Study on the Adsorption of Heavy Metal by Extracellular Polymeric Substances Extracted from *Proteiniphilum acetatigenes* PSB-W

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**Abstract**

The negatively charged coordination groups on the structure of these biomolecules, which is the main components of extracellular polymeric substances (EPS), have good adsorption efficiency for heavy metals. In this paper, EPS form *Proteiniphilum acetatigenes* PSB-W which was extracted by EDTA method, and the effects of pH, EPS dosage, adsorption time and initial metal concentration on the adsorption regularity of Cu$^{2+}$ and Pb$^{2+}$ ions on EPS structure were studied respectively, as well as the adsorption efficacy of EPS on Cu$^{2+}$ and Pb$^{2+}$ heavy metals was also studied. The results showed that the adsorption of Cu$^{2+}$ and Pb$^{2+}$ by EPS was favorable when the pH value was 6. The adsorption equilibrium time of Cu$^{2+}$ and Pb$^{2+}$ by EPS was about 50 min. When EPS concentration was 96.6 mg/L, EPS had the best adsorption effect on Cu$^{2+}$ and Pb$^{2+}$ heavy metals was also studied. The results showed that the adsorption of Cu$^{2+}$ and Pb$^{2+}$ by EPS was favorable when the pH value was 6. The adsorption equilibrium time of Cu$^{2+}$ and Pb$^{2+}$ by EPS was about 50 min. When EPS concentration was 96.6 mg/L, EPS had the best adsorption effect on Cu$^{2+}$ and Pb$^{2+}$. The adsorption efficiency of EPS to Pb$^{2+}$ was higher than that of Cu$^{2+}$. EPS extracted from *Proteiniphilum acetatigenes* PSB-W can be used in the treatment of heavy metal pollution, which has great significance in the removal of heavy metals from sewage.

**Subject Areas**

Bioengineering, Environmental Sciences, Microbiology

**Keywords**

Extracellular Polymeric Substances, Microorganism, Heavy Metals

**1. Introduction**

Heavy metal pollution has always been an environmental concern. In many wastewater treatments, the adsorption of heavy metals by microbial biofilms also
occupies a place, in which the extracellular polymer is used as the matrix to connect the surrounding microorganisms together [1] [2], forming an organized and dynamic environment with complex structure [3] [4]. The extracellular polymeric substance (EPS), which is secreted by the cells, forms an embedding matrix that serves both as a binding agent for the colony (thus directly affecting its morphology) and a shield against external attacks. EPS molecules are generally complex mixtures of proteins, carbohydrates, lipids, DNA, nucleic acids, amphiphilic polymers, etc. [5]. Previous studies have shown that EPS has a good adsorption capacity for heavy metals, because the functional groups in EPS, such as hydroxyl, carboxyl, amino and phosphorus groups, combine with heavy metal ions, thus achieving the purpose of microbial resistance to heavy metals [6]. EPS has good adsorption performance, and many functional groups on EPS can provide adsorption sites for heavy metals and various organic pollutants [7]. As EPS is a material that microorganisms play a major role in the adsorption process, it has no activity or metabolic activity, and has a high adsorption capacity for heavy metals, with good adsorption effect for heavy metal ions. Therefore, the method of absorbing heavy metal ions in water by EPS alone has attracted more and more attention.

In recent years, the study on the adsorption of extracellular polymers extracted from microorganisms on different heavy metals has become a hot spot. Studies have found that EPS has different adsorption capacity for different single heavy metals. The reason may be that different components of EPS affect its adsorption effect, among which polysaccharides and proteins in EPS have the biggest impact on the adsorption effect [8]. Although extracellular polymers are mainly generated by microorganisms, more studies have been done on extracellular polymers from activated sludge [9], whereas relatively few studies have been done on extracellular polymers extracted from pure bacteria. Although the core mechanism of metal binding and transformation using microbial explorers is the same, there are significant differences in the presence and complexity of EPS in pure bacterial culture, biofilm, biofilm and activated sludge systems, which affect the interaction between EPS and metals [10]. Therefore, the study on the adsorption of heavy metals by extracellular polymers cultured by pure bacteria should be paid more attention.

