



Understanding key constituents and feature of the biopolymer in activated sludge responsible for binding heavy metals



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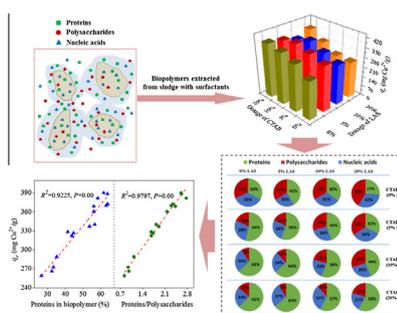
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HIGHLIGHTS

- Key constituents and feature of the biopolymer binding heavy metals were disclosed.
- Biopolymers with different proportions of constituents were extracted from sludge.
- Adsorption capacities of the biopolymers for Cu²⁺ varied with their constituents.
- Proteins were the key constituents of the biopolymer for the adsorption of Cu²⁺.
- High proteins/polysaccharides ratio was beneficial to the adsorption of Cu²⁺.

GRAPHICAL ABSTRACT



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ABSTRACT

The biopolymer in activated sludge plays an important role in the removal of heavy metals, and it can also be extracted to serve as biosorbent for heavy metals. It is of big interest to understand the key constituents and feature of the biopolymer. In this study, the biopolymers with quite different proportions of constituents were extracted from activated sludge after the conditioning by cetyl trimethyl ammonium bromide (CTAB) and linear alkylbenzene sulfonate (LAS), and then were used to test their adsorbability for metal ions. CTAB was prominent in increasing proteins (PN) content, and LAS was prominent in increasing polysaccharides (PS) and nucleic acids (NA) content. The biopolymers with different proportions of PN, PS and NA showed different adsorption capacities for Cu²⁺ (ranging from 258.94 to 389.92 mg/g). Their adsorption capacities significantly correlated with proteins ($R^2 = 0.9225$, $P = 0.00$), proteins/polysaccharides ($R^2 = 0.9797$, $P = 0.00$), polysaccharides ($R^2 = 0.6739$, $P = 0.01$), and nucleic acids ($R^2 = 0.6960$, $P = 0.00$). The adsorption capacities showed significant positive correlation with proteins and proteins/polysaccharides, but negative correlation with polysaccharides and nucleic acids. Proteins and high proteins/polysaccharides were identified to be the key constituents and feature of the biopolymer responsible for binding heavy metals, respectively. The findings denote that all biomass with high protein content could be an excellent adsorbent for metal ions.

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1. Introduction

Heavy metals undergo important cycles in the environment between their abiotic and biotic elements, but the indiscriminate

discharge of toxic metals has also posed toxicity to the environment and human health [1]. Since heavy metals are non-renewable resources and non-degradable pollutants, a possible recovery and reuse should be the best way to reduce their loss and harm to the latter groups [2]. Owing to biodegradability, non-toxicity and good efficacy, biopolymers are regarded as an cost-effective material to remove metal ions from wastewater [3] or even to reclaim some valuable metal resources [4]. Various biomass could be directly used or used as the raw material of biopolymers, including plants, bacteria, fungi, marine algae, activated sludge, and digested sludge etc [5]. Especially, activated sludge has drawn particular interest recently due to its good metal-sequestering property, abundant availability and cost free nature [6].

Various types of sludge have been used as biopolymer for binding heavy metals (Table 1), such as sewage activated sludge [7,8], dried waste activated sludge [9,10], aerobic digested activated sludge [11,12], alkali modified sewage sludge [8], and aerobic granular sludge [13]. Macromolecular biopolymers in the sludge play an important role in the adsorption of heavy metals by the sludge [14]. They are a complex mixture of biomolecules, e.g., proteins (PN), polysaccharides (PS), nucleic acids (NA), humic-like substances, uronic acids, and lipids [14]. The major functional groups in the biopolymers, which are closely related to the adsorption of heavy metals, contain carboxyl, hydroxyl, amine and phosphoric groups [11]. Several studies aimed at explaining the functional groups and adsorption mechanisms between heavy metals and biopolymer. Yuan et al. [15] revealed that carboxyl and hydroxyl groups have been found to be primarily involved in the heavy metal complexation by forming stable complexes under neutral pH solution. Phosphoric amine and amidocyanogen of proteins, polysaccharides and phospholipids could also become negatively-charged and serve as effective metal binding ligands. Ozdemir et al. [16] also found that the carboxyl groups were negatively charged in neutral pH solution, and attracted positively-charged cations through electrostatic interaction and form organometal complexes. Fang et al. [17] further mentioned that nitrogen in amino-sugars and oxygen in hydroxyl and carboxyl were the main electron donor atoms, which were prone to preferentially bind with soft metal cations of strong covalent characteristics and then form inner-sphere complexes. We also found that the adsorption mechanisms of the biopolymer obtained from waste activated

sludge for Pb^{2+} and Zn^{2+} contained complexation (between amino group and metal ions) and ion-exchange (between carboxyl group and metal ions) [11].

