 °C temperature. Well-isolated colony was selected from the plate and inoculated in SR medium, inoculated flasks were incubated on rotary shaker at 150 rpm and 30 ± 2 °C temperature. After seven days of incubation, culture was harvested from the flasks and the plates and studied for morphological and 16S rRNA genes analysis.

2.3. Development of L. ferrooxidans for enhanced IOR

Actively growing 10 mL isolated *L. ferrooxidans* SRPCBL (2×10^8 cells/mL) culture was exposed to UV radiation at 5 μJ mm^2 in sterile petridishes for 30–120 s for the selection of efficient population (Menon and Dave, 1994). The UV exposed culture was immediately added in 90 mL SR medium and the flasks were covered with black paper to protect the UV exposed culture from light. The experiment was repeated five times for each exposure. All the inoculated and uninoculated (control) flasks were incubated on rotary shaker at 150 rpm and 30 ± 2 °C temperature.

To study the influence of initial oxidation–reduction potential of the medium on improvement in ferrous iron biooxidation ability of *L. ferrooxidans* SRPCBL, initial oxidation–reduction potential of the medium was adjusted between 370 and 400 mV by addition of desired ferrous–ferric iron ratio, and experiment was done as mentioned above.

2.4. Biofilm development

A glass column of 25 cm height and 4.2 cm inner diameter was packed with 450 g of acrylic pieces having dimension of 2:2:3 mm length:width:height. SR medium (150 mL) inoculated with 10% v/v an actively growing developed *L. ferrooxidans* was circulated for five days for biofilm development. Thereafter, fresh uninoculated SR medium was circulated for five cycles, each cycle with 24 h contact time.

2.5. Ferric regeneration from the leachate

Chalcopyrite concentrate leachate containing 12.8, 15.7 and 42 g/L of ferrous iron, ferric iron and copper, respectively was treated in the developed airlift biofilm column and 2.0 L stirred tank reactor (STR) with working volume of 1 L in batch process. Only in STR 10% v/v (2.0×10^8 cells/mL) actively growing developed *L. ferrooxidans* was added as inoculum, where as in the airlift biofilm column the developed biofilm served as inoculum, so separate addition of the inoculum was not needed.

3. Results and discussion

Iron biooxidation profiles in terms of ferrous iron concentration, total iron, IOR, total cell count and achieved oxidation–reduction potential at the end of different cycles are shown in Table 1. The observed very low IOR during the 1st cycle was certainly due to the change in the growth medium as the inoculum was grown on polymetallic bulk concentrate during the bioleaching process (Tipre and Dave, 2004). The major constituents of polymetallic concentrate was in g/kg: zinc 300; lead 132; copper 25; iron 92; sulphur 271 and other metals in the range of 25–850 ppm and insoluble matter 117 g/kg. There was a gradual increase in IOR up to 25 cycles, thereafter, it remained constant till the end of the study. This could be initially due to an increase in the actively growing ferrous iron oxidizing organisms, adaptation of the organisms to ferrous iron as

Table 1
Ferrous biooxidation profiles with bioleachate of polymetallic concentrate as an inoculum

Cycle number	Ferrous sulphate concentration (g/L)	Total iron (g/L)	IOR (g/L/h)	Redox potential at the end (mV)	Total cell count
1	20	20	0.03	620	2×10^6
10	20	200	0.25	690	1×10^7
20	20	400	0.35	750	3×10^8
25	20	500	0.40	760	7×10^8
30	20	600	0.40	760	5×10^8