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Characterization and fouling properties of exopolysaccharide produced by *Klebsiella oxytoca*

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ABSTRACT

Klebsiella oxytoca produced a type of exopolysaccharide (EPS) with the average molecular weight (Mw) of 116,018 Da and the average size of 260 nm. The EPS monosaccharide components contained rhamnose, fucose, arabinose, xylose, mannose, galactose and glucose and the molar ratio among them was 0.033:0.0411:0.0147:0.0051:0.2393:0.0986:0.1304. Typical EPS absorption peaks in FT-IR spectrum and pseudoplastic properties were also revealed. The polyvinylidenefluoride (PVDF) membrane showed a relatively larger flux decline resulted from the EPS fouling. The EPS filtration was dominated by more than one mechanism at the beginning phase and mainly by the cake formation at the later phase for both membranes. The pore blocking resistance had a predominant contribution to the filtration resistance and the cake resistance played a secondary role for both the membranes. The EPS adsorption resulted in a weak membrane fouling. The PVDF membrane exhibited a larger adsorption resistance than the polypropylene (PP) membrane.

1. Introduction

Membrane bioreactor (MBR) systems have been successfully used in many wastewater treatment plants in recent years to provide excellent effluent qualities. However, their widespread applications have been hindered due to excessive membrane fouling which resulted in reduced performance, severe flux decline, high-energy consumption and frequent membrane cleaning or replacement (Bailey et al., 1994; Cote and Thompson, 2000; Miura et al., 2007).

With respect to membrane fouling, biofouling especially had a significant limitation on the widespread application of MBR, not only because of pore blocking but also microorganism erosion of membranes. Membrane biofouling was the formation of mature biofilms on the surfaces of membrane modules in MBR systems resulting in significantly increased operating costs (Zhang et al., 2006). Some previous studies have shown that exopolysaccharide (EPS), which had high molecular weight (Mw) distributions and viscous characteristics, presented with a relatively large amount in extracellular polymeric substances and frequently played a critical role in the process of biofilm formation (Weiner et al., 1995; Danese et al., 2000; Frank, 2000). EPS not only may directly cause membrane fouling through forming complex slime layers on membrane surfaces or pores, but also serve as a condition affecting membrane attachment of other biomasses. Previous studies have already proved the important relationship between MBR fouling and EPS fraction presented in supernatant (Nagaoka et al., 1996; Clech et al., 2005; Chen and Lee, 2006). Therefore, investigations on filtration characterization and fouling mechanism of EPS will provide useful information for controlling complex membrane biofouling.

So far, most of studies have focused on the fouling of extracellular polymeric substances (Nagaoka et al., 1998; Janga et al., 2007) which are mainly composed of EPS, protein and nucleic acids. Although these studies involved the effects of EPS on membrane fouling, these investigated effects were multiple concerned in the mixtures of EPS and microorganisms, substrates, extracellular proteins and nucleic acids, etc. That can not present a profound and actual understanding of EPS membrane biofouling mechanism, which is important and valuable for the MBR application. Some model EPS (sodium alginate, cellulose and dextrans) have been often used to investigate the effect of EPS fouling on the membrane because of the difficulty in isolating, purifying and characterizing EPS which are liable to adsorb on the membrane in an actual filtration process. For example, dynamic filtration experiments of sodium alginate were conducted to identify fouling mechanisms using microfiltration and ultrafiltration membranes (Ye et al., 2005a,b, 2006; Katsoufidou et al., 2007). Aqueous solutions containing dextran and cellulose were used to investigate the

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influence of operating parameters on the ultrafiltration (Garcia-Molina et al., 2006a,b). A detailed investigation of fouling mechanisms was reported for ultrafiltration membranes with dextrans obtained by studying membrane–solute (static adsorption) and membrane–solute–solute interactions (ultrafiltration) (Susanto and Ulbricht, 2005, 2006). From these studies, some useful references to EPS fouling mechanism were obtained even if these model compounds could not completely represent real EPS. However, it is not comprehensive to reveal EPS fouling mechanism of MBR only through these model EPS, especially several of them even secreted by algae or plants. As a long chain macromolecular polymer, EPS originating from different microorganisms have different physicochemical properties such as sugar composition, glycosidic bond configuration and rheology, which have significantly impacts on EPS adsorption to membrane materials. Therefore, more EPS secreted by microorganisms, especially dominant strains from MBR biofouling layers, should be investigated for a better understanding of membrane fouling nature.

In this study, EPS produced by *Klebsiella oxytoca*, a dominant strain extracted from the membrane biofouling layer, was isolated and purified. We investigated the physicochemical and rheological properties of the EPS, the filtration flux and fouling resistance during microfiltration. Our aim is to provide some available and valuable information on evaluation of membrane biofouling mechanism in process of MBR wastewater treatment.

2. Methods

2.1. Model microorganism

The *K. oxytoca* strain used in this work was isolated from a biofouled microfiltration membrane module in a pilot submerged MBR, which has been described in detail in our previous study (Li et al., 2007). Tables 1 and 2 showed the operation conditions of the MBR and synthetic wastewater components respectively. The isolated strain was phylogenetically identified by 16S rDNA sequencing analysis and the GenBank Data homology research result was 99% (data not shown).

