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Contribution of extracellular polymeric substances fractions to the adsorption of silver nanoparticles by activated sludge

Yanghui Xu^{a,b}, Qin Ou^a, Qiang He^a, Zhengsong Wu^a, Jun Ma^c, Xiaoliu Huangfu^{a,*}

^a Key Laboratory of Eco-environments in Three Gorges Reservoir Region, Ministry of Education, Faculty of Urban Construction and Environmental Engineering, Chongqing University, Chongqing 400044, China

^b Section of Sanitary Engineering, Department of Water Management, Faculty of Civil Engineering and Geosciences, Delft University of Technology, Stevinweg 1, 2628 CN Delft, the Netherlands

^c State Key Laboratory of Urban Water Resource and Environment, School of Municipal and Environmental Engineering, Harbin Institute of Technology, Harbin 150001, China

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ABSTRACT

The extracellular polymeric substances (EPS) from activated sludge played significant roles in the removal of nanoparticles from wastewater. A series of batch experiments were carried out to determine the adsorption mechanism of three nano-Ag by activated sludge, as well as the contributions of EPS fractions including dissolved EPS (DEPS), loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS). The results demonstrated that the adsorption of nano-Ag by sludge biomass agreed with pseudo-second-order kinetic reaction model and Freundlich isotherm model. About 26.0–41.2% of nano-Ag was trapped by the bound EPS (BEPS) matrix of activated sludge (especially LB-EPS) and 42.5–52.6% of them was adsorbed onto the inner cells after the adsorption. Moreover, the interaction energy contributions of EPS fractions followed the order of $E_{DE} > 0 > E_{TB} > E_{LB}$, suggesting DEPS in wastewater went against the removal of nano-Ag due to steric repulsion while LB-EPS and TB-EPS were positive to nano-Ag adsorption by modifying biomass surface and providing extensive binding sites. Besides, EPS fractions played significant roles in the adsorption of nano-Ag with low initial concentrations but had limited effect at high concentrations. Overall, this study investigated the effect of EPS fractions on the adsorption behaviors of nano-Ag by activated sludge biomass, which is meaningful to understand the removal mechanism of nanoparticles in sewage and the potential role of EPS fractions.

1. Introduction

With increasing production and application in numerous commercial field, silver nanoparticles (nano-Ag) have been found to be increasingly released into wastewater treatment plants (WWTPs) [1]. As barriers of most pollutants, WWTPs played important roles in preventing nanoparticles from entering the natural environments [2]. Conventional or modified activated sludge processes in WWTPs exhibited a considerable removal efficiency to nanoparticles via aggregation, precipitation, biosorption, and so on [3–6]. Accumulations of nanoparticles in activated sludge because of their high affinity to sludge biomass might be one of the primary physical removal mechanisms [4,7]. As one of the most common nanoparticles, nano-Ag have been studied the fate and influence in WWTPs [1,5,8]. Kiser et al. quantified and compared biosorption of eight nanoparticles exposed to wastewater biomass, and the removal

rates of nonfunctionalized and functionalized nano-Ag were 97% and 39%. They found that functionalization of nano-Ag seemed to hinder the interaction between nanoparticles and biomass surface [4]. Gu et al. made a comparison of nano-Ag removal by flocculent and granular sludge. The results showed that flocculent sludge favored the adsorption and enmeshment of nano-Ag, resulting from the specific loose “network” structure of extracellular polymeric substances (EPS) [1]. However, the removal mechanism of nano-Ag by activated sludge and the specific roles of EPS have not been investigated.

EPS in activated sludge are composed of protein, polysaccharide, nucleic acid, humic acid and other complex mixtures of high molecular weight [9]. In terms of the structure and composition, EPS can be categorized into three parts: dissolved EPS (DEPS), loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS) (Yu 2008, [10]). The presence of EPS played a significant role in the removal of nanoparticles, which

* Corresponding author.

E-mail address: hxfu@cqu.edu.cn (X. Huangfu).

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originated from the interaction between nanoparticles and EPS such as van der Waals forces, steric interactions, electrostatic interaction, complexation, and hydrophobic interaction [3,6]. Wei et al. studied the adsorption of ZnO and CuO NPs on extracted hydrophobic and hydrophilic EPS from activated sludge and found that hydrophobic EPS adsorbed more nanoparticles [11]. Moreover, experiments showed that hydroxyl, amino, carboxyl, amide groups and C-O-C groups of EPS favored nanoparticles binding/removal [11]. Sudheer Khan et al. revealed that electrostatic attraction contributed to the adsorption of exopolysaccharides on nano-Ag under neutral condition, while electrostatic repulsion occurred under $\text{pH} < 6$ [12]. Park et al. also conducted the removal of Ag, TiO_2 , and SiO_2 nanoparticles by activated sludge and found that the entrapment of nanoparticles by EPS enhanced their removal efficiencies by activated sludge [7]. However, in another article, NOM and EPS took no effect on the removal of Ag, TiO_2 and fullerol nanoparticles but reduced the removal of fullerenes from the bulk water phase by hindering the interaction between C_{60} nanoparticles and sludge cells [4]. Similar results were found in Yang et al.'s experiments that dissolved organic matter in wastewater could inhibit C_{60} nanoparticles adsorption on activated sludge by electrostatic and steric effect [13]. The removal characteristics of several nanoparticles by activated sludge have gained considerable attention, and both positive and negative effect of EPS on the removal of nanoparticles have been reported in previous studies. Therefore, whether can promote or inhibit the removal efficiency of nanoparticles by activated sludge and its reasons remain controversial. In addition, it is not clear whether different EPS fractions including DEPS, LB-EPS and TB-EPS can play different roles in the removal of nanoparticles, especially nano-Ag.

