

High efficient mixed culture screening and selected microbial community shift for bioleaching process

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Abstract: To screen the high efficient mixed culture and understand the bioleaching behaviors of mixed culture for low-grade copper sulfide ore bioleaching, ten mixed cultures were collected and screened from different acid mine drainages obtained from sulfide mines of China. The leaching rate was set as criterion to screen the mixed culture and the metagenomic approach. Community genome array (CGA) was used for analyzing the mixed culture microbial community shift during the bioleaching process. The results indicate that the mixed culture obtained from Yinshan(YS) lead-zinc mine in Dexing of Jiangxi province in China reaches the maximum copper extraction (68.89%) during the one bioleaching period of 24 d. CGA results show that YS culture contains nine kinds of bacteria which are belong to six divisions, and the microbial community structure is changing during the bioleaching process. This provides a good way to accelerate the bioleaching process and reveals the microbial community shift during the bioleaching process.

Key words: bioleaching; high efficient mixed culture; community genome array (CGA); microbial community shift

1 Introduction

Bioleaching has been used commercially and offers many cost advantages over traditional techniques, such as energy-efficiency, environmentally friendliness, cost-saving, economic, pollution-free process and low capital requirements. It is quite suitable for treating low-grade ores, waste ores, small and complicated ore bodies and ores that are hard to be recovered by traditional techniques[1]. A lot of studies were carried out in different copper mines around the world by selected bioleaching strains or mixed cultures from acid mine drainage (AMD)[2–4], and the results indicated that the bioleaching capability of mixed culture is obviously better than pure stains[5–8]. However, no research work has focused on comparing bioleaching of low-grade copper sulfide ore using the mixed cultures to choose the high efficient mixed culture.

The understanding of the microbial community structure in the bioleaching systems is very important for the optimization of microbial community by controlling the operating conditions in bioleaching systems, enhancing the bioleaching rate[9]. The microarray has

been developed and evaluated for bacterial detection and microbial community analyses in bioleaching systems by ZHOU[10], which has high density, high sensitivity and high quantitative capability.

In this study, ten mixed cultures of acidophilic microorganisms were collected and screened at 30 °C from different AMDs of sulfide mines of China. By comparison experiments in the shake flask, the copper ion bioleaching capabilities of the ten mixed cultures were studied. By the metagenomic approach, the microarray was used for analyzing the mixed culture microbial community shift during the bioleaching process.

2 Materials and methods

2.1 Site description and microorganisms isolation

The mixed cultures of acidophilic microorganisms were collected from AMD samples of sulfide mines of China. The site locations where the samples were collected are shown in Table 1. The indigenous mixed culture is BC and the others are exotic cultures. The pH and temperature of origin samples were 2–3 and 32–35 °C, respectively.

Table 1 Site of samples collected

Abbreviation	Sampling point
SLS	Dropsy of Dexing mining area, Jiangxi province
LS	AMD of Longshan lead-zinc mine, Hunan province
LM	AMD of Qibashan copper mine, Hunan province
DY	AMD of Daye copper mine, Hubei province
ZJ	Zhujia acid water reservoir, Dexing copper mine, Jiangxi province
DMT	Acid water reservoir of Jiujiang copper mine region, Jiangxi province
YTW	Acid water reservoir of Yangtaowu, Dexing copper mine, Jiangxi province
YS	The second heap bioleaching liquor of Yinshan lead-zinc mine bioleaching plant, Jiangxi province
DBS	AMD of Dabaoshan lead-zinc mine, Guangdong province
BC	Indigenous AMD of Dongguashan mine, Anhui province

The AMD samples were filtered by 0.2 μm -nylon filtration membrane (Biobasic Inc.), then the mixed cultures were collected within 24 h after collection and preserved at $-20\text{ }^{\circ}\text{C}$ for use. The ten mixed cultures were cultured in the 9 K culture medium under the same condition, containing 0.5 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 3.0 g/L $(\text{NH}_4)_2\text{SO}_4$, 0.5 g/L K_2HPO_4 , 0.1 g/L KCl and 0.01 g/L $\text{Ca}(\text{NO}_3)_2$. 5%(m/V) Dongguashan copper sulfide ore was replaced by FeSO_4 as the energy source. It was incubated in rotary shakers with a speed of 180 r/min at $30\text{ }^{\circ}\text{C}$. The initial pH of the cultures was adjusted to 2.0 with 2.5 mL 1:1 (V/V) H_2SO_4 . After 30 d, the biomass reached 1×10^7 cells/mL, then microorganisms were collected by centrifugation at speed of 10 000 r/min for 15 min. The sedimentary cells were washed twice with H_2SO_4 (pH=2.0), and diluted to the same concentration of 1×10^7 cells/mL for the next experiments.