The biopolymer in activated sludge plays an important role in the removal of heavy metals, and the sludge with different biopolymers showed different removal efficiencies of metal ions [14]. When the biopolymer was extracted to serve as biosorbent for heavy metals, their specificity and adsorbability also varied with the sludge source [18]. It is of big interest for both activated sludge system and biosorption process to understand the key constituents and feature of the biopolymer responsible for binding heavy metals.

In this study, the biopolymers with quite different compositions were extracted from activated sludge after the conditioning by cetyl trimethyl ammonium bromide (CTAB) and linear alkylbenzene sulfonate (LAS), and then were used to test their adsorbability for metal ions. The macromolecular biopolymers including PN, PS and NA were determined as the major constituents of the biopolymer. The adsorption capacities of the extracted biopolymers for metal ions (Cu^{2+}) were comparatively investigated. Then the Pearson correlations between adsorption capacity and the biopolymer constituent parameters (PN, PS, NA, proteins/polysaccharides ratio (PN/PS), proteins/nucleic acids ratio (PN/NA), and polysaccharides/nucleic acids ratio (PS/NA)) were modeled, and the key constituents and feature of the biopolymers responsible for binding heavy metals were identified.

2. Materials and methods

2.1. Chemicals and sludge samples

$Cu(NO_3)_2 \cdot 3H_2O$ (>99% purity) was obtained from Runjie Chemistry Reagent (Shanghai, China) and the stock solution of Cu^{2+} was prepared by dissolving $Cu(NO_3)_2 \cdot 3H_2O$ in distilled water at an initial concentration of 1000 mg/L. LAS and CTAB (99% purity), with a molecular weight of 326.49 and 364.45 g/mol, respectively, were purchased from Shanghai Chem. Co. Ltd., China.

Activated sludge samples were obtained from the secondary settling tank return sludge from a full-scale municipal wastewater treatment plant in Shanghai, China. Anaerobic-anoxic-aerobic process was used in the domestic wastewater treatment plant. The

Table 1
Biosorption of heavy metals by various sludges.

Sludge	Heavy metal	Operation conditions				q_e (mg/g)	Ref.
		pH	T ($^{\circ}C$)	C_0 (mg/L)	t		
Activated sludge	Cd^{2+}	5.0	RT	50	–	28.10	[8]
	Cu^{2+}	5.0	RT	50	–	19.07	[8]
	Ni^{2+}	5.0	RT	50	–	8.80	[8]
	Pb^{2+}	5.0	RT	50	–	142.97	[8]
	Zn^{2+}	5.0	RT	50	–	15.69	[8]
	Cu^{2+}	5.0	30	–	48 h	80.60	[9]
	Cd^{2+}	5.0	30	–	48 h	69.20	[9]
	Ni^{2+}	5.0	30	–	48 h	40.60	[9]
Dried waste activated sludge	Cr^{3+}	4.5	25	–	24 h	95.20	[10]
	Ni^{2+}	4.5	25	–	24 h	238.10	[10]
	Cd^{2+}	6.0	25	–	1 h	84.30	[11]
	Pb^{2+}	4.0	25	–	1 h	131.60	[11]
Aerobic digested activated sludge	Zn^{2+}	6.0	35	20	2 h	182.42	[12]
	Pb^{2+}	6.0	35	20	2 h	239.90	[12]
	Cu^{2+}	5.5	25	20	4 h	328.10	[13]
Alkali modified sewage sludge	Cu^{2+}	5.0	30	–	48 h	133.30	[9]
	Cd^{2+}	5.0	30	–	48 h	91.70	[9]
	Ni^{2+}	5.0	30	–	48 h	58.80	[9]
Aerobic granular sludge	Cd^{2+}	7.0	26	50	3 h	127.60	[14]

collected samples were transported to our laboratory within 30 min after sampling. The samples were subsequently screened through a 1.2 mm sieve to remove grit and then gravity concentration for 30 min. The main parameters of concentrated sludge were as following: suspended solids (SS) 8.7 ± 0.6 g/L, the ratio of volatile suspended solids to suspended solids (VSS/SS) $68 \pm 3\%$, and pH 6.7–7.4. Concentrated sludge was stored at 4 °C in fridge and used within 2 days.

