Utilization of extracellular polymeric substances (EPS) immobilized in epoxy polymer as double ion exchanger biosorbent for removal of chromium from aqueous solution

Lilis Kistriyani\textsuperscript{a}, Zainus Salimin\textsuperscript{a}, Achmad Chafidz\textsuperscript{a,b,*}

\textsuperscript{a}Chemical Engineering Department, Universitas Islam Indonesia, Yogyakarta 55584, Indonesia
\textsuperscript{b}Center for Material Science and Technology Studies, Chemical Engineering Department, Universitas Islam Indonesia, Yogyakarta 55584, Indonesia

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Abstract
Extracellular Polymeric Substance (EPS) can be used as a biosorbent and ion exchange bioresin replacing organic resins. It is because EPS contains organic functional groups i.e. cation and anion absorbers. Additionally, epoxy resin also has a sorption capability for chromium ions. In this study, the EPS was immobilized in epoxy polymer to improve usability of the biosorbent. The objective of this research is to study the utilization of EPS immobilized in epoxy polymer as a double ion exchanger biosorbent to remove chromium ions from aqueous solution. The EPS was extracted from activated sludge bacterial by centrifugation at 9000 rpm for 20 minutes at 4°C. The functional group analysis results with Fourier Transform Infra-Red (FT-IR) Spectroscopy showed that the EPS containing chemical bonds such as –CH, -OH, -NH, and -C=O which proved that EPS were exchanging cations and anions. Additionally, the epoxy polymer was prepared by mixing bisphenol A monomers and epichlorhydrin at weight ratio of 1:1. The Immobilized EPS double ion exchange bioresin in epoxy polymers was prepared by mixing 200 mg EPS and 1800 mg epoxy at pH 5, 6 and 7. The analysis results showed that the optimum result gave the chromium ion sorption efficiency of 89.20% at pH 5.

Keywords: Extracellular polymeric substance (EPS), activated sludge bacteria, epoxy polymer, biosorbent, chromium

1. Introduction
Several industries (such as electroplating, textile, metallurgy, mining, battery, or ceramic) release wastewater which contains heavy metals into environment, mostly aquatic ecosystem [1]. These heavy metals are difficult to be degraded and tend to accumulate and this is why they are considered as the persistent environmental pollutant [2]. They are also harmful to humans and animals due to their toxicity. Therefore, the presence of these heavy metals in the environment becomes a great concern of researchers in the world [3]. Heavy metals, such as chromium - if not treated using adequate treatment - might threat human health and environment. Cr(IV) is more toxic than Cr(III) due to its mobility [4].

There are many different methods for the removal of heavy metal ions from the aqueous solution, such as adsorption, evaporation, chemical precipitation, electrochemical treatment, ion exchange, and membrane technologies [5]. However, these methods disadvantageously are high energy consuming, needs high amount of reagent, generates floc residues as secondary pollution, and has low removal efficiency when the concentration of heavy metal pollutants in the range of 1-100 mg/L [6]. Biosorption has been considered as a promising alternative method for the removal heavy metal pollutants due to its effectiveness and eco-friendly [7]. One type of biosorbent that has attracted interest among researchers is Extracellular polymeric substances (EPS), resulted from microorganisms, i.e. living and dead bacteria cells. The EPS contain a great amount of negatively charged functional groups, such as carboxyl (-COOH), or phosphate (-PO\textsubscript{4}H\textsubscript{3}), sulphate (-SO\textsubscript{3}H) and the positively charged functional groups such as hydroxyl (-OH), and amino carboxylic (R-C(NH\textsubscript{2})H-COOH); hence, they can interact/react with heavy metals ions as ligands [6, 8, 9]. Based on the literatures, EPS has a significant potential for the removal of heavy metals from aqueous solution [6, 7, 10].

