Article

Effects of Extracellular Polymeric Substances and Specific Compositions on Enhancement of Copper Bioleaching Efficiency from Waste Printed Circuit Boards

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Abstract: Bioleaching has been proven to be an efficient and environment-friendly method for processing metalliferous ore and waste printed circuit boards (PCBs), a type of urban mine waste. Extracellular polymeric substances (EPS) play a major role in the attachment of bacteria to the surface of sulfide minerals. However, there are few reports on the effects of EPS components on the bioleaching of metals from PCBs. In this study, synthetic EPS were used to investigate the effects of the composition of exo-polymers on the bioleaching of copper from waste PCBs, including the process efficiency. The copper extraction rate in bioleaching assays with synthetic EPS was 11.7% greater than in those without synthetic EPS. Moreover, the composition of EPS was proven to be a crucial factor affecting the efficiency of copper bioleaching, with EPS containing arginine yielding the highest recovery (95.2% copper). Under the condition of 0.5 g/L synthetic EPS added at the early stage of log phase, the copper leaching efficiency from waste PCBs was highly improved. This study provides important insights into how to analyze the working mechanisms of EPS for a better recovery efficiency.

Keywords: waste electrical and electronic equipment; printed circuit boards; *Acidithiobacillus ferrooxidans*; extracellular polymeric substances; bioleaching; copper

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1. Introduction

With economic development and the improvement of people's living standard, a drastic increase in waste electrical and electronic equipment (WEEE) around the world has taken place [1]. The global amount of WEEE generation was estimated to rise to 52.2 million tons in 2021 [2]. The core component of e-waste is printed circuit boards (PCBs), which contain brominated flame retardants, high concentrations of metals, and other materials [3,4]. Therefore, this may cause negative impacts on both natural eco-systems and human health, especially when improper treatment is adopted. On the other hand, PCBs can be considered as secondary resources of metals such as Cu, with a higher concentration in PCBs than in natural ores. Many approaches have been developed for the recovery of metal from PCBs, such as thermal treatment, as well as mechanical, chemical, and biological methods [5–7]. Bioleaching as an effective, low-cost, and eco-friendly method has been widely used to extract metals from not only PCBs, but also mineral ores and solid wastes with the help of microorganisms [8,9]. As a result, there are many reports on the biological leaching of waste PCBs. However, during the bioleaching process, the interactions between

the bacteria and waste PCBs are still unclear, especially the interfacial processes that are mediated by extra cellular polymeric substances (EPS) [10]. Most studies investigating bioleaching processes have focused on optimizing the process conditions, selecting suitable types of microorganisms, improving the cultivation techniques for microbial growth, and testing catalyzers, etc. [11–13]. In this study, EPS was proposed to affect the leaching efficiency, the best use conditions of EPS were optimized, and the components of EPS that could have effects on leaching efficiency were analyzed. These could help us to understand the interactions between the bacteria and waste PCBs during the bioleaching process, and to provide insights into promoting the bioleaching process.

Acidithiobacillus ferrooxidans (*A. ferrooxidans*) is a broadly used bioleaching bacterium that catalyzes the oxidation of ferrous iron to ferric, which indirectly oxidizes metal in PCBs to metal ions [14]. *A. ferrooxidans* strain CCTCC NO. M 2013102 was selected as the bioleaching bacterium in this study.

The EPS produced via bacterial secretion (but it can also contain lysis products) plays a pivotal role in bioleaching [15]. During the bioleaching of metals from pyrite by bioleaching bacteria (such as *A. ferrooxidans*), the synthesis of EPS is a prerequisite for the attachment of bacterial cells to the metal sulfide surfaces [16,17]. The initial attachment of *A. ferrooxidans* to the negatively charged surface of pyrite appears to be electrostatic. EPS concentrates ferric ions through uronic acids, leading to the net positively charged surface of *A. ferrooxidans* [18]; the ferric ions chelated by EPS show a greater capacity for oxidative attack on the metal sulfides [19], resulting in enhanced metal dissolution.

