

Copper (II) Removal from Industrial Effluent Using a Coagulation-Flocculation Process Employing Rhizobium Extracellular Polysaccharide

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Abstract

The ability of *Rhizobium sp.* extracellular polysaccharide (EPS) – polyethyleneimine (PEI) flocculant system to sequester cupric ions from a semiconductor company effluent was studied. Flocs characteristics and Cu²⁺ removal efficiencies were found to be influenced by the sequence of polymer addition, pH, and EPS/PEI mass ratio. Superior flocs characteristics and Cu²⁺ removal efficiencies occur when addition of EPS to the wastewater preceded addition of PEI. Cupric ion sequestration by the EPS-PEI flocculant system was most significant at pH 4-6. The best and workable EPS/PEI mass ratios were found to be 26.67 and 25-30.77, respectively. At these ratios, firm floc formations allowed sludge separation to be achieved by mere decanting of the supernatant. The EPS-PEI flocculant system was found capable of reducing the Cu²⁺ concentration of the industrial wastewater to regulatory standards. Its maximum capacity was found to be about 28 mg Cu/ g EPS-PEI. Essentially complete Cu²⁺ removal was achieved.

Keywords: Coagulation, flocculation, heavy metals, industrial effluent, extracellular polysaccharide, *Rhizobium*

Introduction

Among the various environmental problems today, heavy metal pollution is considered to be one of the most life-threatening and needful of serious attention. Heavy metals are dangerous because they are highly toxic at low concentrations (Kowalczyk, Hubicki, & Kolodynska, 2013), and are not amenable to natural degradation (Karnib, Kabbani, Holai, & Olama, 2014). Heavy metals persist in the environment and are simply redistributed in various forms and through various media. They are highly soluble in water and, if ingested beyond allowable concentrations,

can cause debilitating health disorders and diseases (Kurniawan, Chan, Lo, & Babel, 2006).

Heavy metals that are being strictly regulated in industrial wastewaters include zinc, copper, nickel, mercury, cadmium, lead, and chromium (Fenglian & Wang, 2011). The typical effluent standard in mg/L for some of these heavy metals is 0.5 for hexavalent chromium, 1.0 for trivalent chromium, 0.5 for copper, 0.1 for lead, 1.0 for zinc, and 0.5 for cadmium (Lund, 1971).

Implementation of regulatory standards for heavy metals in industrial effluents is necessitated by the fact that industrial effluents are usually discharged into bodies of water

that have important ecological and economic functions. One such body of water is the Laguna Lake which is surrounded by industrial hubs that house, among others, semiconductor processing and electroplating plants. The Laguna Lake also serves as fish farm and a source of irrigation to many nearby rice fields. In such scenario, heavy metals discharged into the lake can easily get their way into the food chain.

For the removal and recovery of heavy metals, several traditional and advanced technologies may be employed such as chemical precipitation, coagulation-flocculation, ion exchange, adsorption, reverse osmosis, membrane filtration, flotation, ultrafiltration, and electrochemical treatment techniques (Kurniawan et al., 2006). Among these technologies, those involving precipitation processes are most widely used since the procedures and equipment involved are simple and inexpensive. One such process is chemical precipitation with lime to form metal hydroxides. This process, however, cannot always provide adequate metals removal to meet regulatory standards and usually produces high volumes of low density toxic sludge which present dewatering and disposal problems (Fenglian & Wang, 2011). Also, chemical precipitation is often inhibited by the presence of complexing agents and other dissolved chemical contaminants that are present in industrial effluents.

While the more advanced methods such as ion exchange and reverse osmosis can be tapped, their use for large-scale wastewater treatment may not be economically viable. This gives good reason to continue use of precipitation/flocculation methods and to search for effective metal flocculants other than caustics.