The EPS mainly consists of polysaccharides (40-95% of total EPS), protein (1-60%), nucleic acid (1-10%), lipids (1-10%), and other compounds like amino acid polymer and bacteria [9,11]. The EPS can be used as biosorbent that is capable of binding the cations and anions of environmental pollutant, e.g. heavy metals by the mechanism of ion exchange, complex formation, hydrogen bonding and absorption. The cations binding by EPS is done by the

\* Corresponding author.
Email: achmad.chafidz@ui.ac.id
presence of functional groups, such as carboxyl, phosphate, and sulphate as the following reaction [9,12]:

\[
\text{n R-COOH} + \text{M}^{m+} \rightarrow (\text{RCOO})_n \text{M} + \text{n H}^+ \\
\text{n R-OPO}_3\text{H} + \text{M}^{m+} \rightarrow (\text{R-OPO}_3)_n \text{M} + \text{n H}^+ \\
\text{n R-OSO}_3\text{H} + \text{M}^{m+} \rightarrow (\text{R-OSO}_3)_n \text{M} + \text{n H}^+ 
\]

(Mn⁺) is the positively charged cation on the EPS that can be driven out by another cation with the greater selectivity. The greater selectivity is the preference for ion by the ion exchanger of functional group. An ion exchanger tends to prefer [13]:

1. Ions with higher valence
2. Ions with smaller solvated volume
3. Ions with greater ability for polarization
4. Ions with stronger reaction with the ion exchange sites of the ion exchanger solid, and
5. Ions with least participation with other ions to form complexes

For the usual cation exchanger, the preference series for the most common cations are as follows [13,14]:

\[
\text{Ba}^{2+} > \text{Pb}^{2+} > \text{Sr}^{2+} > \text{Ca}^{2+} > \text{Ni}^{2+} > \text{Cd}^{2+} > \text{Cu}^{2+} > \text{Co}^{2+} > \text{Zn}^{2+} > \text{Mg}^{2+} > \text{Ag}^{+} > \text{Cu}^{+} > \text{K}^{+} > \text{NH}_4^+ > \text{Na}^{+} > \text{H}^+ \text{and } \text{UO}_2^{2+} > \text{Cu}^{3+} > \text{Co}^{3+} 
\]

(4)

The anions binding of anion by EPS is performed by the functional groups of hydroxyl and amino carboxylic [9,12]:

\[
\text{R-OH} + \text{CrO}_4^{2-} \rightarrow \text{R}_2\text{CrO}_4 + 2\text{OH}^- \\
\text{R-CH-COOH} \rightarrow \text{R-CH-COO}^- \\
\text{NH}_2\text{NH}_3^+ + \text{NO}_3^- \rightarrow \text{R-CH-COO}^- \\
\text{NH}_2\text{NH}_3^+ 
\]

(5) (6) (7) (8)

The functional groups of amino carboxylic also can bind the cation as shown in Equation 6. Whereas, for the anion exchanger, the following are preference sequences for the most common anions [13,14]:

\[
\text{SO}_4^{2-} > \text{I}^- > \text{NO}_3^- > \text{Br}^- > \text{Cl}^- > \text{OH}^- 
\]

(9)

The functional groups of amino carboxylic, hydroxyl, sulphate and phosphate also can bind the metal ion by complex formation. The complexion between organic ligands and metal ion may occur.

Several literatures have reported the utilization of EPS for the removal of heavy metals from wastewater. Nouha, et al. [15] studied about the heavy metals removal from industrial effluent using EPS produced by Cloacibacterium normanense in activated sludge with glycerol as the supplement. They also focused on EPS extraction by using different methods (i.e. centrifugation, heating, and EDTA). They reported that EPS production by Cloacibacterium normanense was influenced by different glycerol loadings in the medium. It was found that the EPS concentration within 72 hours of fermentation increased from 13 g/L to 21.3 ± 0.7 g/L when added with glycerol 2% (w/v). The optimal removal efficiency of Ni from (primary treated) wastewater was found at 80% when using 35 mg/L of EPS extracted by centrifugation method. Wang, et al. [16] studied about the removal of Pb(II) and Zn(II) from aqueous solution via biosorption using EPS of Rhizobium radiobacter. They found that the optimum pH for biosorption process included pH 5 for Pb(II) removal and pH 6 for Zn(II) removal, respectively. The kinetic analysis results showed that the biosorption followed pseudo 1st order and Langmuir model. The adsorbed heavy metals could be desorbed effectively using HCl. The desorbed EPS could still reach 80% of initial biosorption capacity after being reused five times. These results showed that EPS had potential application for removal Pb(II) and Zn(II).