EPS itself does not have biological activity, can reduce using live bacteria for adsorption of ecological risk, can be in a certain extent, make up for the inadequacy of traditional microbial adsorption method, the EPS as a potential new biological adsorbent, heavy metals in the industrial pollution, heavy metal pollution of heavy metals in wastewater treatment, water, emergency response, etc, has the certain application value. The study of the interaction characteristics and mechanism of heavy metal ions and EPS is of great theoretical value and practical significance for the effective application of biotechnology in treating heavy metal wastewater and remediation of water and soil polluted by heavy metals. In order to better apply EPS to heavy metal pollution treatment technology, it is necessary to further understand the interaction between EPS and heavy metals.
2. Materials and Method

2.1. Materials

2.1.1. Cultivation of Strains

The activated *Proteiniphilum acetatigenes* PSB-W strain was inoculated in basic fermentation medium with 10% inoculation amount, the edge of the bottle was sealed with sealing film, the light intensity was 2000 Lux, and the seed liquid was obtained after anaerobic culture at 30°C for 5 days [11].

2.1.2. Preparation of EPS

Different extraction methods will affect the quantification of EPS and its components [12]. EDTA extraction method was adopted [13]. 50 mL bacterial culture solution was taken, centrifuged at 12,000 rpm for 10 min at 4°C, and washed with 0.9% NaCl solution twice. The collected bacterial weight was then suspended in 0.9% NaCl solution, and 10 mL of EDTA extract with a concentration of 2% was added at 4°C. The extraction time was 3 hours. After the extraction, the solution was centrifuged at 12,000 rpm for 15 min to remove the remaining cells. The temperature of extraction and centrifugation was kept at 4°C to avoid cell lysis and damage to the structure of EPS. Because EDTA can interfere with the determination of protein, the EPS solution extracted with EDTA should be dialysis for 24 hours, and finally the EPS solution obtained will be filtered through a 0.45 μm acetate membrane for use.

2.2. Experimental Methods

Standard solutions of 1 g/L were prepared with lead nitrate and copper sulfate respectively, and diluted successively by experimental design. With 0.1 mol/L NaOH solution and 0.1 mol/L HCl solution, the pH was adjusted to the required value. Two 150 mL conical bottles were added to lead solution and copper solution with a certain concentration, and a certain amount of EPS was added at the same time. The pH value was adjusted. After oscillating on a thermostatic water bath shaker at 170 r/min for a period of time, it was injected into a dialysis bag, placed in a beaker (50 mL distilled water) for 12 hours dialysis, and the concentrations of lead and copper in the beaker samples were determined.

2.3. Analysis Methods

2.3.1. Determination of Cu²⁺

The content of copper in water was determined by spectrophotometry with 2, 9-dimethyl-1, 10-phenanthroline. The quantitative sample was added to 25 mL colorimetric tube and distilled water was added to 15 mL. Then add 1 mL sulfuric acid, 1.5 ml 100 g/L hydroxylamine hydrochloride solution, 3.0 sodium citrate solution and 3.0 mL (pH = 6.8) acetic acid-sodium acetate solution, and shake well. Finally, 1.5 mL 1.0 g/L o-phenanthroline solution was added and fully mixed. After standing for 5 min, the absorbance was measured with a spectrophotometer at the wavelength of 457 nm using water as a reference.
2.3.2. Determination of Pb²⁺

The content of lead in water was determined by spectrophotometry. A certain amount of sample solution was accurately transferred into a 50 mL colorimetric tube, 0.08 mmol/L dichlorogluconol orange solution, 0.75 mmol/L O-phenylline solution and 0.04 mmol/L hexamethylenetetramine buffer solution (pH = 5.8) were successively added. The solution was shaken at a constant volume with secondary distilled water and placed at room temperature for 15 min. At the wavelength of 576 nm, a 1 cm colorimetric dish was used to measure the absorbance with water as a reference.

2.3.3. Determination of EPS

The main components of EPS protein and polysaccharide were quantitatively analyzed, and the concentration of EPS was characterized by the sum of their contents. The content of protein was determined by Folin-phenol method, and the content of polysaccharide was determined by anthrone-sulfuric acid method. After analysis, EPS concentration was 115.63 mg/L.

