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Assessing the effects of bacterial predation on membrane biofouling

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ABSTRACT

Membrane biofouling is one of the major obstacles limiting membrane applications in water treatment. In this study, *Bdellovibrio bacteriovorus* HD 100, a Gram-negative predatory bacterium, was evaluated as a novel way to mitigate membrane biofouling and its subsequent performance decline. Dead-end microfiltration (MF) tests were carried out on *Escherichia coli* DH5α and *B. bacteriovorus* HD 100 co-culture feed solutions. Predation of *E. coli* was performed at either a low or high multiplicity of infection (MOI), which is defined as the predator to prey cell ratio. The MOIs tested were 2 and 200, and the viability of both the *E. coli* prey and the predator was monitored over 48 h. The higher MOI (high predator, HP) culture showed a nearly 6-log loss in *E. coli* number after 24 h when compared to both the control and low MOI (low predator, LP) cultures, whereas the *E. coli* population within both predated cultures (HP and LP) became nearly identical at 48 h and 4-log lower than that of the control. The unpredated cultures led to significant loss in water flux at 12, 24, and 48 h of culture, but the HP and LP membranes showed less loss of flux by comparison. Analysis of the total membrane resistance showed a similar trend as the flux decline pattern; however, irreversible resistance of the membrane was much higher for the 48 h LP culture compared to the unpredated and HP cultures at 48 h. This increase in irreversible resistance was attributed mainly to *E. coli* debris, which accumulated in the medium after the predator lysed the prey cells. These results show that pretreatment of wastewater using a suitable concentration of predatory bacteria such as *B. bacteriovorus* can enhance membrane performance.

1. Introduction

A membrane is a semi-permeable layer between two phases and its application has grown remarkably in water treatment and other industries, particularly with the introduction of low pressure microfiltration (MF) and ultrafiltration (UF) membranes (Huang et al., 2009). The MF process, with a membrane pore size range of 0.1–10 μm, has been broadly applied for

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diverse chemical and biochemical processes to remove particulate matter (Keller et al., 2001). Sieving is generally considered to be a major filtration mechanism of the MF process (Hwang and Sz, 2011; Veerasamy et al., 2009).

However, one of the most crucial drawbacks of membrane processes is membrane biofouling, which results in a decline in membrane performance and increased operating cost (Magara and Itoh, 1991). Membrane biofouling is generally triggered by the attachment of microorganisms on the membrane surface which then start to multiply and develop a biofilm containing extracellular polymeric substances (Kolari et al., 2001). Numerous studies have focused on mitigating biofouling problems by: (i) modifying the membrane surface with various antimicrobial materials, such as silver nanoparticles (Sawada et al., 2012; Mollahosseini et al., 2012) as well as (ii) improving feed water quality through coagulation (Gamage and Chellam, 2011), ozonation (Hwang et al., 2010), sonification (Veerasamy et al., 2009; Hakata et al., 2011), and chlorination (Rajagopal et al., 2003), etc. Although these methods enhance membrane performance, they are not environmentally-friendly due to the dosing chemicals and sludge-waste produced. Furthermore, the disinfecting chemicals employed, such as chlorine, have an adverse impact on most commercial polymeric membranes. One of the most widely used membranes in water treatment, the polyamide membrane, deteriorates easily in chlorine containing agents as the amide bonds (–CO–NH–) of the membrane are extremely susceptible to chlorine attack (Kwon et al., 2011a; Gu et al., 2012; Hong et al., 2013). Additionally, oxidation pretreatments lead to the production of harmful disinfection by-products, such as bromate, aldehydes, trihalomethanes, and carcinogenic haloacetic acids (Huang et al., 2005; Boorman et al., 1999).

