Fluorescence approach for investigating binding properties between metals and soluble microbial products from a biological wastewater treatment plant

Juan Xu, Hong-Wei Luo, Yun-Kun Wang, Guo-Ping Sheng *

CAS Key Laboratory of Urban Pollutant Conversion, Department of Chemistry, University of Science and Technology of China, Hefei 230026, China

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ABSTRACT

The interactions between three metals (Cu^{2+}, Fe^{3+} and Al^{3+}) and soluble microbial products (SMP) from the effluent of wastewater treatment plant (WWTP) were investigated using three dimensional excitation–emission matrix (EEM) fluorescence spectroscopy coupled with self-weighted alternating trilinear decomposition (SWATLD) analysis. Two main components in the SMP were identified as protein-like substances at Ex/Em (230–235/345–350) nm and humic-like substances at Ex/Em (340–350/425–430) nm. The SWATLD approach could discriminate SMP into different meaningful components and estimate their relative concentrations. The conditional stability constants ($\text{Log}\,K_c$) were calculated to evaluate the metal complexing properties of the SMP by Ryan–Weber Model. The two components in SMP was found to be strong ligands for the three metals based on their conditional stability constants, in an order of $Cu^{2+} > Al^{3+} > Fe^{3+}$. The interactions between SMP and metals also can be affected by solution conditions, such as pH, ionic strength and temperature. The results of this study would provide an approach to characterize the SMP complexing properties and are useful to understand the transformation of metals in aqueous environments.

1. Introduction

Effluents from biological wastewater treatment contain many complex and soluble organic compounds, including refractory compounds, residual degradable substrates, intermediates and soluble microbial products (SMP), etc. SMP, which are defined as the pool of organic compounds, are released into solution from microbial metabolism and biomass decay [1,2]. They are found to be the major component of the soluble organic matter in effluents from municipal wastewater treatment plants (WWTPs) [3]. The compositions of SMP are variable and can interact with metal ions to form organometallic complexes through various functional groups, i.e. carboxylates, hydroxyls, sulfydryls, phenols and amines [4]. Previous study have demonstrated that fractions isolated from SMP contain organic ligands for metals [5]. Holakoo et al. [6] clarified that accumulated SMPs in the bioreactor could bind with copper, contributing more than one-half of the total ligand concentration. Consequently, SMP is recognized to effectively control the speciation of metals in aquatic environments and has been shown to be critical in processes such as metal mobilization and dispersion [7].

Therefore, the discharge of SMP in the effluent from WWTPs will affect the bioavailability and mobility of the metals in the natural waters. However, limited data are available on the complexing properties between SMP from WWTPs and metals.

Since the SMP concentration is low in the effluents from WWTPs, sensitive analytical methods such as fluorescence spectroscopy will be an alternative tool for the analysis. It relies on the quenching of the fluorescence signal with metal-organic matter complex formation [8,9]. However, the compositions of SMP is too complex, traditional fluorescence technique is not appropriate to determine the binding properties of SMP, because quenching of fluorescence properties at a single wavelength would not account for different types of fluorophores coexisting in SMP. Three-dimensional excitation–emission matrix (EEM) fluorescence spectroscopy, which collects the whole profiles of fluorescence intensity responses along both excitation and emission wavelengths, can entirely give the fluorescence characteristics of SMP [10–12]. Different regions of EEM spectra were found to be quenched (or enhanced) to a different degree by metals, indicating various degrees of interaction between different fluorophores and metals [13].

However, the EEM fluorescence spectra of complex solutions (e.g. SMP) are often composed of several overlapping fluorophores, thus better resolution is required to obtain accurate

* Corresponding author. Tel.: +86 551 63607453; fax: +86 551 63601592.
E-mail address: gpsheng@ustc.edu.cn (G.-P. Sheng).

fluorescence characteristics of the various fluorophores in SMP samples for calculating their complexing characteristics. EEM fluorescence spectroscopy combined with parallel factor analysis (PARAFAC), which is able to statistically decompose EEM spectra into different independent groups of fluorescent components, has been used to assess the interaction of dissolved organic matter with metal ions [14,15]. Although PARAFAC has been successfully applied for EEM spectra decomposition, the requirement of an accurate estimation of the number of underlying factors is an obstacle to its wider applications, because it is difficult to accurately estimate the number of underlying factors under all circumstances. Recently, an algorithm called self-weighted alternating trilinear decomposition (SWATLD) has been designed to analyze three-way data arrays [16]. Experiments show that SWATLD has the features of fast convergence and is insensitive to the excess factors used in calculation [17]. This algorithm can not only resist the influence of the excess factors used in calculation, but also largely reduce the number of iterations required [18]. Its computational efficiency and resistance to a wrong estimation of the model's rank may make this method an excellent candidate for the EEM spectra decomposing.

Thus, in this study, the complexing properties between SMP and commonly found metals (Cu^{2+}, Fe^{3+} and Al^{3+}) were elucidated using EEM fluorescence spectroscopy coupled with the SWATLD analysis. The complexations were also explored at various pH values, ionic strengths and temperatures. The results can provide more information about the SMP complexing properties and are useful to understand the transformation of metals in aquatic environments.