2.2. Isolation and purification of the EPS

K. oxytoca were cultivated in shake flasks at 30 °C with shaking at 200 rpm. A defined liquid medium which had the same composition as the synthetic wastewater in MBR was used for the cultivation. *K. oxytoca*'s EPS from the cultivation broth was isolated and purified according to a procedure previously described by Allen et al. (2004) with minor modifications. The obtained EPS was frozen at −80 °C.

Table 1
Stable operation conditions of MBR.

Parameters	Values	Parameters	Values
DO (mg L^{-1})	4.0 ± 0.5	Temperature (°C)	25 ± 1
pH	7.0 ± 0.2	Influent COD (mg L^{-1})	600 ± 20
Flux (L m^{-2} h^{-1})	5.0 ± 0.2	Effluent COD (mg L^{-1})	35 ± 5
HRT (h)	8.0 ± 0.3	Influent NH_4-N (mg L^{-1})	25 ± 2
SRT (d)	No discharge	Effluent NH_4-N (mg L^{-1})	1.0 ± 0.2
MLSS (g L^{-1})	10 ± 1		

Table 2
Components and quality of synthetic wastewater (mg L^{-1}).

Components	Concentration (mg L^{-1})	Components	Concentration (mg L^{-1})
Industrial glucose	600 ± 10	$CaCl_2$	10 ± 2
Yeast extract	30 ± 4	$MnCl_2$	2 ± 0.5
Peptone	20 ± 4	$FeSO_4$	0.3 ± 0.1
$MgSO_4 \cdot 7H_2O$	10 ± 2	KH_2PO_4	20 ± 3
NH_4Cl	80 ± 5		

2.3. Characterization of the EPS structure

2.3.1. Molecular weight

The EPS Mw was measured by size-exclusion gel permeation chromatography (waters 600) equipped with an ultrahydrogel linear column (300 × 7.8 mm ID). The mobile phase (0.9 ml/min) was made of 0.1 M $NaNO_3$. Six dextran standards (Sigma–Aldrich) of Mw 180, 4600, 21,400, 41,100, 133,800 and 2,000,000 Da were injected for column calibration, all within the linear range of retention times. The detection was carried out at 25 °C with a double array UV detector (waters 2487 Series) at 280 nm and simultaneously with a Refractive Index Detector (waters 2410 Series). All samples were filtered with 0.45 μm polyvinylidenefluoride (PVDF) filters prior to injection.

2.3.2. Measurement of particle size distribution (PSD)

The EPS particle size was measured by a dynamic light scattering (high performance particle sizer, model HPP5001, Malvern Instruments), which is equipped with a Helium–Neon laser at a wavelength of 633 nm. All the samples were allowed to settle for approximately half an hour in order to remove any large particles that would produce much high intensity value during DLS measurements for estimating PSD measurements. The correlograms of PSD analysis were evaluated with Malvern DTS v3.00 software.

2.3.3. Monose compositions analysis of the EPS

The EPS sample (10 mg) obtained in this work was dissolved in 2 ml of 2 M trifluoroacetic acid (TFA) and hydrolyzed at 120 °C for 2 h. After reduction with 20 mg of sodium borohydride, monosaccharide alditol acetate was prepared using a method described previously (Blakeney et al., 1983). The alditol acetates were analyzed by a gas chromatograph (Shimadzu GC-14A, Japan) with an OV1701 flexible quartz capillary column (30 m × 0.32 mm) and a flame-ionization detector (FID).

Pure nitrogen was used as the carrier gas at a flow of 1.5 ml/min. The column temperature was programmed up to 190 °C from 150 °C at 10 °C/min switching to 3 °C/min to 240 °C. Peaks were identified and estimated using *myo*-inositol as the internal standard. The fraction quantity was determined according to peak areas, using response factors.

2.3.4. FT-IR spectral analysis of the EPS

The EPS FT-IR spectra were analyzed by a Nicolet Nexus 470 FT-IR spectrometer (Thermo Electron Corporation). The EPS sample was pressed into KBr pellets at a sample: KBr ratio of 1:100. The spectra were recorded in transmittance mode over the wave range of 4000–400 cm.

2.3.5. Rheological measurement of the EPS

The lyophilized EPS was dissolved in distilled water at 30, 50 and 100 mg L^{-1}, respectively. Measurements were carried out with a Brookfield RVDV-3 rheometer fitted with a small sample adapter (Brookfield Engineering Laboratories Inc., Stoughton, MA, USA). The viscosity of the solutions was determined at 25 °C under different shearing rates. The relationship between the shearing rate and the apparent viscosity was characterized by assuming the simple power law model (Moreno et al., 2000):

$$\eta = kr^{n-1} \quad (1)$$

where η is the viscosity and r is the shearing rate. The constants, k and n represent the consistency index and the flow behavior index respectively.