Some studies have shown the potential of bacterial extracellular polysaccharide (EPS) for the removal metal ions in water. In their work using EPS-producing cyanobacteria, De Philippis & Micheletti (2009) report that the overall negative charge of cyanobacterial EPS aid in sequestering metal cations that are essential for cell growth and/or prevent direct contact between the cells and toxic heavy metals dispersed in the environment. Other bacteria exhibiting biosorption of toxic heavy metals by virtue of EPS include *Enterobacter* and *Pseudomonas* species (Pal & Paul, 2008). The application of EPS in heavy metal removal is

commonly accomplished through film formation in biofilm reactors (Singha, 2012), while a few researchers have employed EPS in cell-free mode such as EPS extracted from *Rhizomonas* sp. which was converted to powder form and used as bioflocculant material (More, Yadav, Yan, Tyagi, & Surampalli, 2014).

In this study, a coagulation-flocculation technique was employed using extracellular polysaccharide (EPS) from a locally isolated *Rhizobium* species to sequester dissolved copper from an industrial effluent. *Rhizobium* had previously been determined to be capable of tolerating high concentrations of Pb and Hg due to its heavy production of mucilaginous exopolysaccharides (Mamaril, Capuno, Trinidad, & Lales, 1990). EPS is a complex sugar polymer containing the organic functional groups $-\text{OH}$, $-\text{NH}_2$, $-\text{NH}$, $-\text{CONH}_2$ and RCOO^- which are responsible for the negative charge of the polymer (Mamaril, Paner, Capuno, & Trinidad, 1989). Because EPS is slightly water soluble and forms small and gel-like slow-settling flocs, a secondary polyelectrolyte, polyethyleneimine (PEI), was added to increase floc size and to facilitate the separation of the EPS- Cu^{2+} complex from the wastewater. PEI was used as secondary polyelectrolyte due to its high charge density which means that only a small amount of PEI will be enough to neutralize the EPS- Cu^{2+} complex. While it is highly possible that other metal ions present in the actual wastewater may also be removed during flocculation, sequestration of Cu^{2+} was given focus in this research. The industrial effluent to which the coagulation-flocculation technique was applied was obtained from a semiconductor processing plant in Laguna, Philippines.

Materials and Methods

Reagents

Polyethyleneimine $(-\text{NHCH}_2\text{CH}_2-)_x[-\text{N}(\text{CH}_2\text{CH}_2\text{NH}_2)\text{CH}_2\text{CH}_2-]_y$ (MW 70,000) was purchased from Wako Pure Chemicals Co., Japan. NaOH pellets and concentrated HCl were purchased from Univar. All reagents were analytical grade.

Equipment

Equipment used in the experiments were: Magnetic Stirrer, Analytical Balance (model: AT261 Delta Range) d: ± 0.01 mg, Orion EA 940 pH meter, laboratory mill, and Perkin-Elmer AA Analyst 100 Spectrophotometer.

Extraction of EPS from Rhizobium sp.

Bacterial exopolysaccharide was extracted from fresh *Rhizobium* (1469 BJVr 12) fermentation product cultivated at the Environmental Biotechnology Laboratory at BIOTECH, UPLB. Three liters of 95% ethanol were added to 1 L of *Rhizobium* fermentation product. This required slow, gentle mixing and leaving the mixture to stand for at least 30 minutes to allow for complete lysis of cells. Separation of the polysaccharide from the supernatant liquid was accomplished by filtering with cheesecloth. The filtered material was dried at 70°C, cooled to room temperature, then ground to fine particles using a laboratory mill. EPS mixtures for the whole experiment were prepared from a single batch of powdered EPS.

Preparation of 100X diluted semiconductor company wastewater

The semiconductor company wastewater was analyzed of its initial Cu^{2+} composition by atomic absorption spectrophotometry (AAS). It was found to contain about 2825 mg/L of Cu^{2+} at pH 1.23. To approximate the Cu^{2+} concentration of electroplating rinse water, the wastewater was diluted 100 times using triple distilled water. The pH of the diluted wastewater was 3.01.