Generally, EPS are composed of various organic compounds [20]. The composition of EPS can differ based on the bacterial strain(s) and various environmental factors, such as the substrate types and incubation time [21]. EPS extracted from the bioleaching of a chalcopyrite concentrate mainly consists of sugars, lipids, proteins, and ferric ions [22]. In comparison, EPS produced by *A. ferrooxidans* grown on sulfur were found to contain a lower concentration of sugars, a higher concentration of lipids, and virtually no chelated ferric ions [16]. While no relationship has been found between the amounts of EPS produced and bacterial metabolic activity, a correlation was found between the ferric ion concentration in the EPS and the level of bacterial activity [21]. It was reported that the EPS can facilitate ferric ions by complexation through uronic acids or sugars, resulting in a net positive charge to the cells [23,24]. In addition, EPS contains hydrophobic substances, such as lipids and proteins. The hydrophobic force could enhance the attachment of bacteria to the metal surface [25,26].

As extraction methods can affect the quantity and composition of the extracted EPS [27], we used synthetic EPS in this study to investigate functions of EPS during the bioleaching of copper from metals concentrated by *A. ferrooxidans*. Moreover, EPS could be produced by bacterial species during almost the entire bioleaching process. In summary, our research can provide references for improving the bioleaching efficiency of waste PCBs and other WEEE, and help understand the bioleaching mechanisms, especially contact mechanisms.

2. Materials and Methods

2.1. Microorganisms and Culture

The bacterial strain of *A. ferrooxidans* (CCTCC NO. M 2013102) in this study was isolated from acid mine drainage and has been shown to have a high leaching efficiency in bioleaching copper from waste PCBs [28]. The growth medium used was the 9K medium containing 3.00 g/L $(\text{NH}_4)_2\text{SO}_4$, 0.10 g/L KCl, 0.50 g/L K_2HPO_4 , 0.50 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01 g/L $\text{Ca}(\text{NO}_3)_2$, and 44.3 g/L $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, serving as an electron donor for *A. ferrooxidans* [29]. The pH of the medium was adjusted to 2.0 using 1 M H_2SO_4 . *A. ferrooxidans* was grown in 9K medium at 30 °C under 165 rpm for 2 days. After washing (with fresh medium) to remove precipitates, the cells were harvested by centrifugation at $10,000 \times g$ for 20 min. The harvested cells were resuspended in pH = 2.0 sulfuric acid.

2.2. Metal Concentration of Waste PCBs Samples

Waste PCBs were obtained from a computer recycling shop in the Tianhe Computer City electronic market in Guangzhou. The disassembled PCB scraps were shredded using stainless steel blades and then crushed to a powder by a high-speed universal pulverizer (FW-400A, Beijing Zhongxing Weiye Instrument Co., Ltd., Beijing, China; run for 2 min). Next, powders under 0.425 mm were treated by floatation. A foaming agent was added in the powders to adhere to the non-metallic particles with low density (mainly plastics), which were then removed from the metallic particles using their density differences. The metal samples were air dried and then sieved, and those with particle sizes between 0.180–0.425 mm were kept for the bioleaching experiments.

In order to determine the chemical composition of the metal concentrates of waste PCBs, the metal powder needed to be digested first. First, 1.00 g waste PCB metal powder sample was weighed and added into the digestion tank, then 20 mL aqua regia, 5 mL H₂O₂, and 5 mL HF (HCl-HNO₃-H₂O₂-HF, volume ratio is 3:1:1:1) were added. Then, the digestion tank was sealed for microwave digestion, and 10 mL 4% H₃BO₃ was added after digestion and cooling, and the digestion tank was sealed again for digestion. The obtained digestion solution was analyzed using inductively coupled plasma-optical emission spectrometry (ICP-OES; China, PerkinElmer, Optima 5300 V) and the results are shown in Table 1. Furthermore, the PCB powder was sterilized for 30 min under UV before its use in the bioleaching experiments.

