Thiobacillus Acidophilus Removes Lead and Copper From Wastewater Containing Low Concentration of Heavy Metals

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Abstract. One of the main source of heavy metal wastewater is the electronics industry. If treated ineffectively, it may be discharged into water bodies, causing water body pollution and endangering human health. Traditional treatment methods have a poor quality of wastewater and high treatment process costs. This subject takes the low concentration of lead and copper wastewater from a lead storage battery factory as the research object, and uses the principle of the precipitation of Thiobacillus acidophilus to remove lead and copper from the wastewater. The results show that at a temperature of 30°C, pH of 2.5, and rotation speed of 130r/min, acidophils have the highest removal rate of 74.69%, 38.48% for lead and copper, respectively.

Keywords: Acidithiobacillus ferrooxidans; Acidithiobacillus thiooxidans; heavy metals; removal.

1. Introduction

In the electronic industry wastewater, heavy metal pollution has attracted people's attention because of its high toxicity and difficulty in biodegradation. Lead (Pb) is one of the most widely used non-ferrous metals by mankind and is widely used in the lead storage battery manufacturing industry. Lead ions (Pb (II)) can damage the kidney, liver, brain function, reproductive system, and basic cell processes of humans, and pose a threat to fetuses, infants, and young children [1-6]. Copper is also widely used in electronic industry, and the main source of copper pollution contains brass manufacturing, petroleum refining, copper-based agricultural industries, and smelting. When the concentration of copper is greater than 5 mg/L, it will produce toxicity [7-8]. Some researchers have extensively explored the ability of various methods to remove heavy metal ions (M (II)), such as electrochemical treatment, oxidation and reduction, precipitation, ion exchange, reverse osmosis, and adsorption, which can be used to purify metal-contained wastewater [9-10]. Most of them are expensive and difficult to achieve in practice.

For the purpose of reclaiming wastewater, this paper uses acidophilus thiobacillus to remove lead and copper in low concentration of heavy metals wastewater sourced from electronic industry.

2. Materials and Method

The strains used in the experiment named a mixture of Acidithiobacillus ferrooxidans (hereinafter referred to as A.f bacteria) and Acidithiobacillus thiooxidans (hereinafter referred to as A.t bacteria), which were collected from Dexing Copper Mine and self-screened in laboratory subsequently [11].
The strains of (A.f+ A.t) bacteria were cultured in the medium of the energy sources (Fe$^{2+}$+S mixture), and the pH was adjusted to 2.5. The chemical compositions of the media are shown in Table 1. Combined with the characteristics of wastewater from the lead storage battery plant, simulation and preparation of 10 mg/L of lead and copper wastewater were carried out. Use Origin 8.0, Excel 2019 software to process the experimental data.

### Table 1 Culture medium compositions of A.f bacteria and A.t bacteria mixture (g/L)

| Ingredient             | Mixed medium |
|------------------------|--------------|
| (NH$_4$)$_2$SO$_4$     | 3.0          |
| KCl                    | 0.1          |
| K$_2$HPO$_4$          | 0.5          |
| MgSO$_4$·7H$_2$O      | 0.5          |
| Ca (NO$_3$)$_2$       | 0.01         |
| FeSO$_4$·7H$_2$O      | 22.3         |
| CaCl$_2$·2H$_2$O      | 0.13         |
| S                      | 5.0          |

### 3. Experimental program

In this experiment, mixed culture of A.t bacteria and A.f bacteria were used to treat wastewater containing low concentrations of lead and copper. A total of 20 treatment groups were designed for the mixed culture of A.t bacteria and A.f bacteria and the uninoculated treatment control group. The (A.t+A.f) bacteria were inoculated into a conical flask with 10% (v/v) of inoculum amount, and the pH is 2.5. Each group was operated in triplicate. Finally, the average value is taken to reduce the error in the experiment. A blank control group is set for each corresponding time in the time gradient. The inoculation treatment experimental group and the non-inoculation treatment control group were placed in a constant temperature shaker incubator with a temperature of 30°C and rotating speed of 130 rpm/min for 60h. During the incubation period, the culture solution was measured every 12h, 24h, 36h, 48h, and 60h. Changes in pH and ORP, and sampled and analyzed changes in lead and copper content in the treatment solution. The initial concentration of lead and copper in the treatment solution was 10 mg/L. The experimental conditions are set in Table 2.

