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Impact of conditioning film on the initial adhesion of *E. coli* on polysulfone ultrafiltration membrane

Krizel D. Garrido [a], Ronna Jane S. Palacios [a], Chaeyoung Lee [b], Seoktae Kang [a,*]

[a] Department of Civil Engineering, Kyung Hee University, 1732, Deokyoungdaero, Giheung-gu, Yongin-si, Gyeonggi-do 446-701, Republic of Korea
[b] Department of Civil Engineering, The University of Suwon, San2-2 Wau-ri, Bongdam-eup, Hwaseong-si, Gyeonggi-do 445-743, Republic of Korea

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ABSTRACT

The impact of conditioning films on the initial adhesion of bacterial cells on polysulfone ultrafiltration membrane was investigated. Interestingly, the clean membrane possessed higher adhesion of both latex particles (CML) and *Escherichia coli* than the membrane conditioned with bovine serum albumin (BSA), alginate (SA), and natural organic matter (NOM). While CML and *E. coli* had similar zeta potentials, the motility of the *E. coli* increased initial adhesion on to membrane surfaces than CML. The addition of calcium ion increased the adhesion of CML and *E. coli* with the great impact on the SA-coated membrane as compared to BSA and NOM.

1. Introduction

In recent years, the demand of membrane-based technology for water and wastewater treatment has been steadily increasing. Almost all conventional water sources include mainly bacteria, debris, silt, clay or any other organic matters; and these substances attach to the surface leading to membrane fouling, moreover, serve as a precursor for biofilm formation on the membrane surface. Membrane fouling can cause severe flux decline and affects the quality of water being produced. The mitigation of fouling is the main objective and most difficult aspect in membrane-based water treatment processes [1].

The formation of biofilm on membrane surface is initiated by the attachment of microorganisms to surfaces and the development of biofilm starts when the attached microorganisms begin to reproduce and grow. These biofilms are even more difficult to eliminate as compared to free-floating bacterial cells [2]. Moreover, biofilm can cause further changes of membrane surface properties that may shorten life of membranes. The biofilm formation is influenced by different factors like solution chemistry and surface characteristics of both microorganisms and membrane [3,4].

Bacterial adhesion to surface is primarily due to the transport and attraction of the cells to the surface followed by adsorption and attachment. Adhesion of bacteria to surfaces in general involves a complex interplay of physical, chemical and biological factors [5]. Bacterial cells are attracted to the surface through the physicochemical properties such as Brownian motion, van der Waals attraction forces, gravitational force, electrostatic forces, and hydrophobic interactions with additional biological factors [2,6]. Such difference reflects the biological affinity of the membrane toward bacteria. Cell surface properties, development of conditioning film on membrane surfaces, co-adhesion and biological changes in attaching bacteria may well affect the prerequisites for adhesion to such an extent that prediction of the adhesion process is virtually impossible if based only on the physicochemical factors experimented by colloidal particles [7,8].

As one example, organic molecules are spontaneously and rapidly adsorbed onto the surface and forms a conditioning film when membrane surfaces are exposed to the feed water [9]. The conditioning step may last for few seconds to minutes after the membrane is exposed to the feed water. In previous studies, it was demonstrated that the presence of alginate and Suwannee River natural organic matter (SRNOM) conditioning films significantly improved bacterial initial adhesion onto glass surface at low ionic strength [10]. Also, humic acid films on the surface increased colonization of *Escherichia coli* bacteria as compared to the clean substratum [11]. After the bacterial transport, interactions between bacterial surface structures and the membrane become predominant. This implies firmer adhesion of bacteria to a surface

* Corresponding author. Tel.: +82 31 201 3326; fax: +82 31 202 8854.
E-mail addresses: krizel.garrido@gmail.com (K.D. Garrido), chaeyoung@suwon.ac.kr (C. Lee), seoktae.kang@khu.ac.kr, seoktae.kang@gmail.com (S. Kang).

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by the selective-bridging function of bacterial surface polymeric structures, which include capsules, fimbriae, or pili and slime [6]. For motile cells, the impact of the flagella on deposition is dependent on the presence of the conditioning film. In addition, flagellar motility is suggested to be necessary for initial attachment, probably to overcome repulsive forces [12]. Meanwhile, conditioning film may affect the attachment of cells that the steric interactions between flagella and extended polyelectrolytes of the conditioning film hinder cell deposition [13]. Protein films were also found to reduce adhesion of bacteria to inanimate susbtrata caused by an increase in surface net negative charge [14]. It has been also reported how to prevent the formation of biofilms on steel surfaces through the N-acetyl-L-cysteine (NAC) conditioning layer [15]. NAC was confirmed not only to reduce adhesion but also detached adhered cells from the steel surface. NAC has been reported to decrease EPS production for *K. pneumonia* bacteria.

The presence of the conditioning film still varies on each condition and properties of both particles and cells. Although many studies have shown relationships of membrane surface hydrophobicity, charge or roughness with bacterial adhesion, it is not clearly shown the adsorption of a conditioning film affect initial bacterial adhesion on membranes [5,16,17].