2.2. Biopolymer extraction from sludge

Series of bench-scale experiments were conducted to extract the biopolymers with quite different compositions. In order to obtain the biopolymers with quite different compositions from activated sludge, cetyl trimethyl ammonium bromide (CTAB) and linear alkylbenzene sulfonate (LAS) were used as the chemical conditioning agents [19]. When the mass ratio of CTAB/SS (0%, 5%, 10%, or 20%) was set, the mass ratio of LAS/SS was increased from 0% to 20%. Sludge conditioning was conducted in 250-mL beakers with the concentrated sludge sample of 100 mL. The conditioning was initiated immediately by adding various content of LAS into the beakers once a specified constant dose of CTAB was applied, and then mixing the sample at 250 rpm for 2 min, followed by slow stirring at 150 rpm for 8 min with a temperature of 298.15 ± 1 K using a constant temperature –magnetic stirrer (RCT/RET, Guangzhou Boletai. Co., China). Biopolymer extraction was carried out

with centrifugation. After conditioning, sludge samples were centrifuged twice at 10,800g with a constant temperature of 277.15 K for 10 min. The supernatant was the raw biopolymer, which was further purified via cold acetone method [11].

2.3. Adsorption of Cu^{2+} by the biopolymer

Adsorption experiments were carried out by adding the biopolymer into 250-mL Erlenmeyer flasks containing 50 mL of $\text{Cu}(\text{NO}_3)_2$ solutions with the initial concentration of 10 mg Cu^{2+} /L. After adding biopolymer with the weight ratio of biopolymer/ Cu^{2+} at 2.0, the flasks were sealed immediately to decrease the evaporation of solution. Reaction conditions were controlled as follows: temperature 303.15 K, initial pH 5.5. After agitation for 30 min in an isothermal shaker with the speed of 150 rpm, samples were taken from the solutions and separated from the precipitates prior to analysis through centrifugation at 10,800g for 10 min with a temperature of 277.15 K, and then filtrated using 0.22- μm cellulose nitrate membrane filters. Filtrate was acidified with HNO_3 and then analyzed the residual concentration of Cu^{2+} using inductively coupled plasma atomic emission spectrometry (ICP-AES, Agilent 720ES, USA). The amount of Cu^{2+} adsorbed by the biopolymer (q_e , mg/g) is calculated using Eq. (1).