2.2 Minerals composition and structure

Dongguashan copper sulfide mine, located in Tongling, Anhui Province, is the deepest underground copper mine of Asia. The proved reserve is about 102.18 million tons (containing 1 046 800 t copper), and the average grade of Cu is 1.02%.

The main copper minerals of Dongguashan copper sulfide ore are chalcopyrite, pyrite and pyrrhotite. 89.42% of copper minerals are primary copper sulfides. The ore is composed of two main characteristics, fine-grained copper sulfide minerals (0.02–0.002 mm) and medium-fine grained minerals (2–0.02 mm). The main chemical compositions are listed in Table 2. Table 3

shows that 89.42% of total Cu is primary copper sulfide. Table 4 shows that the main minerals are chalcopyrite, pyrite and pyrrhotite. The ore was ground so that over 90% is less than 0.074 mm.

Table 2 Main chemical compositions of Dongguashan copper sulfide ore(mass fraction, %)

Cu	S	Fe	As	Au
1.07	18.13	32.61	0.002	0.28
MgO	CaO	SiO_2	Al_2O_3	Ag
4.70	7.33	20.96	1.63	3.80

Table 3 Copper mineral compositions of Dongguashan copper sulfide ore (mass fraction, %)

Free oxidized copper	Conjunction oxidized copper	Secondary copper sulfide	Primary copper sulfide	Total copper
1.86	2.84	5.88	89.42	100.00

Table 4 Mineral compositions of Dongguashan copper sulfide ore (mass fraction, %)

Chalcopyrite	Pyrite	Pyrrhotite	Valleriite	Magnetite	Siderite
2.61	11.20	17.40	0.62	7.5	2
Andradite	Dolomite	Calcite	Plagioclase	Talc	Sericite
6	6	8	3	1.5	2
Serpentine	Chlorite	Diopside	Actinolite	Quartz	Others
6	5.35	6	4	10	0.82

2.3 Bioleaching experiment methods

The bioleaching experiments of high efficient mixed culture screening were carried out in 250 mL-Erlenmeyer flasks that were shaken in an air-conditional shaker. At the beginning of each experiment, 100 mL 9K culture medium containing 0.5 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 3.0 g/L $(\text{NH}_4)_2\text{SO}_4$, 0.5 g/L K_2HPO_4 , 0.1 g/L KCl and 0.01 g/L $\text{Ca}(\text{NO}_3)_2$ was prepared. The initial pH, temperature, rotation, pulp density and bacteria load of mixed cultures were 2.0, $30\text{ }^{\circ}\text{C}$, 180 r/min, 10% (m/V) and 10%(m/m) respectively. Two parallel comparative experiments were designed. In control experiment, pure *Acidithiobacillus ferrooxidans* was inoculated.

Distilled water was added into the flasks on time every day in order to compensate for evaporation losses. Copper concentrations in solution were analyzed every 4 d by atomic absorption spectrophotometry (AAS). Solid residues were filtered, washed, dried, and finally analyzed by X-ray diffractometry (X-RD). Free bacteria in solution were counted in a Petroff-Hausser chamber under an optical microscope.

2.4 Analysis of community genome array (CGA)

YIN et al[11] have reported that a CGA could measure the microbial community structure and the

community succession of the bioleaching system successfully. So this CGA was used for measuring the changes in bioleaching system of the mixed culture with the highest copper bioleaching rate. At the beginning of the bioleaching, the 10th day and the 20th day, the samples were taken. The extraction of nucleic acids of the best mixed culture and purification, the oligonucleotide probe (50-mer) design, the microarray construction, the fluorescent labeling of DNA, the hybridization, and the image processing and data analysis were carried out according to the procedure described by YIN et al[11].

3 Results and discussion

3.1 Comparison of bioleaching experiments

Figure 1 shows the copper extraction rate of Dongguashan copper sulfide ore by ten mixed cultures and pure *Acidithiobacillus ferrooxidans* strain (A.f).