Determination of the effective polymer addition sequence

Two separate batches of flocculation experiments were conducted to determine the effective polymer addition sequence to be used in succeeding experimental runs. In the first batch, 30 mL samples of 100X diluted wastewater (pH 5) were treated first with 0.5 mL 2% EPS, then with increasing volumes of 0.5% PEI. In the second batch, the same volume combinations of EPS and PEI were used except that PEI was added

into the wastewater prior to adding EPS. These experimental runs were done in two trials.

In terms of Cu^{2+} removal and flocs characteristics, the first batch, i.e. EPS added before adding PEI, yielded superior results in terms of floc size and copper removal. This sequence of polymer addition was therefore used in all succeeding runs.

Evaluation of Cu^{2+} removal efficiency and flocs characteristics at different pHs

Six samples of 30 mL 100X diluted wastewater were adjusted to pH 2-7, one sample corresponding to one pH, using 1.0 M NaOH and/or 1.0 M HCl. Each sample was treated with 1.0 mL 2% EPS and 0.16 mL 0.5% PEI. Treated samples were filtered and their residual Cu^{2+} concentration analyzed by AAS. Qualitative evaluation of flocs and filtrate characteristics was performed. All runs were done in two trials.

Determination of the best and workable EPS/PEI mass ratios at pH 5

Thirty mL samples of 100X diluted wastewater (pH 5) were treated with 0.5 mL of 2% EPS and increasing amounts of 0.5% PEI. The pHs of the samples were checked and found to be essentially the same after treatment, hence, readjustment to a common pH was no longer necessary. Treated samples were filtered and analyzed of their residual Cu^{2+} concentration by AAS. Flocs and filtrate characteristics were evaluated qualitatively. These experimental runs were done in two trials.

Evaluation of the scavenging of Cu^{2+} by the EPS-PEI flocculant system at pH 5 and an EPS/PEI mass ratio of 25

To evaluate the scavenging ability of the EPS-PEI flocculant system, 30 mL samples of 100X diluted wastewater (pH 5) were treated with increasing amounts of the flocculant system while maintaining an EPS/PEI mass ratio of 25. The pHs of treated samples were readjusted to pH 5 using 1.0 M HCl and 1.0 M NaOH. Treated samples were filtered and analyzed of their residual Cu^{2+} concentration by AAS. These experimental runs were done in two trials.

Analytical methods

Cu^{2+} concentrations were determined by AAS. Samples were acidified to contain 1.16 N HCl prior to AAS analysis.

Results and Discussion

Determining the effective polymer addition sequence

A comparison of Cu^{2+} removal efficiencies between the two modes of polymer addition is shown in Figure 1. Results show that superior flocs characteristics and Cu^{2+} removal efficiencies are achieved when EPS is added to the wastewater prior to adding PEI. Flocs resulting from this sequence are bigger and easier to separate from the water. On the other hand, a reversed sequence of adding the polymers, i.e. PEI is added first followed by EPS, resulted to the formation of small, scattered flocs that are difficult to separate from the water. This polymer addition sequence also results to a bluish supernatant indicating that soluble PEI- Cu^{2+} complexes are retained in solution. This is probably due to the strong Cu^{2+} -chelating property of PEI which prevents the binding of EPS with the PEI- Cu^{2+} complex because of the tendency of PEI to fill all binding sites in the Cu^{2+} coordination sphere. Figure 2 illustrates the proposed mechanisms of Cu^{2+} sequestration arising from the two polymer addition sequences. The observations indicate that the appropriate sequence of polymer addition is EPS first, then PEI.

