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Development of *Leptospirillum ferriphilum* dominated consortium for ferric iron regeneration and metal bioleaching under extreme stresses

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HIGHLIGHTS

- ▶ Developed consortium SR-BH-L gave iron oxidation rate 1954 mg/L/h in SDB1 medium.
- ▶ It oxidized Fe^{2+} under major stress of 7.47 M ionic strength, 2.3 pH and 393.8 TDS.
- ▶ It showed 78.0% and 70.0% copper and zinc extraction from PBC.
- ▶ DGGE analysis indicated the presence of 11 OTUs in the consortium.
- ▶ RE digestion analysis of 16S rDNA showed dominance of *Leptospirillum ferriphilum*.

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ABSTRACT

Activated iron oxidizing consortium SR-BH-L enriched from Rajpardi lignite mine soil sample gave iron oxidation rate 1954 mg/L/h. Developed novel polystress resistant consortium oxidized ferrous iron under 11cP viscosity, 7.47 M ionic strength, 2.3 pH and g/L of 0.50 cadmium, 3.75 copper, 0.20 lead, 92.00 zinc, 6.4 sodium, 5.5 chloride, 154 sulphate and 393.8 TDS. The developed consortium showed 78.0% and 70.0% copper and zinc extraction from polymetallic bulk concentrate in monophasic bioleaching process. The bioregenerated ferric by the consortium in leachate showed 80.81% and 54.0% copper and zinc leaching in only 30 and 90 min. The DGGE analysis indicated the presence of 11 OTUs in the consortium. 16S rRNA gene sequence (JN797729) of the dominant band on DGGE shared >99% similarity with *Leptospirillum ferriphilum*. RE digestion analysis of the total 16S rRNA gene also illustrated the dominance of *L. ferriphilum* in the consortium.

1. Introduction

The leaching of nonferrous metals like Co, Cu, Ni and Zn by microorganisms is a quite promising and environmentally secure process. However, the commercial applications of such processes are suffered by the low extraction rates, the mesophilic range of temperature and long duration. The conventional chemical leaching (i.e. using the acidic ferric sulfate solution as leaching agent) gives a considerably higher rate of metal dissolution from the sulphidic minerals as compared to routine single stage bioleaching processes (Fomchenko and Biryukov, 2009). However, this process results in the generation of ferrous iron having an extremely acidic pH and high toxic metal ion concentration, where chemical regeneration of ferric iron is extremely slow or insignificant. As mentioned by Ehrlich (2004), Rawlings (2004) and Rossi (1990), the ferrous iron oxidation rate in acidic condition is increased half a million to a million times by *Acidithiobacillus ferrooxidans* as compared to chemical oxidation of ferrous iron. In bioleaching processes, metal extraction reactions are chiefly driven by the biologically generated ferric iron and redox potential of the medium (Dave et al., 1980; Mousavi et al., 2008). The resulting ferrous iron in the leaching stage can be re-oxidised to ferric iron at ambient temperature and pressure under highly acidic condition by the application of iron oxidising acidophilic chemolithoautotrophic bacteria such as *A. ferrooxidans*, *L. ferrooxidans*, *Acidimicrobium ferrooxidans*, *Acidithiobacillus albertensis*, *Alicyclobacillus tolerans* etc., and regenerated ferric iron can be reused for the chemical leaching of metals (Carranza et al., 1997; Dave, 2008; Palencia et al., 1998; Romero et al., 1998). *A. ferrooxidans* (formerly *Thiobacillus ferrooxidans*), being first biomining organism isolated from acidic leaching environment (Kelly and Wood, 2000; Temple and Colmer, 1951), remained a centre of research for many years. Although *A. ferrooxidans* was considered to be the leading biological candidate in bioleaching operations, leptospirilla found to be the dominant iron-oxidizing bacteria in biooxidation processes (Dave, 2008; Rawlings et al., 1999). The ferric to ferrous ratio (redox potential) is one of the major factors which determine the

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