Table 1. Metal content in waste PCBs.

Metal	Cu	Sn	Pb	Zn	Fe	Ni	Mg	Rest
Contents (%)	68.62 ± 1.01	7.48 ± 0.19	2.27 ± 0.08	0.91 ± 0.05	0.28 ± 0.10	0.17 ± 0.01	0.09 ± 0.06	20.18 ± 0.96

The data represent the mean value ± standard deviation (SD) obtained from the duplicate analysis.

2.3. Synthetic EPS

As mentioned above, the composition and recovery of EPS can vary with different extraction methods; therefore, synthetic EPS were used to analyze the effect of their composition on the bleaching processes. The composition of the synthetic EPS agreed with that of the biogenic EPS synthesized by *A. ferrooxidans* grown on iron (II) sulfate, comprising of (*w/w*) 42.4% lipid, 20.5% fucose, 13.9% rhamnose, 11.4% glucose, 4.4% uronic acid, 0.9% xylose, 0.4% mannose, and 0.7% ferric ions. The remaining fraction of EPS (5.4%) was allotted to the proteins, which is another constituent that has previously been detected in extracted biogenic EPS. All of the substances were of analytical grade. The pH value of the synthetic EPS solution was adjusted to 2.0 using 1 M H₂SO₄.

2.4. Leaching Experiments

Leaching in the presence/absence of synthetic EPS: Bioleaching of copper from waste PCBs was conducted by adding synthetic EPS (1 g/L) into 500 mL conical flasks, each of which contained 200 mL 9K medium, 10% inoculum (20 mL) of *A. ferrooxidans*, and 12 g/L sterilized waste PCBs. Synthetic EPS (1 g/L) was also added into the flasks containing 200 mL 9K medium and 10% (20 mL) pH = 2.0 sulfuric acid, which were set up as the abiotic leaching control. Additionally, bioleaching and abiotic leaching groups were set up in a similar manner, respectively, in the absence of synthetic EPS.

Bioleaching in the presence of synthetic EPS with varying composition: To investigate the effect of different chemical compositions of EPS on the copper bioleaching, *A. ferrooxidans* cultures (in 200 mL 9K medium, 10% inoculum) were pre-grown in flasks within a shaker at 30 °C and 165 rpm. After 24 h, 12 g/L of waste PCBs was added into all of the flasks. Synthetic EPS (1 g/L) was added into the flasks intended for bioleaching in the presence of synthetic EPS, while equal amounts of one of the following compounds were added into four other bioleaching groups: glucose, uronic acid, stearic acid, and arginine.

Bioleaching under different concentrations of synthetic EPS: Bioleaching experiments were conducted in 500 mL conical flasks containing 200 mL 9K medium, and the harvested cells (10%) were inoculated. All the flasks were cultivated in a shaker at 30 °C at 165 rpm for 24 h. Then, sterilized waste PCB powder (12 g/L) was added, together with 0, 0.1, 0.5, 1.0, or 5.0 g/L of synthetic EPS, separately.

Bioleaching of copper from waste PCB with synthetic EPS at different time points: The bioleaching experiments were conducted in the 500 mL flasks containing 200 mL 9K medium, and 20 mL (10% *v/v*) *A. ferrooxidans*. After 24 h, waste PCB (12 g/L) was added into the flasks and they were returned to be cultivated at 30 °C and 165 rpm. The additions of 1 g/L synthetic EPS were done at different time points: at inoculation, early log phase, middle log phase (in a control experiment), and late log phase.