In this study, to investigate the effect of EPS sub-fractions on the adsorption of nano-Ag by activated sludge, the different activated sludge samples were harvested via extracting different EPS sub-fractions. We investigated and compared the adsorption kinetics and equilibrium of bare Ag (Bare-Ag), citrate coated Ag (CIT-Ag), polyvinylpyrrolidone coated Ag (PVP-Ag) nanoparticles with batch sorption experiments. The distribution of nano-Ag in different EPS fractions and sludge cells was measured to analyze the role of EPS fractions. Additionally, to quantify the contribution of EPS sub-fractions to the adsorption of nano-Ag to activated sludge, XDLVO theory was also employed to estimate the interaction energy between nano-Ag and different sludge. The systematic investigation on the contribution of different EPS sub-fractions to nano-Ag adsorption can contribute to understanding the role of different EPS sub-fractions in the removal of nano-Ag by activated sludge.

2. Materials and methods

2.1. Preparation and characterization of activated sludge samples

The activated sludge was collected from Dadukou wastewater treatment works in Chongqing. Collected samples were stored at 4°C in the laboratory. The sludge samples were rinsed three times with a buffer solution (10 mM NaCl, 4 mM NaHCO_3). For experiments of the adsorption kinetics and mechanism of nano-Ag, to avoid the interference of soluble microbial products (SMP) or DEPS, the activated sludge was centrifuged at 2000g for 5 min to remove DEPS. To further investigate the contributions of EPS fractions to the adsorption behaviors, activated sludge biomass was required to extract different EPS fractions [6,14]. The detailed method could be found in Supporting Information (Text S1). The solution pH was adjusted to 6.8 using 0.1 mM HCl and NaOH. The mean radius and zeta potentials of sludge samples were determined by a Malvern Zetasizer (Nano ZS, Malvern, UK). The hydrophobicity of different activated sludge samples was determined by measuring the relative hydrophobicity (RH) and contact angles (Text S2 and S3) [6].

2.2. Preparation and characterization of different Ag nanoparticles

Three types of nano-Ag were synthesized according to previous

studies [15,16]. The detailed synthesis methods were provided in the Supporting Information (Text S3). The mean radius and zeta potentials of sludge samples were determined by a Malvern Zetasizer (Nano ZS, Malvern, UK). The contact angle was measured as described in Text S4.

2.3. Adsorption batch experiments

The adsorption batch experiments were carried out by adding a certain concentration of nano-Ag (25 mL) to 25 mL sludge suspension (2 g SS/L) in a 100-mL flask. The pH and temperature were constant ($\text{pH} = 6.8$, $T = 25^\circ\text{C}$) and the flasks were stirred at 125 rpm in a water bath shaker. Short contact times of 500 min and 200 min were chosen to minimize nano-Ag aggregation and transformation, as well as growth or other changes in biomass. For the kinetics experiments, the initial nano-Ag concentrations were 5, 10 and 50 mg/L, and the samples were taken at a series of time intervals. For the isotherm experiments, the initial nano-Ag concentrations were 0.5, 1, 2, 5, 10, 20, 50, 100 and 150 mg/L and the equilibration time was 200 min.

2.4. Determination of nano-Ag concentration

The concentration of unadsorbed nano-Ag in the adsorption experiments was determined using inductively coupled plasma optical emission spectrometry (ICP-OES, Agilent 5100-VDV). Each extracted sample was centrifuged at 2000g for 5 min and the supernatant was harvested to determine unadsorbed nano-Ag concentration. To quantify the distribution of nano-Ag in sludge biomass, each sludge sample was required to extract LB and TB-EPS, and the harvested supernatant with EPS fractions was used to determine and calculate the amount of nano-Ag captured by LB-EPS, TB-EPS and inner cells. Avoidably, the heating and centrifugation procedure when extracting LB and TB-EPS might lead to the change in nano-Ag distributions in activated sludge [17]. Although there is a certain bias in this approach, it is reasonable to study the distribution of nano-Ag when there is no further feasible method. The harvested supernatant was first digested with concentrated nitric acid, and then filtered using a $0.45\text{-}\mu\text{m}$ syringe filter. Herein, the method of digestion followed by filtration was chosen to avoid the loss of nano-Ag as much as possible. The diluted samples were stored at 4°C prior to determining Ag^+ concentrations. The amount of nano-Ag adsorbed by activated sludge was calculated according to the following equation [17]:

$$q_t = \frac{C_0 - C_e}{\text{SS}} \quad (1)$$

Where q_t is the concentration (mg/g) of nano-Ag adsorbed by activated sludge at time t , C_0 (mg/L) and C_e (mg/L) are the initial and equilibrium nano-Ag concentrations in solution.

2.5. Adsorption isotherms and adsorption kinetics

Two of the most common adsorption models including Langmuir model and Freundlich model are widely applied to model the adsorption of dyes and heavy metals on activated sludge [17,18]. In this study, they were also applied to fit the experimental data according to the following equations:

$$q_e = \frac{q_m b C_e}{1 + b C_e} \quad (2)$$

$$q_e = K_F C_e^{1/n} \quad (3)$$

where q_m is the maximum adsorption capacity of nano-Ag on activated sludge under a given condition and b is a Langmuir constant which reflects the affinity of the adsorbent to the adsorbate. K_F and n are Freundlich constants related to the maximum adsorption capacity and adsorption intensity, respectively. The favorability of the adsorption

process can be indicated by the equilibrium parameter R_L in the Langmuir isotherm. The value of R_L can provide information about whether the adsorption is irreversible ($R_L = 0$), favorable ($0 < R_L < 1$), linear ($R_L = 1$) or unfavorable ($R_L > 1$).

$$R_L = \frac{1}{1 + bC_0} \quad (4)$$

Pseudo-first-order kinetic model, pseudo-second-order kinetic model, and intra-particle diffusion model which are used to analyze the adsorption kinetics characteristics of nano-Ag by different activated sludge biomass are expressed as follows:

$$\ln(q_e - q_t) = \ln q_e - k_1 t \quad (5)$$

$$\frac{t}{q_t} = \frac{1}{q_e^2 k_2} + \frac{t}{q_e} \quad (6)$$

$$q_t = k_i (t^{0.5}) + C \quad (7)$$

where q_e and q_t are the amounts of nano-Ag adsorbed by biomass at equilibrium and at time t , respectively. k_1 , k_2 , and k_i are the corresponding rate constant of each model, respectively.

