Overview of Extracellular Polymeric Substances

Subjects: Environmental Sciences

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Extracellular polymeric substances (EPS) are complex extracellular polymers with high molecular weight, which are metabolic products of microorganisms and result from effluent organic matter and microbial lysis or hydrolysis. EPS were proved to have the ability to absorb and biodegrade certain substances. The main components in EPS were found to influence the properties of microbial floccules, such as transfer, surface hydrophilicity/hydrophobicity, and aggregate stability.

wastewater   extracellular polymeric substances   nitrogen removal

1. Fundamentals of Extracellular Polymeric Substances

1.1. Extracellular Polymeric Substances Structure and Distribution

Extracellular polymeric substances (EPS) have a three-dimensional structure with a gel-like and highly hydrated matrix; the microorganisms are embedded and more or less immobilized in EPS [1]. Owing to the distinct structure, EPS possess a vast surface area and carry numerous functional groups (e.g., carboxyl, phosphoric, amine, and hydroxyl) [2], significantly affecting the physico-chemical characteristics of sludge flocs, such as adhesion, hydrophobicity, settling, and dewatering. Two forms of EPS can be subdivided outside of microorganisms cells, including bound EPS (sheaths, capsular polymers, condensed gels, loosely bound polymers, and attached organic materials) and soluble EPS (soluble macromolecules, colloids, and slimes) [3]. Although the interaction between soluble EPS and cells is weak, the soluble EPS are found to have impacts on microbial activity and sludge properties [4]. However, the research on soluble EPS is limited, and the EPS mentioned in this research without being specified are bound EPS. Bound EPS surrounding bacteria or in sludge flocs likely possess a dynamic double-layered structure where tightly bound EPS (TB-EPS) forms an inner layer, and loosely bound EPS (LB-EPS) diffuses in the outer layer [5]. LB-EPS are highly hydrated and tend to form a dispersible and loose slime layer without a significant edge (dispersible part). Sludge settleability and dewaterability were found to be more strongly correlated with LB-EPS than TB-EPS [6], while TB-EPS were found to have effective flocculation activity compared to LB-EPS [7].

1.2. Methods of Extracting Extracellular Polymeric Substances

Several EPS extraction methods can be employed from various wastewater sludge (biofilm and aerobic or anaerobic activated sludge). Centrifugation is the most common extraction method for soluble EPS. Notably, lots of extraction methods for bound EPS have been generated, and new approaches are being developed [8]. These extraction methods can be classified as physical methods and chemical methods, listed in **Table 1**. The physical extraction methods usually adopt external forces, which are created by ultrasonic, centrifugation, or heating, to encourage the EPS to detach from cells and then dissolve in solution. The chemical extraction methods involve adding chemical compounds to disrupt the binding interactions between the EPS and the cells to accelerate the dissolution of the EPS. In general, the extraction efficiencies of the physical extraction methods are lower than those of the chemical extraction methods. As LB-EPS loosely bind with cells, mild methods, such as high-speed centrifugation, heating at low temperatures, or high-rate shear, should be utilized to prevent TB-EPS release. In addition to various physical methods, chemical methods, and enzyme treatments, the extraction of TB-EPS usually adopts more harsh combination methods, including CER + sonication, alkaline + heating, and formaldehyde + heating.

Table 1. Different Tightly Bound-Extracellular Polymeric Substances Extraction methods.

Methods		Mechanism	Features	Disadvantages	References
Physical	Centrifugation	EPS are separated from cell surface and then dissolve to solution under the centrifugal force.	• Comparatively less cell lysis. • Separate the soluble EPS from the cellular biomass.	• The lowest amounts of EPS are extracted. • Bound EPS cannot be extracted significantly.	[9][10][11][12][13]
	Heating	EPS dissolution will be accelerated by enhancing molecular movement with heating.	• Loosen the sludge structure by heating. • Separate EPS from microbial cells easily.	• Significant lysis and cells disruption. • Partial EPS hydrolysis.	[10][11][14][15]
	Sonication	EPS of biofilm matrix are extracted under different impulsive pressures	• Effectively disintegrate sludge flocs and release enzymes.	• EPS stripping is not complete and the number of extractions is low.	[10][11][16][17][18]