Additionally, there have also been several literatures that particularly reported about the use of EPS for the removal Chromium. Long, et al. [4] reported the use of EPS produced from Pseudochrobactrum saccharolyticum for the reduction of Cr(IV). They reported that the optimum reduction of Cr(IV) by using EPS was about 81.5%. Chug, et al. [17] studied about the effect of growth phase, temperature, and pH on the production of EPS by Azotobacter beijreinkii and Bacillus subtilis and their performances to remove Cr(IV) from aqueous solution. The test results found that using Azotobacter beijreinkii could produce optimum EPS after 24 hours at 30 °C and pH 7, while Bacillus subtilis could produce optimum EPS after 96 hours at 37 °C and pH 7. Furthermore, from the biosorption tests, they revealed that from initial Cr(IV) concentration of 10 ppm, the optimum reduction of Cr(IV) by using EPS were 26 % and 48 % for EPS produced by Azotobacter beijreinkii and Bacillus subtilis, respectively. There have been many other literatures that studied the utilization of EPS for removing chromium from aqueous solution [11,17-19].

It is well known that activated sludge is one of the most widely used processes in treating wastewater. This method has disadvantage of great amount production of activated sludge waste, which is also considered as pollution. To decrease such pollution, it needs to reduce the volume and mass of activated sludge. The activated sludge taken from wastewater treatment contains millions of bacterial colonies. Based on the literatures, EPS can be extracted from activated sludge [9, 15, 20]. Hence, the use of EPS has two purposes or advantages: to remove heavy metals and to utilize activated sludge, which may reduce the mass and volume of the sludge [9].

The EPS can be extracted from activated sludge using centrifugation or solvent extraction method. The EPS can be directly dispersed or immobilized on the polymer matrix as solid biosorbent, which can be applied in column operation process. The immobilized EPS on the polymer matrix has more advantages than the dispersion operation. For instance, the immobilized EPS can be employed in the packed-tower or fluidized-bed reactor. Here, the higher the loading capacity of biomass on the column, the easier the regeneration of saturated biomass [21].

Epoxy resin is the mixture of the monomers of bisphenol A and epichlorohydin having the chemical formula as shown in Figure 1. Epoxy resin has good mechanical properties and
chemical resistance. The hardener of resin functions as the catalytic of polymerization reaction between the monomers. Additionally, epoxy resin has a sorption capability for chromium ions [22].

![Chemical structure of bisphenol A, epichlorhydrin and epoxy resin](image)

The relation between mass ratio of resin and hardener to epoxy polymer qualities has been studied in our previous work [23], as shown in Table 1 in which the weight ratio of resin and hardener that gave the best quality of epoxy was the ratio of 1:1, giving the compressive strength of epoxy of 37.5 kN/Cm².

Table 1. Relation between mass ratio of resin and hardener to epoxy polymer quality [23]

| Weight ratio % | Resin ratio/Hardener | Quality of polymerization result |
|---------------|----------------------|---------------------------------|
| Resin Hardener | Density (gr/cm³) | Compressive strength (kN/cm²) |
| 25 75         | 0.33                 | -                               |
| 30 70         | 0.43                 | 1.02                            | 35 |
| 40 60         | 0.67                 | 1.04                            | 36 |
| 50 50         | 1.00                 | 1.08                            | 37.5 |
| 60 40         | 1.50                 | 1.08                            | 36.5 |
| 70 30         | 2.33                 | 1.07                            | 36.3 |

2. Materials and Methods

2.1. Material

Materials for the experiment included industrial activated sludge, Whatman 41 filter paper big size pore, sodium hydroxide, chromium (III) nitrate nanohydrate [Cr(NO₃)₃.9H₂O], and potassium chromate [K₂CrO₄].