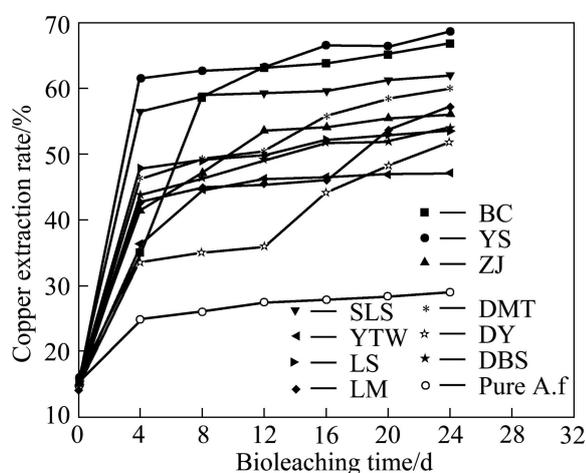


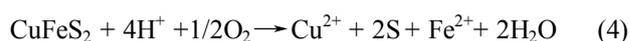
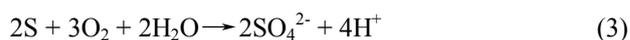
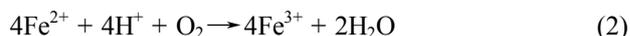
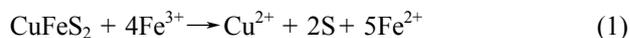
Fig. 1 Copper extraction rate by ten mixed cultures and pure *A.f*

The results reveal that ten mixed cultures of microorganisms presented in AMD have different capabilities. The mixed culture BC and the mixed culture YS are found to be more efficient than other groups. The mixed culture YTW collected from the acid water reservoir of Yangtaowu, Dexing copper mine, Jiangxi province, has the minimum bioleaching capability of 47.08%. But it is better than the control experiment by pure *Acidithiobacillus ferrooxidans* strain (30.94%). Furthermore, YS shows the highest extraction rate of Cu. The highest bioleaching rate is 68.69% by the YS. The mixed culture YS is collected from the second heap leach liquor of Yinshan bioleaching plant, Jiangxi province.

89.12% of the Dongguashan copper sulfide ore is primary copper sulfide. Due to the slow kinetics and the high activation energy of chalcopyrite, leaching of chalcopyrite has encountered limited success. However, 68.69% Cu is leached out by YS. In the first 4 d, the

bioleaching rate increases faster than in the last 20 d. The possible reasons are as follows.

Firstly, the ore is a kind of high-iron sulfide copper. The total Fe and sulfur are 32.61% and 18.13%, respectively. The iron-oxidizing bacteria in the mixed culture could oxidize Fe (II) to Fe (III) and utilize electrons in their metabolic processes [12]. Fe (III), a powerful oxidant, is able to chemically oxidize the majority of sulfide minerals [13].



Secondly, the relation of the magnetite in a lean magnetite ores to gangue is not close; ball milling not only can improve the mineral liberation degree of copper notably but also can improve the particle size characteristics of the ground particles, enhancing the leaching rate.

Finally, this kind of ore is rich in pyrite (11.2%) and pyrrhotite (17.4%). The effect of galvanic interaction between chalcopyrite and pyrite or pyrrhotite [14] can greatly improve the bioleaching rate of chalcopyrite.

3.2 Results of CGA

Analysis of CGA shows that nine kinds of bacteria presented in the bioleaching system of mixed culture of YS. Table 5 shows the results of mutative microbial community structure and foundations of single roles of YS.

In the bioleaching system, nine kinds of bacteria, *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*, *Acidithiobacillus caldus*, *Sulfobacillus* spp., *Alicyclobacillus* spp., *Arthrobacter globiformis*, *Acidisphaera* spp., *Ferromicrobium* spp.S-4 and *Leptospirillum* spp., belong to six divisions, α -Proteobacteria, β -Proteobacteria, γ -Proteobacteria, Firmicutes, Actinobacteria and Nitrospira [2, 15].

For many years, *Acidithiobacillus ferrooxidans* has been assumed to be the most important microorganism in the bioleaching of sulfide ores at temperature lower than 40 °C, as a iron-oxidizer and sulfur-oxidizer. Using the analysis of the 16S rDNA amplification products of total DNA, the sulphur-oxidising bacteria, *Acidithiobacillus thiooxidans* and the iron-oxidising bacteria *Leptospirillum ferrooxidans*, have been found as the dominant populations in bioleaching tanks. At temperatures higher than 40 °C, the moderately thermophilic *Acidithiobacillus caldus* was also present [16].

