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Synergetic effects of *Ferroplasma thermophilum* in enhancement of copper concentrate bioleaching by *Acidithiobacillus caldus* and *Leptospirillum ferriphilum*

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ABSTRACT

Assistance of introduction of *Ferroplasma thermophilum* in improving copper concentrate bio-dissolution was investigated. Results in pH-controlled stirred tank reactors showed that addition of *F. thermophilum* into the defined microbial consortium (*Acidithiobacillus caldus* and *Leptospirillum ferriphilum*) improved the dissolution and conversion of iron and meanwhile caused significant difference in the planktonic and attached population dynamics. Thereby, copper extraction was accelerated and increased by 22.7% after 24 days in comparison to the control without *F. thermophilum*. The intensive study of relationships between the modified bioleaching performance and augmentation of *F. thermophilum* conducted in shake flasks indicated that (1) *F. thermophilum* was capable of bio-catalytically regenerating the sulfide-attacking agent—ferric iron in bioleaching; (2) more importantly, mixed cultures involving *F. thermophilum* collaboratively resisted the inhibitory effects of environmental stress conditions on cells, such as 0.04% yeast extract, 5 g L^{-1} Cu^{2+} and low initial pH 1.2 demonstrated in the simple growth medium, and furthermore increased the total cell numbers and accelerated the iron or sulfur oxidation compared to pure cultures. In conclusion, these findings revealed that there were synergetic acts between *F. thermophilum* and *At. caldus* or *L. ferriphilum*, which contributed to an improvement in copper leaching rate and level.

1. Introduction

Bioleaching of sulfide minerals is a promising technology to extract valuable metals (such as gold, copper, cobalt, nickel, zinc, uranium etc.) due to its low capital investment, environment friendly, mild reaction and low energy consumption [1]. Diverse groups of acidophilic microorganisms were found and employed in the bioleaching system, especially moderate thermophiles [2–6]. The main functional acidophiles involved in the bioleaching processes are able to oxidize ferrous iron or inorganic reduced sulfur compounds to regenerate the metal sulfide-attacking agents, such as ferric irons and protons [7–10]. However, it is worth noting that heterotrophic or chemomixotrophic acidophiles also have been found in the most acidic, metal-rich natural environments known as well as commercial bioleaching tanks by removing soluble organic materials that might inhibit some chemotrophic bacteria [11,12]. As reported in the previous studies [6,13–15], limited moderate thermophiles, such as *Leptospirillum* spp., *Acidithiobacillus caldus*, *Sufobacillus* spp., and *Ferroplasma* spp., dominated and played significant roles in the bioleaching stirred-tank reactors of copper concentrate operated at 45–50 °C.

Species of the genus *Ferroplasma* were cell wall-deficient, extremely acidophilic and iron-oxidizing mixotrophic or organotrophic archaea [16]. To date, most efforts were directed in exploring the phenotypic and genotypic characteristics of *Ferroplasma* isolates [17–21] and/or found the enhancement in sulfide mineral dissolution by microbial consortium with *Ferroplasma* spp. [13,22]. Our previous studies [23,24] isolated and characterized a novel extremely acidophilic, moderately thermophilic *F. thermophilum* L1 and also found some promising results to extract copper from chalcopyrite concentrate in 500-mL shake flasks inoculated by the defined mixed cultures of moderate thermophiles

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involving this strain [24]. These results confirmed the conclusions of previous reports [22,25] that mutualistic interactions between physiologically distinct moderately thermophilic acidophiles would be of great importance in promoting chalcopyrite dissolution. However, the data on how *Ferroplasma* sp. cooperated with the other iron/sulfur oxidizing bacterium and thereby improved copper leaching are very limited. Particularly, the combined effects of *Ferroplasma* sp. and *At. caldus* or *Leptospirillum ferriphilum* on the bioleaching performance, iron/sulfur functional conversion or population dynamics of microbial community in the stirred tank reactor or shake flasks lacked the detailed knowledge.

Thus, to obtain a complete comprehension, we went on to investigate the influences of augmentation of *F. thermophilum* L1 on the copper extraction and community dynamics of the free and sessile cells in the stirred tank reactors. Moreover, further studies to probe into the microbial interactions between *F. thermophilum* and *At. caldus* or *L. ferriphilum* were carried out in shake flasks by comparing the variations of iron/sulfur oxidation and microbial growth between the pure cultures and co-cultures grown in the simple growth media with different stress conditions. These results will together reveal the close relationships between the bioleaching performance, microbial interaction and existence of *F. thermophilum*. Furthermore, such information in the present study may facilitate the understanding and monitoring of the enhancement in copper concentrate dissolution inoculated by artificial bioleaching consortia of moderate thermophilic acidophiles [12].

2. Material and methods

2.1. Microorganisms and culture conditions

One sulfur-oxidizing bacterium *At. caldus* s2, one iron-oxidizing bacterium *L. ferriphilum* YSK and one chemomixotrophic archaeon *F. thermophilum* L1 were used in this study [23,26,27]. Pure cultures of *L. ferriphilum* YSK and *At. caldus* s2 were separately cultured in the defined growth medium [28] at 45 °C and initial pH 1.5, with $FeSO_4{\cdot}7H_2O$ (30 g L^{-1}) or sulfur (10 g L^{-1}) as energy source. *F. thermophilum* L1 was grown chemomixotrophically with 20 g L^{-1} $FeSO_4{\cdot}7H_2O$ and 0.02% (w/v) yeast extract in growth medium at 45 °C (acidified to initial pH 1.0 with 50% (v/v) H_2SO_4).

