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Original Research

Adsorption Characteristics between Different Sizes of Microplastics and EPS Fractions of Anaerobic Granular Sludge

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Abstract

The adsorption characteristics between extracellular polymeric substances (EPS) and microplastics polystyrene (PS) in anaerobic granular sludge were studied. In anaerobic granular sludge, the smaller the particle size of microplastics, the more obvious the increase of total EPS. 0.5 um PS promotes an increase in EPS to 1.14 times that of the blank reactor, 200 um PS reduces the EPS to 0.91 times. Among the five fractions of EPS, the hydrophilic fraction (HPI) fraction can resist the toxicity of small size microplastics, so the increment is the most obvious. 5 um PS promotes an increase in the relative intensity of HPI from 55.5 mg/gVS to 87.2 mg/gVS. The adsorption behavior of the five fractions of EPS on microplastics PS can be well described by pseudo-second-order kinetics model and Freundlich model. Hydrophobic interaction, π-π electron-donor-receptor interaction, chemical adsorption and electrostatic attraction simultaneously affect the adsorption of five fractions of EPS by PS. The analysis of infrared spectroscopy, which showed that O-H stretching vibration, carbonyl C-O group, amide-1, amide-2 absorptivity, and phenol C-O bond have a great contribution to the adsorption of microplastics. It can be seen that small particle sizes of PS have a more significant impact on sludge EPS.

Keywords: sludge, extracellular polymeric substances (EPS), microparticles (MPs), polystyrene (PS)

Introduction

Environmental problems caused by wastewater have attracted more and more attention in recent years [1]. However, as a by-product of wastewater treatment plants (WWTPs), the pollution of sludge to the environment is often ignored. This is mainly because sludge is not only difficult to be effectively treated and high-cost [2], but also has the risk of secondary pollution [3]. Sludge anaerobic digestion technology is one of the most popular technologies for sludge reduction, stabilization and recycling at present, and is widely used by large WWTPs [4]. Due to improper disposal, there were a large number of unmanaged plastic wastes in the natural environment that were gradually transformed into microplastics of

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different sizes through the action of wind, light, heat and microorganisms in the natural world [5]. It was found that 95%~99% of microplastics in wastewater were captured by sludge [6], resulting in a dry weight of about 1×10^3 n/kg-2.40×10^4 n/kg of microplastics enters the sludge of the sewage plant [7]. The intensive physical, chemical, and biological processes in sewage treatment plants can alter the physical and chemical properties of MPs, thereby enhancing their adsorption potential for pollutants. In addition, multiple studies had shown that MPs can significantly affect the performance of anaerobic digestion, which was closely related to the content of MPs [8]. Wei et al. found that both polyvinyl chloride particles and polyethylene particles can induce reactive oxygen species and have adverse effects on the microbial community, thereby inhibiting the anaerobic digestion reaction of sludge [9]. Therefore, the inhibition of microplastics on anaerobic digestion efficiency of sludge has become a current research focus [10].

The extracellular polymeric substances (EPS) is the skeleton of anaerobic granular sludge. EPS can not only agglomerate into micro gel that affect carbon flux, but also participate in the adhesion of microorganisms and pollutants to affect the migration of pollutants [11]. On the one hand, it supports the formation of sludge aggregates as a skeleton, and on the other hand, it acts as a protective agent to protect cells from external shocks [12]. Microplastics can inhibit anaerobic digestion by adsorbing on EPS of anaerobic granular sludge. The shape of microplastics was an important basis for determining its surface charge and bonding behavior. In theory, the smaller the size of the microplastics, the higher the binding efficiency with the sludge EPS surface. In general, the types, size, reaction temperature and physical and chemical properties of microplastics all affect their adsorption characteristics on sludge EPS. On the other hand, the chemical composition of sludge EPS and its adsorption on microplastics have also received considerable attention [13]. Existing research has found that smaller sized plastics can trigger protein rich EPS [14]. The presence of MPs can lead to the generation of different components in EPS [15]. Most previous studies focused on the inhibition of single size microplastics on anaerobic granular sludge [16]. At present, the influence of the size of microplastics on the different fractions of EPS, the main substances of anaerobic granular sludge, is urgently to be studied.

