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Differential ultraviolet–visible absorbance spectra for characterizing metal ions binding onto extracellular polymeric substances in different mixed microbial cultures

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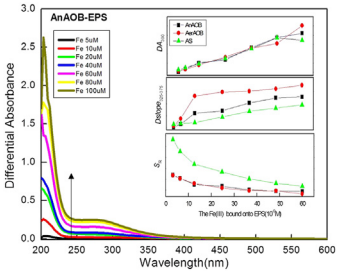
HIGHLIGHTS

- The metal binding properties of EPS was explored by DAS.
- The complexation ability of EPS was positive with DA_{300} and $DSlope_{325\text{-}375}$.
- The complexation ability was of EPS negative with the S_R.
- AS-EPS interacted with Fe(III) and Cu(II) readily than AnAOB- and Aer-AOB-EPS.

GRAPHICAL ABSTRACT



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ABSTRACT

Ultraviolet–visible (UV–vis) absorbance spectra was adopted to quantify the binding of major metal ions (e.g., Na(I), Ca((II)), Fe(III), Cu(II), and Pb(II)) on extracellular polymeric substances (EPSs) extracted from different mixed cultures. The results showed that the differential absorbance spectra (DAS) provided discernible features for revealing the changes in optical properties of EPSs induced by metals, i.e., the intensity of DAS increased largely with incrementally increased metal concentrations (Fe(III), Cu(II), and Pb(II)). It can be assumed attributable to the changes in the conformations and inter-chromophores of the EPS biomolecules. In addition, the changes in spectral parameters of $DSlope_{325\text{-}375}$ (spectral slope in the range of wavelengths 325–375 nm) and DA_{300} (differential absorbance at 300 nm) were found to be closely related to the amounts of metals bound onto all extracted EPSs, particularly for Fe(III) and Cu(II). The decreased S_R (the ratio of $slope_{275–295}$ to $slope_{350\text{-}400}$) of the EPS solutions after dosage of metals suggested increased molecular weight or size of the EPS biomolecules. Deconvolution of the DAS yielded six Gaussian bands, which were present in all of the EPS samples with various metals. Moreover, the relative contributions of different Gaussian bands in the DAS were determined by the nature of EPS-metal ions interactions good correlated with the covalent-bonding index. This study concluded that DAS and selected spectral parameters (DA_{300}, $DSlope_{325\text{-}375}$ and S_R) can be used to successfully characterize the binding of metals onto EPS at environmentally relevant concentrations.

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1. Introduction

Extracellular polymeric substances (EPSs) originating from cell growth and lysis in both engineered (Liu and Fang, 2003; Liu et al., 2015) and natural systems (Zhang et al., 2012) are mainly composed of proteins, carbohydrates and humic-like substances (Liu and Fang, 2002; Wang et al., 2014). EPSs play a crucial role in the characteristics or behavior of microbial cells, such as the formation of microbial three-dimensional matrix aggregates (Hou et al., 2015), adhesion of cells to media (Adeleye et al., 2014), bio-flocculation processes (Yu et al., 2009), and settleability and dewatering properties of activated sludge (Liu and Fang, 2003). Studies have also shown that EPSs are of importance in bio-sorption behaviors of various contaminants such as dyes (Sheng et al., 2008), organic pollutants (Pan et al., 2010), and micropollutants (Pasquini et al., 2013). More importantly, EPSs are highly capable of adsorbing heavy metals (Sheng et al., 2013b; AG and F, 2016). In fact, metal binding onto an EPS can have a strong impact on the speciation and transport of heavy metals in aquatic systems and wastewater treatment facilities(Comte et al., 2008). Furthermore, the complexation of heavy metals by EPSs and their precipitation on microbial cell surfaces can protect the microbial cells against harm caused by heavy metals and contain the sludge flocs to a compact structure(Salehizadeh and Shojaosadati, 2003; Wang et al., 2015).

To date, a number of methods have been applied to investigate the binding of heavy metals to EPSs, e.g., by using titration experiments Guibaud et al. studied the complexation ability of Cd(II), Pb(II), and Ni(II) onto the EPS produced from a mixed culture of activated sludge and eight pure cultures of bacteria isolated from the activated sludge (Guibaud et al., 2005). A study based on equilibrium dialysis has also revealed the strong binding capacity of an EPS isolated from a species of *Marinobacter* on Pb(II) and Cu(II) (Bhaskar and Bhosle, 2006). EPS precipitation, with the addition of cold ethanol, could also help to reveal the adsorption of Pb(II), Cu(II), and Zn(II) on the carbohydrates produced by *Bacillus firmus*(Salehizadeh and Shojaosadati, 2003). Furthermore, isothermal titration calorimetry showed that the complexation between Cu(II) and an EPS extracted from activated sludge is exothermic and thermodynamically favorable (Sheng et al., 2013b). Yet, the above methods have all indicated that EPSs are highly capable of adsorbing metal ions, possibly attributable to the presence of numerous binding sites of negatively charged functional groups, such as carboxyl, phosphoric, amine, and hydroxyl groups of EPSs (Liu and Fang, 2002; Sheng et al., 2013b). However, all these methods are either lab–intensive and/or require considerable amounts of EPS sample for analysis (5 g dry weight per liter (Guibaud et al., 2005)). These EPS solution concentrations are often unrealistic when compared to the concentration of EPSs in natural waters (i.e., 0.81 mg dry weight/L (Yin et al., 2011)) and wastewater treatment systems (i.e., 20.8 and 27.9 mg/g-VSS for aerobic and anaerobic sludge, respectively (Sheng et al., 2008)). Therefore, the results obtained by these previous methods cannot reveal the EPS-metal interactions in environmentally relevant conditions. It is necessary to consider an alternative technique to obtain metal ion binding data for EPSs under realistic environmental conditions.