Methods		Mechanism	Features	Disadvantages	References
		created by sonication.	• Mild and effective. • EPS are separated using the shear force generating from ultrasound and the pressure formed by the cavity rupture.	• Disruption of the extracellular matrix.	
	Sonication/centrifugation	EPS will dissolve into solution under the impulsive pressure created by the sonication and centrifugal force.	• Mild and effective. • Widely used method. • Ultrasound and centrifugation techniques are repeatedly used to extract different grades of EPS.	• Disruption of the extracellular matrix and partial cell breakage.	[11][19]
Chemical	Acidic treatment	EPS are fallen away from the cell surface, as the interaction between EPS and cells is disrupted by the repulsive force.	• Rich in chemical groups (hydroxyl, peptide bond, polysaccharides, phosphate, and sulfur functional groups).	• The extraction efficiency is low.	[9][10][11]
	Alkaline treatment	Alkaline treatment with NaOH addition	• Effectively sever cell lysis and the	• Neither extract more EPS nor get more	[9][10][11]

Methods	Mechanism	Features	Disadvantages	References
	causes the groups to be ionized, resulting in a strong repulsion between EPS and cells.	disruption of macromolecules.	chemical groups.	
CER	CER removes the divalent cations resulting in EPS falling apart.	• High extraction efficiency. • Widely used method. • The products obtained from the processing facilitate subsequent analysis.	• Insignificant cell lysis using low amount of CER.	[9][10][11][20]
EDTA	EPS matrix will fall apart, because divalent cations for the cross-linking of charged compounds are removed using EDTA.	• Cause a low degree of cell lysis. • Mild and acceptable extraction efficiency.	• Form complexes with EPS generating interfere in the colorimetric analysis.	[9][10][11][21]
Enzymatic extraction	The carbohydrate and protein-hydrolyzing enzymes are used to disrupt the structure of sludge and dissolve EPS.	• Stable humus contents in the extracted EPS. • Mild and effective.	• Specific for polymers. • Represent only a minority of EPS. • Underestimation of the total	[10][11][22]

Methods	Mechanism	Features	Disadvantages	References
			polymer.	
HCHO/NaOH	HCHO reduces the cell lysis caused by NaOH addition.	• Extract carbohydrate, protein and uronic acid for all sludges. • The highest amounts of EPS extracted from sludges.	• Formaldehyde alters the structure and properties of proteins in EPS. • Change the polymer composition at pH > 9.	[24] [10][18][23]

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2.2. Adhesion Ability

The formation of biofilm in a liquid environment is related to micro-surface characteristics. EPS present on cell surfaces could enhance microbial deposition [27]. Some functional groups in EPS contribute to microbial adhesion onto surfaces [28][29]. In the previous study, the number of adherent bacteria on the sludge surface was found to be reduced after EPS removal [30], and EPS-rich strains have stronger adhesion to bacteria than the EPS-deficient strains under similar surface charge conditions [31]. Thus, the adhesion of cells created by EPS can help to generate bioflocculation [32].

2.3. Biodegradability

Carbon and energy from EPS can be used by activated sludge [33]. Proteins and polysaccharides are the main substances in EPS, and the degradation enzymes for these polymers are adequate in biological wastewater treatment reactors. The excreted EPS were found to be utilized by bacteria for metabolic activity when there was a nutrient shortage [34][35]. However, some studies showed that some EPS could not be degraded by microbes [3]. EPS located in the inner layer of aerobic granular sludge were reported to be biodegradable, but the situation was on the contrary in the outer layer [36]. Although some EPS can be used as carbon resources, the degradation can cause deflocculation in the activated sludge system. Moreover, the non-degradable EPS can be discharged along with the effluent and bring negative effects on the effluent quality [37].

3. Contaminant Removal

3.1. Organic Matter Removal

Synthetic organic matter is extensively used in many fields, and the unintended release into the environment poses a potential risk to human health and ecological systems [38]. EPS, as a kind of synthetic organic matter, play an important role in organic pollutant removal [39]. EPS contain large quantities of negatively charged functional groups with strong capabilities to adsorb organic pollutants [40], including phenanthrene [41], benzene [42], humic acids [43], dye [44], and antibiotics [45]. A previous study reported that more than 60% of benzene, toluene, and m-xylene could be absorbed by EPS and that only a small part was performed by cells [46]. Because the binding strength and capability of proteins are higher than that of humic substances, soluble EPS with more proteins have a greater ability to bind organic pollutants than the bound EPS [47].

It was pointed out that EPS was important for organic pollutant adsorption, especially for some special organic matter, such as antibiotics [44]. Large amounts of antibiotics from hospitals and pharmaceutical factories have been discharged into the sewage plant every year [48][49]. Sorption was regarded as the primary way to remove antibiotics in activated sludge systems [50][51], and the adsorption capacity would decrease significantly after EPS removal [52]. Notably, sulfamethazine, one typical antibiotic, could be effectively removed by EPS adsorption, which was beneficial for the subsequent biodegradation [53].