In this paper, we used ammonium hydroxide solution to extract EPS from anaerobic granular sludge, and then fractionate it into five fractions. In this paper, the effects of typical microplastics Polystyrene (PS) on the yield of EPS in anaerobic granular sludge. In addition, due to the excellent decontamination effect of the adsorption method [17], the adsorption characteristics of five fractions of EPS on PS and the chemical structure changes of each fraction of EPS during the adsorption process were also studied. It is expected to provide theoretical support for the treatment of microplastics in the environment.

Materials and Methods

Characteristics of Microplastics

Small size PS microplastics (0.5 um, 5 um and 50 um) purchased from Hug biotechnology Co., Ltd. (Shanghai, China), while large PS microplastics (100 um and 200 um) purchased from Zhongcheng Co., Ltd. (Guangzhou, China).

Effect of Microplastics Size on EPS

Since the anaerobic granular sludge samples used in this experiment were from the laboratory scale Up-flow Anaerobic Sludge Bed/Blanket (UASB). Firstly, 2 g TSS/L of anaerobic granular sludge and 100ml of synthetic wastewater prepared from glucose, NH_4Cl and KH_2PO_4 as the main sources of carbon, nitrogen and phosphorus and added with various trace elements were put into the serum bottle [18]. Secondly, add 0.5 μ m, 5 μ m, 50 μ m, 100 μ m and 200 μm PS particles 10 mg/L (mass concentration) [19] into 5 serum bottles in turn. The last bottle was used as a blank test without adding PS particles of any particle size. Finally, after nitrogen was introduced for more than 5 minutes to maintain the anaerobic condition of the serum bottle, all the bottles were sealed. All serum bottles were incubated in a shaker (35ºC, 150 rpm) until the resulting gas content reached a saturation level.

Extraction and Fractionation of EPS from Anaerobic Granular Sludge

Anaerobic granular sludge EPS was extracted with ammonium hydroxide solution Chen et al. [20]. First, the centrifuged anaerobic granular sludge was taken out for 30 min (4000 g), took 10 g of sludge at the bottom of the centrifuge tube, 20 mL of Ammonium hydroxide solution was added (w/w 28%), and slowly stirred for 24 h. Then the mixture of sludge and ammonium hydroxide was centrifuged for 30 min (4000 g). The upper solution of centrifuged tube was taken and passed 0.45 um filter membrane, the EPS content in anaerobic granular sludge could be obtained. The EPS sample was fractionated into five fractions through XAD resin: hydrophobic acid (HPO-A), hydrophobic neutral (HPO-N), transphilic acid (TPI-A), transphilic neutral (TPI-N) and hydrophilic fraction (HPI). Zhong et al. [21] described the EPS fractionation procedure in detail.

Adsorption Experiment of Microplastics

According to He et al.'s [22] research, intermittent adsorption was adopted in this paper. Firstly, according

to the research of Li et al. [23], a small number of PS was weighed and putted into ultrapure water to prepare PS suspension solution, and the suspension solution obtained was only used once. Then, an equal amount of PS suspension was added into the five fraction solutions of EPS, and the mixed solution was stirred (20±1ºC) on a reciprocating vibrating screen (Huaneng Electronic Technology Co., Ltd., China). After 6.0 h of equilibrium time, the mixture of EPS fractions and PS suspension was collected. The collected mixture was centrifuged at 10000 rpm for 10 minutes, then passed 0.22 um cellulose acetate membrane filter (Haining Zhongli Filter Equipment Factory, China). The TOC value of EPS fractions was measured, and the number of EPS fractions adsorbed on PS was calculated according to the change of TOC value of EPS fractions before and after adsorption. The same operation was performed for EPS fraction solutions in the blank reactor without PS addition. In this paper, the adsorption kinetics characteristics of anaerobic granular sludge EPS and PS were described by a pseudo first-order model and a pseudo second-order kinetic model, and the adsorption isotherm results of EPS fractions were simulated by Langmuir isotherms and Freundlich equation. An overall description of the pseudo first-order model and pseudo second-order kinetic model in detail, see the paper Yu et al. [24].