2.5. Analytical Methods

Changes in pH over time were monitored using a S-3C 153 pH meter. The copper in the solution was measured using an atomic absorption spectrophotometer (AAS; Shanghai Tianmei Scientific Instrument Co., Ltd., Shanghai, China) after appropriate dilution. The total dissolved iron and ferrous ion were measured using the o-phenanthroline method at an absorption wavelength of 510 nm (UV759, SPS, Guangzhou Huaxing Scientific Instrument Co., Ltd., Guangzhou, China). Each condition was carried out in duplicate and the mean of the two duplicates was reported. Copper accounted for about 70% of the metals in the circuit board, so it had the most significant value for recycling. As the main metal component, the data from copper can more intuitively reflect the change of its leaching rate. Therefore, the copper leaching efficiency was used to represent the metal leaching efficiency.

The solid residues at the end of the bioleaching process were collected by centrifugation, washed three times with de-ionized water, and dried in the oven at 60 °C for 12 h. The surface of the PCB residue was investigated using a scanning electron microscope (SEM).

3. Results

3.1. Synthetic EPS on Leaching Metal Concentrates of Waste PCBs

The function of synthetic EPS on the leaching metal powders of waste PCBs by *A. ferrooxidans* can be seen in Figure 1. The variation of pH value with synthetic EPS is shown in Figure 1a. There was a big difference between bioleaching and abiotic leaching trends. At the initial leaching of 3 days, the pH of bioleaching with synthetic EPS was slightly higher than that of bioleaching without synthetic EPS. During these 3 days, the pH value of bioleaching rose quickly, while the abiotic leaching only showed a slight rise. The pH rise of abiotic leaching was mainly because the H⁺ of the leaching solution can directly react with metals and metal oxides in the metal powders of waste PCBs [29], as shown in Formulas (1) and (2).

and the pH of the bioleaching was significantly higher than that of the abiotic leaching, mainly because, in addition to the above-mentioned reactions, *A. ferrooxidans* could consume protons when turning ferrous ions into ferric ions during the bioleaching process [14], as shown in the following formula:

Figure 1. (a) pH, (b) ferrous ion concentrations, (c) total iron concentrations, and (d) copper leaching efficiencies of abiotic leaching and bioleaching with synthetic EPS.

It was clear that *A. ferrooxidans* accelerated the consumption of protons through the leaching process. After 3 days of leaching, the pH value of the bioleaching groups with or without synthetic EPS remained stable around 3.60 and 3.62, respectively. The abiotic leaching groups with or without synthetic EPS remained stable around 3.54 and 3.52 after 7 d of leaching, respectively.

Meanwhile, the concentration of ferrous ions in bioleaching with synthetic EPS decreased slightly faster than that of bioleaching without synthetic EPS, especially at the initial leaching stage, possibly due to the higher ferrous ion oxidation rate in the presence of synthetic EPS. Furthermore, it was implied that the activity level of acidophilic bacteria with synthetic EPS was higher because the rate of growth of bioleaching strains was largely proportional to the oxidation of ferrous ions [30]. The speed of bioleaching with synthetic EPS was much faster than that of bioleaching without synthetic EPS at the earlier stage, which suggests that EPS could be a prerequisite for the attachment between the surface and the cells [21].

The copper leaching efficiencies with leaching time are shown in Figure 1d. The copper leaching efficiencies of bioleaching and abiotic leaching with synthetic EPS were 92.2% and 50.8%, respectively, and the control groups of bioleaching and abiotic leaching without synthetic EPS were 80.5% and 49.8%, respectively. To better understand the input and output of copper and ferrous, their mass balances are presented in Figure S1. The reason copper leaching efficiencies of abiotic leaching with or without synthetic EPS could reach about 50% was that some ferrous ions might be oxidized by oxygen in the air to ferric ions, thereby leaching copper. The results indicate that synthetic EPS could enhance the dissolution of copper with the aid of *A. ferrooxidans*. However, synthetic EPS can only lead to a slight increase from 49.8% to 50.8% in abiotic leaching without the presence of

A. ferrooxidans. This implies that synthetic EPS could improve the activity of *A. ferrooxidans* in the bioleaching process, which may lead to more copper being extracted from the metal powder of waste PCBs.