$$q_e = \frac{(C_0 - C_e)V}{W} \quad (1)$$

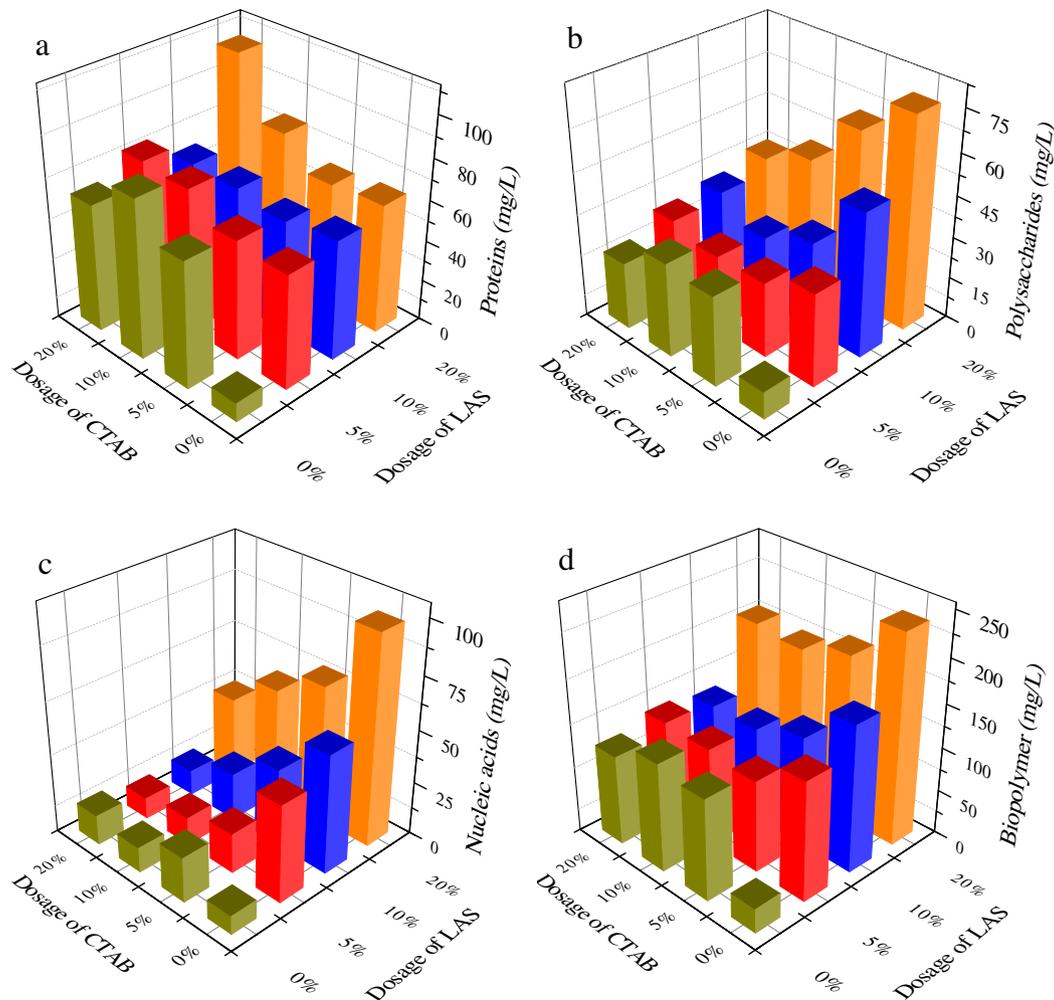


Fig. 1. Concentration variations of (a) proteins, (b) polysaccharides, (c) nucleic acids and (d) total biopolymer extracted from activated sludge after conditioning under the co-effect of cetyl trimethyl ammonium bromide and linear alkylbenzene sulfonate.

where C_0 (mg/L) and C_e (mg/L) are the initial and equilibrium concentration of Cu^{2+} in the solution, respectively; V (L) is the volume of the solution, and W (g) is the weight of the biopolymer.

2.4. Characterization assay

PN, PS and NA were examined as the major constituents of biopolymer using the following colorimetric methods: Bradford method for PN content, bovine serum albumin as the standard [20]; phenol-sulfuric acid method for PS content, glucose as the standard [20]; diphenylamine colorimetric method for NA content, calf thymus deoxyribonucleic acid as the standard [20]. SS and VSS of activated sludge were analyzed following the standard methods [21].

2.5. Statistical analysis

Statistical analysis with SPSS software for Windows (SPSS, Chicago, Illinois, USA) was used to identify the strength of the relationship between PN, PS, NA, PN/PS, PN/NA, PS/NA and the adsorption capacity of the biopolymers for Cu^{2+} . Pearson's correlation coefficient was used to evaluate the linear correlation between two parameters. Pearson's coefficient is always between -1 and $+1$, where -1 means a perfect negative correlation, $+1$ a perfect positive correlation, and 0 the absence of a relationship. Correlations were considered statistically significance with a 95% confidence interval ($P < 0.05$).

3. Results and discussion

3.1. Contents of PN, PS, NA in the extracted biopolymers

The content variations of PN, PS, NA and total biopolymer of the biopolymers extracted from activated sludge after CTAB and LAS conditioning are present in Fig. 1. Chemical conditioning with the surfactants showed obvious influence on the contents of PN, PS, and NA in the biopolymers. The variations of constituent

