Available online at www.sciencedirect.com
ScienceDirect
Bioresource Technology 99 (2008) 7514–7520
BIORESOURCE TECHNOLOGY

Characterization of arsenic resistant and arsenopyrite oxidizing *Acidithiobacillus ferrooxidans* from Hutti gold leachate and effluents

Shailesh R. Dave *, Kajal H. Gupta, Devayani R. Tipre

Department of Microbiology, School of Sciences, Gujarat University, Ahmedabad, Gujarat 380 009, India

Received 8 October 2007; received in revised form 10 February 2008; accepted 14 February 2008
Available online 25 March 2008

Abstract

Four arsenic resistant ferrous oxidizers were isolated from Hutti Gold Mine Ltd. (HGML) samples. Characterization of these isolates was done using conventional microbiological, biochemical and molecular methods. The ferrous oxidation rates with these isolates were 16, 48, 34 and 34 mg L^{-1} h^{-1} and 15, 47, 34 and 32 mg L^{-1} h^{-1} in absence and presence of 20 mM of arsenite (As^{3+}) respectively. Except isolate HGM 8, other three isolates showed 2.9–6.3% inhibition due to the presence of 20 mM arsenite. Isolate HGM 8 was able to grow in presence of 14.7 g L^{-1} of arsenite, with 25.77 mg L^{-1} h^{-1} ferrous oxidation rate. All the four isolates were able to oxidize iron and arsenopyrite from 20 g L^{-1} and 40 g L^{-1} refractory gold ore and 20 g L^{-1} refractory gold concentrate. Once the growth was established pH adjustment was not needed inspite of ferrous oxidation, which could be due to concurrent oxidation of pyrite. Isolate HGM 8 showed the final cell count of as high as 1.12×10^8 cells mL^{-1} in 40 g L^{-1} refractory gold ore. The isolates were grouped into one haplotypes by amplified ribosomal DNA restriction analysis (ARDRA). The phylogenetic position of HGM 8 was determined by 16S rDNA sequencing. It was identified as *Acidithiobacillus ferrooxidans* and strain name was given as SRHGM 1.

Keywords: Arsenic; Arsenopyrite; *Acidithiobacillus ferrooxidans*; Biooxidation; Refractory gold ore

1. Introduction

Arsenic is a toxic metalloid carcinogen found world wide in surface and ground water from natural and anthropogenic sources (Ambika and Natarajan, 2006). World average concentration of arsenic in the earth's crust ranges from 1.5 to 5 mg kg^{-1} (DeMarco et al., 2003). Arsenic is frequently found as mineral in combination with sulphur viz arsenopyrite (FeAsS), realgar (As_4S_4) and orpinment (As_2S_3). It is also found in the sulfide ores of other metals like refractory gold ore, copper as tennantite ($4Cu_2SAs_2S_3$), silver as proustite ($3Ag_2SAs_2S_3$), nickel as niciolite (NiAs) and gerdorffite (NiAsS) and cobalt as smalite ($CoAs_2$) (Cassity and Pesic, 1999; Malatt, 1999; Ning, 2002). World health organization has recently recommended the permissible limit standards for arsenic in drinking water 10–20 μg L^{-1} instead of 40–50 μg L^{-1} (Katsoyiannis et al., 2004).

Low grade refractory sulphide gold ore is preferentially associated with high amount of pyrite and arsenopyrite. In such ore, gold is dispersed as submicroscopic particles and efficient recovery of this finely dispersed gold is very difficult by the conventional method used without pretreatment (Cassity and Pesic, 1999). Separation of pyrite from arsenopyrite becomes important for economical extraction of gold. Pre-biooxidation of arsenopyrite from refractory gold ore by micro-organisms helps in gold recovery by cynaidation method from such refractory ore and concentrates (Malatt, 1999).

The oxidation of arsenopyrite results in a range of products with varying final sulfur and arsenic oxidation states depending upon the oxidizing condition. Various mechanism of arsenopyrite biooxidation that helps in gold recovery is illustrated in literature (Mandl et al., 1992; Nyashanu et al., 1999).

* Corresponding author. Tel.: +91 79 26303225; fax: +91 79 26301919.
E-mail address: shaileshrdave@yahoo.co.in (S.R. Dave).

0960-8524/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2008.02.019

Arsenopyrite biooxidation also leads to the dissolution of the arsenic in the form of As^{+3} and generate ferrous sulphate in the medium. This biological pretreatment plays important role in increased gold recovery, but at the same time it is also responsible for increased arsenite pollution. Indigenous mineral oxidizing bacteria such as *Acidithiobacillus ferrooxidans*, *Leptospirillum ferrooxidans* and *Acidithiobacillus thiooxidans* present in gold ores are reported to be better suited for extraction of gold from its ore (Morin, 1995).

No data are available regarding the isolation and characterization of arsenic resistant *A. ferrooxidans* from Indian habitats. Thus, the systematic study on isolation and characterization of arsenic resistant *A. ferrooxidans* was undertaken and it was extended to check its ability for arsenopyrite biooxidation.

