Bioleaching of Heavy Metals from Sludge by Mixed Strains

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Abstract. Microbial removal of heavy metals in sludge was investigated. Effect of bacterial strains on bioleaching of heavy metals were also studied. Mixed cultures of thellobacillus ferrooxidans, t.thelloooxidans and acid resistant heterotrophic bacteria were used separately to compare efficiency of metal solubilization. The results showed that mixed cultures with acid resistant heterotrophic bacteria were more efficient than mixed cultures without acid resistant heterotrophic bacteria.

1. Introduction

The components of sewage sludge vary because of the differences of sewage sources, composition, and wastewater treatment techniques [1]. Recently, sludge production is large, and the composition is complex in China, and this required adequate treatment [2]. The ultimate goals of sludge treatment are reduction, stabilization, detoxification, and recycling, and one of the major obstacles in sludge recycling is high heavy metal content of sludge [3,4].

Biological leaching has been widely concerned in the removal of heavy metals from sludge [5]. It is an environmentally friendly process compared with physical and chemical processes and rarely generates atmospheric contaminants. Bioleaching operations are relatively inexpensive and simple to operate [6,7]. The bacteria commonly employed in the bioleaching processes are genus Acidithiobacillus and Thiobacillus, such as Acidithiobacillus ferrooxidans, A. thiooxidans and Thiobacillus thioparus. Among them, A. thiooxidans uses reduced forms of inorganic S instead of Fe$^{2+}$ as energy source. In addition, they are highly acidophilic (pH 0.5 to 5.5, optimum pH 2 to 3.5), and can make pH of the leaching medium decreases to 1.5 to 1.0 or even lower. On the other hand, A. ferrooxidans differs from A. thiooxidans by the fact that they derive energy from the oxidation process of Fe$^{2+}$ as an electron donor in addition to reduced S compounds. In the absence of oxygen, A. ferrooxidans is still able to grow on reduced inorganic S compounds using Fe$^{3+}$ as an alternative electron acceptor. Organics such as putrescine in activated sludge can inhibit the growth of thiobacterium and inhibit the normal process of biological leaching. There are a large number of heterotrophic bacteria in activated sludge that can take organic substances such as putrescine as substrate. The biodegradable strains that can withstand the acidic environment can coexist with the acidophilic thiobacterium, and remove the harmful organisms in the activated sludge through their own biodegradability, thus improving the bioleaching efficiency [8-10].

The objective of this research are to study the growth characteristics of an acid-resistant heterotrophic bacterial species isolated from electroplating sludge and the effect of reaction time and removal efficiency on the bioleaching of mixed cultures of thellobacillus ferrooxidans, t.thelloooxidans and acid resistant heterotrophic bacteria leaching system.
2. Materials and methods

2.1. Sample Sludge Characterization

The sludge used in this experiment was from the concentrated sludge of the secondary sink of Yonghe sewage treatment plant. Wastewater received by the plant is composed of 70% of domestic sewage and 30% of industrial wastewater. The main characteristic parameters of the concentrated sludge used in the experiment were: water content, organic matter content (TS), heavy metal content (Cu, Pb, Cd). The physical properties of sludge used in this experiment were shown in Table 1.

Table 1. Sample Sludge Characterization

| pH  | Water content % | TS% | Cu mg/kg | Pb mg/kg | Cd mg/kg |
|-----|-----------------|-----|----------|----------|----------|
| 5.57 | 83.54           | 63.45 | 2559     | 1282     | 32.8     |

2.2. Growth Characteristics of Acid Resistant Heterotrophic Bacteria

A strain of acid resistant heterotrophic bacteria, bacillus, NS-1 was isolated from activated sludge of electroplating sewage treatment plant. Inoculated NS-1 in heterotrophic medium (10g peptone, 5g yeast powder, 5g NaCl, 1000mL H2O), the culture conditions were: temperature 30°C, pH 7.0, growth curves were determined. The growth status of NS-1 were measured at different temperatures (10°C, 15°C, 20°C, 25°C, 30°C, 35°C, 40°C) and different pH values (2, 3, 4, 5, 6, 7, 8).

2.3. Bioleaching Experiments by Mixed Strains

Thellobacillus ferrooxidans, T.thelloooxidans and acid resistant heterotrophic bacteria NS-1 were selected as inoculation strains. the solid content of the concentrated sludge was diluted to 2% with distilled water, and 142ml and 138ml of this concentration of sludge were transferred to 250ml conical flasks. 8ml enrichment cultures of Thellobacillus ferrooxidans and T.thelloooxidans mixture (volume ratio :1:1, pH < 2) and 12ml of enrichment cultures of Thellobacillus ferrooxidans, T.thelloooxidans and acid resistant heterotrophic bacteria NS-1 mixture (volume ratio :1:1, pH < 2) were added respectively. The content of sulfur powder and Fe2+ in the substrate were 6g/L and 4 g/L. The biological leaching temperature, rotating rate and leaching time of sludge samples were 28°C, 150r/min and 14d.