2.2. Equipment

The equipment used on the experiment included pH meter, centrifuge, atomic absorption spectrophotometer (AAS), and Fourier Transform Infra Red (FTIR) Spectroscopy.

2.3. Method

2.3.1. Preparation of Artificial Liquid Waste

The artificial liquid waste was prepared similar to tannery industrial waste containing total chromium of 15.9 ppm (trivalent of chromium 15.2 ppm and hexavalent of chromium 0.7 ppm). It has been done by dissolving 0.1368 g of chromium nitrate nanohydrate and 0.003g of potassium chromate on 1 liter of water.

2.3.2. Extraction of EPS from Activated Sludge

Activated sludge from tannery industrial wastewater treatment was filtered using filter paper with big pore size. Afterward, the cake was washed using distilled water for cleaning its impurities. The clean cake was suspended using distilled water with 5 times volume of the cake. The suspension was then heated at 80 °C for 10 minutes and then cooled down until reaching the ambient temperature. The suspension was then centrifuged at 6,000 rpm and 4 °C for 20 minutes. Afterward, the resulted water supernatant was filtered to clean the remaining impurities and the filtered supernatant was the EPS. The EPS solution was then stored in the refrigerator. The EPS was also analyzed to evaluate the contents of protein, polysaccharides, and lipid. The solution of EPS was then extracted again by adding ethanol 96% in the solution until the ethanol concentration achieving 70% on the temperature of 4 °C. The EPS precipitate was then separated using centrifuge at 5000 rpm for 15 minutes. After precipitation process, the EPS was analyzed by FTIR (Fourier Transform Infra-Red) to determine the presence of functional groups such as hydroxyl, carboxyl, and amino carboxylic.

2.3.3. Formation of Epoxy Polymer

The epoxy polymer was prepared by mixing the epoxy resin of 1700 mg with hardener on its ratio of 1:1. Its homogeneous mixture was molded. The dry mixture was then crushed using rocklabs and sieved using a sieve shaker to obtain the particle size of 30-40 mesh.

2.3.4. Formation of Immobilized EPS Epoxy Polymer Biosorbent

The result of EPS from the extraction of activated sludge on the quantity 300 mg was mixed with 1700 mg of epoxy resin and hardener having ratio of 1:1. Its homogenous mixture was molded. The dry mixture was then crushed using rocklabs and sieved using a sieve shaker to obtain the particle size of 30-40 mesh.

2.3.5. Chromium Sorption Capability of Epoxy Resin

The epoxy polymer of 1700 mg with a particle size of 30-40 mesh was submerged and mixed with the prepared artificial liquid waste containing 15.9 ppm of chromium for 5 hours. After the solution was homogenized, it was analyzed to determine its chromium content by using Atomic Absorption Spectrophotometer (AAS).

2.3.6. Chromium Sorption Capability of Immobilized EPS-Epoxy Resin Biosorbent

The immobilized EPS – epoxy polymer biosorbent of 2000 mg with a particle size of 30-40 mesh was mixed homogeneously in the prepared artificial liquid waste containing 15.9 ppm of chromium on pH 5 for 5 hours. After the test, the solution was analyzed to determine its chromium content by using AAS. This procedure was repeated for pH solution of 6 and 7.
3. Results and Discussion

The solution of EPS extracted from activated sludge had the dry weight of 2.96 g/L, so the weight of 300 mg was equivalent to EPS solution volume of 101.35 mL. To ensure that the resulted sludge supernatant from the extraction of activated sludge was the EPS, it was necessary to determine the contents of polysaccharides (carbohydrate), protein and lipids (fat) on the EPS. The analysis result of the contents of carbohydrate, protein and fat were 60%, 77% and 11% respectively, as shown in Table 2.