proportion in the biopolymers are shown in Fig. 2. The PN proportion in the biopolymer increased with increasing CTAB dosage, but it increased first and then decreased with LAS dosage (above 5%). The PS content in the biopolymer ranged from 21% to 35% under various conditioning processes. In general, the NA proportion in the biopolymer increased with increasing LAS dosage, but decreased with increasing CTAB dosage. The NA content in the biopolymer ranged from 9% to 41% throughout the conditioning process. High NA content meant the cell lysis, which should be resulted by CTAB and LAS as mentioned above. The variation of PN, PS, and NA proportion in the biopolymer could be related to the physiochemical properties of CTAB and LAS. On the one hand, both CTAB and LAS have a non-polar linear hydrocarbon groups which can form micelles and increase the solubility of macromolecule organic matter in aqueous solution [19]. On the other hand, as an excellent PN denaturing agents, LAS could reach the active center of PN to form complexes and cause the inactivation of PN, which resulted in the decrease of PN proportion in biopolymer [22]. As an excellent cationic detergent, CTAB could precipitate the NA and acidic PS in low ionic strength solution, which resulted in the decrease of PS and NA proportion in the biopolymer [19]. The extracted biopolymers with quite different compositions would be used in the following studies.

3.2. Adsorption capacities of metal ions by the biopolymers

In order to examine the adsorption capacity variations of the biopolymers for metal ions, the extracted biopolymers were used to adsorb Cu^{2+} . As shown in Fig. 3, the adsorption capacities of the biopolymers for Cu^{2+} ranged from 258.94 to 389.92 mg/g. All of them are higher than the reported maximum Cu^{2+} adsorption capacities of various biopolymers: 216.54 mg/g for acid treated *Sphaeroplea* algae under pH value of 4.0 [23], 52.08 mg/g for NaOH treated sour orange under pH value of 4.5 [24], and 19.05 mg/g for activated sludge under pH value of 4.0 [7]. These results further confirm that the biopolymer extracted from activated sludge is an excellent adsorbent for heavy metals. The adsorption capacity

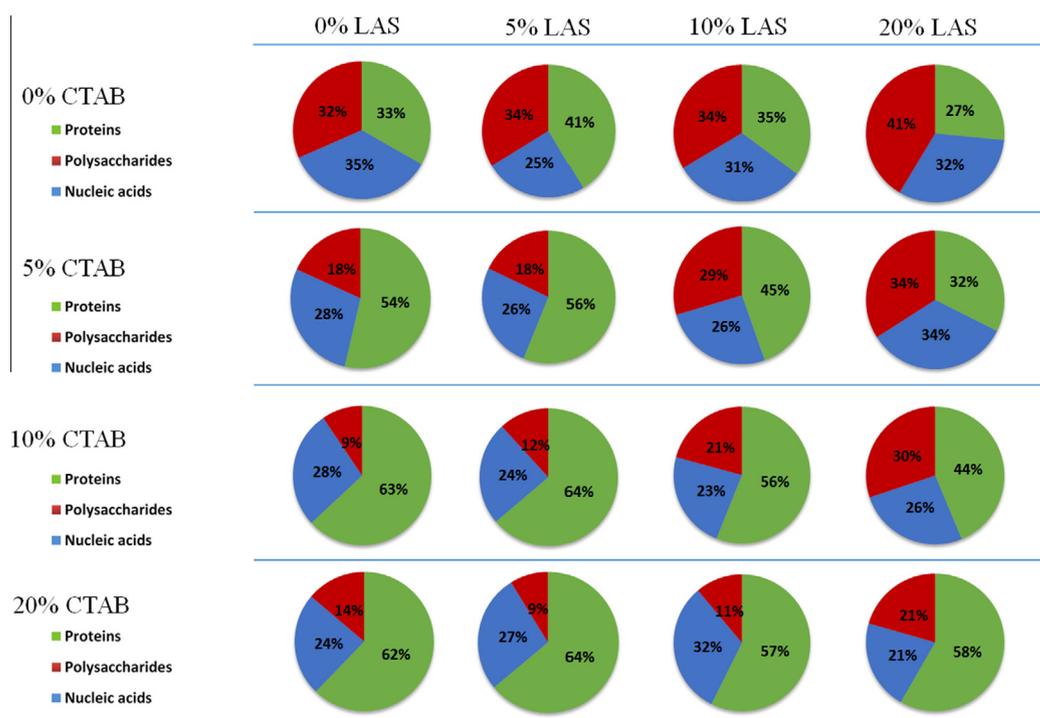


Fig. 2. Constituent proportion variations of the biopolymer extracted from activated sludge after conditioning under the co-effect of cetyl trimethyl ammonium bromide and linear alkylbenzene sulfonate.