3.2. Function of Different Synthetic Compositions of EPS

The leaching efficiencies of synthetic EPS with different compositions during the bioleaching process are shown in Figure 2. The copper leaching efficiencies of synthetic sugar, uronic acid, lipid, and protein were 83.3%, 85.7%, 93.0%, and 95.2%, respectively. The efficiency of the control group with synthetic EPS was 92.9%. Synthetic sugar and uronic acid systems have lower efficiencies than the control group. This could be a result of the effect that sugar and uronic acid can chelate ferric ions, which leads to a high concentration of ferric ions on the surface [16,22]. These EPS could easily induce the passivation layer of jarosite, shielding the surface of metal concentrates and greatly influencing the efficacy of bioleaching [31]. Thus, the copper leaching efficiency was lower than the bioleaching group without synthetic EPS. However, the fatty acid and protein exhibited higher leaching efficiencies compared to that of the synthetic EPS, because lipids and proteins are hydrophobic or amphipathic substances that contain organic functional groups that could benefit the attachment of cells to the metal powder's surface under the hydrophobic force [32]. As a result, the extraction efficiency of copper in waste PCBs was improved by adding lipids and proteins.

Figure 2. Copper leaching efficiencies of chemical components of synthetic EPS with respect to time.

In the process of leaching pyrite or waste PCBs by *A. ferrooxidans*, ferric ions were also detected in EPS [16,19]. This was mainly due to the uronic acids from EPS that can compound ferric ions. In this experiment, ferric ions were not included for the reason that ferric ions have been shown to play roles during the bioleaching process in the presence of *A. ferrooxidans* [33].

3.3. Effect of Different Concentrations of Synthetic EPS

In order to investigate the effect of concentrations of synthetic EPS through the biochemical process, 0.1 g/L, 0.5 g/L, 1.0 g/L, and 5.0 g/L were added into the leaching solution. The solution without synthetic EPS (0.0 g/L) was considered as the control. The results are exhibited in Figure 3. After 7 days of bioleaching, the copper extraction efficiencies of 0.0 g/L, 0.1 g/L, 0.5 g/L, 1.0 g/L, and 5.0 g/L synthetic EPS were 85.1%, 86.9%, 92.7%, 90.7%, and 89.1%, respectively. It was found that the copper leaching efficiencies were improved gradually with the increase of the concentration of synthetic EPS (0.0 g/L, 0.1 g/L, and 0.5 g/L). However, the bioleaching efficiencies decreased when the concentrations increased (1.0 g/L, 5.0 g/L). This implies that concentrations of synthetic EPS can largely affect the bioleaching process.

Figure 3. Copper leaching efficiencies of different concentrations of synthetic EPS with respect to time.

Because low concentration of synthetic EPS could mediate the attachment between the cell and the metal powder, the dissolution of copper was enhanced as a result. However, EPS could compound ferric ions, which could easily lead to the formation of jarosite. The higher the concentration of synthetic EPS, the more precipitate during the bioleaching process. The passivation layer on the surface of the metal powder could hinder the attack to the metal concentrates by the bacteria. As a result, the leaching efficiency will decline when the concentration of synthetic EPS reaches certain threshold.

During the bioleaching process, the major component of the passivation layer was jarosite, as found through XRD analysis in previous research [34]. The SEM image and EDS analysis of the dried precipitate are shown in Figure 4. It was shown that the precipitate consisted of circular particles with some crevasses on the surface. The EDS results indicated that the major elements were 2.77% K, 6.59% S, 19.66% Fe, and 69.39% O. In addition, 1.59% of copper was also detected in these particles.

Figure 4. SEM and EDX image of precipitation after 7 d bioleaching: (a) SEM of precipitate and (b) EDX analysis of precipitate. Bars: 5 μ m.