2. Materials and methods

2.1. Sampling and pretreatment

The SMP samples were obtained from the mixed overflow outlet of the secondary sedimentation tank in the Wangtang WWTP (Hefei, China) before effluent discharging. The samples were collected using clean polyethylene bottles, filtered through 0.45-μm acetate cellulose membranes. The filtrated samples were stirred with H^+-saturated cation exchange resin for 2 h to remove both trace metals and major cations. Afterwards the solutions were settled for 3 min to separate the resin, and then filtrated through 0.45-μm membranes again. The obtained filtrate was used as the SMP fraction for EEM fluorescence spectral analysis. The concentrations of various SMP components were measured according to previous study [19].

2.2. Experimental design

Unless otherwise specified, all chemicals were purchased from Chemical Reagent Corp., Shanghai, and were of analytical grade. Metals used in this study were dosed in the forms of $Cu(NO_3)_2$, $Fe(NO_3)_3$ and $Al(NO_3)_3$. The pH of solution was controlled with dosages of 0.1 M NaOH or HCl solution, while the ionic strength (1–1000 mM) was adjusted with 2.5 M NaCl solution. The experimental temperatures were controlled at a predetermined value (4, 25 or 35 °C). The SMP sample was initially added into each tube, and then different volume of metal solution or distilled water (control) were dosed to make the total volume to 10 mL. Afterwards, the solution was mixed with oscillator and balanced for 4 h until spectral.

2.3. EEM fluorescence spectroscopy

All EEM spectra were measured using a luminescence spectrometer (LS-55, Perkin-Elmer Co., USA). In this study, EEM spectra were collected with subsequent scanning emission spectra from 300 to 550 nm at 0.5 nm increments by varying the excitation wavelength from 220 to 400 nm at 10 nm increments. Excitation and emission slits were maintained at 10 nm and the scanning speed was set at 1200 nm/min for all the measurements. A 290 nm emission cutoff filter was used in scanning to eliminate second order Raleigh light scattering. The spectrum of double distilled water was recorded as the blank. The software Matlab 7.0 (MathWorks Inc., USA) was employed for handling EEM data [20].

The SWATLD was used to resolve the EEM fluorescence data. Before analysis, the raw EEM fluorescence spectra data were corrected with the EEM fluorescence spectra of the background [21]. As light scatter effects can form diagonal lines in the landscapes and it does not follow the tri-linear structure required for the SWATLD to work, the EEM data close to the Rayleigh scattering line were set as zero to eliminate the interfere of the Rayleigh scattering on the SWATLD analysis [22]. The detailed descriptions about the SWATLD could also be found in previous studies [16,17]. The algorithm used in this work is available from the N-way Toolbox for MATLAB at the website http://www.models.kvl.dk. The fluorescence intensity score of SMP components in each EEM can be obtained from SWATLD analysis followed by the complex modeling.

2.4. Complexing modeling

The Ryan–Weber model [23] was used to determine the binding parameters between the metals and the different components in SMP. This model is based on the assumption that metal ion binding occurs at identical and independent binding sites to form 1:1 complex [15]. This assumption simplifies the theoretical analysis and has been widely used to characterize the interaction between metals and dissolved organic matter [9,15]. The conditional stability constant, K_c, and the complexation capacity, L_t, could be determined using a nonlinear fitting of the following equation:

$$\frac{F}{F_0} = 1 + \left(\frac{F_{MeL}}{F_0} - 1\right) \frac{1}{2K_c L_t}$$
$$\left(1 + K_c L_t + K_c[Me]_t - \sqrt{(1 + K_c L_t + K_c[Me]_t)^2 - 4K_c^2 L_t[Me]_t}\right) \tag{1}$$

where F is the fluorescence intensity score at the metal concentration $[Me]_t$ and F_0 is the fluorescence intensity score in the absence of added metal obtained from SWATLD analysis.

However, the use of nonlinear fitting to estimate the three fitting parameters may lead to overly small estimates of L_t. Thus, Luster [24] reduced the number of fitting parameters to two using a constant value for F_{MeL}/F_0 which could be obtained from nonlinear fitting of the following equation:

$$\left|\frac{F}{F_0} - 1\right| = \left|\frac{F_{MeL}}{F_0} - 1\right|(1 - e^{-\alpha[Me]_t}) \tag{2}$$

where $F_{MeL}/F_0 - 1$ and α are the fitting parameters. Such a modification leads to reasonable estimates of K_c.

The humic-like substances and proteins were the main components in SMP based on chemical analysis (humic-like substances 6.7 mg/L, proteins 5.5 mg/L), with dominant contribution to the total COD of SMP due to their high COD equivalents [25]. Compared with humic-like substances and proteins, the complex abilities of other components such as carbohydrates in SMP was relatively weak due to low contents and low binding constant [26]. Thus, in this study the proteins and humic-like substances in SMP were investigated using the Ryan–Weber model, while other components were assumed to have no significant influence on binding characteristics of SMP.