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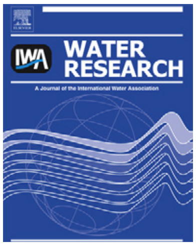

Thermodynamic analysis on the binding of heavy metals onto extracellular polymeric substances (EPS) of activated sludge

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A B S T R A C T

Metal binding to microbial extracellular polymeric substances (EPS) greatly influences the distribution of heavy metals in microbial aggregates, soil and aquatic systems in nature. In this work, the thermodynamic characteristics of the binding between aqueous metals (with copper ion as an example) and EPS of activated sludge were investigated. Isothermal titration calorimetry was employed to estimate the thermodynamic parameters for the binding of Cu^{2+} onto EPS, while three-dimensional excitation-emission matrix (EEM) fluorescence spectroscopy with parallel factor analysis was used for quantifying the complexation of Cu^{2+} with the EPS. The binding mechanisms were further explored by X-ray absorption fine structure (XAFS) and Fourier transform infrared (FTIR) spectroscopy analysis. The results show that the proteins and humic substances in EPS were both strong ligands for Cu^{2+}. The binding capacity N, binding constant K, binding enthalpy ΔH were calculated as 5.74×10^{-2} mmol/g, 2.18×10^{5} L/mol, and -11.30 kJ/mol, respectively, implying that such a binding process was exothermic and thermodynamically favorable. The binding process was found to be driven mainly by the entropy change of the reaction. A further investigation shows that Cu^{2+} bound with the oxygen atom in the carboxyl groups in the EPS molecules of activated sludge. This study facilitates a better understanding about the roles of EPS in protecting microbes against heavy metals.

1. Introduction

Microbial extracellular polymeric substances (EPS), which mainly consist of proteins, carbohydrates and humic substances etc (Wingender et al., 1999; Sheng et al., 2010), are the major composition of the activated sludge flocs in biological wastewater treatment systems. In nature, metal binding to microbial EPS considerably influences the distribution of heavy metals in microbial aggregates (Wingender et al., 1999). The complexation of heavy metals by EPS and their precipitation on microbial cell surface also could protect the cells against heavy metal inhibition (Salehizadeh and Shojaosadati, 2003). Previous studies have revealed that EPS have a high capacity to bind with metals (He et al., 2000; Guibaud et al., 2005; Guine et al., 2006; D'Abzac et al., 2010), but the binding mechanisms and the binding strength between EPS and heavy metals are not well documented.

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Because of the complex nature of EPS, it is difficult to get the thermodynamic information about their binding with heavy metals. EPS contain large quantities of aromatic structures and unsaturated fatty chains with fluorescence characteristics, thus three-dimensional excitation–emission matrix (EEM) fluorescence spectroscopy has been used for their characterization (Esparza-Soto and Westerhoff, 2001; Sheng and Yu, 2006). The intrinsic fluorescence characteristics can be entirely acquired to provide useful information about the structure, functional groups, configuration, and heterogeneity of EPS. The binding of heavy metals would alter the structure and configuration of EPS, and correspondingly their EEM spectrum would be changed. In this way, the binding process could be characterized. This technique has been successfully used to investigate the interactions between natural organic matters and heavy metals (Baker, 2001; Lu and Jaffe, 2001; Chen et al., 2003).

From fluorescence spectroscopy analysis, we can obtain the adsorption kinetics and binding strength information, but other thermodynamic parameters like binding enthalpy remain unknown and the binding mechanism between metals and EPS can't be elucidated. In this respect, microcalorimetry may be a solution to sort out this problem. Calorimetry, as the principal source of thermodynamic information, is a universal method for thermodynamic analysis because all physical, chemical and biological processes are accompanied by heat exchange. The thermodynamic characteristics of the binding between heavy metals and macromolecules can be directly obtained by the microcalorimetry method. Especially, isothermal titration calorimetry (ITC), as a principal microcalorimetric technique, is now widely used to obtain thermodynamic information about biochemical binding processes at constant temperature (Perry et al., 2005; Tan et al., 2009). For this method, the reaction heat released or absorbed after each addition during the titration is monitored by an isothermal titration calorimeter. Thus, thermodynamic analysis can be used to characterize the energetic processes associated with the binding reaction. Through fitting experimental data with a binding model related with equilibrium constants, molar enthalpy of binding and reactants concentration, the thermodynamic parameters could be quantitatively determined.