In order to extract copper from the precipitate, citric acid was used to dissolve the precipitate, and then copper entrapped in the precipitate could re-dissolve into the leaching solution. Hence, the bioleaching efficiency of copper was increased [29]. The addition of sodium chloride could accelerate the extraction of chalcopyrite by reducing the accumulation of elemental sulfur layers on the mineral surface, resulting in an increased concentration of copper ions from 2.37 g/L to 2.67 g/L [35]. The diluted dissolution of sodium and ammonium carbonate was added to remove the surface passivation products by breaking the jarosite structure and enhancing the extraction efficiency of copper [36].

These suggested that the passivation layer is a major factor hindering the leaching rate. Once the influence of the passivation layer is reduced, the leaching rate could be greatly improved. There are two main ways to improve the copper leaching efficiency with the passivation layer:

1. Dissolve the precipitate that covers the surface of waste PCBs or sulfide minerals, then bacteria and ferric ion can re-contact and react with copper;
2. Re-dissolve the precipitate that entrap leached copper, thus the copper is released back into the bioleaching solution.

3.4. Effect of Addition of Synthetic EPS at Different Time Points

To investigate the effects of the different addition time points during the bioleaching process, 0.5 g/L synthetic EPS was added into the bioleaching medium at the inoculation time, early stage of the log phase, or the later stage of log phase, separately. The result of the bioleaching efficiencies of copper from waste PCBs is shown in Figure 5. After the first two days, it was found that adding EPS at the beginning of inoculation showed 48.6% copper leaching efficiency, which was a little higher than that of adding EPS at the early or later stage of the log phase (46.0% and 44.2%, respectively). EPS could aggregate bacterial cells and lead to the development of high cell densities [24]. Thus, the ferrous ions oxidation rate increased and resulted in a higher extracting efficiency. Subsequently, the group of inoculation times showed a lower leaching efficiency than the log phase of adding time. However, after 7 days of bioleaching, the early stage of the log phase time points showed a maximum efficiency of 91.9%, while the later stage of the log phase and the inoculation time points had efficiencies of 88.8% and 87.7%, respectively. This suggests that adding synthetic EPS in the early stage of the log phase could benefit the leaching of copper from waste PCBs.

Figure 5. Copper leaching efficiencies of different adding times of synthetic EPS with respect to time.

4. Discussion

4.1. EPS Promote the Bioleaching Process

Based on the leaching experiments, it was clarified that the difference between acid leaching with synthetic EPS and bioleaching with synthetic EPS was apparent. The images of SEM analysis (Figure 6) showed that the metal concentrates of waste PCBs were flat prior to the leaching experiments. After 3 days of leaching, some cracks were exhibited on the surface of the metal powder from acid leaching with synthetic EPS. In contrast, the surface of the metal powder from bioleaching was not flat and corrosion pits also appeared. What is more, the precipitate from bioleaching with synthetic EPS had some spherical particles on the surface that were similar to jarosite (Figure 4), while the same particles were not found in the precipitate from bioleaching without synthetic EPS. The difference on the surface implied that synthetic EPS could contribute to the bioleaching process, resulting in an acceleration of the oxidation rate of metal concentrates.

Figure 6. SEM images of metal powder after 3 days of leaching: (a) abiotic leaching without synthetic EPS; (b) abiotic leaching with synthetic EPS; (c) bioleaching without synthetic EPS; (d) bioleaching with synthetic EPS.

It is now generally accepted that the dissolution mechanism of metals in bioleaching waste PCBs was mainly the indirect mechanism, which comprised of a non-contact and contact mechanism [37,38]. The non-contact bioleaching mechanism attributed the dissolution to the reaction between the free ferric ions generated by bacteria and the metal concentrates of waste PCBs. In the contact mechanism, EPS secreted by bacteria could complex ferric ions and made an attack on the surface of the metal powder. Thus, the extraction of metal took place at the interface between bacteria cells and the surface of metal concentrates. Therefore, Figure 6 as well as the result that bioleaching with synthetic EPS showed a higher leaching efficiency than bioleaching without synthetic EPS indicates that EPS could promote the bioleaching process of copper from metal concentrates of waste PCBs through the contact mechanism.