2.6. Interaction energy contribution of EPS fractions

The removal of nano-Ag was attributed to the adsorption of activated sludge biomass including LB-EPS, TB-EPS and sludge cells while free DEPS did not contribute this removal. The surface characteristics of sludge surface changed significantly with the removal of the LB-EPS and TB-EPS, which may lead to their contributions to the interaction between nano-Ag and biomass surface were removed step by step. XDLVO theory including van der Waals interaction, electrostatic interaction, Lewis acid-base interaction and steric repulsion was applied to calculate the interaction energy between nano-Ag and biomass surface (Text S5). Surface heterogeneity of activated sludge with and without LB and TB-EPS might change the interaction energy but it is difficult to distinguish. Here, since the surface of activated sludge was much larger than that of nano-Ag, the surface heterogeneity was not considered and surface of activated sludge was assumed to be smooth. The steric repulsion considered only one side polymeric layer from nano-Ag and the other side from activated sludge was considered same for different types of nano-Ag. The sludge cells, LB-EPS, TB-EPS and DEPS were assumed to be responsible for the total interaction energy of this system, which can be expressed as follows:

$$E_{S+DE} = E_S + E_{DE} = E_{S-TB} + E_{LB} + E_{DE} = E_{S-TB} + E_{TB} + E_{LB} + E_{DE} \quad (8)$$

Where E_{S+DE} and E_S are the total interaction energy between nano-Ag and raw sludge in the presence and absence of DEPS, respectively. E_{S-LB} , E_{S-TB} are the total interaction energy between nano-Ag and sludge with TB-EPS extracted. E_{DE} , E_{LB} and E_{TB} are the contribution of interaction energy from DEPS, LB-EPS and TB-EPS, which can be calculated according to the following equations:

$$E_{DE} = E_{S+DE} - E_S \quad (9)$$

$$E_{LB} = E_S - E_{S-LB} \quad (10)$$

$$E_{DE} = E_{S-LB} - E_{S-TB} \quad (11)$$

3. Results and discussions

3.1. Effect of contact time

In order to more conveniently characterize the adsorption of nano-Ag by activated sludge microorganisms, the activated sludge used in the experiment was needed to remove soluble microbial products (SMP)

by a simple centrifugation. SMP dissolved in water were organic matter secreted by activated sludge microorganisms, also known as DEPS. In this study, when exploring the adsorption experiment of activated sludge, we did not consider the free DEPS as part of activated sludge with biological adsorption function. First, the effect of contact time on nano-Ag adsorption was experimentally studied when the initial nano-Ag concentration was 5, 10 and 50 mg/L and the activated sludge concentration was 2 g/L SS. The relation between removal efficiency and adsorption time is shown in Fig. 1. For the addition of all the initial concentrations of different nano-Ag, it found that a large amount of nano-Ag could be removed rapidly within 5 min, and the adsorption of activated sludge reached equilibrium within 20–100 min. The contact time was further increased to 500 min, and the adsorption amount did not increase significantly. The initial rapid absorption of nano-Ag may be due to the high initial concentration of nano-Ag and abundant binding sites on the activated sludge surface. The saturation of the binding sites resulted in the subsequent slow adsorption. Moreover, with the increase of the initial concentration, the removal efficiency of nano-Ag gradually decreased, which proved that the role of biological adsorption by activated sludge was limited. Additionally, the removal efficiency of different types of nano-Ag was different, and the order of removal efficiency followed Bare-Ag > CIT-Ag > PVP-Ag. Particularly, the removal efficiency of exposed Bare-Ag at low concentration (5 mg/L) was about 95%, while that of PVP-Ag was about 65% (Fig. 1). The exposed Bare-Ag was more easily adsorbed on the surface of activated sludge due to its high hydrophobicity. The concentration of Bare-Ag used in the experiment was also low because of its low dispersion in water. CIT-Ag was more hydrophilic than Bare-Ag, so the adsorption efficiency was lower than that of Bare-Ag. Nano-Ag modified by the PVP layer had the lowest removal efficiency due to the increased steric hindrance. Previous studies also reported that the removal rates of unfunctionalized and functionalized nano-Ag were 97% and 39%, respectively [4]. Furthermore, the XDLVO theory was applied to calculate the interaction energies between nano-Ag and biomass surface (Fig. S1). The largest energy barrier existed between PVP-Ag and sludge surface, indicating that there was a strong repulsive force between them. The lowest energy barrier between Bare-Ag and sludge surface suggested the adsorption of Bare-Ag by activated sludge was the most favorable.

3.2. Adsorption isotherms and adsorption kinetics

The adsorption isotherms of three nano-Ag by activated sludge at the initial pH value of 6.8, temperature of 25 °C and stirring speed of 125 rpm are shown in Fig. 2. Two commonly used adsorption isotherms, Langmuir and Freundlich model, were selected to fit the data. The Langmuir model was commonly used for monolayer adsorption, which was assumed to occur at specific homogeneous sites. The Freundlich model was an empirical equation that describes the non-uniform surface energy by multi-layer adsorption, which can estimate the adsorption strength of activated sludge. Fig. 2 and Table 1 show the fitting results of Langmuir and Freundlich models for the adsorption of nano-Ag. Observed from R^2 value, the two models were in good agreement with the adsorption experimental data. The equilibrium parameter $R_L < 1$ in the Langmuir model indicated that the adsorption process was favorable (Table 1). The value of n in the Freundlich model ranged from 1 to 10, indicating that nano-Ag had good adsorption performance on activated sludge. Observed from the experimental data, the adsorption capacity of activated sludge on Bare-Ag should be the maximum, but the maximum adsorption capacity predicted by the Langmuir model was CIT-Ag > PVP-Ag > Bare-Ag. This was due to the error caused by the low concentration of Bare-Ag in this experiment. The value of K_F in the Freundlich model conformed to Bare-Ag > CIT-Ag > PVP-Ag, which was close to the experimental results. This indicated that the Freundlich model was more suitable for simulating the adsorption of nano-Ag on activated sludge than the Langmuir model. Therefore, the adsorption of nano-Ag was likely to occur on the heterogeneous surface of activated