2. Methods

2.1. Samples

Water samples were collected from refractory gold concentrate bioreactor, storage tank of the bioreactor effluent and processed water of Hutti Gold Mine Ltd. (HGML) situated at Raichur district, Karnataka, India. The refractory arsenopyrite containing gold ore and concentrate was provided by HGML. The ore was ground to get the particle size of 44–74 μm.

2.2. Organism

Iron oxidizers from HGML samples were isolated on 9K (Silverman and Lundgren, 1959) modified solid medium (9KMS) where 2.5 g L^{-1} ferrous sulphate was added instead of 44.2 g L^{-1} and was solidified with 8 g L^{-1} agarose. If other wise mentioned 9K modified broth (9KMB) was prepared with 20 g L^{-1} $FeSO_47H_2O$ and pH was adjusted to 1.8 ± 0.2 with 10% H_2SO_4. Isolated colonies were inoculated in 9KMB and the inoculated flasks were incubated on rotary shaker at 30 ± 0.2 °C temperature. Growth of the isolates was also checked on FeTSB broth (Johnson and McGinness, 1991) under identical conditions.

2.3. Iron oxidation, arsenic resistance and bioleaching studies

Iron oxidation rate (IOR) was studied in 9KMB in presence and absence of arsenite. Arsenic resistance of the isolated bacteria was studied using 9KMB where sodium salt of arsenite ($NaAsO_2$) and arsenate ($NaHAsO_4$), were added to achieve 0–200 mM of arsenic. Growth of the isolates was measured in terms of ferrous oxidation.

Arsenopyrite leaching experiments were carried out in 500 mL Erlenmeyer flasks containing 200 mL of 9KMB where ferrous sulphate was replaced with 20 g L^{-1} and 40 g L^{-1} (w/v) refractory gold ore (particle size 44–74 μm) and 20 g L^{-1} refractory gold concentrate (particle size 44–74 μm) separately. The reaction flasks were incubated at 30 ± 0.2 °C on rotary shaker at 150 rpm.

2.4. Chemical analysis

Soluble ferrous iron was estimated at regular interval of time by potassium dichromate titrimetric method (Vogel, 1961). Total soluble iron was estimated from the leachate spectrophotometrically by 1,10 phenanthroline method (APHA, 1995) using Systronics UV–Vis 119 spectrophotometer. The progress of bacterial oxidation of arsenopyrite was determined by measuring concentration of total soluble arsenic in leaching medium by double beam atomic absorption spectrophotometer (model Elico SL 194). The pH and redox potential were measured using saturated calomel electrode (SCE) while redox potential (Eh) was measured in terms of mV by combined SCE platinum electrode (Systronics μ pH system 361). The elemental composition of the ore and concentrate was determined by inductive coupled plasma (ICP) analysis method (Perkin–Elmer, Optima 3300RL).

Bacterial population in the leaching medium was determined by direct microscopic count using Petroff–Hausser chamber.

2.5. Genomic DNA and 16S rDNA analysis

Genomic DNA of all the four iron oxidizers was isolated using phenol chloroform extraction method. PCR amplification of the 16S rDNA was performed in 50 μL reaction mixture containing 5–10 ng bacterial DNA as template, 25 μM of each primer, 1 μL of Taq polymerase, 100 μM of dNTPs, 0.5 mM of $MgCl_2$ and 5 μL of PCR buffer in thermal cycler (PTC100, MJ Research, Walthman, MA). PCR amplification was carried out with using forward primer 8f:5′-AGAGTTTGATYMTGGCTCAG-3′ and reverse primer 1495r:5′-CTACGGCTACCTTGTTACG-3′ (Chowdhury et al., 2004). The PCR conditions consisted of an initial denaturation step of 1 min at 94 °C, followed by 35 cycles of 1 min at 94 °C, 1 min at 52 °C and 1 min at 72 °C with final extension of 5 min at 72 °C. The products were analyzed by 1% agarose gel electrophoresis in 1× TAE (0.04 M tris acetate and 0.001 M EDTA and 57.1 mL of glacial acetic acid, pH 8.0) buffer and the bands were visualized by ethidium bromide stained gel.

ARDRA (amplified ribosomal DNA restriction enzyme analysis) was performed with 10 μl of amplicons obtained from PCR, which were double digested using 2.5 U of A*lu*I and R*sa*I (MBI Fermentas) restriction endonuclease enzymes (Ghosh et al., 2004). The restriction fragments were separated on 2% agarose gel electrophoresis using TAE buffer. *L. ferrooxidans* SRPCBL was used as reference culture in this study (Dave, 2008). The PCR product was purified by PCR purification kit. Direct sequencing was performed in an AB 3730 automated DNA sequencer. Nucleotide sequences were retrieved from the GenBank, EMBL and RDP databases and BLAST probing of the