



Technical Note

Quantification of the interactions between Ca^{2+} , Hg^{2+} and extracellular polymeric substances (EPS) of sludge



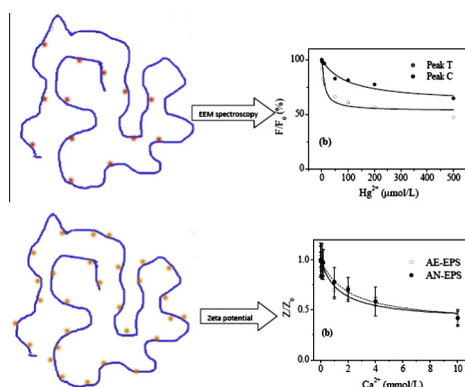
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HIGHLIGHTS

- Ca^{2+} and Hg^{2+} had different effects on EPS EEM spectra and zeta potentials.
- EPS binding constant for Hg^{2+} was two orders of magnitude higher than that for Ca^{2+} .
- Binding mechanism between Ca^{2+} and EPS was different from that between Hg^{2+} and EPS.

GRAPHICAL ABSTRACT



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ABSTRACT

The interactions between metals (Ca^{2+} and Hg^{2+}) and extracellular polymeric substances (EPS) extracted from the aerobic and anaerobic sludge in wastewater treatment reactors were investigated using a combination of zeta potential measurement and 3-dimensional excitation–emission matrix (EEM) fluorescence spectroscopy with parallel factor (PARAFAC) analysis. Results show that Ca^{2+} had no substantial effects on the EEM fluorescence spectra of the EPS, but their zeta potentials increased with the increasing Ca^{2+} dosage. However, Hg^{2+} had a significant effect on the EEM fluorescence spectra of the EPS, while their zeta potentials seemed not to be affected by the dose of Hg^{2+} . The interactions between Hg^{2+} and EPS were elucidated using the fluorescence quenching with PARAFAC analysis, while the interactions between Ca^{2+} and EPS were evaluated by the zeta potential technique. The binding constants for Hg^{2+} and EPS were two orders of magnitude higher than those for Ca^{2+} and EPS, suggesting that the binding mechanisms between Ca^{2+} and EPS were different from those between Hg^{2+} and EPS. The results might be useful for understanding the roles of EPS in bacterial self-protection against heavy metals and the aggregate formation mechanisms through ionic bridging interactions.

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1. Introduction

In biological wastewater treatment reactors, extracellular polymeric substances (EPS) are a complex high-molecular-weight mixture of polymers excreted by microorganisms in sludge and

produced from cell lysis and hydrolysis. Previous studies reveal that EPS have a high capacity to bind with heavy metals (Wingender et al., 1999; Liu and Fang, 2002). The ions bridging between EPS and Ca^{2+} are essential for keeping microbial aggregates in a three-dimensional matrix (Wingender et al., 1999). The adsorption of heavy metals onto bacterial surface by EPS could protect the cells against the inhibition of heavy metals (Salehizadeh and Shojaosadati, 2003; Kantar et al., 2011). Thus, the binding of metals to EPS

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plays an important role in keeping the sludge structure and protecting the sludge cells against the harmfulness of heavy metals. The methods used to evaluate the adsorption characteristics of heavy metals onto EPS include polarography method (Guibaud et al., 2005), equilibrium dialysis method (Bhaskar and Bhosle, 2006), precipitation of EPS with dose of cold organic solvent (Salehizadeh and Shojaosadati, 2003), or microcalorimetry method (Sheng et al., 2013). Results reveal that the carboxylic or phosphoric groups might play a main role in the complexation of heavy metals (Guibaud et al., 2005, 2006; Kantar et al., 2011; Sheng et al., 2013).

Zeta potential measurement is a rapid and sensitive analytical means for interface studies. It is widely used to characterize the surface characteristics of sludge and the conformation of natural organic matters (e.g., humic substances) (Hosse and Wilkinson, 2001; Alvarez-Puebla and Garrido, 2005) under various conditions. The variation of zeta potential reflects the ionization of surface functional groups and thus the quantity of EPS surface charges. The binding between EPS and metals through electrostatic interaction is expected to change the EPS surface charges. Thus, the zeta potential measurement might be able to characterize the interactions between EPS and metals. However, information about the application of zeta potential on exploring such interactions is not available.

The main components of EPS extracted from activated sludge are proteins, humic substances and carbohydrates (Frolund et al., 1996), which have intrinsic fluorescence characteristics (Sheng and Yu, 2006). The intrinsic fluorescence characteristics can provide information related to structure, functional groups, configuration, and heterogeneity of EPS. Three-dimensional excitation–emission matrix (EEM) fluorescence spectroscopy, as a rapid, selective and sensitive technique, should be useful for studying the physical–chemical properties of EPS (Sheng and Yu, 2006; Lee et al., 2013). The main feature of EEM fluorescence spectroscopy is that the fluorescence information can be entirely acquired. It has been successfully used to characterize the natural organic matters, as well as their interactions with heavy metals (Lu and Jaffe, 2001; Wu et al., 2004; Ohno et al., 2008). This technique also be useful to characterize the interactions between metals and EPS. Attributed to the overlap of their EEM fluorescence spectra of different components in EPS, stoichiometric methods, such as parallel factor (PARAFAC) analysis, should be employed to separate the actual fluorescence spectra from the EEM fluorescence spectra (Ohno et al., 2008).

