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Biofouling control: Bacterial quorum quenching versus chlorination in membrane bioreactors

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ABSTRACT

Biofilm formation (biofouling) induced via cell-to-cell communication (quorum sensing) causes problems in membrane filtration processes. Chorine is one of the most common chemicals used to interfere with biofouling; however, biofouling control is challenging because it is a natural process. This study demonstrates biofouling control for submerged hollow fiber membranes in membrane bioreactors by means of bacterial quorum quenching (QQ) using *Rhodococcus* sp. BH4 with chemically enhanced backwashing. This is the first trial to bring QQ alongside chlorine injection into practice. A high chlorine dose (100 mg/L as Cl_2) to the system is insufficient for preventing biofouling, but addition of the QQ bacterium is effective for disrupting biofouling that cannot be achieved by chlorination alone. QQ reduces the biologically induced metal precipitate and extracellular biopolymer levels in the biofilm, and biofouling is significantly delayed when QQ is applied in addition to chlorine dosing. QQ with chlorine injection gives synergistic effects on reducing physically and chemically reversible fouling resistances while saving substantial filtration energy. Manipulating microbial community functions with chemical treatment is an attractive tool for biofilm dispersal in membrane bioreactors.

1. Introduction

Bacteria behave as self-sufficient individuals and maintain a unicellular lifestyle (Costerton et al., 1999), but many of them grow primarily by attaching to biotic or abiotic surfaces (Davey and O'toole, 2000; Kolenbrander, 2000). With time, they grow and spread, forming a biofilm on the attached surface (called biofouling). Biofilm growth on membrane filters impairs filtration performance in membrane bioreactors substantially. It requires high energy consumption, expensive maintenance, and additional downtime costs (Lazarova et al., 2012). Biofouling is thus one of the most challenging unresolved issues for this technology (Meng et al., 2009). Several biofouling control strategies have been developed and applied to membrane bioreactors, including optimizing system operating conditions (Ahmed et al., 2007; Huang et al., 2011; Trussell et al., 2007), modifying membrane filter properties (Yu et al., 2006a, 2008, 2006b), and changing mixed liquor characteristics (Trussell et al., 2007; Wang et al., 2006). However, these approaches are still insufficient for battling against the natural processes associated with microbial growth. Alternatively, it has been recently demonstrated that bacterial biofilm formation and quorum sensing are inextricably linked for some species (Cvitkovitch et al., 2003; Parsek and Greenberg, 2005). Quorum sensing is a bacterial cell communication process that involves the synthesis, release, and detection of signal molecules called autoinducers (Waters and Bassler, 2005). In this context, strategies utilizing quorum sensing inhibition (quorum quenching (QQ)) as an effective tool for biofouling control have attracted significant attention.

A previous study evaluated the relationship between membrane biofouling and autoinducers by using a laboratory-scale membrane bioreactor (Yeon et al., 2009a). Three different types of *N*-acyl homoserine lactone signal molecules were identified in the biofilm. The addition of a QQ enzyme (Porcine kidney acylase I) at a concentration of 10 mg/L reduced the signal molecule concentration in the biofilm and effectively mitigated biofouling. This technology was further developed by immobilizing the acylase enzyme on magnetic particles (Yeon et al., 2009b). Considerably less biofouling was observed in the system with the magnetic particles compared

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to that in the system operated with free enzyme. Jiang et al. (2013) successfully immobilized acylase in sodium alginate capsules for use in a membrane bioreactor. The membrane in the control reactor fouled twice within 110 h (i.e., the transmembrane pressure build-up reached 40 kPa), whereas the transmembrane pressure of the QQ reactor reached only 15 kPa within the same operation time. Oh et al. (2012) enclosed a QQ bacterial strain, *Rhodococcus* sp. BH4, in a microbial vessel for treatment of a laboratory-scale membrane bioreactor. The transmembrane pressure of the control reactor reached 70 kPa within 3 d, whereas the transmembrane pressure of the QQ reactor reached 40 kPa after the same operating period. It was found that the QQ bacterium, *Rhodococcus* sp. BH4, produced the enzyme acyl homoserine lactonase, which can hydrolyze the lactone ring of *N*-acyl homoserine lactone signal molecules and subsequently mitigate biofouling. When entrapped in alginate beads, this QQ bacterium was reported to control biofouling more effectively than the previous QQ carriers (Kim et al., 2013). Additionally, QQ was able to substantially reduce the energy required for filtration and aeration in membrane bioreactors (Weerasekara et al., 2014). To date, studies have concentrated on investigating the effects of different QQ strategies and carriers on membrane biofouling.

Due to the potential for broad impacts, it is important to know whether QQ plays a role in anti-biofouling in the presence of chlorine, which is one of the most commonly used disinfectants. Therefore, this study focused on biofouling control behaviors in membrane bioreactors with densely populated mixed culture microbes when QQ was coupled to chlorine injection. The activity of QQ bacteria and the degree of biofouling were monitored and compared with physical/chemical cleaning strategies. This research evaluated whether blocking microbial communications would be an attractive and effective alternative to physicochemical approaches as a strategy for biofouling control.