### Table 2 Experimental conditions

| Time(h) | Temperature(℃) | Rotating speed(r/min) | pH | Lead content(mg/L) | Copper content(mg/L) |
|---------|-----------------|-----------------------|----|--------------------|----------------------|
| CK      | 12,24,36,48,60  | 30                    | 130| 2.5                | 10                   |
| A       | 12              | 30                    | 130| 2.5                | 10                   |
| B       | 24              | 30                    | 130| 2.5                | 10                   |
| C       | 36              | 30                    | 130| 2.5                | 10                   |
| D       | 48              | 30                    | 130| 2.5                | 10                   |
| E       | 60              | 30                    | 130| 2.5                | 10                   |

### 4. Results and discussion

#### 4.1. Changes in pH and ORP under different experimental conditions

Using (A.f+At) bacteria to remove lead and copper from low concentration of heavy metal wastewater, the pH and ORP changes with different times are shown in Fig. 1. The pH in the inoculated (A.f+At)
bacteria group mainly showed a downward trend. This is caused by the acid production of strains in the culture solution. The pH of the uninoculated control group also showed a downward trend, but it was kept in a higher value range compared with the experimental group. It can be seen from Fig. 1(b) that the ORP in the system was on the rise. The increase in ORP is mainly due to the oxidation of the energy material $S^0$ to sulfuric acid and the oxidation of $Fe^{2+}$ to $Fe^{3+}$ under the catalytic oxidation of Thiobacillus acidophilus.

![Fig. 1](a) pH changes with different times; (b) ORP changes

4.2. Substrate electron microscopy analysis
From the analysis of Fig. 2 and Table 3, it can be seen that the substrate in the culture solution after treatment is mainly composed of O, S, Pb, and Cu elements.

![Fig. 2](a) Sulfur powder morphology; (b) The substrate morphology of the treatment solution in the inoculated experimental group
Table 3 The composition and proportion of sediment elements

| element | weight percentage | atom percentage |
|---------|-------------------|-----------------|
| O       | 57.48             | 49.68           |
| S       | 34.96             | 40.27           |
| Pb      | 6.71              | 8.74            |
| Cu      | 0.85              | 1.31            |
| Total   | 100.00            |                 |

4.3. Removal of Lead and Copper in Wastewater by Thiobacillus Acidophilus

The removal effect of (A.t+A.f) bacteria on lead and copper in low concentration of heavy metal wastewater within 60 h was monitored. From the analysis of Fig.3(a), it can be seen that at 24 h, (A.t+A.f) bacteria affect lead in low concentration of heavy metal wastewater. The maximum removal rate was 74.69%, and the removal of lead in the treatment solution of the uninoculated control group also reached a relatively high level because the treatment solution used concentrated sulfuric acid when adjusting the pH, which resulted in the uninoculated control group containing sulfuric acid. The root ions combine with the lead ions in the treatment solution to produce lead sulfate precipitation to achieve the removal effect. However, due to the oxidation effect of the bacterial strains in the inoculation experimental group, it produces sulfuric acid, which increases the sulfuric acid in the treatment solution. The removal rate of the inoculation treatment experimental group is higher than that of the unvaccinated control group. It can be seen from (b) that the highest removal rate of copper in the treatment group with (A.t+A.f) bacteria is only 38.48%, which may be due to the presence of both lead and copper heavy metals in the solution. (A.t+A.f) bacteria pair The treatment rate of lead in the treatment solution has priority over copper.

![Fig. 3](image)

Fig. 3 The effect of (A.t+A.f) bacteria on the removal of lead and copper over time

4.4. Mechanism analysis

The activity of Thiobacillus acidophilus is closely related to the size of the removal rate and the changes of pH and ORP in the treatment solution and can reflect the mechanism of the removal of heavy metals in the treatment solution by Thiobacillus acidophilus. In the experiment, the surface structure changes of the blank group and the experimental group were observed by SEM, and the element composition of the precipitate was analyzed by EDS, and combined with the changes of pH and ORP under different experimental conditions, it was concluded that (A.t+A.f) bacteria treated low-
concentration heavy metals The mechanism of lead and copper in wastewater may be: Thiobacillus acidophilus oxidizes the energy material S0 to sulfuric acid and combines with lead to form lead sulfate precipitation, thereby achieving the removal of low-concentration metals in the solution. From the analysis of SEM and EDS, it can be known that the elemental composition of the substrate after the reaction is mainly O, S, Pb, Cu, which further verifies the fact that lead sulfate is produced in the treatment system. The reaction equation may be as follows:

\[
2S + 3O_2 + 2H_2O \rightarrow 2SO_4^{2-} + 4H^+
\]  
\[
Pb^{2+} + SO_4^{2-} \rightarrow PbSO_4 \downarrow
\]

5. Conclusions
The effect of (A.t+A.f) bacteria on the removal of lead and copper in low concentration of heavy metal wastewater under different single factor conditions was explored. The following conclusions were drawn:

(1) The maximum removal rate of lead in wastewater is 74.69%, and the maximum removal rate of copper is only 38.48%. This may be due to the presence of both lead and copper heavy metals in the solution. The processing rate takes precedence over copper.

(2) In the process of removing low-concentration heavy metal lead and copper by A.f+A.t bacteria, the decrease of pH value is accompanied by the increase of ORP.

(3) A.t + A.f bacteria oxidize the energy substance S\(^0\) to sulfuric acid and lead to the formation of lead sulfate precipitation, thereby removing low-concentration metals in the solution, and the treated low-concentration heavy metal wastewater to achieve the effect of reclaiming water.

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