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The impact of varying abiotic humification conditions and the resultant structural characteristics on the copper complexation ability of synthetic humic-like acids in aquatic environments

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ABSTRACT

Humic acid (HA) has a high complexation ability with metal ions due to its functional groups. In this study, 11 synthetic humic-like acids (SHLAs) were prepared under varying abiotic humification conditions: precursor species (glycine-catechol and glycine-catechol-glucose), precursor concentrations (from 0.25 M:0.25 M to 1 M:1 M), pH (6–8), temperature (25–45 °C) and mass of MnO_2 catalyst (1.3–2.5% w/v). The effect of the varying humification conditions on the complexation ability of the SHLA for Cu^{2+} were investigated together with the relationships between Cu complexation ability and the structure of the SHLAs (elemental composition, type and content of functional groups, AL/AR, E_4/E_6). Conditional stability constants (log *K*) of the SHLAs ranged from 6.00 to 6.42 and complexation capacities ranged from 1.76 mmol/g to 2.61 mmol/g. SHLAs synthesized at lower temperature (25 °C), pH 8, low precursor concentrations (glycine:catechol = 0.25 M:0.25 M) and a larger proportion of catalyst (2.5% w/v) had a larger copper complexation ability. Log *K* values of SHLAs had significant positive correlations with carboxylic carbon ($r = 0.671$, $p < 0.05$), carboxylic group content ($r = 0.890$, $p < 0.01$) and O/C ratio ($r = 0.618$, $p < 0.05$), and significant negative correlations with aliphatic carbon ($r = -0.616$, $p < 0.05$), total C ($r = -0.685$, $p < 0.05$) and total H contents ($r = -0.654$, $p < 0.05$). Complexation capacities had a significant positive correlation with total N ($r = 0.826$, $p < 0.01$) and a significant negative correlation with C/N ratio ($r = -0.823$, $p < 0.01$).

1. Introduction

Hazardous metal pollution is one of the most serious environmental issues throughout the world (Li et al., 2014; Nagajyoti et al., 2010; Zhang et al., 2017a, 2017b). Hazardous metals are of great concern because, unlike organic pollutants, they are non-biodegradable and can accumulate in living organisms (Lesmana et al., 2009; Mosayebi and Azizian, 2016). Copper is a potentially hazardous metal (Qing et al., 2016). Although copper is an essential metal to living organisms, long-term exposure to excess copper ions can cause gastrointestinal problems, kidney damage, cramps, hair loss, convulsions, anemia, hypoglycemia, and even death for animals and humans (Fu and Wang, 2011; Perera et al., 2014; Tang et al., 2014; Yin et al., 2016).

Humic substances (HS) are natural organic compounds (Güngör and Bekbölet, 2010; Pehlivan and Arslan, 2006) that are commonly classified as: 1) humic acid (HA, soluble in alkali but insoluble in acid); 2) fulvic acid (FA, soluble at all pH values) and; 3) humin (insoluble at any pH value) (Santosa et al., 2007; Yabuta et al., 2008). HA has a high complexation ability with metal ions due to its functional groups, especially carboxylic and phenolic-OH groups (Güngör and Bekbölet, 2010). The interaction between HA and metal ions plays an important role in metal mobility and bioavailability in the environment. Humic acids have been considered as complexation agents for use in both metal-contaminated soil remediation and wastewater treatment. (Fu and Wang, 2011; Perminova and Hatfield, 2005). Many studies have demonstrated that humic acids can form stable complexes with Cu^{2+}, with stability constants ranging from 4.58 to 5.48 (Cao et al., 1995; Fuentes et al., 2013; He et al., 2016; Paradelo et al., 2012; Plaza et al., 2005a, 2005b; Vidali et al., 2011; Xu et al., 2016).

HSs are known to be formed via the polycondensation of humification precursors derived from plants and microbial biopolymers, such as phenols, quinones, reducing sugars and amino acids (Okabe et al., 2011). Humification reactions between humic precursors can be enhanced by materials containing a metallic oxide (e.g., Mn-, Fe-, and Al-oxides), which is abiotic humification (Fukuchi et al., 2012; Fukushima et al., 2009a, 2009b; Nishimoto et al., 2013; Qi et al., 2012a, 2012b;

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Wang and Huang, 2000). To a certain extent, the structural characteristics and composition of the humic acid can be controlled by varying the abiotic humification reaction conditions, such as pH, temperature, the species and ratio of precursors, duration of the reaction and the amount of catalyst (Jokic et al., 2004; Yang and Hodson, 2018a; Zhang et al., 2015). Abiotic humification reactions can provide a promising approach to synthesize a humic acid with a stronger copper complexation ability.

Previously Yang and Hodson (2018b) demonstrated that a SHLA had better Cu complexation properties than a commercially available humic acid (CAS:1415-93-6 from Sigma- Aldrich). However, which structural characteristics in SHLAs result in a good complexation ability is not well defined in the literature. Thus to extend our previous study, here we aim to determine the impact of varying conditions during the humification reactions on the structural characteristics of SHLAs and their Cu complexation ability. To achieve these aims, 11 synthetic humic-like acids (SHLAs) were prepared via a range of abiotic humification conditions: varying precursor species (glycine-catechol system and glycine-catechol-glucose system), precursor concentrations (from 0.25 M:0.25 M to 1 M:1 M), pH (6 − 8), temperature (25–45 °C) and mass of MnO_2 catalyst (1.3–2.5% w/v). The conditional stability constant (log K), complexation capacity (CC) and chemical characteristics (element composition, acid functional groups, E_4/E_6, FTIR and ^{13}C NMR) of the 11 SHLAs were determined.