4.2. The Mechanism of Enhancing Bioleaching Process by EPS

In the absence of EPS, the adhesion would decrease as well as the attachment, and a positive correlation was exhibited between the adhesive force and attached cells [39]. Research has also shown that in the bioleaching process of sulfide minerals, the presence of EPS (called contact substances) was regarded as the pre-requisite for attachment [21]. Furthermore, in the early period of bioleaching, the contact mechanism played a major role in the leaching process [23]. This implies that the attachment process of bacteria to the metal surface of waste PCBs was predominantly mediated by EPS.

Due to the complex composition and structure of EPS, the process of EPS mediating the adsorption of bacteria to the surface of the solid phase material is accomplished through a combination of electrostatic attraction and hydrophobic force. According to the results of the efficiencies achieved from various synthetic EPSs with different compositions in the bioleaching experiments, the initial attachment of iron-grown cells to the metal concentrates of waste PCBs could be explained by the same mechanism. It was suggested that both electrostatic forces and hydrophobicity were the adhesion forces between the cells and metal concentrates.

4.3. Interaction between EPS and Environment

During the leaching period, heavy metals were leached out in the leaching solution and the leftover toxic substances could deteriorate the living environment of the bacteria. EPS as a protection layer could adapt to adverse environmental conditions and protect the microbial community [40,41]. Under higher concentrations of cupric ions, the production of EPS was increased [33]. It has also been reported that mere shear stress for the dissolution product of pyrite induces higher EPS production [42]. It is evident from the study that a

good amount of EPS helps maintain a favorable living environment for bacteria. In the waste PCBs, more kinds of toxic metals were present. It is possible that much more EPS were produced during the leaching process in order to protect the microorganisms under the disadvantageous conditions.

The EPS matrix plays a role not only in protecting the microbial community from toxic metals, but also in the adsorption of metal elements [40,43]. The presence of many organic functional groups in EPS, such as carboxyl, phosphoric, sulfhydryl, phenolic, and hydroxyl groups, could combine with heavy metals [44]. Based on the estimated numbers of the available carboxyl and hydroxyl groups, EPS were regarded as having a very high binding capacity and the adsorption obeyed the Langmuir or Freundlich equations [45]. The bio-sorption of the metal fraction was the sum of several physico-chemical processes: ion exchange reactions, complexation, adsorption, and precipitation [15].

Besides base metals, other metals were also detected in the waste PCBs. It has been suggested that other types of metals could also be adsorbed in the EPS because of the metal binding ability of EPS [46]. A more detailed characterization of the functional groups of EPS is needed, especially for elucidating the exact chemical mechanism of metal binding by EPS in the bioleaching process of metal concentrates of waste PCBs. What is more, further investigations are needed to figure out the function of metal-binding EPS in the bioleaching of waste PCBs.

5. Conclusions

Synthetic EPS could enhance the recovery efficiency of copper with *A. ferrooxidans*. Tweaking the chemical compositions, such as proteins in EPS, could improve the bioleaching process due to the increases in the attachment forces (e.g., electrostatic and hydrophobic force). In the presence of 0.5 g/L synthetic EPS, the highest copper bioleaching efficiency was achieved after 7 days. Adding synthetic EPS in the early stage but not in the later stage contributed to bioleaching. EPS play a vital role in the bioleaching of metal concentrates from waste PCBs, and the metal binding ability of EPS in the bioleaching metal concentrates from waste PCBs needs more investigation.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/su14052503/s1>, Figure S1: Mass balance of copper and ferrous. (a) Mass balance of copper. (b) Mass balance of ferrous.

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