This part of the experiment provides insight into the mechanism of Cu^{2+} sequestration by the EPS-PEI flocculant system when the appropriate polymer addition sequence is employed. Removal of Cu^{2+} from the wastewater can be considered to occur in two steps: (1) binding of Cu^{2+} onto EPS functional groups, particularly the carboxylate groups which are the most common negatively-charged groups in anionic polyelectrolytes, and (2) binding of the EPS- Cu^{2+} complexes with PEI which results to the formation of firm flocs with excellent settling characteristics.

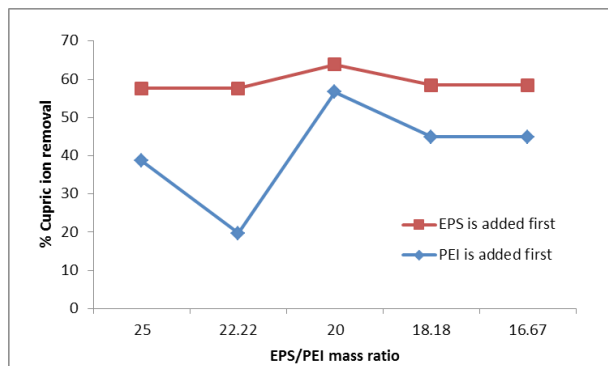


Figure 1. Influence of polymer addition sequence on Cu^{2+} removal.

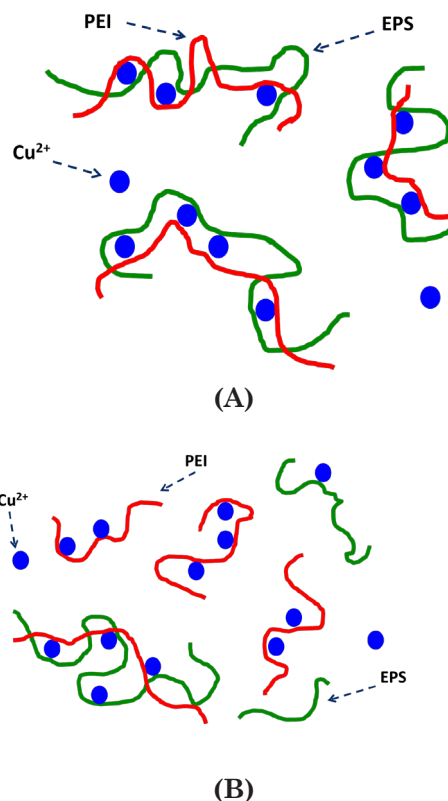


Figure 2. Proposed mechanisms of Cu^{2+} sequestration by the EPS-PEI flocculant system. **(A) Addition of EPS precedes addition of PEI.** Flocculation of the EPS- Cu^{2+} complex by PEI results to effective separation of the sequestered Cu^{2+} from the aqueous phase. This results to high Cu^{2+} removal efficiency. **(B) Addition of PEI precedes addition of EPS.** Flocculation of the PEI- Cu^{2+} complex by EPS is hindered due to the tendency of PEI to fill all binding sites in the Cu^{2+} coordination sphere. This results to low Cu^{2+} removal efficiency because the PEI- Cu^{2+} complex is soluble and is retained in the aqueous phase.

Copper removal at different pHs

The acidic to neutral pH region (pH 2-7) was considered for the study to highlight the role of the EPS-PEI flocculant system on Cu^{2+} sequestration and to minimize the contribution of metal hydroxide precipitation. Working in this acidic region also ensures minimal interference from metal hydroxides in the formation of EPS- Cu^{2+} complexes.

Figure 3 illustrates the resulting trend of copper removal using the EPS-PEI flocculant system at increasing pH. Low Cu^{2+} removal occurs at low pHs (e.g. 2-3) which may be attributed to hydronium ions competing for negatively charged oxygen binding sites along the EPS chain. At pH 2-6, Cu^{2+} removal may be attributed essentially to sequestration by the EPS-PEI flocculant system. Copper hydroxide precipitation could not have played a major role because at these pHs copper exists mainly as Cu^{2+} . Precipitation of Cu^{2+} to $\text{Cu}(\text{OH})_2$ occurs chiefly at pH 6-8.

