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Influence of extracellular polymeric substances on rheological properties of activated sludge

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ABSTRACT

Influence of extracellular polymeric substances (EPS) on rheological properties of activated sludge (AS) was conducted by comparing properties of AS before and after EPS extraction at a TSS of 54 g/L. Slime, loosely and tightly bound EPS (LB- and TB-EPS) were stratified by centrifugation and ultrasound method. The results showed that sludge after LB-EPS extraction produced a higher hysteresis loop area (Hla), limiting viscosity (η_∞), yield stress (τ_y), energy of cohesion of network sludge (E_c), and storage modulus (G'_0), than three other sludge samples, indicating that sludge with TB-EPS exhibited stronger network structure. Strain amplitude sweep (SAS) and frequency sweep (FS) tests revealed that AS before and after EPS extraction produced a gel-like structure in the linear viscoelastic (LVE) domain. TB-EPS appeared to have a positive effect on the gel-like structure of AS.

1. Introduction

Extracellular polymeric substances (EPS) are large molecular weight components of activated sludge (AS) flocs originating from bacteria secretion, cell lysis and hydrolysis, leakage of exocellular constituents, and absorbed organic matter from wastewater [1,2]. EPS are considered to be of great importance in understanding the structure, function, properties and development of microbial flocs/aggregates [3]. The presence of EPS alters the hydrophobic/hydrophilic characteristics of sludge, charge density and other surface properties [4]. They also entrap water and play an important role in sludge dewatering [4]. In recent years, stratification of EPS is of considerable interest. Yu et al. [5] empolyed centrifugation and ultrasound method to stratify EPS into slime, loosely and tightly bound EPS (LB- and TB-EPS).

Wastewater sludge is a non-Newtonian fluid, which possesses both viscous and elastic properties, also called viscoelatic (VE) properties. These VE properties can often be described by rheological models and analyses [6]. Rheology is a powerful tool because it can scientifically describe and predict deformation and flow behaviors in real processes [7]. Understanding rheological properties of sludge is not only important for choosing parameters concerning storage, transportation, and landfill, but also crucial for stabilization and dewatering [8]. Rheology can generally characterize the network strength of sludge [9]. The network structure of sludge flocs formed by three-dimensional, gel-like, highly hydrated EPS with multivalent cations and other particulate materials [10], is of significance in maintaining the structural and functional integrity of flocs/aggregates [11] and in restricting water mobility [12]. Hence, to improve the dewatering efficiency, weakening or destroying the network structure of AS might be a possible solution. Effects of EPS concentrations on rheological properties of sludge flocs [1,4,13] and studies on rheological properties of EPS [14–16] have been reported. However, research focusing on the effects of different EPS fractions on rheological properties of AS is scare.

This study explores the effects of different EPS fractions on rheological properties of AS by comparing properties of AS before and after EPS extraction. We wish to determine a specific EPS fraction, which mainly affects the network structure of AS. This EPS fraction might have a marked effect on sludge dewatering and drying processes. Hence, these results will provide further information on the influence of EPS on the dewatering properties of AS.

2. Materials and methods

2.1. Characteristics of AS

AS was collected from a municipal wastewater treatment plant (WWTP) in Beijing, China. The WWTP treats 6.0×10^5 m^3/day of wastewater using an anaerobic-anoxic-oxic (A^2O) process.

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Table 1
Characteristics of AS.

pH	Conductivity (mS/cm)	TSS (g/L)	VSS (g/L)	VSS/TSS (%)	COD (mg/L)	SCOD (mg/L)	Zeta potential (mV)
7.13 ± 0.02	1.45 ± 0.01	8.18 ± 0.78	5.64 ± 0.52	69.00 ± 0.30	6630.0 ± 32.5	81.6 ± 7.8	−18.9 ± 1.1

Collected sludge samples were transferred to the laboratory within 2 h and immediately screened through a 1.2 mm sieve. Filtered samples were then stored at 4 °C. Table 1 presents the main characteristics of the AS sample. The pH was measured using a pH meter (PB-10, Sartorius Stedim Biotech Co., Ltd., Beijing, China). Conductivity was measured using a conductivity meter (EC215, Beijing Kanggaote Science and Technology Co., Ltd., China). Total suspended solids (TSS, g/L) and volatile suspended solids (VSS, g/L) were assessed from the weight loss of suspended sludge samples dried at specific temperatures and durations according to the standard method [17]. To obtain a consistent TSS concentration around 54 g/L from sludge samples for rheological testing, sludge samples resuspended after EPS extraction were first centrifuged at 1000 × *g* for 10 min, after which the bulk solution and solid phase were obtained separately. Collected sediments were successively diluted with supernatant. Chemical oxygen demand (COD) of the filtrate is referred to as soluble COD (SCOD). COD and SCOD analyses were conducted using a COD expedited testing apparatus (HATO CTL-12, Huatong Environmental Protecting Instruments Co., Ltd., Chengde, China). Zeta potential was recorded by a Malvern Zetasizer instrument (Nano Z, Malvern Co., UK). All tests were conducted within a week. All chemical analyses were carried out in triplicate using chemicals of analytical grade.