2. Materials and methods

2.1. Feed wastewater and quorum quenching bacteria

Simulated wastewater was prepared every day in the laboratory as described elsewhere (Jung et al., 2005) and the chemical composition of the wastewater is provided in Table S1. It was always stored in a refrigerator at 4 °C during the entire experimental period. The quorum quenching bacteria vessel containing *Rhodococcus* sp. BH4 was prepared as described elsewhere (Oh et al., 2012) and a photo of the microbial vessel is shown in Fig. S1.

2.2. Laboratory-scale membrane bioreactor operation

Two identical laboratory-scale submerged membrane bioreactors with a working volume of 2 L were constructed (Fig. S2) and operated under predetermined operating conditions as given in Table 1. The overall study was composed of four phases with different QQ and chlorine doses. For each phase, new membranes were used and the whole mixed liquor present in the two reactors was collected, homogenized, and split back into two parts after equilibration. The membrane modules used were prepared in the laboratory using polyvinyl difluoride (PVDF) hollow fibers (Kolon, Korea) with a nominal pore size of 0.1 μm and an effective surface area of 94.2 cm^2. The two membrane bioreactors were operated at a flux of 30 L/(m^2-h): one was the control reactor and the other was the QQ reactor. The influent was supplied using a peristaltic pump controlled by a level sensor. The hydraulic retention time and solid retention time were set to 7 h and 100 d, respectively, and the reactor temperature was maintained at 25 °C. Air supply to each reactor was performed continuously through an air diffuser underneath the membrane module at a flow rate of 0.5 L/min (velocity gradient = 51 s^{-1}). The transmembrane pressure between the membrane feed and permeate sides was continuously recorded on a laptop computer using a digital pressure transducer (ZSE 40F, SMC, Japan) and a digital multimeter (M-3850D, Metex, Korea). Two types of periodic maintenance cleaning were conducted: physical backwashing using permeate and chemically enhanced backwashing using NaOCl solutions. As a physical cleaning method, permeate backwashing was carried out at 20 kPa for 1 min after every 19 min of filtration. The chemically enhanced backwashing was applied using NaOCl solution, with a concentration of 10 and 100 mg/L as Cl_2, during phases C and D, respectively. The chemically enhanced backwashing was performed once every 24 h while injecting 50 mL of the above NaOCl solutions for ~7 min using a stirred cell (Model 8200, Millipore, USA) under pressure (20 kPa).

2.3. Membrane recovery cleaning procedure

When the transmembrane pressure reached ~50 kPa, the reactor was stopped and the membrane module was taken out for recovery cleaning, which was composed of several physical and chemical cleansing steps. The physically and chemically reversible fouling layers were cleansed as follows. (i) The fouled membrane was placed in a beaker containing 200 mL of a 0.9% NaCl solution and sonicated at 60 Hz for 10 min using a sonicator (2010P, KODO, Korea). The solution collected from this step was used to quantify the amount of biomass attached to the membrane. (ii) The physically cleansed membrane was further cleansed chemically while soaking it in a 0.1% NaOCl solution for 2 h. (iii) After washing the membrane with deionized water (600 mL), the membrane was soaked in a 1.0% citric acid solution for another 6 h. (iv) Finally, the chemically cleansed membrane was rinsed by filtering deionized water (600 mL) through the membrane at a flux of 30 L/(m^2-h).

2.4. Estimation of the hydraulic filtration resistances

The hydraulic filtration resistances at the end of each phase were determined as follows:

$$J = \frac{\Delta P}{\mu\left(R_m + R_p + R_c + R_i\right)} \quad (1)$$

where J is the permeate flux (L/(m^2-h)), ΔP is the transmembrane pressure (kPa), μ is the permeate viscosity (Pa-s), R_m is the intrinsic membrane resistance (m^{-1}), R_p is the physically reversible fouling resistance (m^{-1}), R_c is the chemically reversible fouling resistance (m^{-1}), and R_i is the irreversible fouling resistance remaining after chemical cleaning (m^{-1}). R_m was determined using pure water before use of a virgin membrane. The R_p portion was determined after physical cleaning (i.e., after step (i) stated above). The R_c portion was then determined after chemical cleaning (after step (iv) above). The R_i was the portion of fouling left over after chemical cleaning (i.e., the fouling resistance after the full recovery cleaning).

2.5. Calculation of energy consumption

The specific filtration energy (E_f) was calculated by integrating the relevant transmembrane pressure profiles as follows:

$$E_f = \frac{1}{\eta t_f}\int_0^{t_f} \Delta P dt \quad (2)$$

where t_f is the filtration time (d) and η is the pump efficiency (which was assumed as 0.6).