Thus, it is expected that a combined use of the ITC technique and the EEM fluorescence spectroscopy may offer deeper and more comprehensive insight into the thermodynamic characteristics of the binding between metals and EPS. In this study, copper ions (Cu^{2+}), as one of the main heavy metals with a relatively high toxicity in environments (Hu et al., 2007; Olsson et al., 2007), is adopted as the representative metal, and the two methods above are used for the thermodynamic investigation. To further characterize the binding mechanisms between EPS and Cu^{2+}, X-ray Absorption Fine Structure spectroscopy (XAFS) and Fourier transform infrared spectroscopy (FTIR) are also used to investigate the local structure of EPS-Cu^{2+} complex, including bond distance, coordination number, and type of near-neighbors surrounding copper atom. This study facilitates a better understanding about the roles of EPS in protecting microbial cells against heavy metals in biological wastewater treatment systems.

2. Materials and methods

2.1. *EPS extraction*

Activated sludge was collected from an aeration tank in the Wangxiaoying Municipal Wastewater Treatment Plant, Hefei, China. The ratio of volatile suspended solids/suspended solids of the sludge was 66.3%. The suspended solids and volatile suspended solids of sludge were determined according to the Standard Methods (APHA, 1995).

The EPS of the activated sludge was extracted using the cation exchange resin (CER) technique, which enables a high extraction efficiency with little cell lysis and polymer disruption (Frolund et al., 1996). The resin can be removed readily, and does not interfere the subsequent analysis. The effective removal of multivalent cations from the EPS solution using the CER can provide actual binding characteristics between EPS and Cu^{2+}. The detailed procedures of EPS extraction are described in a previous study (Sheng et al., 2008). Before use, the extracted EPS were filtrated through 0.45-μm acetate cellulose membrane, and dried by freeze drying.

2.2. *EPS binding tests*

An EPS solution of 15.7 mg/L was prepared before the binding experiments. Then, 0.1 mL of double distilled water or Cu^{2+} solution with various concentrations was added to 10-mL EPS solution and mixed immediately. The pH was adjusted to 6.0 using 0.2 M HCl or 0.2 M NaOH to avoid precipitation of Cu^{2+}. Thereafter, the solution was placed at room temperature for 2 h to ensure the binding equilibrium. These solutions were then used for EEM spectral analysis. The bindings of Cu^{2+} to bovine serum albumin (50 mg/L, pH 6.0) were also evaluated for comparison.

2.3. *EEM fluorescence spectroscopy*

EEM fluorescence spectra were obtained using a luminescence spectrometry (LS-55, Perkin–Elmer Co., USA). The scanning wavelength range was set as: excitation wavelength (Ex) 250–400 nm, emission wavelength (Em) 300–550 nm. Because EPS have complex compositions, the overlap of their fluorescence spectra can be serious. To solve this problem, parallel factor (PARAFAC) analysis was employed to separate the actual fluorescence spectra from the EEM fluorescence spectra (Hall et al., 2005). Before analysis, the spectrum of double distilled water was recorded as the blank, and had been subtracted from the EEM spectra of samples. The EEM data close to the Rayleigh scattering line were set as zero to eliminate the interference of the Rayleigh scattering (Rinnan et al., 2005). The software MatLab 6.5 (MathWorks Inc., USA) was employed for handling the EEM data.

To quantify the complexation of Cu^{2+} with the sludge 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 equation has been widely used to characterize the interaction between metal and dissolved organic matters (Luster et al., 1996; Monteil-Rivera and Dumonceau, 2002; Kumke et al., 2005):