3.2. Nitrogen and Phosphorous Removal

Nitrogen and phosphorous are common nutrients in domestic wastewater [54]. The biological phosphorus and nitrogen removal process has been developed for treating wastewater and protecting water against eutrophication [55]. The activated sludge process is the wildly used and well-established biological nutrient removal process [56]. Notably, the EPS of sludge also play an important role in phosphorus and nitrogen removal because of the special roles in mass transfer [57][58][59]. The differences in surface functional groups of EPS lead to variations in hydrophilicity and hydrophobicity, influencing nutrient removal [60].

Cloete and Oosthuizen indicated that phosphorous could be accumulated significantly in sludge EPS, and the role of EPS in phosphorous removal could not be negligible in biological wastewater treatment systems [59][61]. The removal process of phosphorous by EPS through transformation and transportation was considered to occur simultaneously with the phosphorus-accumulating organism metabolism and phosphorous precipitation [59][60][62]. Wang et al. indicated that 27% of phosphorus were adsorbed by EPS in the denitrifying phosphorus removal process [63].

EPS adsorption also has positive effects on biological nitrification/denitrification [63], especially for promoting the adsorption of ammonium [64]. Respectively, 32.94% and 72.29% of total nitrogen were removed by EPS adsorption in the processes of heterotrophic denitrification and anammox [58]. However, as EPS are highly complex, the roles of nutrient removal need to be further studied. In particular, knowledge of pollutant removal pathways by EPS in activated sludge systems is very limited [60][62].

3.3. Metal Ion Removal

Ecotoxicological risks of heavy metals are widely investigated and have been a global concern [65]. The existence of heavy metals in the aquatic system can be detrimental to various living species because these materials are non-biodegradable and tend to accumulate in living organisms, causing diseases and disorders [66]. Owing to the non-biodegradable characteristics of heavy metals, biological sludge in municipal or industrial wastewater facilities can be effectively used in heavy metal removal processes [67]. Sludge consists of numerous organisms and several organic substances, such as EPS and cell flocs [10], and has been proven to adsorb heavy metal ions in wastewater [68][69][70][71]. EPS also display acid-base and metal-ion binding properties [72][73][74][75]. The investigation has shown the adsorption-desorption behaviors of Hg(II) and Sb(V) on EPS [67]. Other authors also studied the sorption of Pb(II) and Cd(II) by EPS extracted from pure bacterial strains or activated sludge and showed EPS adsorbed over 80% Pb(II) and 30% Cd(II) at pH 7 [76]. Mayer et al. found that Ca(II) and Mg(II) could be removed in activated sludge [77]. Furthermore, a previous study reported that some trace elements (e.g., Cu, Zn, and Ni) might be trapped by the organic matrix of EPS [78].

Functional groups of EPS, such as carboxyl, phosphoric, amine, sulfhydryl, phenolic, and hydroxyl groups [79], harbored by cell walls, govern the metal-ion sorption [74][75][80]. These functional groups represent potential binding sites for the sequestration of metal ions [81]. Proteins, carbohydrates, and nucleic acids in EPS all have the ability to bind with heavy metals [82]. Proteins and humic substances in EPS were both strong ligands for Cu(II), and further investigation showed that Cu(II) was bound with oxygen atoms in the carboxyl groups of EPS [69]. Additionally, the abundant charged groups in EPS can react with metal ions [58].

Several parameters, such as temperature, ionic strength, pH, biosorbent size, biosorbent dosage, initial solute concentration, and agitation rate [83], will influence the ability of biosorbents to bind with metal ions. Because the metal affinity order to EPS changes with pH [84], the biosorption for metal ions can be significantly influenced by pH [85]. For example, the number of EPS binding sites for Cu(II), Pb(II), and Cd(II) could increase with pH increasing. It was reported that EPS produced by *Parapedobacter* sp. ISTM3 strain removed 70% Cr(VI) at pH 4.0 and 95% Cr(VI) at pH 5.0 [86]. However, knowledge of how pH affects EPS binding sites is very limited [84]. Higher temperatures usually enhance sorption due to the increased surface activity and kinetic energy of the solute [87]. EPS have different binding capacities for different metal ions. The binding ability to Pb(II) is better than that to Cd(II) [76], while Cu(II) can be adsorbed more easily than Zn(II) [88].

The mechanisms of metal sorption, such as chelation, ion exchange, proton exchange, coordination, and precipitation, are involved during metal and EPS interactions [76][89]. The adsorption of heavy metals onto the unfractionated and hydrophobic EPS could be better described by the Langmuir isotherm, while Freundlich models are more suitable for hydrophilic EPS [88]. However, further work is needed to obtain a full understanding of the precise roles of EPS on heavy metals removal in biological treatment systems.

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