2. Materials and methods

2.1. Materials

All chemicals were analytical reagent grade (for detail please see Yang and Hodson, 2018b).

2.2. Preparation of 11 synthetic humic-like acids by abiotic humification

Sterile conditions were maintained throughout the experiments to guarantee the humification process was abiotic. Prior to use, all glassware and solutions were autoclaved at 121 °C under 0.12 MPa for 27 min (Model MLS-3751, SANYO, Japan). To synthesize the SHLAs, a 1-litre aliquot of phosphate buffer (autoclaved, pH 6, 7 or 8, 0.2 M Na_2HPO_4/NaH_2PO_4) containing 0.2 g (0.02% w/v) thimerosal was placed in a 3 L beaker, and then either 13.33 g or 25.00 g of MnO_2 was added inside. Combinations of powdered catechol, glycine and glucose were added to the suspensions to give a range of concentrations for each of the chemicals. The mixture was stirred in the dark (IKA C-MAG hotplate stirrers, Germany) at 25 °C, 35 °C or 45 °C for 240 h. The abiotic humification reaction conditions and concentrations of the precursors for the 11 SHLAs are listed in Table S1.

After incubation SHLAs were extracted and purified using the standard method of the International Humic Substances Society (IHSS) (Swift, 1996); details are given in Yang and Hodson (2018a) and Yang and Hoson (2018b).

2.3. Characterization of synthetic humic-like acids

The elemental composition (C, H, N, O), total acidity, carboxylic group content, phenolic-OH content, E_4/E_6 ratio, FTIR spectra and solid-state CP-MAS ^{13}C NMR spectra of the SHLAs were determined. Details of characterization method (e.g. instrument model, operation procedures and conditions, quality control, software used, etc) are given in Yang and Hoson (2018b). The aromaticity and the ratio of alkyl to aromatic carbons (AL/AR) were calculated according to the following equations based on ^{13}C NMR data (Qi et al., 2012a; Yang and Hodson, 2018b).

$$\text{Aromaticity} = \frac{A_{Ar}(110\text{–}160\text{ mm})}{A_{Ar}(110\text{–}160\text{ mm}) + A_{Alk}(0\text{–}110\text{ mm})} \quad (1)$$

$$\text{AL/AR} = \frac{A_{Alk}(0\text{–}110\text{ mm})}{A_{Ar}(110\text{–}160\text{ mm})} \quad (2)$$

where A_{Alk} are alkyl-C peak integration values and A_{Ar} are aromatic-C peak integration values.

2.4. Determination of conditional stability constants (log K), complexing capacities (CC) and complexation efficiency

For the complexation experiments, 100 mg SHLA was dissolved in 50 ml of 1 M NaOH, and then 50 ml of 1 M HNO_3 was added. The SHLA solution was then diluted with ultrapure water to a volume of 1 L. 50 ml of the SHLA solution and 50 ml of $Cu(NO_3)_2$ (2 μM, 10 μM, 20 μM, 100 μM, 200 μM, 250 μM and 300 μM) in 0.2 M $NaNO_3$ were mixed. This produced solutions at seven Cu^{2+} concentrations (1 μM, 5 μM, 10 μM, 50 μM, 100 μM, 125 μM and 150 μM) each with an SHLA concentration of 50 mg/L in a background electrolyte of 0.1 M $NaNO_3$ (Yang and Hodson, 2018b). All experiments were conducted at 25 °C and pH of 6.

All solutions were shaken orbitally at 150 rpm for 24 h and then free Cu^{2+} concentrations were measured using an Orion 9629BNWP Ion-Selective Electrode (ISE, Thermo Scientific, USA). All the measurements were performed in triplicate. Details of the calibration of the Cu-ISE are given in Yang and Hodson (2018b).

A 1:1 stoichiometric model was used to describe the reaction between humic acid and Cu (Eq. (3), Ružić, 1982; Tipping, 2002).

$$Cu + HA \Leftrightarrow CuHA \quad (3)$$

The conditional stability constant (K) is calculated as:

$$K = \frac{[CuHA]}{[Cu][HA]} \quad (4)$$

And the apparent complexation capacity (C_t, Plaza et al., 2005a, 2005b) is calculated as:

$$C_t = [CuHA] + [HA] \quad (5)$$

$[CuHA]$ is then calculated as:

$$[CuHA] = Cu_0 - [Cu] \quad (6)$$

where Cu_0 is initial concentration of Cu^{2+}; $[Cu]$ is concentration of free Cu^{2+} after 24 h.

Combining and rearranging Eqs. (4)–(6), the Eq. (7) is given as:

$$\frac{1}{Cu_0 - [Cu]} = \frac{1}{K \cdot C_t \cdot [Cu]} + \frac{1}{C_t} \quad (7)$$

Linear regression analysis was performed for $1/(Cu_0\text{-}[Cu])$ and $1/[Cu]$ in order to calculate K and C_t (Ružić, 1982).

2.5. Statistical analysis

The log K and CC values of SHLAs produced under various reaction conditions were analyzed using Kruskal-Wallis and Mann-Whitney tests. The significance level was set to $p < 0.05$ (SPSS 23.0, IBM, USA). Pearson (all but two properties) and Spearman rank (for aromatic C content and AL/AR) correlations were used to investigate the relationship between structural properties and Cu complexation ability (SPSS 23.0, IBM, USA).

3. Results

3.1. Characterization of 11 synthetic humic-like acids (SHLA)

3.1.1. Elemental composition

The C, N, H and O contents of the 11 SHLAs were 53.48–56.58%, 3.26–4.59%, 2.83–3.16% and 36.60–39.36%, respectively (Table 1) similar to previous studies of synthetic humic-like acids produced by abiotic humification (Okabe et al., 2011; Qi et al., 2012a).