2.2. Extraction and analytical methods of different EPS fractions

Slime, LB- and TB-EPS of AS were extracted by centrifugation and ultrasound method [5]. Slime was obtained from the supernatant after low-speed centrifugation. LB- and TB-EPS were dissolved in a buffer solution (pH 7) containing Na_3PO_4, NaH_2PO_4, NaCl, and KCl at a molar ratio of 2:4:9:1. conductivities of buffers were adjusted with distilled water to match those of the filtrated sludge samples presented in Table 1. Membranes (0.45 μm) were used to filter out the particulates present in slime, LB- and TB-EPS solutions after all EPS fractions had been extracted.

Proteins (PN) and humic-like substances (HS) of different EPS fractions were measured by the modified Lowry method using bovine serum albumin (Beijing Aoboxing Biotechnology Co., Ltd., China) and humic acid (Sigma, America) as standards, respectively. Polysaccharides (PS) and DNA were determined by the anthrone method, using glucose as the standard, and the diphenylamine colorimetric method, using 2-deoxy-D-ribose (Beijing Ruibo Biotechnology Co., Ltd., China) as the standard, respectively. All chemicals used were of analytical grade, and all tests were performed in triplicate.

2.3. Rheological testing

Rheological tests were performed using a rheometer (Physica MCR 300, Anton Paar, Austria) in conjunction with US 200 software which recorded the rheology data. Temperature was maintained at 25 °C by a Peltier control. A PP 50 plate and plate sensor with 49.94 mm diameter and 2.0 mm gap was used.

Measurements were initially carried out for the first rheological testing mode in steady flow: (1) increasing shear rate from 0.1 to 1000 s^{-1} in a logarithm manner; (2) maintaining constant shear rate at 1000 s^{-1} in 30 s; (3) decreasing shear rate in a logarithm manner from 1000 to 0.1 s^{-1}. Rheograms of shear stress (τ) as a function of shear rate ($\dot{\gamma}$) were recorded and analyzed for raw AS and sludge after EPS extraction. In general, 30 s is sufficient to ensure that the equilibrium point of the stationary state can be reached [18,19]; while during this controlled shear rate (CSR) test, a shorter time span of 5 s for AS response was employed to indicate "network strength" of the sludge at each shear rate [7]. Based on these rheograms, the parameter hysteresis loop area (Hla) can be calculated as follows [9]:

$$\mathrm{Hla} = \int_0^t \tau \cdot d\dot{\gamma}(t) \quad (1)$$

where τ (Pa) is the shear stress and $\dot{\gamma}$ (s^{-1}) is the shear rate.

The second mode was a type of transient rheological test-strain amplitude sweep (SAS) test, in which strain amplitude varied in a sinusoidal manner over time while the frequency remained fixed at 1 Hz. This test was carried out to exploit the linear viscoelastic (LVE) domain. The rheogram of the moduli (storage modulus G', loss modulus G'', complex modulus G^*) as a function of strain on the logarithmic coordinates can present that rheological parameters t (including the above moduli) are independent of strain until a critical strain level (γ_c), above which the linear domain is reached. Beyond this value, the G^* or G' values decline as the material loses its structural integrity [7,20,21]. Subsequently, its nonlinear behavior appears, and the transition can be used to determine γ_c and the critical storage modulus (G'_0). G'_0 represents the critical elasticity of the sludge network. The product between G^* on the plateau and the critical deformation (γ_c) directly provides the value of the yield stress: $\tau_y = G^*\gamma_c$. In addition to leaving the sample in a more intact state, the dynamic mechanical test is also advantageous in its simplicity and independence from any model assumptions [7,18,20]. Provided the values of τ_y and γ_c determined from the aforementioned SAS test, the corresponding value of E_c, is also equal to the half product of τ_y and γ_c [19,22]. After determing the LVE range by SAS test, frequency sweep (FS) test was conducted at 0.1% of the strain value. Corresponding G' and G'' values were measured.

Rheological tests were performed in duplicate.

3. Results and discussion

3.1. Characteristics of different EPS fractions in AS

Characteristics of different EPS fractions in AS are displayed in Table 2. The sum of DNA content from slime, LB- and TB-EPS accounted for only 0.09% of total EPS (the sum of PN, PS, HS, and DNA from all EPS samples), indicating that EPS extraction in this study did not destroy the cell [23]. PN and PS were mainly distributed in TB-EPS (45.5% and 37.4%, respectively) and slime (43.7% and 41.9%, respectively), with the lowest percentage (10.8% and 20.7%, respectively) detected in LB-EPS. HS were mainly found in slime fraction (72.7%), followed by the TB-EPS fraction (21.8%). In comparison to other fractions, LB-EPS had the lowest concentration

Table 2
Contents and characteristics of the different EPS fractions of AS.

	Slime	LB-EPS	TB-EPS
PN (mg/g-VSS)	22.53 ± 2.62	5.59 ± 0.31	23.45 ± 1.67
PS (mg/g-VSS)	28.82 ± 0.35	14.22 ± 0.10	25.69 ± 0.24
HS (mg/g-VSS)	0.40 ± 0.05	0.03 ± 0.01	0.12 ± 0.02
DNA (mg/g-VSS)	0.24 ± 0.03	0.28 ± 0.01	0.45 ± 0.01
Zeta potential (mV)	−13.3 ± 2.16	−10.4 ± 1.24	−17.0 ± 1.08