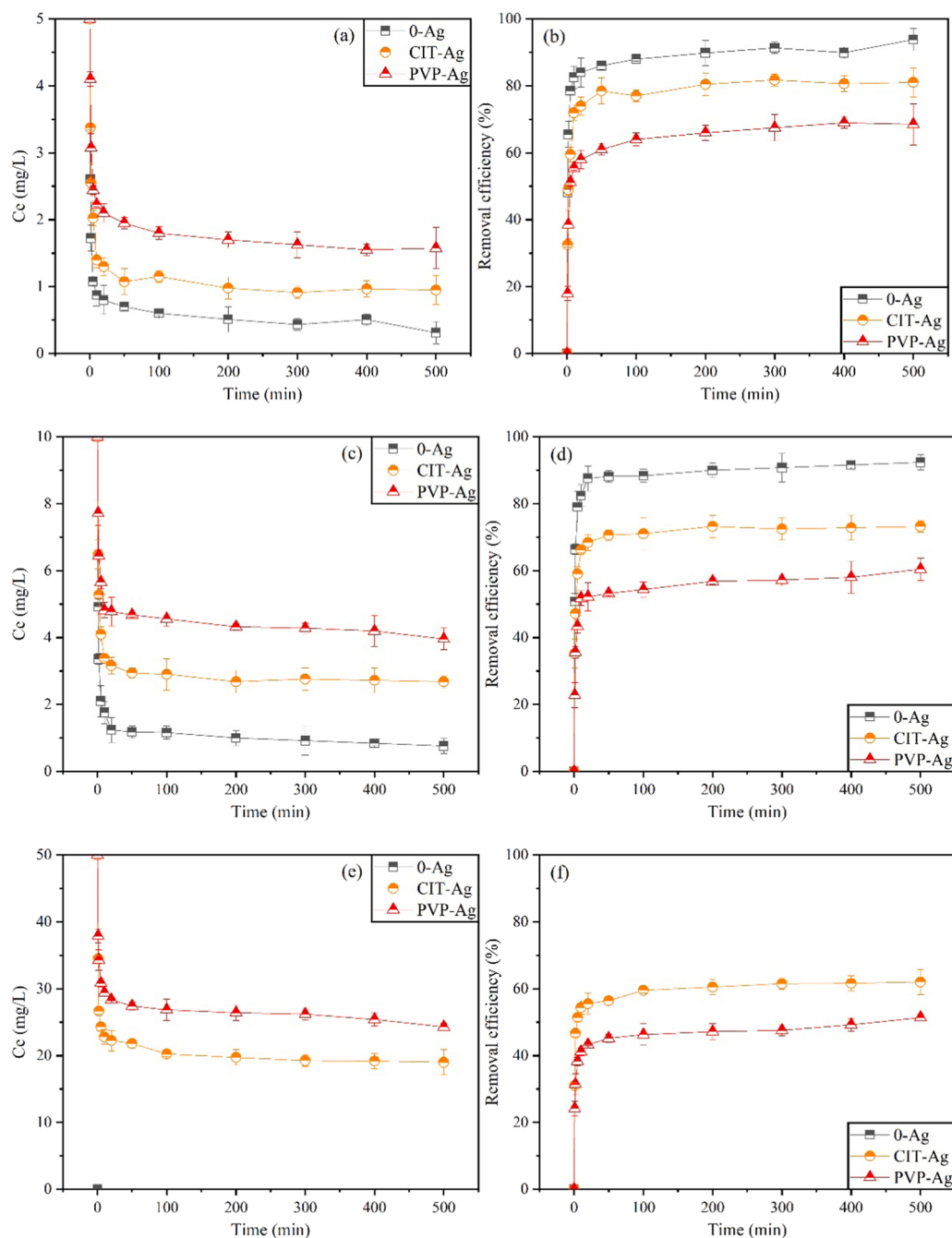


Fig. 1. The change of the equilibrium concentration and removal efficiency of nano-Ag with time. Bare-Ag: (a) and (b); CIT-Ag: (c) and (d); PVP-Ag: (e) and (f).

sludge [13,19].

The reaction mechanism of nano-Ag adsorption on the surface of activated sludge was investigated by three kinetic models: pseudo-first-order kinetic model, pseudo-second-order kinetic model, and intra-particle diffusion model. Conditions of the adsorption process are shown in Fig. 1 and the modeling results are shown in Table 2. The pseudo first order reaction kinetic model was the most widely used biological adsorption kinetic model. The rate constant k_1 and the calculated equilibrium capacity ($q_{e\text{ cal}}$) were calculated from the slope and intercept of the $\ln(q_e - q_t)$ - t curve, which should be a straight line to confirm the applicability of the first-order kinetic model. The results showed that the correlation coefficient (R^2) of all nano-Ag samples was low, and the $q_{e\text{ cal}}$ value did not match the $q_{e\text{ exp}}$ value (Table 2). The pseudo-first-order equation is not a true first-order equation; the constant k_1 does not represent the total number of available sites, and the

adjustable parameter $\ln(q_e)$ is usually not equal to the intercept of the curve. Therefore, the adsorption of nano-Ag on the surface of activated sludge did not conform to pseudo-first-order kinetic. The pseudo-second-order kinetic model suggests that chemisorption is rate-controlling mechanism. The predicted maximum adsorption capacity was in good agreement with the experimental results. Moreover, all the R^2 were greater than 0.998, indicating that the pseudo-second-order kinetic could well simulate the adsorption of nano-Ag on activated sludge. A lower k_2 indicated a faster adsorption process. k_2 value decreased with the increase in the initial concentration of nano-Ag (Table 2), indicating that high initial concentration of nano-Ag favored the adsorption. Moreover, the adsorption rates of Bare-Ag and PVP-Ag were faster than that of CIT-Ag, which may be related to the high negative surface charge and hydrophilicity of CIT-Ag. The kinetic model of intra-particle diffusion can be expressed as multiple linearity.