3. Results and discussion

3.1. Growth Curve of Acid Resistant Heterotrophic Bacteria

Growth of bacteria can be divided into 4 phases: lag phase, logarithmic phase, stationary phase and decline phase, which depend on the growth rate and bacterial activities. Monitoring of growth curve is crucial in study growth pattern of bacteria. Through the growth curve, the different growth status of bacteria can be observed and bacteria collection would due to different objectives, for example, bacteria in logarithmic phase is collected for rapid oxidation of elemental sulfur, and bacteria in lag phase is best for preservation.

The OD600 growth curve of bacillus NS-1 in 30°C and pH=7 is shown in fig. 1.

In Fig.1, the first 0.5 day is lag phase, and the bacteria is adjusted to adapt new environment, and collect energy and material for growth. The bacteria growth entered logarithmic phase from 0.5 day, and went to stationary phase in about 2.5 days. The increase rate of bacteria declined, and reproduction and death rate equilibrant in stationary phase.
3.2. Growth Temperature Range of Acid Resistant Heterotrophic Bacteria

The growth of NS-1 at different temperature was shown in Fig. 2. The optimal growth temperature of NS-1 was 30 °C, can maintain normal growth in 15 ~ 35 °C. This is good for an outdoor biological leaching system, because the growth metabolism of the strains will not be blocked by outdoor high and low temperature. To keep the reactors temperature at around 30 °C can get the best effect.

3.3. Growth pH Range of Acid Resistant Heterotrophic Bacteria

pH is an important parameter in leaching. The pH requirements vary in different leaching processes, and the optimal pH of different bacteria are also different. So that, the effect of pH to growth of bacteria should be know to control leaching. Growth of NS-1 in different pH was shown in Fig. 3.
The best pH for NS-1 is 6, and which could be kept growth in pH 3~8, so NS-1 shows acid tolerance. At the beginning of bioleaching, pH in the system decrease rapidly, and the heterotrophic local bacteria which adapt in neutral environment dead extensively, and only the acid tolerant heterotrophic bacterial can co-growth with Thioabacillus.

3.4. Effect of mixed bacteria bioleaching on removal of heavy metals

Bioleaching is to leach heavy metals from sludge into leaching solution by the acid production and strong oxidation of *Thelloobacillus* species. Therefore, pH is an important indicator of biological leaching process. NS-1 was mixed with *Thelloobacillus ferrooxidans* and *T.thelloooxidans* in the same proportion and inoculated into the bioleaching system, and the change of pH in leaching process was monitored.

![Figure 4. The pH changes in different groups of leaching system](image)

The change of pH can dynamically reflect the status of sludge biological leaching process. When the pH reached 2, it indicated that the bioleaching starts successfully. It can be seen from the Fig 4 that the mixed cultures of *Thelloobacillus ferrooxidans* and *T.thelloooxidans* leaching system can be activated in about 6 days, mixed cultures of *Thelloobacillus ferrooxidans, T.thelloooxidans* and acid resistant heterotrophic bacteria leaching system can be activated in less than 4 days. Addition of acid resistant heterotrophic bacteria significantly shortening the system startup time.

|                   | Cd       | Cu       | Pd       |
|-------------------|----------|----------|----------|
|                   | 4d       | 10d      | 4d       | 10d      | 4d       | 10d      |
| Leaching rate of  |          |          |          |          |          |          |
| *Thelloobacillus* | 6.11     | 49.17    | 6.47     | 63.77    | 4.87     | 51.69    |
| bioleaching       |          |          |          |          |          |          |
| system(%)         |          |          |          |          |          |          |
| Leaching rate of  | 28.98    | 60.97    | 37.26    | 75.42    | 31.26    | 63.76    |
| heterotrophic     |          |          |          |          |          |          |
| bacteria mixed    |          |          |          |          |          |          |
| with *Thelloobacillus* |          |          |          |          |          |          |
| bioleaching       |          |          |          |          |          |          |
| system(%)         |          |          |          |          |          |          |

According to the results shown in table 2, heavy metal elimination efficiency, including heavy metal dissolution rate and percentage, of the acid resistant heterotrophic bacteria mixed group increase significantly compared with the *Thelloobacillus* group. The addition of acid resistant heterotrophic bacteria can metabolize toxic organic compounds in sludge, so that inhibition of the toxic organic compounds to *Thelloobacillus* can be eliminated, the reaction time is decreased, and the efficiency is increased. This is a typical case of co-metabolization of functional microorganisms. Although cultivation of acid resistant heterotrophic bacteria would increase the cost, the economic benefit due to reduction of reaction time is still the obvious advantage of acid resistant heterotrophic bacteria addition.
4. Conclusion
A strain of acid resistant heterotrophic bacteria, bacillus, NS-1 was isolated from activated sludge of electroplating sewage treatment plant. NS-1 can be normal growth in 15°C~35°C, pH 3~8. The optimum growth conditions for NS-1 was 30°C and pH = 7. After mixed culture NS-1 with *Thelloobacillus*, the start-up time of leaching system can be significantly shortened, and the efficiency of heavy metal removal can be improved.

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