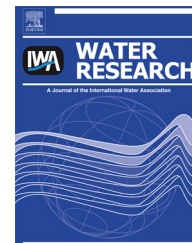




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Hydration interactions and stability of soluble microbial products in aqueous solutions

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ABSTRACT

Soluble microbial products (SMP) are organic compounds excreted by microorganisms in their metabolism and decay and the main constituents in effluent from biological wastewater treatment systems. They also have an important contribution to the dissolved organic matters in natural aqueous systems. So far the interactions between SMP colloids have not been well explored. In this work, the interactions between SMP colloids in water and salt solutions were studied by using a combination of static and dynamic light scattering, Fourier transform infrared spectra, Zeta potential and acid-base titration techniques. The second osmotic virial coefficient had a larger value in a 750-mM salt solution than that in a 50-mM solution, indicating that repulsion between SMP colloids increased with an increase in salt concentration, which is contrary with the classic Derjaguin, Landau, Verwey, and Overbeek (DLVO) theory. Such a repulsion was attributed to water structuring and enhanced by the accumulation of hydrophilic counter ions around SMP colloids and the formed hydration force. The repulsion and hydration effect led to the dispersing and deeper draining structure, accompanied by a decreased hydrodynamic radius and increased diffusion coefficient. This hydration force was related to so-called ion specific effect, and electrolyte sodium chloride had a more substantial effect on hydration force than KCl, CsCl, NaBr and NaI. Our results provide an experimental approach to explore the SMP structures, inter-colloid interactions and confirm the non-DLVO forces.

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1. Introduction

Soluble microbial products (SMP) are soluble organic compounds that are released by microorganisms during their metabolism and decay (Barker and Stuckey, 1999; Laspidou and Rittmann, 2002; Ni et al., 2011). SMP are crucial for determining the effluent quality of biological water treatment systems, as they are found to comprise the majority of soluble organic materials in effluents (Ni and Yu, 2012). However, the interactions between SMP colloids in water system have been rarely studied, especially in salt solution. Salts are ubiquity and ion effects depend on not only the individual properties

(i.e., charge, size, charge distribution and polarizability), but also its counter ion (Boström et al., 2001). Salts are present more or less in water and wastewater treatment systems, e.g., desalination membrane systems. Therefore, it would be valuable to understand the interactions between SMP colloids in salt solutions with respect to ion specific effect and interpret the complicated phenomena, such as the dependence of deposition or flocculation of SMP on salt species and concentrations.

With dose of salts, ions can cause a degree of electrostatic ordering of neighboring waters to hydrate and interfere the water–water structure (Hribar et al., 2002). On the other hand,

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Glossary of terms

A_2	second virial coefficient
C	SMP concentration
C^*	inner concentration
D_0	translational diffusion coefficient
DLS	dynamic light scattering
dn/dC	specific refractive index increment
FTIR	Fourier transform infrared spectra

LLS	laser light scattering
M_w	weight-averaged molar mass
R_{ex}	excess Rayleigh ratio
$\langle R_g \rangle$	z-average root-mean-square radius of gyration
$\langle R_h \rangle$	mean hydrodynamic radius
SLS	static light scattering
SMP	soluble microbial products
ρ	ratio of $\langle R_g \rangle$ to $\langle R_h \rangle$
ZP	zeta potential

ions can bind to molecular chain to affect the SMP colloid conformations. The water–water structure, ion–water structure and ion–SMP structure are highly dependent on the ion specific effect. Usually, interactions and stability of colloids in biological systems at a low salt concentration, usually <50 mM, could be well described by the Derjaguin, Landau, Verwey, and Overbeek (DLVO) theory, i.e., balance between van der Waals attraction and electrostatic repulsion (Boström et al., 2001). In the DLVO theory the screening of the double-layer repulsion increases with the increasing salt concentration. However, since the ion-specific differences at identical ionic strengths and other present forces are not accounted for by the DLVO theory, this theory fails to describe the experimentally observed stability behaviors at higher salt concentrations (Boström et al., 2001; Israelachvili, 1995; Parmar and Muschol, 2009). Thus, an insight into the response of SMP interactions to ion specific effect will be beneficial for understanding the behaviors of SMP in aqueous solutions with a high level of salts, e.g., the saline wastewater treatment and fouling layers on nanofiltration or reverse osmosis membranes.