At pH 7, copper removal can be attributed mainly to copper hydroxide precipitation. The formation of faint blue flocs, however, mean that there remains substantial contribution by the EPS-PEI flocculant system on copper removal.

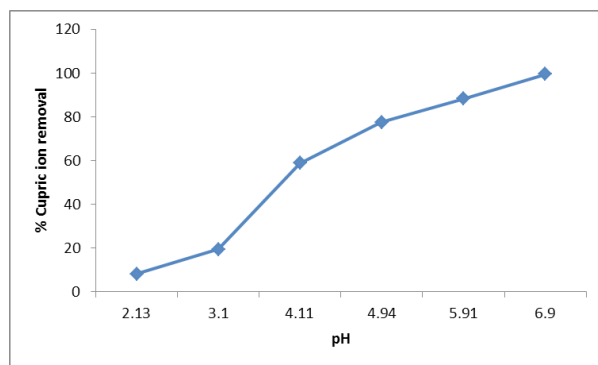


Figure 3. Cupric ion removal by the EPS-PEI flocculant system at increasing pH and an EPS/PEI mass ratio of 25.

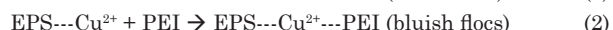
Flocs characteristics at different pHs

Flocs characteristics were found to vary at different pHs. At very acidic pHs (e.g. pH 2-3), flocs formed were loose with the filtrate presenting some turbidity. This observed turbidity may have been due to the presence of residual EPS in the

supernatant. However, flocculation of the EPS- Cu^{2+} complex by PEI was still evident even with the absence of the distinctive bluish coloration of the flocs because of the relative ease by which the supernatant can be filtered.

At pH 2, the binding site of PEI may mostly be the negatively charged oxygen atoms along the EPS chain. This explains the lack of blueness of the flocs. At pH 3, flocs exhibited a faint blue coloration suggesting a second binding mechanism of PEI on the EPS- Cu^{2+} complex, that is, amine groups in PEI form coordinate covalent bonds with sequestered Cu^{2+} . Through this mechanism, flocculation of EPS- Cu^{2+} complexes is possible even when EPS is saturated with Cu^{2+} .

The proposed binding mechanisms of PEI which result to floc formation can therefore be summarized as follows:



The second mechanism could account for the faint blue coloration of the flocs because amine groups in PEI form blue-colored complexes with copper. This amine- Cu^{2+} complex forms because Cu^{2+} prefers a coordination number of four, enabling it to participate in one or more covalent bonds aside from the ionic bonds with negatively charged oxygen atoms on the EPS chain. PEI bonds with Cu^{2+} by displacing one or more water molecules in the Cu^{2+} coordination sphere.

At this point, it is important to note that the two proposed binding mechanisms may occur randomly and are only indirectly related to pH. The type of mechanism that prevails depends only upon the relative abundance of sequestered Cu^{2+} or unreacted oxygen binding sites.

At pH 4-6, flocs formed are firm, bluish, and whole. Because at these pHs less hydronium ions are present to compete with Cu^{2+} for binding sites, higher removal efficiencies were possible. Floc formation could still be explained by the two mechanisms defined previously but the second mechanism dominates. With higher amounts of sequestered Cu^{2+} , and the binding sites possibly saturated, there is greater chance for PEI to attach to sequestered Cu^{2+} rather than to unreacted negatively charged oxygen binding sites along the EPS chain. At pH 7, prominent loss of blueness and firmness of flocs were observed. This may be

explained by the scarcity of Cu^{2+} binding sites for PEI since at this pH, much of the Cu^{2+} could have already precipitated as $\text{Cu}(\text{OH})_2$.