2.2. Copper concentrate

The copper concentrate used in this study was obtained from Meizhou Jinyan Copper Company Ltd. in Guangdong province (China), and mainly consisted of chalcopyrite (32.7%), bornite (29.8%) and pyrite (25.1%) together with small amount of galena. Major elemental composition of the concentrate was 30.2% Cu, 24.8% Fe, 37.7% S and 7% Pb. Prior to bioleaching experiments, the finely ground mineral sample was passed through a sieve with a pore size of 75 μm.

2.3. Bioleaching experiments of copper concentrate with two different defined mixed cultures in the stirred tank reactors

Batch experiments were performed in a 3-L glass cylindrical reactor at 4% (w/v) pulp density, initial pH of around 2.0, 45 °C, 500 rpm stirring, 20 L h^{-1} aeration and 2000 mL iron-free sterile growth medium. The copper concentrate sample was sterilized by autoclaving before bioleaching. During the whole leaching processes, the pulp pH was adjusted by addition of H_2SO_4 (50% v/v) when it exceeded the initial pre-set value (pH 2.0). Sterile distilled water was added into the reactor through a peristaltic pump in order to compensate for evaporation losses.

Two different bioleaching groups were designed: Consortium A (*At. caldus* s2 and *L. ferriphilum* YSK, as the control group) and Consortium B (*At. caldus* s2, *L. ferriphilum* YSK and *F. thermophilum* L1). Prior to bioleaching, pure cultures of *At. caldus* s2, *L. ferriphilum* YSK and *F. thermophilum* L1 were stepwise acclimatized to 4% (w/v) copper concentrate over a prolonged adaptation period by observing microbial growth in the different pulp density. After that, cells were harvested by centrifugation at 12,000 × *g* for 15 min and washed twice with sterile water (pH 2.0), finally re-suspended in sterilized fresh medium (pH 2.0). According to the experimental design, equal cell numbers of each strain (final cell density of approx. 1×10^7 cells mL^{-1}) was inoculated into the stirred tank reactors. Leaching experiments lasted for 24 days. Samples were withdrawn at regular intervals and analyzed for variations in bioleaching performance and microbial community of free and attached cells in the solution and on the mineral.

2.4. Studies of enhancement in copper extraction and exploration of microbial interactions

2.4.1. Ability of pure cultures to bioleach copper in shake flasks

Bioleaching experiment by pure culture of *F. thermophilum* L1 was carried out in 250 mL shake flasks containing 2% (w/v) of copper concentrate and 100 mL iron-free growth medium [28]. It was acidified to the initial pH 1.0 with H_2SO_4 (50% v/v) and cultured on the rotary incubator shaker at 180 rpm and 45 °C, which was compared to the one of pure culture of *L. ferriphilum* YSK with initial pH 1.6. Abiotic control test was also performed in the same experimental set up. Each experimental group was accomplished in triplicate and sterilized by autoclaving for 25 min at 121 °C and 103.4 kPa. Samples were removed at regular intervals from the flasks and analyzed for copper recovery.

2.4.2. Synergetic experiments by co-cultures including F. thermophilum L1 in simple growth medium with some stress condition

In order to further understand how microorganisms interact with each other and thereby assisted in improving copper extraction, experiments of pure cultures and co-cultures involving *F. thermophilum* L1 were carried out in simple modified growth medium with different stress conditions. Table 1 showed the experimental designs used for the synergetic tests. (1) In experiments of pure cultures (*F. thermophilum* and *L. ferriphilum*) and their co-cultures, stress conditions of organic compounds (0.04% (w/v) yeast extract) and heavy metals (5 g L^{-1} Cu^{2+}) were separately studied (Table 1). Both pure cultures and co-cultures of *F. thermophilum* L1 and *L. ferriphilum* YSK were grown at initial pH 1.5 with addition of 50 g L^{-1} $FeSO_4{\cdot}7H_2O$ as energy source. (2) As for *F. thermophilum* and *At. caldus*, their synergetic acts on heavy metals (5 g L^{-1} Cu^{2+}) or high acid concentration (initial pH 1.2) were investigated (Table 1). Energy source of 20 g L^{-1} $FeSO_4{\cdot}7H_2O$ and 10 g L^{-1} sulfur were supplied in both pure cultures and their co-cultures.

All experiments were performed in 250 mL shake flasks containing 100-mL growth medium with some stress condition. They were sterilized by autoclaving for 25 min at 121 °C and 103.4 kPa. Three strains used were previously grown to exponential growth phase and harvested as described in Section 2.3. Equal cell numbers for each strain was inoculated into the shake flasks of pure or mixed cultures. Finally, these cultures were incubated on a rotary shaker at 180 rpm and 45 °C. Unless otherwise stated, all experiments were performed in triplicate, with results reported as means ± standard deviations (SD). Samples were collected from each flask at predetermined time intervals to assess the influences of organic compounds (0.04% yeast extract), heavy metals (5 g L^{-1} Cu^{2+}) or high acid concentration (initial pH 1.2) on the total cell numbers and iron or sulfur oxidization by comparison between pure cultures