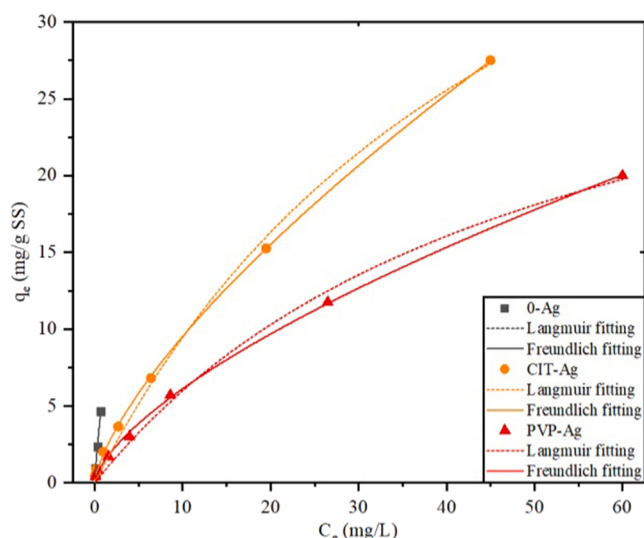


Fig. 2. Adsorption isotherms of nano-Ag onto activated sludge (25 °C).

Table 1

A comparison of the Langmuir and Freundlich adsorption isotherm constants.

Ag samples	Langmuir constants				Freundlich constants		
	q_m	b	R_L	R^2	K_F	n	R^2
Bare-Ag	21.39	0.397	0.201	0.999	6.365	1.147	0.999
CIT-Ag	59.00	0.019	0.345	0.984	1.876	1.418	0.999
PVP-Ag	36.56	0.020	0.333	0.983	1.330	1.509	0.999

The whole process usually consists of two or three steps [17]. The constants of the adsorption process simulated by the intra-particle diffusion model are shown in Table 2. Obviously, the adsorption of nano-Ag occurred on the surface of activated sludge, and the adsorption can quickly reach saturation (Fig. 1). Then, nano-Ag entered the pores of activated sludge and was absorbed by the inner cells. As the concentration of nano-Ag in the solution increased, the value of k_{i2} increased. The predicted result of the intra-particle diffusion model were not in good agreement with the experimental data. Therefore, the adsorption of nano-Ag by activated sludge generally conformed to the pseudo-second-order kinetic model.

3.3. Distribution of nano-Ag after adsorption

Previous experiments showed that the adsorption of nano-Ag occurred on the heterogeneous surface of activated sludge. The microbial surface of activated sludge was surrounded by BEPS, presenting a dynamic bilayer structure. In order to study the effect of BEPS on the adsorption of nano-Ag by activated sludge, we investigated the distribution of nano-Ag in the components of activated sludge after the

adsorption of three nano-Ag by activated sludge, and the results are shown in Fig. 3. In general, the distribution of nano-Ag can be divided into three parts, including aqueous solution ($[Ag]_{Free}$), EPS ($[Ag]_{EPS}$) and inner cell ($[Ag]_{Cell}$). When the initial concentration of nano-Ag was 5 mg/L, EPS, especially LB-EPS, contributed a lot to the removal of nano-Ag (Fig. 3). About 26.0–41.2% of nano-Ag was trapped by the EPS matrix of activated sludge. Nano-Ag captured by EPS was not easy to diffuse to the inner cells, and 42.5–52.6% of nano-Ag was adsorbed to the inner cells. Moreover, the content of the three nano-Ag adsorbed by EPS and inner layer cells decreased in the order of Bare-Ag > CIT-Ag > PVP-Ag, which was consistent with the adsorption capacity of the three nano-Ag (Fig. 1). Notably, by calculating the ratio of $[Ag]_{Cell}$ to $[Ag]_{Total}$, it can be known that the amount of adsorbed Bare-Ag in contact with inner cells (54.8%) was a little lower than that of CIT-Ag (58.3%) and PVP-Ag (62.0%). This was contrary to the adsorption capacity of nano-Ag, indicating that the stronger the interaction between EPS and nano-Ag was, the less likely the nano-Ag diffused to the inner layer of activated sludge.

Fig. 3(b) shows the distribution of nano-Ag at the initial Ag concentration of 50 mg/L. Only 10.3–15.5% of nano-Ag was distributed in EPS, and 45% of nano-Ag was absorbed into the inner cells. At higher initial concentrations, the proportion of nano-Ag distributed in EPS and inner cells decreased, but the amount of adsorption increased. In addition, it can be inferred that EPS contributed more when the initial concentration of nano-Ag was lower. It mainly depended on the adsorption of internal cells under high initial concentrations. Therefore, the interception capacity of EPS was actually limited as the sludge concentration and EPS content were low.