3. Results and Discussion

3.1. Main Conditions for the Adsorption of Heavy Metals by EPS

The optimum adsorption conditions of Cu²⁺ and Pb²⁺ on EPS were investigated by controlling pH value, reaction time and EPS dosage.

3.1.1. Effects of pH on the Adsorption of Heavy Metals by EPS

When the temperature was kept at 25°C, the added amount of EPS was 0.5 mg, the initial mass concentration of metal ions was 30 mg/L, and the adsorption time was kept unchanged for 1 hours, the pH value of the solution was adjusted to 3, 4, 5, 6, 7, 8, 9, respectively. The adsorption experiment on heavy metals by EPS was carried out. The effect of pH on the adsorption of heavy metals on EPS is shown in Figure 1.

![Figure 1. Effect of different pH on the adsorption of heavy metals by EPS.](image-url)
The pH value ranged from 3.0 to 6.0. With the increase of pH, the adsorption rate of EPS on Cu\(^{2+}\) and Pb\(^{2+}\) gradually increased, among which the change was more obvious in lead ion. When pH = 6.0, the adsorption capacity reaches the upper limit. At pH > 6.0, the adsorption rate decreases gradually and the adsorption efficiency decreases gradually. At pH > 6, with the increase of pH, the adsorption rate of EPS to Cu\(^{2+}\) and Pb\(^{2+}\) gradually decreased. The analysis shows that the adsorption of metal ions is affected by the precipitation of lead hydroxide and copper hydroxide due to the combination of hydroxide ions in the solution with lead ion and copper ion. Therefore, the adsorption rate decreases significantly at pH > 7. When the pH value is between 5.0 and 7.0, the adsorption effect of metal ions is significant.

3.1.2. The Influence of Adsorption Time on the Adsorption of Heavy Metals by EPS

The initial mass concentration of metal ions was 30 mg/L. The adsorption time was respectively 20 min, 30 min, 40 min, 50 min, 60 min and 70 min. The experiment of heavy metal adsorption was carried out. The effect of adsorption time on the adsorption of heavy metals by EPS is shown in Figure 2.

The adsorption of Cu\(^{2+}\) and Pb\(^{2+}\) by EPS was similar with time. The whole adsorption process can be roughly divided into three stages: fast adsorption - slow adsorption - adsorption equilibrium. EPS had obvious adsorption of Cu\(^{2+}\) and Pb\(^{2+}\). At the time of 50 min, EPS reached adsorption equilibrium for Cu\(^{2+}\) and Pb\(^{2+}\), and the adsorption rates were 26.68% and 37.59% respectively. According to the analysis, the adsorption of Cu\(^{2+}\) and Pb\(^{2+}\) by extracellular polymer is mainly through complexation, chelation and ion exchange, etc. Functional groups combine with heavy metals in this way, which does not require a lot of energy and the reaction goes on very fast. In addition, the adsorption equilibrium can be reached quickly.

The rapid adsorption stage may be attributed to the fact that EPS, as an inactive body, has abundant binding sites on it and adsorbed through complexation, chelation and ion exchange, etc. This binding mode is basically energy-free, so the reaction proceeds very quickly [8]. After entering the slow adsorption stage, the adsorption site is gradually occupied by metal ions, and the remaining metal ions become less and less, so the adsorption becomes inefficient. In order to reach the maximum adsorption capacity in a relatively short time, the reaction time of the adsorption test was set as 50 min.