Table 5 Mutative microbial community structure and foundations of single role of YS

Microorganism	Role of bioleaching	Thermal classification	Percent/%		
			The 1st day	The 10th day	The 20th day
<i>Acidithiobacillus ferrooxidans</i>	Iron-and sulfur-oxidizers [2–3, 15–17]	Meso	14.47	26.63	21.46
<i>Acidithiobacillus thiooxidans</i>	Sulfur-oxidizers [3, 15–17]	Meso	20.00	24.32	17.11
<i>Sulfobacillus</i> spp.	Iron-oxidizers/reducers and sulfur-oxidizers [3]	Meso and mod thermo	7.35	7.62	18.42
<i>Alicyclobacillus</i> spp.	Heterotrophic acidophiles [3]	Meso	27.35	15.42	15.18
<i>Arthrobacter globiformis</i>	Heterotrophic acidophiles [3]	Meso	8.13	13.10	8.00
<i>Acidisphaera</i> spp.	Heterotrophic acidophiles [3]	Meso	6.17	5.80	8.49
<i>Acidithiobacillus caldus</i>	Sulfur-oxidizers [3]	Mod thermo	7.73	0.00	3.74
<i>Leptospirillum</i> spp.	Iron-oxidizers [3, 15]	Meso	4.36	5.25	2.76
<i>Ferromicrobium</i> spp.S-4	Iron-oxidizers [2–3]	Meso	4.45	1.85	4.83

Meso—Mesophiles ($t_{\text{optimum}} < 40\text{ }^{\circ}\text{C}$); Mod thermo—Moderate thermophiles ($t_{\text{optimum}} 40\text{--}60\text{ }^{\circ}\text{C}$)

In this study, the dominant bioleaching bacteria were *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*, *Alicyclobacillus* spp. and *Sulfobacillus* spp.. In the first 10 d of bioleaching, the percentage of *Acidithiobacillus ferrooxidans* increased from 14.47 % to 26.63%, and it played the most important role. The percentage of three kinds of bacteria, *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*, *Alicyclobacillus* spp., increased from 61.82% to 66.47%. The total biomass increased significantly, too. In the second 10 d, the bioleaching process trended to cease, so the biomass began to decrease, and the percentage of three dominant bacteria decreased to 53.75%. The synergistic effect of autotrophic microorganisms and aerobic-anaerobic microorganisms in the bioleaching system increases the bioleaching rate of the sulfide ore. *Alicyclobacillus* spp. decreases the toxicity of organic matter accumulation, and *Acidithiobacillus thiooxidans* avoids the element sulfur membrane of mineral surface, and the copper is leached by *Acidithiobacillus ferrooxidans* continuously.

4 Conclusions

1) Ten mixed cultures enriched from different places have different capabilities of copper extraction. The mixed cultures BC and YS are more efficient than the others, the mix culture obtained from Yinshan lead-zinc mine (YS) reaches the maximum copper extraction of 68.89% during the one leaching period of 24 d.

2) CGA results show that YS contains nine kinds of bacteria, which belong to six divisions and the microbial community structure is changing during the bioleaching process. The main bioleaching bacteria are *Acidithiobacillus ferrooxidans*, *Acidithiobacillus*

thiooxidans, *Alicyclobacillus* spp. and *Sulfobacillus* spp.. Sulfide-oxidizing bacteria, iron-oxidizing bacteria, the autotrophic bacteria and aerobic-anaerobic bacteria result in synergistic effect of bioleaching process.

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高效浸矿混合菌种的筛选及其在浸矿过程中的微生物群落结构演变

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摘要: 为了筛选可高效浸出低品位硫化矿的混合菌株, 从 10 个典型的硫化矿矿区的酸性矿坑水中分离富集到混合菌株。以浸出率作为筛选的主要标准, 筛选后的高效菌株, 利用群落基因组芯片分析混合菌群的组成和过程变化。浸矿持续进行 24 d 后, 采集自江西德兴银山铅锌矿的混合菌浸出率最高, 为 68.89%。群落基因组芯片结果表明银山菌群包含 9 种菌, 可被分为 6 类, 在浸出过程中群落始终在变化。该研究为加速浸出和了解浸出过程中群落演替提供了一个较好的方法。

关键词: 生物浸出; 高效混合菌群; 群落基因组芯片; 微生物群落演替

(Edited by LI Xiang-qun)