In this paper, we have investigated the influence of the organic conditioning film formed on membrane surface together with changing solution chemistry, as these are the factors affecting biofilm formation. Carboxylated modified latex (CML) particles and *E. coli* cells were used in the experiment as the surrogate of colloids and bacterial cells. This study will provide better insight in explaining the effects of protein, carbohydrates and soluble microbial products in bacterial adhesion which can help in formulating effective antifouling strategies.

2. Materials and methods

2.1. Preparation of membrane, CML particles and bacteria cells

A commercial polysulfone ultrafiltration membrane (GE Osmonics, USA) was used in the experiment. Fluorescent carboxylated modified latex (CML) particles (1 μm in diameter; Magsphere, USA) were used as model colloidal particles. For the model bacterial cell, fluorescent *E. coli* BL21 pET-25 prepared from Korea Research Institute of Bioscience and Biotechnology was used with the Kanacycin-resistant plasmid. *E. coli* cells were incubated at 37 °C in Luria–Bertani (LB) broth with 50 mg L^{-1} Kanamycin solution. The cells were then harvested after 5 h in the exponential growth stage. The harvested bacterial suspension was centrifuged at 13,500 rpm for 2 min (Wisespin, CF-10), and then, washed and re-suspended in 0.9% NaCl solution for three times to remove any impurities present in the solution. Upon re-suspension, each vial was vortexed carefully for a homogenous mixture before repeating the centrifuge process. The *E. coli* cell pellets were finally re-suspended in their respective ionic concentration to be used in the experiment using KCl solution.

The zeta potentials of CML and *E. coli* were determined by zeta potential analyzer (ZetaPlus; Brookhaven Instruments Corporation, USA) under three different ionic strengths (1, 10 and 100 mM). The pH was adjusted from pH 2 to pH 11 by titration with 0.05 M NaOH and HCl solution.

2.2. Membrane conditioning

Three model organic compounds were used in conditioning the membrane surface. Bovine serum albumin (BSA; Sigma–Aldrich, USA), sodium alginate (SA; Sigma–Aldrich, USA), Suwannee River natural organic matter (NOM; IHSS, USA) were used as surrogates for proteins, carbohydrates and soluble microbial products, respectively. For conditioning of membranes, stock solutions of 10 mg/10 ml were prepared by dissolving each organic compound in deionized (DI) water and membrane coupons were dipped for 30 min on each solution type and then washed twice with the corresponding ionic strength solution to be used in the experiment to remove weakly bound organic matters. The FT-IR spectrum of the membranes was analyzed to see the different functional groups existing before and after conditioning the membranes.

2.3. Adhesion experiment

After the membrane coupons had been prepared, 3 pieces were attached on each microscopic slide and were submerged in a 20-ml solution using conical vials for 1 h. The experiment was conducted at different ionic strength of 1, 10 and 100 mM KCl with varying pH of 4, 7 and 10. To test the effect of calcium ions on the adhesion of CML and *E. coli*, 1 mM $CaCl_2$ with 7 mM KCl (total ionic strength of 10 mM) solution was used in comparison with 10 mM KCl solution. Each solution contains 2.5×10^6 ml^{-1} CML particles or *E. coli* cells at ambient pH (6.0 ± 0.2). After the adhesion, membranes were washed twice with the corresponding ionic strength solution and observed under the fluorescent microscope (Olympus BX43, Japan). At least ten photos were taken on each sample, and attached CML particles or *E. coli* cells were directly counted within the observed area.

3. Results and discussion

3.1. Material characterization

3.1.1. Zeta potential measurements

Zeta potentials of CML particles and *E. coli* cells were examined under 2.5×10^5 ml^{-1} concentration at different pH and ionic strength as shown in Fig. 1. There are similarities between the zeta

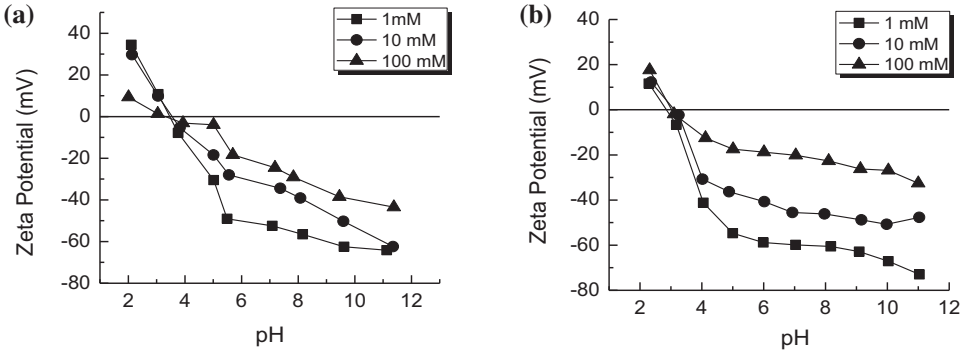

Fig. 1. Zeta potential of (a) CML particles and (b) *E. coli* cells at various pHs and ionic strengths.