3.1.3. Effect of the Added Amount of EPS on Its Adsorption of Heavy Metals

Take 25 mL metal solution with a concentration of 30 mg/L, keep the pH at 6, the temperature at 25°C, and the adsorption time at 1 h unchanged. Add it according to the EPS dosage of 0.2 mg, 0.3 mg, 0.4 mg, 0.5 mg, 0.6 mg, 0.7 mg, respectively, and do the heavy metal adsorption experiment. The effect of EPS on the adsorption of heavy metals is shown in Figure 3.
When the EPS dosage was 0.2 mg - 0.5 mg, the adsorption rate of Cu$^{2+}$ and Pb$^{2+}$ increased significantly. When the EPS dosage was increased from 0.2 mg to 0.5 mg, the Cu$^{2+}$ and Pb$^{2+}$ increased from 15.35% and 20.87% to 26.21% and 37.13%, respectively. When the added amount of EPS was higher than 0.5 mg, the adsorption efficiency of EPS to metal ions gradually tended to be stable. It is speculated that the reason may be that, when EPS concentration is high, some adsorption sites are embedded because the polymer chain structure cannot be fully developed in water, which does not play an adsorption role. In addition, the number of unadsorbed metal ions in the solution is small, and the reaction impetus is reduced. After the EPS dosage reached 0.5 mg, the adsorption rate of EPS continued to level off without significant improvement. Therefore, the EPS dosage was selected as 0.5 mg in the subsequent adsorption test.

3.2. Studies on the Adsorption Efficiency of EPS on Heavy Metals

In the holding temperature of 25˚C, pH 6, Cu$^{2+}$ and Pb$^{2+}$ initial mass concentration of 15 mg/L, 25 mg/L, 35 mg/L, 45 mg/L, 55 mg/L metal solutions were put into 0.5 mg EPS, heavy metal adsorption experiments were carried out, and the adsorption efficiency of different concentration of metal ions with time (20 min,
30 min, 40 min, 50 min, 60 min). The adsorption efficiency of EPS on Cu\textsuperscript{2+} is shown in Figure 4, and the adsorption efficiency of EPS on Pb\textsuperscript{2+} is shown in Figure 5.

With the increase of metal ion concentration, the adsorption rate of EPS to copper ion and lead ion also increases gradually, and the adsorption state is about the same. Among them, when the concentration of copper ion and lead ion is less than 30 mg/L, the adsorption state of EPS metal ion is always in the fast adsorption state. When the concentration of copper and lead ions was 30 - 40 mg/L, the metal ions adsorbed by EPS showed a slow adsorption state. When the time was 50 min and the concentration of copper ion and lead ion was 50 - 60 mg/L, EPS reached adsorption equilibrium for Cu\textsuperscript{2+} and Pb\textsuperscript{2+}, the adsorption rates were 30.62% and 41.06%, respectively. With the increase of metal ion concentration, the adsorption rate of EPS to copper ion and lead ion increased from 19.56% and 25.47% to 30.62% and 41.06% respectively, and the adsorption rate of EPS to Pb\textsuperscript{2+} was higher than that to Cu\textsuperscript{2+}. Due to the different types and quantities of functional groups on EPS, the binding ability of EPS to different heavy metals is different.

![Figure 4. Studies on the adsorption effect of EPS on Cu\textsuperscript{2+}.](image)

![Figure 5. Studies on the adsorption effect of EPS on Pb\textsuperscript{2+}.](image)
4. Conclusions

1) The adsorption pH value of Cu$^{2+}$ and Pb$^{2+}$ by *Proteiniphilum acetatigenes* PSB-W extracellular polymer extracted by EDTA method is 6; the equilibrium time is about 50 min. When the added amount of EPS was 0.5 mg, EPS had the best adsorption effect on Cu$^{2+}$ and Pb$^{2+}$, which could make EPS more effective in the adsorption of heavy metals.

2) When the concentration of copper ions and lead ions was less than 30 mg/L, EPS was always in the rapid adsorption stage, higher than 30 mg/L, and the adsorption efficiency of EPS gradually tended to balance.

3) The adsorption capacity of EPS on Cu$^{2+}$ and Pb$^{2+}$ is different. In terms of the adsorption of single metal by EPS, the adsorption rate of EPS on Pb$^{2+}$ is higher than that on Cu$^{2+}$, which may be caused by the coordination or covalent binding of different chemical groups with heavy metals, resulting in the difference of the adsorption of EPS on different metals.

Acknowledgements

This work was financially supported by the Natural Science Foundation Project of Shanxi Province (201801D121206) and the Natural Science Foundation of Modern College of Humanities and Sciences of Shanxi Normal University (2018JCYJC01).

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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