Bioresource Technology 268 (2018) 480–487

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Disentangling effects of temperature on microbial community and copper extraction in column bioleaching of low grade copper sulfide

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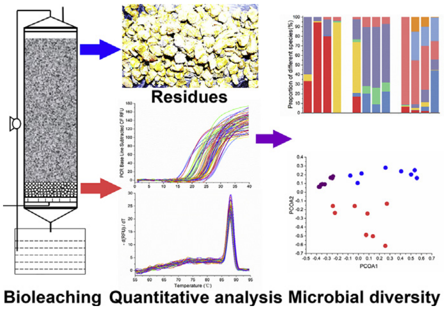
GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Low grade copper sulfide
Bioleaching
Temperature
Community dynamics
Copper extraction

ABSTRACT

The lack of knowledge about responses of microbial community to temperature in copper sulfide bioleaching, and subsequent effects on copper extraction hampered understanding of how to improve bioleaching efficiency. This study presents first detailed quantitative data on microbial diversity and dynamics during bioleaching of low grade copper sulfide at different temperatures. The results demonstrate that temperature had significant effects on microbial community and copper extraction. The microbial structures on the ore surfaces were independent of communities in the leachates. Different species dominated the communities at different temperatures and portions of laboratory scale heap column. Moderate thermophiles rather than extreme thermophiles dominated the communities at 65 °C. The height of ore bed was sufficient to affect microbial communities at 30 °C and 65 °C. Sulfur-oxidizers were very important to improve copper extraction. High microbial diversity also were beneficial to enhance copper extraction within a certain temperature range in the final stage.

1. Introduction

Acid mine drainage (AMD) is one of the most severe environmental problems throughout the world (Jones and Johnson, 2016; Bonilla et al., 2018). It is derived from the mining wastes such as mill tailing, waste rock and low-grade ore. A large amount of effort has been employed to remediate AMD (Jones and Johnson, 2016). However, the conventional technologies have produced economic and sustainable pressures (Simate and Ndlovu, 2014). Overall, it is generally believed that solving the detrimental effects of AMD will require the use of new strategies. One approach is to develop technological processes to recover dissolved metals from AMD instead of remediating it, for example

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https://doi.org/10.1016/j.biortech.2018.08.031
Received 5 July 2018; Received in revised form 7 August 2018; Accepted 9 August 2018

Table 1
The culture conditions of bacteria and archaea used in this study.

Acidophiles	Temperature (°C)	pH	Energy sources (g/L)	Yeast extract (g/L)	Culturing	Basal salt medium (g/L)
Acidithiobacillus ferrooxidans	30	2.0	$FeSO_4·7H_2O$, 44.7 or S, 10	–	For preparation of inoculum, each species was inoculated into 250 mL shake flasks containing 100 mL medium, and incubated in a rotary shaker at 170 rpm.	$(NH_4)_2SO_4$, 3; KCl, 0.1; K_2HPO_4, 0.5; $MgSO_4·7H_2O$, 0.5; $Ca(NO_3)_2$, 0.01
Leptospirillum ferrooxidans	30	2.0	$FeSO_4·7H_2O$, 30	–		
Acidithiobacillus thioxidans	30	2.5	S, 10	–		
Ferroplasma acidiphilum	35	1.5	$FeSO_4·7H_2O$, 20	0.2		
Leptospirillum ferriphilum	45	1.6	FeSO4·7H_2O, 44.7	–		
Acidithiobacillus caldus	45	2.0	S, 10	–		
Sulfobacillus acidophilus	45	1.8	$FeSO_4·7H_2O$, 30 or S, 10	0.2		
Sulfobacillus thermosulfidooxidans	45	1.8	$FeSO_4·7H_2O$, 30 or S, 10	0.2		
Ferroplasma thermophilum	45	1.0	$FeSO_4·7H_2O$, 20	0.2		
Ferroplasma cupricumulans	50	1.0	$FeSO_4·7H_2O$, 20	0.2		
Acidianus brierleyi	65	2.0	S, 10	0.2		
Acidianus manzaensis	65	2.0	S, 10	0.2		
Sulfolobus metallicus	65	2.0	S, 10	0.2		
Metallosphaera sedula	65	2.0	S, 10	0.2		

copper (Park et al., 2015). Some commercial projects have been developed to selectively extract and/or concentrate dissolved metals from AMD (Simate and Ndlovu, 2014). However, these technologies still cannot effectively treat AMD with 100% efficiency (Simate and Ndlovu, 2014). Another alternative approach is to directly recover metals from the byproducts of the mining industry, which can prevent the formation of AMD. Heap bioleaching has already been widely employed to extract metals from mill tailing, waste rock and low-grade ore (Pathak et al., 2017).