The workable and best EPS/PEI mass ratio

Flocculation of EPS- Cu^{2+} complexes in the industrial wastewater is perceived to occur by chargeneutralization and/or bridging mechanisms when using PEI as flocculant. This flocculation mechanism coincides with the observations that (1) there exists an optimum stoichiometric proportion of the EPS- Cu^{2+} complexes and the polycation PEI, and (2) overdosing of the polycation results to re-stabilization of the colloids. Table 1 provides the summary of data supporting these observations.

Data from Table 1 point to a range of EPS/PEI mass ratios where effective flocculation and Cu^{2+} sequestration could occur. For convenience, this range is referred to as the *workable* EPS/PEI mass ratio. This workable range, which is characterized by easy filterability, clear filtrates and firm flocs that settle quickly, occurs somewhere between the EPS/PEI mass ratios 25 to 30.77. Ease of filterability was qualitatively determined by passing the treated water through a Whatman Grade 1 (11 μm) filter paper.

For systems where a workable range is noticeable, it is important to determine the width of workable ratios because it indicates the flexibility of the polymer dosing proportions where effective flocculation and Cu^{2+} sequestration could take place.

Table 1. Data for finding the best and workable EPS/PEI mass ratios at pH 5.

mL 0.5% PEI	EPS/ PEI	Filterability	Filtrate	Flocs	%Cu Removal
0.000	Infinity	Hard	Clear	Loose	56.23
0.040	50.00	Hard	Clear	Faint blue, loose	63.02
0.045	44.44	Moderate	Clear	Faint blue, loose	66.81
0.050	40.00	Moderate	Clear	Faint blue, loose	61.39
0.055	36.36	Easy	Clear	Faint blue, loose	57.77
0.060	33.33	Easy	Clear	Faint blue, loose	61.92
0.065	30.77	Easy	Clear	Faint blue, firm	60.47
0.070	28.57	Easy	Clear	Faint blue, firm	64.25
0.075	26.67	Easy	Clear	Bluish, firm	72.73
0.080	25.00	Easy	Clear	Bluish, firm	57.74
0.085	23.53	Easy	Clear, VFMB	Bluish, firm	55.56
0.090	22.22	Easy	VFMB	Bluish, firm	59.71
0.095	21.05	Easy	VFMB	Bluish, firm	56.27
0.100	20.00	Easy	VFMB	Bluish, firm	52.11
0.105	19.05	Easy	VFMB	Bluish, firm	58.97
0.110	18.18	Easy	VFMB	Bluish, firm	58.24

VFMB = very faint misty blue

Operating with a low initial colloid concentration made the best ratio occur at a narrow range, almost a point. In terms of copper removal, floc firmness and supernatant filterability, the best EPS/PEI mass ratio is 26.67. This outcome is expected because the width of the flocculation region or optimum dose is directly related to the initial colloid concentration (Chaudhari & Tare, 1999). At high initial colloid concentrations, the best ratio is expected to broaden.

At the best EPS/PEI ratio, the hexose/ethyleneimine mole ratio is found to be 5.715. This means that 1.0 mole of ethyleneimine is needed to flocculate 5.715 moles of Cu²⁺-bearing hexose.

A graph showing the trend of Cu²⁺ removal at different EPS/PEI mass ratios is shown in Figure 4. This graph incorporates only the EPS/PEI ratios within the range enclosing the workable EPS/PEI ratios because ratios above and below this range are already irrelevant.

At the best EPS/PEI mass ratio, Cu²⁺ removal is at maximum because at this ratio, binding sites along the EPS chain are kept saturated upon addition of the secondary flocculant, and most, if not all, of the EPS-Cu²⁺ complexes are flocculated and recovered from the mixture. Also at this ratio, the EPS-Cu²⁺-PEI complex is essentially electrically neutral; hence, the possibility of colloid re-stabilization to occur is at minimum.