In this work, to investigate the ion-specific effects on the interactions among SMP, five different salts, i.e., NaCl, KCl, CsCl, NaBr, and NaI, were used. To avoid more complicated interactions, e.g., bridging, the multivalent ions and polyatomic ions, were not taken into account. The interactions among SMP colloids and related structures in water and the five salt solutions were explored with a combined use of laser light scattering (LLS), Fourier transform infrared spectra (FTIR), Zeta potential (ZP) and acid-base titration techniques. This work could be used to elucidate the role of small ion components in SMP interactions: They are not only a part of the background responsible for screening the electrostatic forces as the DLVO theory describes, but also may interact with SMP molecules to modify the associated water structures, and alter the inter-molecule interactions and related SMP structures. These results could provide interpretations for the experimentally observed non-DLVO stability behaviors.

2. Materials and methods

2.1. SMP extraction

SMP were extracted from *Bacillus megaterium* TF10, which was used in our previous studies (Wang et al., 2012a, 2012b; Yuan et al., 2011), and the strain was cultured aerobically at

30 °C for 24 h in a sterilized *Bacillus* minimal medium (K_2HPO_4 2.5 g/L, KH_2PO_4 2.5 g/L, $(NH_4)_2HPO_4$ 1.0 g/L, $MgSO_4 \cdot 7H_2O$ 0.2 g/L, $FeSO_4 \cdot 7H_2O$ 0.01 g/L, $MnSO_4 \cdot 7H_2O$ 0.007 g/L) with 10 g/L sucrose as carbon source. The seed culture was grown in a 500-ml flask containing 200 mL media for 24 h and then inoculated with 5% (V/V) into a 5 L fermenter (working volume 4 L, aeration) at 30 °C for 24 h. After removal of insoluble material by centrifugation at 10,000 rpm for 5 min, the supernatant was filtered through a 0.45 μm filter paper. The resulting filtrate was precipitated with four volumes ethanol and left overnight at 4 °C. The precipitate was centrifuged at 10,000 rpm for 20 min and re-dissolved in distilled water, then dialyzed against distilled water at 4 °C, using dialysis tubing with a molar mass exclusion limit of 8000 Da to remove salts and unutilized sucrose. Finally, the purified SMP were collected after lyophilization of the retentate.

2.2. FTIR spectroscopy analysis, acid-base titration and Zeta potential (ZP) measurement

FTIR spectroscopy and acid-base titration were combined to identify the functional groups of SMP. The samples were directly mixed into KBr powder and recorded with a VERTEX 70 FTIR (Bruker Co., Germany). The acid-base titration was conducted using an automated titrator (DL50 Mettler-Toledo Co., Switzerland) at 25 °C according to Wang et al. (2012a). The obtained titration curves were analyzed with the PROTOFIT 2.1 software (Turner and Fein, 2006). Briefly, the pK and binding site density of SMP were optimized with ProtoFit by minimizing the sum of squares between derivative function of Q_{tot} (the total number of protons exchanged with the titration system, normalized to SMP mass, estimated from the raw titration data) and model-derived of F_{tot} (the total number of protons exchanged with the titration system, normalized to SMP mass, estimated from the simulated titration mode).

ZP was measured at 25 °C using a Nanosizer ZS instrument (Malvern Instruments Co., UK). The samples were carefully filled into Zeta potential folded capillary cells (DTS1060) to avoid air bubble formation. Each sample was measured at least 6 times and evaluated by Zeta Quality Report to ensure validity.

2.3. LLS measurements

LLS measurements were conducted on an ALV/DLS/SLS-5022F spectrometer equipped with a multi- τ digital time correlator

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