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Impacts of hydrophilic colanic acid on bacterial attachment to microfiltration membranes and subsequent membrane biofouling

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ABSTRACT

In order to examine the interactions between physicochemical properties of specific extracellular polymeric substances (EPS) and membrane biofouling, we investigated the impacts of hydrophilic colanic acid, as a model extracellular polysaccharide component, on initial bacterial attachment to different microfiltration (MF) membranes and membrane biofouling by using *Escherichia coli* strains producing different amounts of colanic acid. In a newly designed microtiter plate assay, the bacterial attachment by an *E. coli* strain RcsF^{+}, which produces massive amounts of colanic acid, decreased only to a hydrophobic membrane because the colanic acid made cell surfaces more hydrophilic, resulting in low cell attachment to hydrophobic membranes. The bench-scale cross-flow filtration tests followed by filtration resistance measurement revealed that RcsF^{+} caused severe irreversible membrane fouling (i.e., pore-clogging), whereas less extracellular polysaccharide-producing strains caused moderate but reversible fouling to all membranes used in this study. Further cross-flow filtration tests indicated that colanic acid liberated in the bulk phase could rapidly penetrate pre-accumulated biomass layers (i.e., biofilms) and then directly clogged membrane pores. These results indicate that colanic acid, a hydrophilic extracellular polysaccharide, and possible polysaccharides with similar characteristics with colanic acid are considered as a major cause of severe irreversible membrane fouling (i.e., pore-clogging) regardless of biofilm formation (dynamic membrane).

1. Introduction

Membrane bioreactors (MBRs) have been applied in wastewater treatment and reclamation. However, membrane fouling needs to be addressed to reduce the treatment costs. Membrane fouling in MBR systems is complex and occurs as a result of accumulation of bacterial cells, extracellular polymeric substances (EPS) and other soluble microbial products (SMP) on membrane surfaces and membrane pores (Chang

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et al., 2002; Le-Clech et al., 2006; Meng et al., 2009; Drews, 2010; Ni et al., 2011). The EPS and SMP are either deposited from the bulk liquid or produced in biofilms formed on membrane surfaces. The soluble fraction of EPS or SMP, particularly polysaccharides, has been considered as a major cause of membrane fouling because soluble polysaccharides are predominant in EPS in the mixed liquor of MBRs (Lesjean et al., 2005; Fan et al., 2006; Ng et al., 2006; Kimura et al., 2012). An extensive effort has been made to reveal the interactions between EPS and membrane fouling. However, most studies have simply dealt with the impacts of the EPS concentration in the mixed liquor (Chang et al., 2002; Le-Clech et al., 2006; Meng et al., 2009; Drews, 2010) and their characteristics (i.e., biodegradability and size) (Miura et al., 2007a, 2008; Ni et al., 2011; Zhou et al., 2012) on membrane fouling. It should be also noted that some contradictory results could be found in the literatures due to the complex nature of the interactions (Chang et al., 2002; Le-Clech et al., 2006; Meng et al., 2009; Drews, 2010; Ni et al., 2011). Therefore, further studies are obviously required to better understand the interactions between EPS and membranes.

The composition and characteristics of EPS are dependent on growth conditions such as the reactor feed (wastewater) characteristics and growth phase. Numerous studies have suggested the importance of EPS production in biofilm formation (Davey and O'Toole, 2000). Biofilm formation involves complex developmental processes, including initial attachment of microorganisms, formation of microcolonies, and maturation of the microcolonies (Davey and O'Toole, 2000). Cellular characteristics (motility, cell surface charge and hydrophobicity) are considered to play important roles in the initial bacterial attachment to a membrane surface (Pang et al., 2005; Jinhua et al., 2006). Production of certain EPS has been reported to promote the distinct stages of biofilm development (Davey and O'Toole, 2000). For example, during biofilm formation of *Escherichia coli*, poly-β-1,6-GlcNAc (PGA) (Wang et al., 2004) and curli (Pringent-Combaret et al., 2000) are required for both primary surface colonization and subsequent biofilm formation. Interestingly, while colanic acid reduces initial cell attachment (Hanna et al., 2003; Chao and Zhang, 2011), its production is important for development of voluminous three-dimensional biofilms (Pringent-Combaret et al., 2000; Danese et al., 2000; May and Okabe, 2008).