3.4. Contribution of EPS fractions to nano-Ag adsorption

In order to further understand the effect of EPS on the adsorption of nano-Ag, we extracted different EPS fractions from activated sludge and investigated the adsorption experiment of nano-Ag by activated sludge after removing different EPS components. Herein, we included DEPS into the composition of EPS to explore its influence on the adsorption behavior of activated sludge. Fig. S2 shows the change of the equilibrium concentration of CIT-Ag absorbed by activated sludge with different EPS fractions. When the initial concentration of CIT-Ag was 5 mg/L, different EPS components affected the adsorption of activated sludge. The presence of DEPS inhibited the adsorption of nano-Ag, which was similar to the results reported in other literatures [13]. As macromolecules, DEPS may act as depleting agents, providing either attractive or repulsive interactions depending on whether they adsorb to the surface of colloids or are suspended in the solution; if they are suspended freely in the solution, they will function as depleting agents and produce attractive depletion interactions; Conversely, if they are attached to colloidal surfaces, they will function as polymer brushes and lead to steric repulsion [20,21]. In this study, DEPS was likely to adsorb on the surface of nano-Ag, inhibiting the attachment of nano-Ag to activated sludge through steric hindrance [4,13]. However, DEPS had negligible effect on the removal of nano-Ag with high initial

Table 2

Adsorption kinetic models parameters for adsorption of nano-Ag onto activated sludge.

C_0 (mg/L)	Ag samples	q_e exp	Pseudo-first-order kinetics			Pseudo-second-order kinetics			Intra-particle diffusion				
			q_e cal	k_1	R^2	q_e cal	k_2	R^2	q_e cal	k_{i1}	k_{i2}	k_{i3}	R^2
5	Bare-Ag	2.35	0.40	0.005	0.582	2.31	0.020	0.999	2.33	9.33	2.63	0.101	> 0.789
	CIT-Ag	2.03	0.36	0.011	0.717	2.04	0.031	0.999	1.72	8.27	1.92	0.080	> 0.707
	PVP-Ag	1.71	0.46	0.008	0.863	1.73	0.019	0.999	1.28	7.00	1.92	0.125	> 0.820
10	Bare-Ag	4.65	0.80	0.007	0.715	4.61	0.009	0.999	4.76	23.20	5.40	0.193	> 0.796
	CIT-Ag	3.66	0.70	0.009	0.724	3.66	0.018	0.999	3.37	18.52	4.63	0.155	> 0.782
	PVP-Ag	3.02	3.48	0.001	0.427	2.98	0.010	0.999	2.47	14.92	4.52	0.209	> 0.977
50	Bare-Ag	—	—	—	—	—	—	—	—	—	—	—	—
	CIT-Ag	15.3	2.80	0.010	0.877	15.52	0.007	0.999	16.33	31.48	5.55	0.402	> 0.917
	PVP-Ag	11.8	3.16	0.005	0.699	12.60	0.004	0.998	11.29	25.37	4.38	0.430	> 0.929

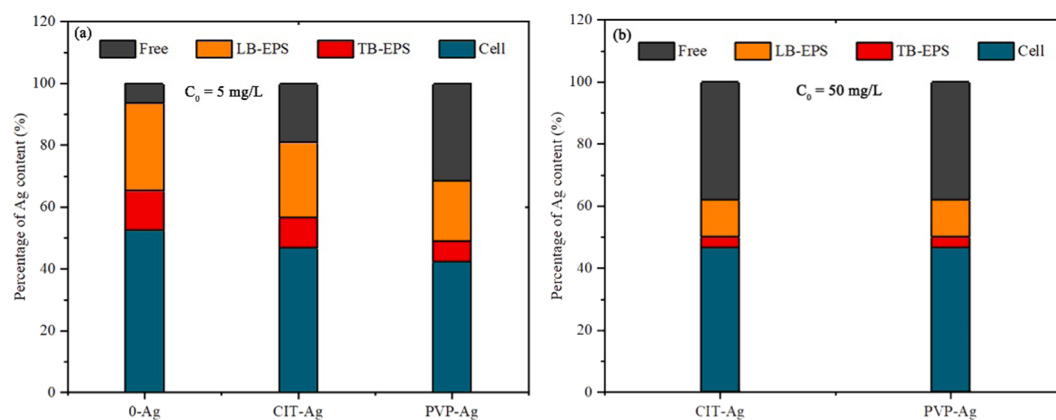


Fig. 3. The distributions of nano-Ag among activated sludge, (a) $C_0 = 5$ mg/L; (b) $C_0 = 50$ mg/L.

concentration (Fig. 4a). As the concentration of activated sludge was low and DEPS content was low, the removal of nano-Ag was not affected. The physicochemical properties and special location of EPS make them important components to maintain the structure and function of microbial aggregates [22]. As the first barrier of microbial cells, BEPS also played an important role in resisting the invasion of external toxic substances [3,23,24]. Observed from Fig. 4a, the adsorption ability of activated sludge to nano-Ag was significantly weakened after removing LB-EPS and TB-EPS (Fig. 4). Compared with high initial concentration of nano-Ag, the effect of BEPS at low concentration was greater (Fig. 4a). Therefore, BEPS may contribute more to the removal of nano-Ag at low concentration while the effect of BEPS at high concentration was limited. The role of BEPS in nano-Ag removal may be attributed to several reasons. BEPS on surfaces of activated sludge contain polysaccharides and proteins as dominant components together with some lipid, nucleic acids, and humic-like substances, which are likely to take part in the removal of nano-Ag by activated sludge [25]. The hydrophobic components, such as proteins, amino acids and lipids, may facilitate the adsorption of nano-Ag by hydrophobic interaction [15,26]. Hydrophilic polysaccharides consist of long chains of monosaccharide units bound together by glycosidic bonds with linear to highly branched structure, determining the “network” structure of EPS [27]. This “network” structure of EPS is related to large specific surface area and surface roughness, which may be beneficial to the attachment and capture of nano-Ag [1,24]. On the other hand, after removing LB-EPS and TB-EPS from activated sludge, the surface negative charge was lower and the surface hydrophobicity was reduced (Table S1). From the perspective of interfacial interaction, the increased electrostatic repulsion and reduced hydrophobic effect may also explain the decrease of

the removal efficiency of activated sludge without LB-EPS and TB-EPS. The XDLVO theory was applied to calculate the interaction energy between CIT-Ag and biomass surface (Fig. S3). There was a maximum energy barrier between nano-Ag and biomass surface in the presence of DEPS due to the additional steric repulsion energy. After the removal of BEPS sub-fractions, it seemed to exhibit higher energy barriers compared with the raw sludge, indicating that the adsorption of nano-Ag by activated sludge decreased in the absence of LB and TB-EPS. Furthermore, the difference value of each XDLVO interaction energy was calculated to determine the contributions of DEPS, LB-EPS, and TB-EPS (E_{DE} , E_{LB} and E_{TB}) to nano-Ag adsorption (Fig. 4b). The values of E_{DE} were positive, E_{LB} and E_{TB} were negative as nano-Ag approached biomass surface, which suggested DEPS had a negative effect on nano-Ag removal while LB-EPS and TB-EPS were positive. Moreover, LB-EPS seemed to contributed more to the attachment of nano-Ag because E_{LB} were more negative than E_{TB} .