The main objective of this work was to employ a combination of the zeta potential measurement and the EEM fluorescence spectroscopy with PARAFAC analysis to characterize the interactions between sludge EPS and Ca^{2+} or Hg^{2+} . The selection of the two cations was mainly based on their importance in environments. In addition, Ca^{2+} is the most important bridging cation in microbial aggregate formation (Wingender et al., 1999), where Hg^{2+} is a heavy metal with a relatively high potential toxicity, and their distribution and transportation in soils and waters have attracted increasing interests (Najera et al., 2005).

2. Materials and methods

2.1. Sludge and EPS analysis

Aerobic sludge was collected from an aeration tank in the Wangxiaoying Municipal Wastewater Treatment Plant, Hefei, China. Anaerobic methanogenic sludge was sampled from a pilot-scale upflow anaerobic sludge blanket reactor. The EPS of the aerobic and anaerobic sludge, designated as AE-EPS and AN-EPS respectively, were extracted using cation exchange resin technique

(Frolund et al., 1996; Sheng and Yu, 2006). After the extraction, the solutions filtrated through 0.45- μm acetate cellulose membranes were used as the EPS fraction.

The sludge suspended solids (SS) and volatile SS (VSS) were determined according to the Standard Methods (APHA, 1995). The content of carbohydrates in EPS was measured with the anthrone method using glucose as standard, while the contents of proteins and humic substances in EPS were determined using the modified Lowry methods using chicken egg albumin and humic acids as standards, respectively (Frolund et al., 1996). The total EPS content was measured as total organic carbon (TOC) (TOC-V_{CPN} analyzer, Shimadzu, Japan).

2.2. EPS binding with metals

Metals used in this study were dosed in the form of CaCl_2 and HgCl_2 . The concentration range for Ca^{2+} and Hg^{2+} were 0–10 mM and 0–500 μM , respectively. The EPS were diluted in 100 mM NaCl solution to maintain a constant ionic strength, and resulting in the TOC concentrations of AE-EPS and AN-EPS of 11.3 and 7.4 mg L^{-1} , respectively. 0.1 mL of double distilled water or metal solutions with various concentrations was added to the 10-mL EPS solutions and mixed immediately. The initial pH was then adjusted to 7.0 using a small volume of 0.2 M HCl and 0.2 M NaOH. These solutions were then used for EEM spectral analysis and zeta potential measurement.

2.3. EEM fluorescence spectroscopy

All of EEM fluorescence spectra were measured using a luminescence spectrometry (LS-55, Perkin–Elmer, USA) (Sheng and Yu, 2006). To solve the overlap problem of the fluorescence spectra of the mixture, the PARAFAC analysis on the EEM fluorescence spectra was performed. A detailed description about the PARAFAC method could also be found elsewhere (Ohno et al., 2008).

To quantify the interactions between heavy metals with the EPS, a non-linear Ryan–Weber equation (Ryan and Weber, 1982) was used to interpret the quenching of the separated fluorescence spectra by PARAFAC analysis. This model is based on the assumption that metal ion binding occurs at identical and independent binding sites to form 1:1 complex. This assumption simplifies the theoretical analysis, and has been widely used to characterize the interactions between metals and dissolved organic matters (Luster et al., 1996; Plaza et al., 2006).

Based on the assumption above, the binding of a metal ion (M) onto EPS (L) at a constant pH and ionic strength could be described using the following equation (Ryan and Weber, 1982):

$$\frac{I}{I_0} = 1 + \left(\frac{I_{ML}/I_0 - 1}{2KC_L} \right) \left\{ (KC_L + KC_M + 1) - \left[(KC_L + KC_M + 1)^2 - 4K^2C_LC_M \right]^{1/2} \right\} \quad (1)$$

where I_0 and I are the fluorescence intensities of the EPS without and with metal; I_{ML} accounts for the residual EPS fluorescence and due to the fluorescence of the complex ML or fluorophores that do not participate in the complexation reaction; C_M and C_L are the stoichiometric concentrations of the metal ion and the binding sites of EPS, respectively; K is the binding constant.

Since EPS are very complex, their fluorescence peaks in the EEM spectra would overlap. It is difficult to obtain the individual peak intensity. In this study, the PARAFAC analysis was used to extract the change of the relative peak intensity (I/I_0) in the EEM spectra of EPS at various metal dosages. From the non-linear regression between I/I_0 and $[M]$ using Eq. (1), the binding strength could be estimated.

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