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Effect of Membrane Surface Properties During the Fast Evaluation of Cell Attachment

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Abstract: The biofouling potential is one of the important factors to design and to select membranes for water and wastewater treatment. In this investigation, the effect of membrane surface properties during the attachment of *S. cerevisiae* cells was examined using a laboratory-scale membrane filtration cell enabling direct microscopic observation of microbial cell deposition. The experimental results from 6 commercially available membranes showed that the initial adhesion rate, k_d, was affected by the zeta potentials, hydrophobicity, and roughness of membrane surfaces. The k_d value was significantly lower at the membrane which had more negative, hydrophilic, and smooth surfaces. The results will be helpful to minimize

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the time for selecting membranes in different situations, and for testing the performance of newly designed membranes.

Keywords: Membranes, biofouling, surface potentials, surface roughness, hydrophobicity, membrane selection

INTRODUCTION

For water and wastewater treatment, removal of particles (colloids, bacteria, etc.) and soluble matters (organics, inorganic nutrients, humic acids, etc.) are critical. Membrane technology can compete with traditional technologies such as gravity separation, air or gas flotation, and chemical flocculation, due to its advantages of lower energy consumption, smaller space occupation, and high quality of permeate (1–3). However, the decrease of permeate flux or membrane fouling is recognized as the main problem in the application of membrane technologies (4–6). Several types of membrane fouling have been introduced including inorganic fouling or scaling, colloidal fouling, organic fouling, and biofouling (7). Of them, the formation of biofilm on the membrane surfaces or membrane biofouling have been regarded as the most serious problem (8, 9). Membrane biofouling is initiated by irreversible adhesion of one or more bacteria to the membrane surface followed by growth and multiplication of the sessile cells at the expense of feed water nutrients (6, 8). Membrane biofouling is a very complicated process that is affected by many factors, including some characteristics of bacteria itself, membrane surface, and the environmental factors such as pH, ionic strength, ion species, etc. (8, 10). Generally, there are two strategies to control biofouling in the membrane process;

1. optimization of operating conditions including pretreatment of feed and cleaning procedures, and
2. new membrane development or modification of existing membranes which have less biofouling potentials.

The development of new membranes and the modification of existing membranes have been extensively studied during past decades. Many membranes have been developed to minimize fouling by electrostatic repulsion, polymer grafting, hydrophilic coating, etc. (2, 11–13). It have been widely accepted that hydrophilic membranes exhibited lower fouling potentials than hydrophobic ones (14, 15). Some research, however, showed that the surface hydrophilicity of a membrane alone did not necessarily indicate whether a membrane would be fouling resistant (16, 17). Brant and Childress (16) found that colloid-membrane and colloid-colloid interaction played important roles during the fouling of hydrophilic membranes. They concluded that it would be necessary to assess the adhesive energy between the

membrane and the colloid to predict the fouling behavior. Ridgway et al. (17) evaluated the biofouling potentials of "hydrophobic" *Mycobacterium* and "hydrophilic" *Flavobacterium* onto nine modified polysulfone membrane surfaces. The results suggested that hydrophobic mycobacteria tended to attach better to more hydrophobic membranes. They also found that physical properties of membranes (pore size, roughness, porosity) affected the attachment of microorganisms. However, the optimization of the membrane process could be hardly examined because there are too many cases to test, and each case takes a long time (7, 18–20). Moreover, characteristics of source waters and the operating conditions are different in each situation. Hence, fast and realistic examination methods for membranes in each of the source water conditions are essential in membrane fouling prediction and new membrane design.

Ridgway et al. (12, 17) developed QSAR to evaluate the rate of biofouling using 23 kinds of membranes with different physico-chemical characteristics. They incubated membranes in solutions and rinsed using DI water followed by enumeration of bacteria on the membrane surfaces. Pasmore et al. (15) also evaluated the biofouling potential of various membrane materials using rotating incubator or "rototorque." However, these methods are time consuming and far from real membrane process. Several past studies have utilized direct measurement via online monitoring techniques to derive mechanistic information about various membrane fouling and scaling phenomena (6, 21, 22). Chen et al. (23) provided an excellent review of in-situ monitoring techniques used to investigate various fouling mechanisms in membrane filtration processes.