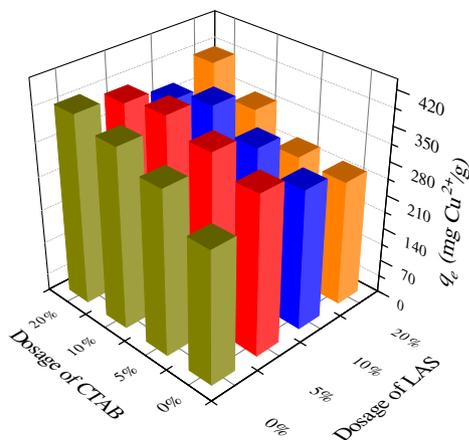


Fig. 3. Adsorption capacity variations of Cu^{2+} by the biopolymer extracted from activated sludge after conditioning under the co-effect of cetyl trimethyl ammonium bromide and linear alkylbenzene sulfonate.

variation of the biopolymers for Cu^{2+} is consistent with the PN proportion variation in the biopolymers. Zhang et al. [5] also revealed that the protein-like substances in the biopolymers from natural biofilm showed excellent binding ability for Hg^{2+} because its anionic properties and amide groups contributes to the electrostatic

interaction, complexation and ion-exchange with metals. Therefore, PN might be the core constituent in the biopolymer for the metal biosorption process.

3.3. Pearson correlations between adsorption capacity and biopolymer compositions

In order to model the relationship between the adsorption capacity of the biopolymer for Cu^{2+} and the biopolymer constituent parameters (PN, PS, NA, PN/PS, PN/NA, and PS/NA), Pearson's correlation coefficient was used to evaluate the linear correlation between two parameters. Fig. 4 showed the Pearson correlations between PN, PS, NA, PN/PS, PN/NA, PS/NA and the adsorption capacity of biopolymer for Cu^{2+} . Adsorption capacity of biopolymer for Cu^{2+} significantly correlated with PN ($R^2 = 0.9225$, $P = 0.00$) and PN/PS ($R^2 = 0.9797$, $P = 0.00$), and also showed some correlation with PS ($R^2 = 0.6739$, $P = 0.01$), NA ($R^2 = 0.6960$, $P = 0.00$), PN/NA ($R^2 = 0.5307$, $P = 0.11$) and PS/NA ($R^2 = 0.2267$, $P = 0.16$).

Increased amounts of PN or/and PN/PS in the biopolymer could promote the binding of Cu^{2+} (Fig. 4(a) and (d)). There were significant positive correlations between PN, PN/PS and the adsorption capacity of biopolymer for Cu^{2+} . Moreover, PS in biopolymer has negative effect on the adsorption of Cu^{2+} . Thus, biosorption of Cu^{2+} by the biopolymer extracted from activated sludge was mainly influenced by PN and PN/PS. PN in biopolymer played a