Adsorption Methods

In this paper, the quantitative method of protein, polysaccharide and carbohydrate in anaerobic granular sludge EPS was shown in Dai et al. [25]. For the detection methods of TOC and DOC of anaerobic granular sludge in this paper, please refer to Standard Methods for the Examination of Water and Wastewater.

The surface groups of EPS and the four fractions after PS adsorption were identified by a Perkin Elmer Spectrum One B Fourier Transform Infrared (FT-IR) spectrometer. The spectrum scanning range was 4000 to 400 cm^{-1} [26].

Results and Discussion

Effect of Microplastics with Different Sizes on EPS

In this experiment, the EPS yield was carefully studied to evaluate the effect of PS with different particle sizes on the total EPS of anaerobic granular sludge. Existing research has found that the main components of EPS are polysaccharides, proteins, and humic acids, which are not only important carbon sources for anaerobic granular sludge, but also able to resist the invasion of external toxic and harmful substances [27]. EPS can combine with cells to form sludge aggregates, forming a huge network "barrier" containing a lot of water, which can resist the inhibition of microplastics to a certain extent [28]. Fig. 1 summarized the impact of microplastics with different particle sizes on EPS, and showed the content changes of five fractions of EPS. The total amount of EPS in anaerobic reactor added 0.5 μm PS increased significantly, which reached 1.14 times of the blank reactor. The total amount of EPS in anaerobic reactor added 200 μm PS decreased to 0.91 times of the blank reactor. Therefore, the production of EPS was negatively related to the size of microplastics exposed to anaerobic granular sludge. The smaller the particle size of microplastics, the more obvious the increase in the total amount of EPS. Large particle size microplastics improve the hydrolysis efficiency of EPS, reduce the content of EPS, and thereby improve the degradation efficiency of sludge. The study found that the limiting factor for efficient operation of sludge anaerobic digestion is the hydrolysis efficiency of EPS [29]. Efficient hydrolysis can degrade 30%~50% of sludge EPS in the anaerobic digestion system. Therefore, the total amount of EPS and digestive activity in anaerobic reactors with added 50um, 100um, and 200um particle size PS were significantly reduced.

Due to the correlation between the content of humic acid and the total generation of EPS, the HPI with the highest humic acid content among the five fractions of EPS showed the most significant changes. Anaerobic granular sludge reactor with smaller size PS (0.5 um and 5 um) produces more HPI, and the relative intensity of HPI in EPS increases from 55.5 mg/gVS in blank reactor to 83.0 mg/gVS (0.5 um) and 87.2 mg/gVS (5 um) (Fig. 1). However, anaerobic granular sludge reactor with larger size PS (50 um, 100 um and 200 um), the relative intensity of HPI in EPS decreased to 38.4 mg/gVS, 32.5 mg/gVS and 35.9 mg/gVS respectively. Liu et al. [19] found that humic acid can reduce the toxicity of microplastics with small particle size, but can do nothing for microplastics with large particle size. This further confirms that adding small particle size microplastics to the anaerobic granular sludge reactor in this experiment can promote the production rate of HPI, while adding large particle size microplastics has almost no change in the production rate of HPI.

In the blank anaerobic reactor, HPO-A and HPI have a significant advantage in the anaerobic granular sludge EPS, which was about 41.5 % and 20.7%. The highest content of the other three fractions was only 18.7% (TPI-A), while the lowest was only 7.6% (TPI-N). Since 50%~90% organic matter in sludge exists in the form of polysaccharide, protein and humic acid in EPS, it can be used as the main carbon source material for anaerobic digestion [30]. It was found that about 121.6 mg g^{-1} VS protein could be extracted from EPS in the blank reactor, and most of them were in the form of HPI (48.6%) and HPO-A (37.7%). Compared with protein, the extraction amount of EPS polysaccharide in the blank reactor was 73.8 mg g^{-1} VS, while the content of carbohydrate was lower, only about 48.6 mg g^{-1} VS. Like protein, most polysaccharides and