It is well known that the microorganisms which predominate in the bioleaching systems are specialized acidophiles including more than 14 genera and 33 species (Kondrat'eva et al., 2012). According to their optimal growth temperatures, acidophiles can be classified into three groups: mesophiles (20–40 °C), moderate thermophiles (40–60 °C) and extreme thermophiles (> 60 °C) (Johnson, 2006; Halinen et al., 2009a). The heap bioleaching system is inevitably heterogeneous (both spatially and temporally), particularly with respect to temperature (Johnson, 2008). It has been observed that temperature could increase to more than 80 °C in the heaps due to exothermic oxidation of sulfides (Olson et al., 2003). Franzmann et al. (2005) indicated that temperature was a major selective pressure for microorganisms involved in bioleaching, which affected bioleaching efficiency in turn. So temperature is one of critical factors in achieving economic metal extraction with high efficiency (Liu and Granata, 2018). Previous studies have demonstrated that temperature had significant effects on microbial community structure and bioleaching efficiency [see review in (Watling et al., 2016), and references therein]. In general, mesophiles were mainly detected at < 40 °C, moderate thermophiles at 40–60 °C, and extreme thermophiles at > 60 °C (Johnson, 1998; Rawlings and Johnson, 2007). However, the knowledge is still limited on responses of microbial community to temperature in bioleaching of low grade copper sulfide, and subsequent effects on bioleaching efficiency. Several questions require to further study as follows: (i) How more complicated microbial community structures change during bioleaching of low grade copper sulfide? Most previous studies used relatively simple mixed cultures to bioleach sulfides at different temperatures, even the inocula did not contain all types of acidophiles as mentioned above (Brierley, 2003; Halinen et al., 2009b; Chen et al., 2014; Watling et al., 2016). (ii) What is the spatiotemporal succession pattern of microbial populations in detail, especially for attached communities? Most analyses of microbial dynamics focused on leachate rather than the ore itself, or microbial structures at the end of bioleaching (Mutch et al., 2010; Norris et al., 2012). (iii) How microbial communities develop during bioleaching if quantitative methods (for example, real-time quantitative PCR) were employed to precisely describe the changes of community structure and dynamics? The molecular techniques adopted by most previous studies such as Restriction Fragment Length Polymorphism (RFLP), Single Strand Conformation Polymorphism (SSCP), Denaturing Gradient Gel Electrophoresis (DGGE) limited their ability to accurately quantify the abundance of specific species, although quantitative data are becoming an essential topic of the link between structure and function (Johnson and Martiny, 2015; Danchin, 2016; Ortiz-Alvarez and Casamayor, 2016; Props et al., 2017). Since it is very vital to define the roles of acidophiles during bioleaching process to improve bioleaching efficiency, a comprehensive awareness of microbial dynamics is required such as quantitatively spatiotemporal succession pattern of microbial communities both in the leachates and on the ore surfaces (Brierley and Brierley, 2013; Jones and Johnson, 2016; Bobadilla-Fazzini et al., 2017).

In this study, column bioleaching of low grade copper sulfide was carried out at different temperatures after inoculation with mesophiles, moderate thermophiles and extreme thermophiles. The microbial diversity and dynamics of predominant species in the leachates and on the ore surfaces of different portions of the column during bioleaching were analyzed by clone libraries of 16S rRNA gene and real-time quantitative PCR. The hypotheses tested in this study were that (i) the microbial structures on the ore surfaces would be independent of community structures in the leachates, and (ii) effects of temperature on microbial communities at different portions of column would be different and would change as bioleaching progressed.

2. Materials and methods

2.1. Inoculum preparation

A mixed microbial culture, comprising 14 species of mesophiles, moderate thermophiles and extreme thermophiles and a moderately thermophlic consortium (Table 1), was assembled and acclimated to gradually increasing pulp densities of low grade copper sulfide (1%, w/v) as described in the previous studies (Wang et al., 2014, 2018). All strains were subcultured every two weeks and maintained as active cultures as shown in Table 1. The moderately thermophlic consortium was maintained as batch culture in stirred tank reactor with chalcopyrite as energy source.

2.2. Minerals components

The low grade copper sulfide used in this study was obtained from the Yulong copper mine in Tibet, China. The main chemical composition of the ore detected by X-ray fluorescence spectrometer (Axios mAX,