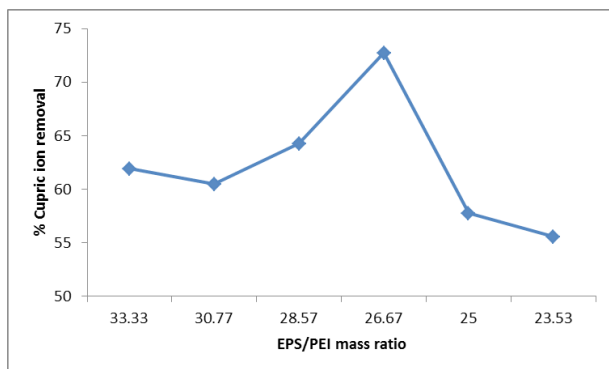


Figure 4. Cupric ion removal efficiencies at different EPS/PEI ratios and pH 5.

At ratios above the best ratio where there is an overabundance of EPS relative to PEI, lower Cu²⁺ removal is due to the presence of unflocculated soluble EPS-Cu²⁺ complexes in the bulk phase. This means that there is lack of the secondary flocculant to neutralize all of the EPS-Cu²⁺ complexes and the resulting electrostatic interactions cause the colloids to resist aggregation.

At ratios below the best ratio where there is an overabundance of PEI relative to EPS, lower Cu²⁺ removal may be attributed to the tendency of the EPS-Cu²⁺-PEI complex to become a positively charged colloid because of the presence of excess PEI. This is a consequence of PEI's high charge density. Stabilized EPS-Cu²⁺-PEI complexes remain in the aqueous phase and impart a faint blue mist in the filtrate.

Ability of the EPS-PEI flocculant system to reduce Cu²⁺ to acceptable concentrations

The ability of the EPS-PEI flocculant system to scavenge Cu²⁺ from the wastewater was studied by increasing the EPS dosage while maintaining an EPS/PEI mass ratio of 25 at pH 5. This mass ratio is within the workable range. Flocs formed were firm and bluish and supernatants were clear and easily filterable.

Results show that substantial removal of Cu²⁺ by the EPS-PEI flocculant system can be attained even with the possible presence of other interfering ions and chemical species in the industrial wastewater. For an initial Cu²⁺ concentration of 25.28 mg/L, almost complete Cu²⁺ removal was achieved at 1792.11 mg/L EPS; regulatory effluent standards for copper were met at lower doses of EPS. At an EPS concentration of 1657.13 mg/L, the Cu²⁺ concentration was reduced from 25.20 mg/L to 0.32 mg/L. This effluent concentration of Cu²⁺ already satisfies typical effluent standards.

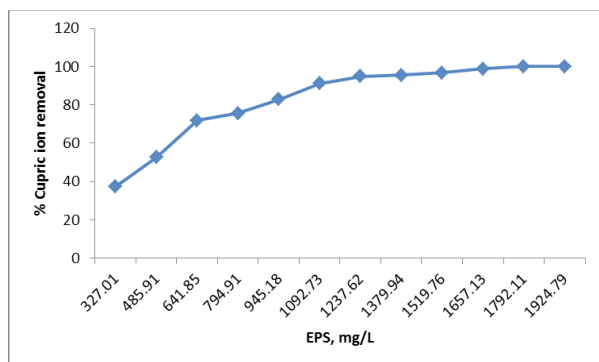


Figure 5. Cupric ion removal efficiencies at increasing doses of EPS at pH 5 and an EPS/PEI mass ratio of 25.