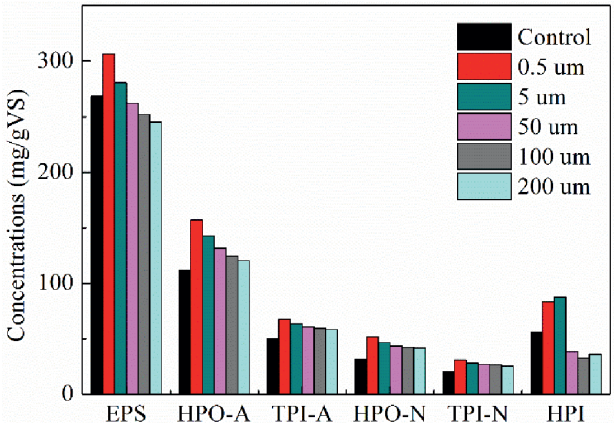

Fig. 1. Effect of microplastics with different sizes on the total amount of EPS.

carbohydrates in blank reactor exist in the form of HPI and HPO-A. Therefore, in the reactors with different particle sizes of PS, the contents of five fractions of EPS gradually decrease with the increase of microplastics particle size. The decline rate of HPI and HPO-A were the fastest, and the decline rate of the other three fractions were also positively correlated with their content in EPS.

Adsorption Kinetics Analysis

According to the experiment, it was found that the particle size of microplastics had different effects on EPS in anaerobic granular sludge. A representative particle size of 50 um was selected to study its adsorption effect on different fractions of EPS. It was found that the five fractions of EPS were rapidly adsorbed within 1 h at the beginning and reached equilibrium within the next 5 h. This is consistent with the adsorption characteristics of EPS for metals, refractory substances [31], etc., which means that rapid adsorption is achieved in the early stage, and the adsorption effect is slow in the middle and late stages. This phenomenon may be due to the fact that PS surface has more active sites in the early stage. With these sites gradually occupied and/or the steric hindrance of EPS previously adsorbed, the adsorption of PS naturally becomes slow and inefficient in the middle and later stages [32]. A pseudo first-order model and a pseudo second-order kinetic model were used to fit PS adsorption of EPS fractions. As shown in Table 1, under all experimental conditions, the kinetic adsorption data of EPS fractions and PS were better matched with a pseudo second-order kinetic model (R^2>0.931). A pseudo first-order model has poor fitting effect on the kinetic experimental data, and the correlation coefficient R^2 was less than 0.915. Compared with hydrophobic fractions (HPO-A and HPO-N), hydrophilic fractions (HPI) show lower adsorption capacity for PS, this can be proved by the much lower q_e and K_2 values when HPI fraction adsorb PS. The pseudo second order rate constant K_2 of HPO-A, HPO-N, TPI-A and TPI-N adsorbed on PS was higher than that of HPI whose main component was humic acid, because the hydrophobicity of these four fractions were higher than that of HPI. When the initial concentration of EPS fractions was low (5 mg·L^{-1}), the K_2 value generated by PS adsorption was significantly lower than the initial concentration was high (10 mg·L^{-1}). This shows that the lower the initial concentration of EPS fractions, the easier to obtain the adsorption sites on PS. The above results showed that the adsorption process of five EPS fractions on the microplastics, not only hydrophobic interaction, but also π-π electron-donor-receptor interaction all played a major role [33]. In addition, the R^2 value of TPI-A and TPI-N fractions in the pseudo second order kinetic equation were also not high, which means that chemical adsorption plays a major role in the combination of microplastics with TPI-A and TPI-N fractions.

Table 1. Kinetic data of EPS fractions obtained from microplastics adsorption at 293 K.