With increasing interest in new, effective, and environmentally safe ways to mitigate membrane biofouling, several researchers have investigated the use of biological treatments as an alternative. *Bdellovibrio*-and-like-organisms (BALOs) are group of Gram negative bacteria which live through attacking other Gram negative species and lysing them to obtain their nutrients. *Bdellovibrio bacteriovorus* HD 100, the most characterized member of this group is a small bacterium which has a typical width of 0.3 μm (Stolp and Starr, 1963). Its life cycle has two main phases; the attack phase in which the predator swims in the medium using its single polar flagellum searching for prey, and the intraperiplasmic phase in which the predator starts upon encountering a suitable prey cell. In this phase, the predator loses its flagellum and penetrates the prey periplasm where it elongates, septates, and lyses the prey from inside before the progeny finally come out and attack other prey in the medium (Dwidar et al., 2012a; Sockett and Lambert, 2004). This intraperiplasmic phase usually takes about 3–4 h and produces 3–6 progeny cells from a single *Escherichia coli* cell (Sockett and Lambert, 2004). In fact, *B. bacteriovorus* was found in several studies to be very effective in killing its prey bacteria and decreasing their populations in the liquid medium by 3–4 orders of magnitude (Dwidar et al., 2012b; Dashiff et al., 2011). Also, unlike the eukaryotic predators which are limited in their abilities to attack biofilms and microcolonies (Derlon et al., 2012; Bohme et al., 2009), this bacterial predator penetrate deeply into the biofilm formed by its preys and effectively eradicate them (Dwidar et al., 2012b; Dashiff et al., 2011; Kadouri and O'Toole, 2005; Fratamico and Cooke, 1996). In addition, another study found that encapsulated prey, which are usually resistant to bacteriophages, are still attacked and predated upon by BALOs (Koval and Bayer, 1997).

The aim of this study was to evaluate the potential of using the bacterial predator *B. bacteriovorus* HD 100 as an environmentally-friendly alternative to decrease the microbial load in feed water and, hence, mitigate membrane biofouling and the subsequent performance decline. Predation on *E. coli* was investigated in the absence or presence of *B. bacteriovorus* at two different initial multiplicities of infection (MOI), which is defined as the predator to prey cell ratio, and the effects of bacterial predation on membrane biofouling were investigated by flux analysis of the co-culture medium and fouling analysis using a resistance-in-series model.

2. Materials and methods

2.1. Microorganisms and culture conditions

E. coli DH5α, obtained from the Korean RDA-Genebank Information Center (genebank.rda.go.kr), was used as the model prey in this study. It was transformed with the pAMCyan plasmid (Clontech, Palo Alto, USA), which contains the *cfp* gene that codes for cyan fluorescent protein (CFP) and the β-lactamase gene that confers ampicillin resistance. This strain was kept as a frozen glycerol stock at −80 °C and was routinely streaked on LB (Difco, Detroit, MI, USA) agar plates supplemented with ampicillin (100 μg/ml) and incubated at 30 °C. *E. coli* is typically rod-shaped, and has a diameter of 0.7–1.0 μm and length of 1.6–3.0 μm (Grossman et al., 1982; Pierucci, 1978). After overnight growth, one colony was inoculated into LB broth supplemented with ampicillin and allowed to grow overnight (16 h) at 30 °C and 250 rpm. This culture was then used as inoculum for the experiments.

B. bacteriovorus HD 100, which was purchased from the German Collection of Microorganisms and Cell Cultures, was used as the model bacterial predator. This predator was also kept as a frozen glycerol stock and was streaked within a top diluted nutrient agar plate containing *E. coli* MG1655 as the prey, as described previously (Monnappa et al., 2013). After 2–3 days of incubation at 30 °C, a small portion was aseptically transferred from the clear area formed around the streak zone and vortexed with 5 ml of HEPES buffer (25 mM HEPES buffer, containing 2 mM $CaCl_2$ and 3 mM $MgCl_2$, pH 7.2). This solution was then filtered through a 0.45 μm filter to remove any prey cells and agar remaining while the predator, due to its small size which is about 0.3 um in average (Stolp and Starr, 1963), was able to pass through. This filtrate (1 ml) was then added to fresh prey cells as described below. The co-culture was incubated in a shaking incubator (250 rpm at 30 °C) until it showed clearance (optical density [OD] < 0.25) and predator cells were clearly seen under a microscope.

B. bacteriovorus HD 100 was sub-cultured every 24 h in 15 ml HEPES buffer (25 mM, pH 7.2) supplemented with 2 mM $CaCl_2$ and 3 mM $MgCl_2$ which contained *E. coli* MG1655 as a prey at an initial OD of 1.0. To prepare these subcultures, the prey (*E. coli* MG1655) was grown in LB broth overnight at 30 °C and

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