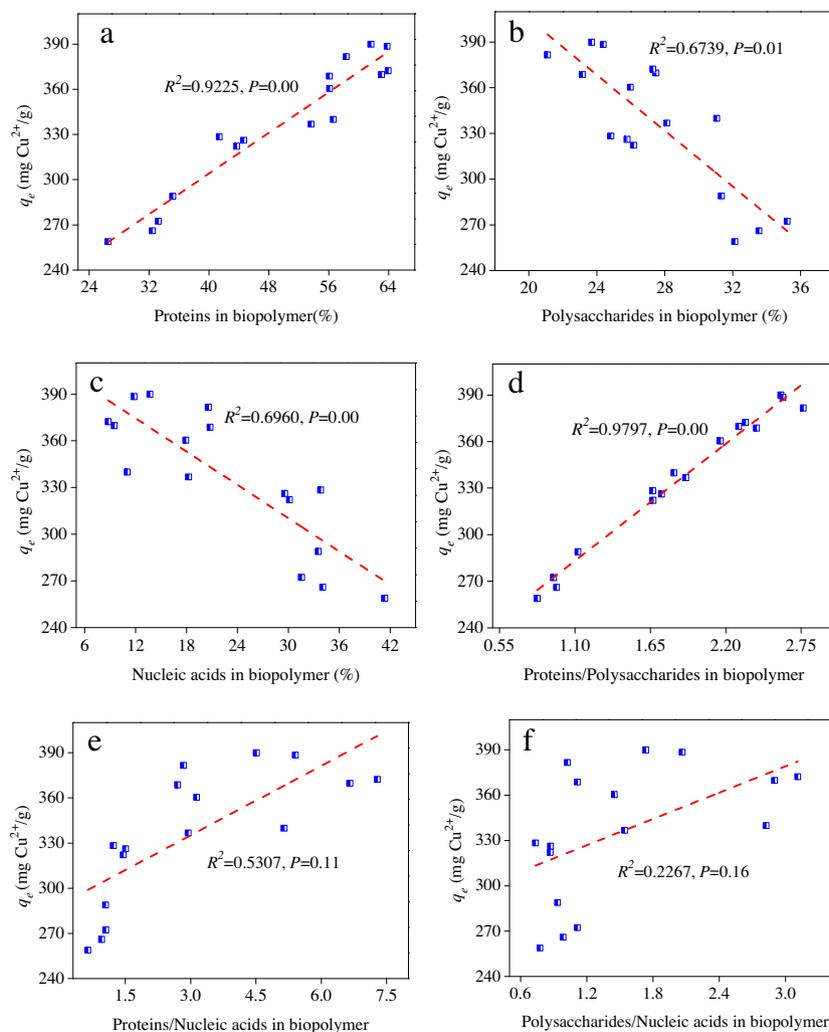


Fig. 4. Pearson correlations between adsorption capacity of Cu^{2+} and (a) proteins, (b) polysaccharides, (c) nucleic acids, (d) proteins/polysaccharides, (e) proteins/nucleic acids, (f) polysaccharides/nucleic acids in the biopolymer extracted from activated sludge after conditioning under the co-effect of cetyl trimethyl ammonium bromide and linear alkylbenzene sulfonate.

key role in binding metal ions. PN contains large number of functional groups, such as amino, carboxyl, and amide, which exhibit excellent metal-binding properties [5,25].

Accordingly, the removal of metal ions via either activated sludge system or biosorption process can be enhanced by improving the PN content in the biopolymer. Some methods can be used to achieve this aim. As for the activated sludge system, extracellular polymeric substances (EPS), which are the major source of the biopolymer, are bonded with cell surface through ion bridging with multivalent metals [14]. High concentration of metal ions could increase the protein content in the EPS of activated sludge [26]. Nutrient levels also have a significant effect on the composition of EPS. Durmaz and Sanin [27] mentioned that a carbon to nitrogen ratio of 5 resulted in the protein enrichment but low content of carbohydrates in activated sludge EPS. However, the content of protein decreased sharply whereas the concentration of carbohydrates increased when the carbon to nitrogen ratio increased to 40 [27]. Liu and Fang [28] also found that a low carbon to nitrogen ratio tends to promote the production of EPS with a high proteins-/carbohydrates ratio by activated sludge. As for the biopolymer extracted as adsorbent, the extraction method of the biopolymer can be adjusted to obtain high PN proportion. Frølund et al. [20] used cation exchange resin (CER), sodium hydroxide and heating treatment for the extraction of EPS from activated sludge, and the protein contents were 325.83, 95.93 and 120.56 mg/g VSS, respectively. Results suggested CER was the best method to extract high protein content biopolymers from activated sludge.

In addition, the findings in this study denote that the proteins and protein-rich biomass might be used as environmentally friendly adsorbents for metal ions. Due to abundance, low cost and zero-emission of CO₂, the biomass with high protein content has been used for adsorbing some metal ions [2,29]. For example, as the byproducts with huge yields in the Japanese food industry, soybean protein, chicken egg-shell membrane and seasoning pollack-roe membrane contain large amounts of proteins (approximately 91%, 90% and 64%, respectively), and all of them could adsorb almost 100% of Au³⁺ and Pd²⁺ (5 mg/g-biomass) [29]. Simon and Azam [30] mentioned that the ratio of cell protein and dry weight in planktonic marine bacteria up to 63%, and this kind of bacteria could be directly used as an excellent heavy metal adsorbent.

4. Conclusions

Biopolymers with different proportions of PN, PS and NA showed different adsorption capacities for Cu²⁺. Adsorption capacities of the biopolymers for Cu²⁺ significantly correlated with PN ($R^2 = 0.9225$, $P = 0.00$), PN/PS ($R^2 = 0.9797$, $P = 0.00$), PS ($R^2 = 0.6739$, $P = 0.01$), and NA ($R^2 = 0.6960$, $P = 0.00$). The adsorption capacity showed significant positive correlation with PN and PN/PS, but negative correlation with PS and NA. PN and high PN/PS in the biopolymer were identified to be the key constituents and feature of the biopolymer responsible for binding heavy metals, respectively.

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