	C_0 mg·L^{-1}	Pseudo first order-model			Pseudo second-order kinetic model		
		k_1 (h^{-1})	q_e (mg·g^{-1})	R^2	k_2 (g·mg^{-1}·h^{-1})	q_e (mg·g^{-1})	R^2
HPI	5	1.279	5.388	0.911	0.584	6.527	0.931
	10	1.298	5.430	0.902	0.596	6.684	0.967
HPO-A	5	1.858	6.145	0.847	0.655	6.863	0.964
	10	1.867	6.270	0.879	0.668	7.026	0.983
TPI-A	5	1.738	6.387	0.886	0.618	6.656	0.956
	10	1.801	6.461	0.915	0.634	6.793	0.972
HPO-N	5	1.656	6.456	0.812	0.637	6.751	0.963
	10	1.686	6.592	0.864	0.657	0.862	0.958
TPI-N	5	1.327	5.381	0.854	0.601	6.620	0.938
	10	1.368	5.563	0.893	0.620	6.788	0.944

Table 2. A comparison of the Langmuir and Freundlich adsorption isotherm constants.

Adsorbent	Langmuir isotherm		Freundlich isotherm		
	K_L ($L \cdot mg^{-1}$)	R^2	K_F ($mg \cdot g^{-1}$)	n	R^2
HPI	5.21	0.904	6.38	3.12	0.927
HPO-A	4.89	0.894	6.68	3.57	0.984
TPI-A	4.95	0.852	6.57	3.44	0.958
HPO-N	4.76	0.873	6.51	3.38	0.976
TPI-N	4.81	0.838	6.42	3.25	0.967

Adsorption Isotherm Analysis

In this paper, Freundlich and Langmuir models were used to analyze the experimental data of PS adsorption by sludge fractions, and the specific values obtained were shown in Table 2. It was found that Langmuir model was usually used for single-layer adsorption, this model has supposed that the adsorption takes place at the specific adsorption sites. The interaction forces between molecules and adsorption sites decreased as these molecules become further from the same surfaces [34, 35], but EPS fractions adsorbed on the surface of microplastics may have multilayer or even more complex adsorption. Therefore, Freundlich model describing multi-layer adsorption was introduced, which can also estimate the adsorption strength of activated sludge. Under experimental conditions, compared with Langmuir isotherm model, the experimental data of adsorption of five fractions of sludge EPS on PS were usually better represented by Freundlich isotherm (R^2>0.927). It shows that the adsorption process of EPS fractions on PS was a chemical adsorption process, and adsorption was likely to occur on the uneven surface of activated sludge [36]. The n value reflects the favorable degree of the microplastics in the sludge to the adsorption of EPS fractions. The higher the n value is, the stronger the heterogeneity of the adsorption surface is, and the adsorption generated is more favorable [37]. The order of n values of five EPS fractions in Table 2 was consistent with that of Freundlich adsorption coefficient (K_F), which was: HPO-A>TPI-A>HPO-N> TPI-N>HPI. This order is consistent with the order of aromatic substances content in the five fractions of EPS, that is, the amount of aromaticity is directly proportional to the adsorption affinity of PS [38]. Since the R^2 value of the hydrophilic fraction in EPS was the lowest and the R^2 value of the hydrophobic fraction was the highest, it indicated that heterogeneous adsorption (physical and chemical process) contributes greatly to the adsorption of hydrophobic fractions [39]. It can be seen that the adsorption of EPS fractions in sludge and microplastics was mainly driven by hydrophobic interaction, n-π and π-π electron-donor-acceptor interaction, which was consistent with the adsorption characteristics of carbon nanomaterials and DOMs [40].