Estimation of Cu^{2+} uptake using the Langmuir isotherm

To approximate the capacity of the EPS-PEI flocculant system for Cu^{2+} sequestration, the Langmuir isotherm was used. The Langmuir isotherm is expressed as:

$$Q = \frac{Q_{\max} b C_{eq}}{1 + b C_{eq}}$$

where Q = concentration of bound metals in the adsorbent (mg/g), C_{eq} = equilibrium metal concentration in the bulk phase (mg/L), b = Langmuir constant (L/mg), and Q_{\max} = adsorption capacity (mg/L). Double reciprocal linearization of this equation yields:

$$\frac{1}{Q} = \left(\frac{1}{Q_{\max} b} \right) \left(\frac{1}{C_{eq}} \right) + \frac{1}{Q_{\max}}$$

Strong linear behavior resulting from using the Langmuir isotherm ($r^2 = 0.977$) means that the EPS- Cu^{2+} complex behaves according to the Langmuir model requirements, i.e., a monomolecular layer of Cu^{2+} and uniform energy of adsorption exists along the EPS chain. With this result it can be said that the maximum capacity of EPS is about $28\text{mgCu}^{2+}/\text{g EPS-PEI}$, with a stability constant of 1.24 Lmg^{-1} . Figure 6 shows the Langmuir isotherm while Figure 7 shows the plot of its linearized form.

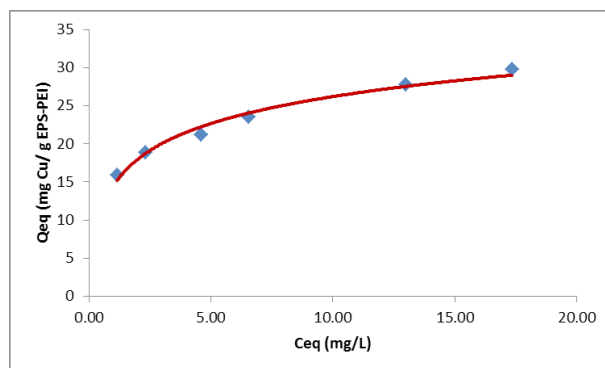


Figure 6. Langmuir adsorption isotherm of Cu^{2+} sequestration by EPS.

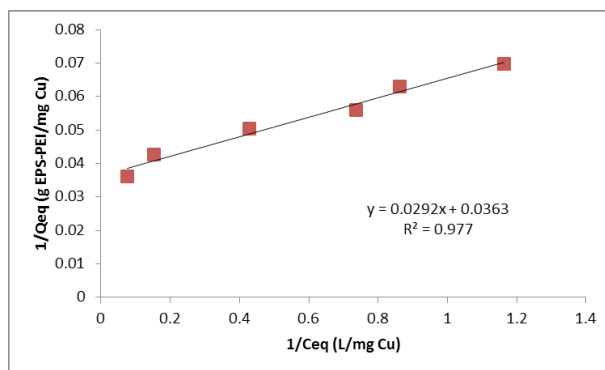


Figure 7. Linearized plot of the Langmuir adsorption isotherm.

Comparison of Cu^{2+} uptake capacity with other systems

To compare the performance of the EPS-PEI flocculant system's Cu -uptake capacity, a literature search was conducted. A summary of gathered data is provided in Table 2. Data shows that Cu^{2+} capacity of the EPS-PEI flocculant system is comparable with that of natural adsorbents and adsorbents derived from natural sources.

Table 2. Copper adsorption capacities of different adsorbents.

Adsorbent	mg Cu / g adsorbent
EPS-PEI flocculant system	28
Bentonite clay (Aljlil & Alsewailem, 2014)	13.22
Marine green algae activated carbon (Abdelwahab, 2007)	32.3
Palm shell activated carbon (Onundi, Mamun, Al Khatib, & Ahmed, 2010)	1.58
Vermiculite (Dizadji, Rashtchi, Dehpouri, & Nouri, 2013)	20
Clinoptilolite (Dizadji et al., 2013)	11.24

Summary and Conclusions

The EPS-PEI flocculant system was found to be an excellent Cu²⁺ scavenger for the industrial effluent studied. Regulatory standards for Cu²⁺ concentration can be achieved using the simple coagulation-flocculation process employed in this study. Formation of firm, quick-settling flocs makes this process superior to conventional chemical precipitation techniques.

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