Bacterial attachment rate is strongly related to biofilm formation rate. Bacterial characteristics involved in cell attachment to membrane surfaces and subsequent biofilm formation have been studied by using pure-culture of bacterial strains but the key characteristics are still not well understood. The relevance of cellular characteristics (motility and cell surface properties) to biofilm formation on reverse osmosis (RO) membranes was investigated using bacterial strains isolated from a fouled RO membrane system (Pang et al., 2005). However, membrane fouling potential was not measured in this study. Since the convective transport of bacteria to the membrane by filtration remains the main cause of biofilm formation, biofilm formation was highly enhanced under filtration conditions (Eshed et al., 2008). Thus, bacterial attachment to membranes and subsequent biofouling potential must be evaluated under filtration conditions. Even under implementation of continuous physical cleaning (air-scrubbing) of membranes, specific microbial populations attached and formed biofilms on the microfiltration (MF) membrane, resulted in severe membrane fouling in MBRs treating real domestic wastewater (Miura et al., 2007b,c).

The impacts of specific EPS components required for biofilm formation on initial bacterial attachment to MF membranes and subsequent membrane fouling are not well understood. The role of bacterial exopolysaccharides in bacterial attachment and biofouling potential of a RO membrane was evaluated using an alginate overproducing (mucoid) *Pseudomonas aeruginosa* under cross-flow conditions (Herzberg et al., 2009). Alginate overproduction increased the hydrophilicity of the mucoid strain, which resulted in the lower cell attachment and consequently decelerated biofouling of the RO membrane (Herzberg et al., 2009). EPS produced by microorganisms are composed of many different components, which can interact with membranes in different ways (Kimura et al., 2012). The effects of other EPS components on membrane fouling potential need to be studied for better understanding of the complex interactions between EPS and membranes.

Although EPSs have been identified as the principal foulants in MBR (Ng et al., 2006; Kimura et al., 2012), little is understood about the effect of each EPS component on membrane biofouling. In this study, we focused on one of EPS, colanic acid, which is a hydrophilic, highly viscous capsular polysaccharide and common antigen produced by many enterobacteria (Rättö et al., 2006), and investigated the effect of colanic acid on membrane biofouling using *E. coli* as a model organism. Because colanic acid reduces initial cell attachment (Hanna et al., 2003; Chao and Zhang, 2011), its production by the primary colonizers may influence the biofouling propensity of MF membranes. We constructed genetically modified *E. coli* K-12 strains producing different amounts of colanic acid to investigate the impacts of colanic acid production on initial cell attachment to different MF membranes and consequent fouling potential. The degree of cell attachment was measured by a newly designed microtiter plate assay. The membrane fouling potential was assessed by bench-scale cross-flow filtration tests. The results of cell attachment and biofouling potential were discussed in correlation with the physicochemical characteristics of bacterial strains and MF membranes.

2. Materials and methods

2.1. Bacterial strains and growth conditions

E. coli K-12 (F^+ λ^+) was used as a model bacterium for cell attachment assay and filtration experiments in this study. Four strains including *E. coli* K-12 harboring an empty cloning plasmid pCA24N (WT), K-12/pCA24N-*rcsF* ($RcsF^+$), K-12 Δ*wcaA*::*kan*/pCA24N ($WcaA^-$), and K-12 Δ*wcaA*::*kan*/pCA24N-*rcsF* ($WcaA^-$ $RcsF^+$) were used as test strains. The *rcsF* gene is a positive regulator of colanic acid synthesis (Gervais and Drapeau, 1992), and this gene was overexpressed by isopropyl-β-D-thiogalactopyranoside (IPTG)-inducible $P_{T5\text{-}lac}$ promoter localized in pCA24N (Kitagawa et al., 2005). The *wcaA*