4. Conclusions

The adsorption behaviors of nano-Ag by activated sludge were studied to investigate the contributions of EPS fractions. The results showed that electrostatic force, hydrophobic interaction and steric repulsion were responsible for the distinct adsorption behaviors of different nano-Ag on the surface of activated sludge, and the adsorption process was in accordance with pseudo-second-order kinetic reaction model and Freundlich isotherm model. A small part of nano-Ag was distributed in BEPS matrix after adsorption by activated sludge, and most of them were still in contact with internal cells. With the increase of the initial concentration of nano-Ag, the proportion of nano-Ag in EPS

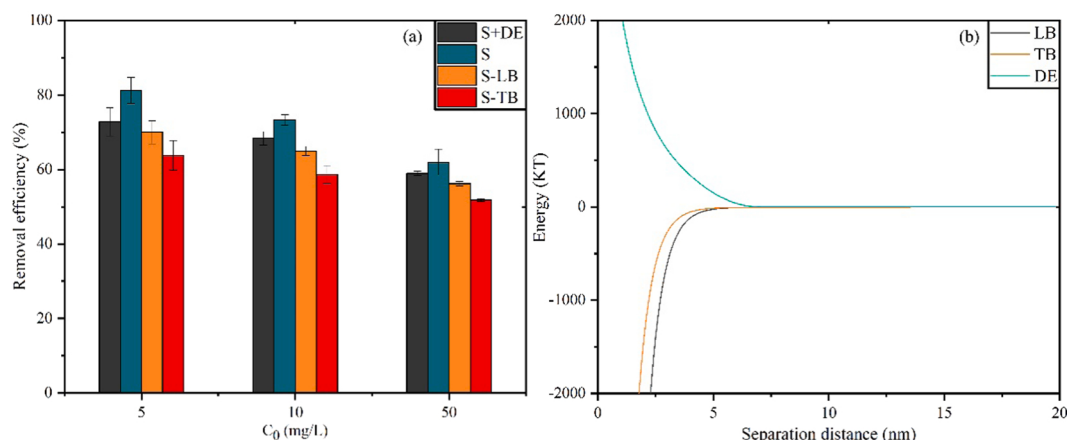


Fig. 4. (a) Effect of EPS fractions on the removal efficiency of CIT-Ag; (b) The contribution of the interaction energy from DEPS, LB-EPS and TB-EPS.

decreased. Furthermore, the contributions of different EPS fractions to the biosorption behaviors of activated sludge were studied, and the result found that DEPS in wastewater was not conducive to the removal of nano-Ag, while BEPS on the surface of activated sludge had a significant effect on nano-Ag adsorption. This was related to the spatial structure and extensive binding sites of BEPS, as well as the change of surface characteristics of activated sludge in the presence of BEPS.

CRedit authorship contribution statement

Yanghui Xu: Data curation, Writing - original draft. **Qin Ou:** Data curation, Writing - original draft. **Qiang He:** Writing - review & editing, **Zhengsong Wu:** Writing - review & editing, **Jun Ma:** Writing- review & editing, **Xiaoliu Huangfu:** Conceptualization, Methodology.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supporting Information

Extraction of different EPS fractions; Relative hydrophobicity measurements; Preparation of different nano-Ag; Contact angle measurements and interfacial energy calculation; The XDLVO interaction energy calculation; Characteristics of the sludge samples and nano-Ag; The XDLVO interaction energies between nano-Ag and sludge surface; Effect of EPS fractions on the adsorption kinetics of CIT-Ag nanoparticles by activated sludge.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jece.2022.108316.