As an alternative method, deconvolution (Yan et al., 2013a; Yan and Korshin, 2014) and log-transformation of ultraviolet–visible (UV–vis) absorbance spectra (Yan et al., 2013b) have been recently developed for studying interpretable characteristics associated with dissolved organic matter and their interactions with metal ions. In addition, the determination of some spectral parameters, such as $DSlope_{325\text{-}375}$ (spectral slope in the range of wavelengths 325–375 nm) and DA_{300} (differential absorbance at 300 nm), can help to quantify the metal ions binding onto dissolved organic matter because their changes are strongly correlated with the concentration of complexation metal ions (Yan et al., 2013b). The ratio of spectra slopes (S_R, the ratio of $slope_{275–295}$ to $slope_{350\text{-}400}$) was found to be effective in identifying the metal-induced changes in the molecular weight (MW) of DOM, e.g., a smaller S_R reflecting a higher molecular weight (MW) (Helms et al., 2008). Obviously, UV–vis absorbance spectroscopy provides a simple, fast, and sensitive technique for characterizing DOM and their interactions with metal ions. Like DOM, EPSs also contain a large number of aromatic structures and unsaturated fatty chains having various types of chromophores; therefore, UV–vis absorbance spectroscopy would enable the study of the binding of metal ions onto EPS.

The present study is therefore aimed at investigating the interactions of Fe(III), Cu(II), and Pb(II) with EPS extracted from three different mixed cultures, including conventional activated sludge and two mixed cultures dominated by aerobic (AerAOB) and anaerobic ammonium-oxidizing bacteria (AnAOB), respectively. The metal ion-induced changes in optical properties of EPS were characterized using the differential UV–vis absorbance spectrum (DAS) method. The binding propensity of metal ions was quantified by $DSlope_{325\text{-}375}$, DA_{300}, and S_R. Because EPS biomolecules have strong fluorescence properties (Esparza-Soto and Westerhoff, 2001), three-dimensional excitation-emission matrix (EEM) fluorescence spectroscopy has thus been employed to study the binding of metal ions onto EPSs (Zhang et al., 2012; Sheng et al., 2013a). Furthermore, the measurements by EEM are sensitive and rapid and can be conducted with a low EPS concentration, so the EEM measurements were employed to verify the reliability of the UV–vis absorbance spectrum method.

2. Materials and methods

2.1. EPS extraction and chemical analysis

In this study, the binding of metal ions onto EPS extracted from three mixed cultures dominated by AnAOB, AerAOB, and ordinary heterotrophic organisms (OHO) in activated sludge (AS) was investigated. The AnAOB- and AerAOB-enriched mixed cultures were obtained from a lab-scale ANAMMOX reactor (Meng et al., 2014) and a lab-scale nitritation membrane bioreactor (Shen et al., 2014). Related content on the feed composition, cultivation and acclimation conditions of AOBs has described in detail in the SI file (see Tables S3–S5). The AS samples were collected from the aeration tank of a local municipal wastewater treatment facility in Guangzhou, China. Then kept the AS samples in the refrigerator at 4 °C in the lab and for EPS extraction.

The EPSs of the three mixed cultures were extracted using the heating method, with some modifications made previously (Li and Yang, 2007). The mixed cultures were centrifuged at 3200 rpm for 15 min to remove bulk solution. After discarding the supernatant, the remaining sludge pellets were washed and re-suspended to the original volume with Milli-Q water, then subjected to a thermostatic water tank at 60 °C for 30 min. Next, the EPS were harvested by centrifugation at 12,000 rpm for 15 min and then filtrated through 0.22-μm membrane.

The concentrations of proteins and carbohydrates were analyzed using a UV–vis spectrophotometer (UV2000, UNICO, Shanghai, China), following the modified Lowry method (Lowry et al., 1951) and the phenol-sulfuric acid method (Dubois et al., 1956), respectively. The suspended solids (SS) and the volatile suspended solids (VSS) of the mixed cultures were determined according to Standard Methods (APHA, 1995). All of the above chemical analyses were carried out in triplicate with chemicals of analytical grade.