Table 2. Analysis Result of EPS Composition

| Analyzed Component | Analysis Result (%) | Reference EPS percentage [9] (%) | Analysis Method |
|--------------------|---------------------|----------------------------------|----------------|
| Polysaccharides    | 40-95               | 50-55                            | Gravimetry     |
| Protein            | 1-60                |                                   | Luff school    |
| Lipid              | 1-10                |                                   | Kjeldahl       |

The example of polysaccharides are chitin, cellulose, starch, carbohydrate, and glycogen. If the polysaccharides content is assumed 5 times of the carbohydrate content (i.e. 12%), so the percent of polysaccharide is 60% of total EPS, which is according to the reference value (i.e. 40-95%). The protein percentage in the EPS is 77%, which is according to the reference value (i.e. 1-60%). The lipid percentage in the EPS is 11%, which is according to the reference value (i.e. 1-10%). Overall, the values are in accordance with the reference of the EPS in activated sludge.

The analysis result of EPS functional group by FTIR Spectroscopy is presented in Fig. 2. As seen in Fig. 2, the prepared EPS contained the chemical bound of CH on medium-strong intensities of 1300-1500 cm⁻¹; and weak intensities of 600 – 900 cm⁻¹, OH on medium-strong intensities of 1200-1500 cm⁻¹, NH on medium-strong intensities of 1500 – 1700 cm⁻¹; and medium intensities of 700 – 900 cm⁻¹ and C=O on the medium intensities of 1600-1900 cm⁻¹. According to the existing bound, it could be indicated that the EPS contained functional groups of carboxyl, hydroxyl and amino carboxylic. This indicated that the EPS could act as the cation and anion exchanger for the removal of chromium waste.

Table 3 presents the data of chromium sorption capabilities of biosorbent. Based on Table 3, it was found that different values of pH gave the different values of chromium sorption capability of the biosorbent. The pH 5 gave the best result for chromium sorption capabilities of biosorbent, on the pH 5, there was the excess of ion H⁺ on the solution.

Table 3. Data of Chromium Sorption Capabilities of Biosorbent

- **Types of biosorbent**
  - Epoxy Resin
  - EPS
  - EPS
  - EPS
  - EPS

- **Mass of Biosorbent (g)**
  - 1.70
  - 2.00
  - 2.00
  - 2.00

- **pH**
  - 2
  - 5
  - 6
  - 7

- **Vol. (L)**
  - 15.9
  - 15.9
  - 15.9
  - 15.9

- **Chromium Concentration (mg/L)**
  - Initial
  - Final
  - Elimination

- **Efficiency of Elimination**
  - 8.80
  - 89.20
  - 50.11
  - 36.50

Additionally, based on the FTIR analysis results, the biosorbent contained the functional groups of carboxyl, hydroxyl and amino carboxylic. The trivalent chromium on the presence of ion H⁺ had the positive catalytic condition for strengthening the expulsion of ion H⁺ on the biosorbent to be replaced by the trivalent chromium. At pH 5, the remaining concentration of chromium had the smallest value of 1.718 mg/L. In other words, this condition resulted in the biggest efficiency of chromium elimination of 89.20%.

4. Conclusion

The EPS extracted from activated sludge of industrial waste treatment contained polysaccharides 60%, protein 77% and lipids 11%. Based on the FTIR analysis results, it was noted that the EPS contained the functional groups of carboxyl, hydroxyl, and amino carboxylic. Hence, the EPS could act as cation and anion exchanger. Additionally, the EPS was immobilized on the epoxy polymer matrix to act as double ion exchangers for removing chromium from the liquid waste. The epoxy resin had the capability of chromium sorption due to its content of hydroxyl. The selectivity ion of Cr₂O₇⁻² was greater than selectivity of ion OH⁻, so the ion OH⁻ on the epoxy resin was driven out and replaced by ion Cr₂O₇⁻², which resulted in the decrease of chromium concentration on the solution from 15.9 mg/L to 14.5 mg/L. The pH of the solution affected the chromium sorption capability of EPS immobilized in epoxy resin biosorbent. The pH 5 gave the

Fig 2. The analysis result of EPS by spectroscopy of FTIR

Based on the literatures review, epoxy resin and EPS have the capability of Chromium sorption. As shown in Fig. 1,
best result for chromium sorption capabilities of EPS-Epoxy Resin Biosorbent. At pH 5, the remaining concentration of chromium had the smallest value of 1.718 mg/L. This means that this condition gave the highest efficiency of chromium elimination of 89.20%.

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