References

- [1] L. Gu, Q. Li, X. Quan, Y. Cen, X. Jiang, Comparison of nanosilver removal by flocculent and granular sludge and short- and long-term inhibition impacts, *Water Res* 58 (2014) 62–70.
- [2] B. Nowack, T.D. Bucheli, Occurrence, behavior and effects of nanoparticles in the environment, *Environ. Pollut.* 150 (1) (2007) 5–22.
- [3] X. Huangfu, Y. Xu, C. Liu, Q. He, J. Ma, C. Ma, R. Huang, A review on the interactions between engineered nanoparticles with extracellular and intracellular polymeric substances from wastewater treatment aggregates, *Chemosphere* 219 (2019) 766–783.
- [4] M.A. Kiser, H. Ryu, H. Jang, K. Hristovski, P. Westerhoff, Biosorption of nanoparticles to heterotrophic wastewater biomass, *Water Res* 44 (14) (2010) 4105–4114.
- [5] Z. Liang, A. Das, Z. Hu, Bacterial response to a shock load of nanosilver in an activated sludge treatment system, *Water Res* 44 (18) (2010) 5432–5438.
- [6] Q. Ou, Y. Xu, X. Li, Q. He, C. Liu, X. Zhou, Z. Wu, R. Huang, J. Song, X. Huangfu, Interactions between activated sludge extracellular polymeric substances and model carrier surfaces in WWTPs: a combination of QCM-D, AFM and XDLVO prediction, *Chemosphere* (2020) 253.
- [7] H.J. Park, H.Y. Kim, S. Cha, C.H. Ahn, J. Roh, S. Park, S. Kim, K. Choi, J. Yi, Y. Kim, J. Yoon, Removal characteristics of engineered nanoparticles by activated sludge, *Chemosphere* 92 (5) (2013) 524–528.
- [8] Y. Wang, P. Westerhoff, K.D. Hristovski, Fate and biological effects of silver, titanium dioxide, and C60 (fullerene) nanomaterials during simulated wastewater treatment processes, *J. Hazard Mater.* 201–202 (2012) 16–22.
- [9] Y. Salama, M. Chennaoui, A. Sylla, M. Mountadar, M. Rihani, O. Assobhei, Characterization, structure, and function of extracellular polymeric substances (EPS) of microbial biofilm in biological wastewater treatment systems: a review, *Desalin. Water Treat.* 57 (35) (2015) 16220–16237.
- [10] G.H. Yu, P.J. He, L.M. Shao, P.P. He, Stratification structure of sludge flocs with implications to dewaterability, *Environ. Sci. Technol.* 42 (21) (2008) 7944–7949.
- [11] L. Wei, J. Ding, M. Xue, K. Qin, S. Wang, M. Xin, J. Jiang, Q. Zhao, Adsorption mechanism of ZnO and CuO nanoparticles on two typical sludge EPS: Effect of nanoparticle diameter and fractional EPS polarity on binding, *Chemosphere* 214 (2018) 210–219.
- [12] Sudheer Khan, S., A. Mukherjee and N. Chandrasekaran, Interaction of colloidal silver nanoparticles (SNPs) with exopolysaccharides (EPS) and its adsorption isotherms and kinetics, *Colloids Surf. A: Physicochem. Eng. Aspects* 381 (1–3) (2011) 99–105.
- [13] Y. Yang, N. Nakada, H. Tanaka, Adsorption of fullerene nC60 on activated sludge: Kinetics, equilibrium and influencing factors, *Chem. Eng. J.* 225 (2013) 365–371.
- [14] Y. Xu, Q. Ou, X. Zhou, Q. He, Z. Wu, R. Huang, J. Song, J. Ma, X. Huangfu, Impacts of carrier properties, environmental conditions and extracellular polymeric substances on biofilm formation of sieved fine particles from activated sludge, *Sci. Total Environ.* (2020) 731.
- [15] J.E. Song, T. Phenrat, S. Marinakos, Y. Xiao, J. Liu, M.R. Wiesner, R.D. Tilton, G. V. Lowry, Hydrophobic interactions increase attachment of gum Arabic- and PVP-coated Ag nanoparticles to hydrophobic surfaces, *Environ. Sci. Technol.* 45 (14) (2011) 5988–5995.
- [16] Y. Yin, M. Shen, Z. Tan, S. Yu, J. Liu, G. Jiang, Particle coating-dependent interaction of molecular weight fractionated natural organic matter: impacts on the aggregation of silver nanoparticles, *Environ. Sci. Technol.* 49 (11) (2015) 6581–6589.
- [17] Y.X. Song, C.H. Lu, P. Liu, X.L. Chai, X. Chen, X.B. Min, C.J. Tang, L.Y. Chai, Insights into the role of extracellular polymeric substances in Zn(2+) adsorption in different biological sludge systems, *Environ. Sci. Pollut. Res Int* 25 (36) (2018) 36680–36692.
- [18] M. Basibuyuk, C.F. Forster, An examination of the adsorption characteristics of a basic dye (Maxilon Red BL-N) on to live activated sludge system, *Process Biochem.* 38 (9) (2003) 1311–1316.
- [19] D. Ding, Y. Zhao, S. Yang, W. Shi, Z. Zhang, Z. Lei, Y. Yang, Adsorption of cesium from aqueous solution using agricultural residue-walnut shell: equilibrium, kinetic and thermodynamic modeling studies, *Water Res* 47 (7) (2013) 2563–2571.
- [20] J. Choi, G. Kim, S. Choi, K. Kim, Y. Han, S. Bradford, S. Choi, H. Kim, Application of depletion attraction in mineral flotation: i. theory, *Minerals* 8 (2018) 10.
- [21] G. Kim, J. Choi, S. Choi, K. Kim, Y. Han, S. Bradford, S. Choi, H. Kim, Application of depletion attraction in mineral flotation: ii. effects of depletant concentration, *Minerals* 8 (2018) 10.
- [22] Chen, Lili, Yang, Ying, Ye, Tingjin, Guo, Wu, Zhen, S. J. J. o. C. Technology and Biotechnology (2015). "Extraction of extracellular polymeric substances in activated sludge using sequential extraction.
- [23] J. Song, Y. Xu, C. Liu, Q. He, R. Huang, S. Jiang, J. Ma, Z. Wu, X. Huangfu, Interpreting the role of NO3(-), SO4(2-), and extracellular polymeric substances on aggregation kinetics of CeO2 nanoparticles: measurement and modeling, *Ecotoxicol. Environ. Saf.* 194 (2020), 110456.
- [24] H. Zhang, Y. Jia, S.K. Khanal, H. Lu, H. Fang, Q. Zhao, Understanding the role of extracellular polymeric substances on ciprofloxacin adsorption in aerobic sludge, anaerobic sludge, and sulfate-reducing bacteria sludge systems, *Environ. Sci. Technol.* 52 (11) (2018) 6476–6486.
- [25] D.Q. Yuan, Y.L. Wang, J. Feng, Contribution of stratified extracellular polymeric substances to the gel-like and fractal structures of activated sludge, *Water Res* 56 (2014) 56–65.
- [26] T. Du, G. Shi, F. Liu, T. Zhang, W. Chen, Sulfidation of Ag and ZnO nanomaterials significantly affects protein corona composition: implications for human exposure to environmentally aged nanomaterials, *Environ. Sci. Technol.* 53 (24) (2019) 14296–14307.
- [27] H.Q. Yu, Molecular insights into extracellular polymeric substances in activated sludge, *Environ. Sci. Technol.* 54 (13) (2020) 7742–7750.