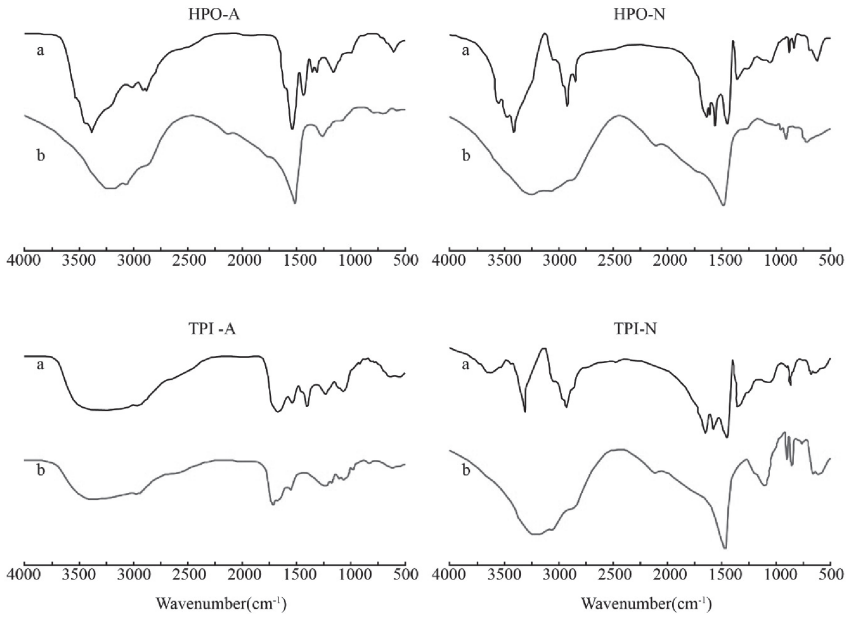

Fig. 2. FTIR spectrum of the EPS pre- and post-microplastics sorption.

FT-IR Structural Variations

Each absorption peak in the FT-IR spectrum corresponds to a type of substance, especially in the high-frequency region of 4000~1300 cm^{-1}, where each absorption peak represents a functional group region [41]. As shown in Fig. 2, FT-IR spectral changes of three fractions of raw sludge HPO-A, HPO-N and TPI-N were very similar. The obvious absorption peak at 3670-3300 cm^{-1} disappeared after the adsorption experiment, and the strong signals at 2950 cm^{-1}, 1720-1700 cm^{-1}, 1652 cm^{-1} and 1550 cm^{-1} disappeared after the adsorption of the microplastics. This result indicating that the O-H stretching vibration, carbonyl C-O component, C-O stretching, amide 1 and 2 groups were all the main forces involved in the chemical adsorption of microplastics. In addition, the disappearance of C-O group and hydrocarbon (1420-1400 cm^{-1}) peak also indicated that the band of sludge HPO-A, HPO-N and TPI-N were inefficient in the chemical adsorption of microplastics. For the TPI-A fraction different from the three fractions, a strong absorption peak (1403 cm^{-1}) of O-H in carboxylic acid fractions and C-O in alcohol fractions was found in the TPI-A fraction of sludge EPS. This is because the main component of TPI-A fraction is low molecular weight organic carboxylic acid compounds, which usually disappear in large quantities after adsorption experiments. In summary, the organic matter in various parts of sludge EPS plays a significant role in the adsorption of microplastics, especially the high content components of HPI and HPO-A, which significantly inhibit the anaerobic digestion of sludge by adsorbing microplastics.

Conclusions

In this study, the adsorption capacity of five fractions of sludge EPS to microplastics was studied for the first time, and the adsorption effect was discussed from the aspects of structure and adsorption theory, and the following conclusions were obtained. (1) The yield of EPS was negatively correlated with the particle size of microplastics, and the smaller the particle size, the more obvious the increase of the total amount of EPS. Among the five fractions, the HPI can resist the toxicity of smaller PS, so the increment was the most obvious. (2) The adsorption behavior of the five fractions of EPS on microplastics PS were similar, which can be well fitted by pseudo-second-order kinetics model and Freundlich model. Hydrophobic interaction, π-π electron-donor-acceptor interaction, chemical adsorption and electrostatic attraction simultaneously act on the adsorption of five fractions of EPS by PS. (3) O-H stretching vibration, carbonyl C-O group, amide-1, amide-2 absorptivity, and phenol C-O bond have a great contribution to the adsorption of microplastics. Further research is needed to determine the specific values of the inhibitory effects of EPS fraction on anaerobic digestion of sludge.

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Conflict of Interest

The authors declare no conflict of interest.

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