In the previous study, we developed direct microscopic observation technique combined with a novel analytical method and showed that it could be applied to rapid identifying fouling potentials of membrane materials. In this study, we will apply the technique to develop a rapid and quantitative method for evaluating the biofouling potential of various membranes. For this, the rate of cell attachment onto membranes which have different physico-chemical properties including hydrophobicity, surface charge, and surface roughness will be investigated using a novel lab-scale crossflow membrane filter enabling direct visual observation of microbial deposition onto membrane surface.

MATERIALS AND METHODS

Preparation and Characterization of Cells

The bacterium used in this study was *Saccharomyces cerevisiae* (obtained as dry yeast from Fleischmann, Inc., USA). It was grown in MYGP medium (malt extract, 0.6%; yeast extract, 0.6%; peptone, 1%; glucose, 2%), and monitored by the measurement of optical density at 570 nm. Cells were harvested during the early stationary phase and washed using distilled water twice. The hydrodynamic diameter of the yeast cells was characterized by a particle size analyzer (Coulter Counter Multisizer, Beckman-Coulter, Inc., USA).

The zeta potentials of *S. cerevisiae* were determined at 22°C by measuring the electrophoretic mobility using an ELS 8000 electrophoretic light scattering spectrophotometer (Otsuka Electronics, Japan). The relative hydrophobicity of cells was evaluated by the microbial adhesion to solvents (MATS) test as previously suggested (24, 25). The chloroform, hexadecane, ethyl acetate, and decane were chosen as non-polar, electron acceptor/donor solvents. Three milliliters of cell suspension (1×10^7 cells/ml, five replicates) in 10 mM NaCl at pH 5.6 were mixed with 1 ml of each solvent, vigorously vortexed for 20 seconds, and then left to separate for 10 minutes. The absorbance of the aqueous phase was measured by spectrophotometry at 570 nm (Beckman, USA) before and after MATS. The MATS was determined by taking the percentage decrease in the absorbance.

For tests of membrane biofouling potential, 0.26 g of cells was placing in 100 ml of isotonic water (0.9% NaCl), stirred for 30 minutes, and centrifuged at 2,000 rpm for 10 minutes. The supernatant was removed and the centrifuging procedure was repeated two more times using DI water. After washing, cells were dyed by adding 1 ml of 6% Coomassie brilliant blue R-250 (Sigma, USA), 10 ml of acetic acid, and 25 ml of isopropanol in 64 ml of deionized water (to the final volume of 100 ml). The mixture was then stirred for three hours, centrifuged, and supernatant was removed. The dyed cells were washed twice to remove the excessive dye. There were negligible differences between wild and dyed cells with respect to measured zeta potential and hydrophobicity by MATS, which indicated there were no significant changes in surface characteristics during cell staining (data not shown).

Characterization of Membranes

The six commercially available UF and MF membranes (A, B, and C from GE-osmonics, D from Saehan, E from Celgard, F from Sumitomo) were used in this study. For the measurement of membrane surface potentials, polystyrene latex particles (particle diameter ~520 nm) were used as internal colloids with ELS 8000 zeta potential analyzer. Three measurements were taken, and the standard deviations of the zeta potentials calculated. The contact angles were determined using the sessile drop technique, with an NRL Contact Angle Goniometer (Rame-Hart, USA). The measurements were carried out at room temperature, with a 10 μl DI water droplet, and five contact angles were measured immediately following the deposition of the water droplet. The surface roughness of membranes was estimated using atomic force microscopy (PSIA, Korea) in the non-contact mode with the NHCP tip.

Direct Visual Observation System

A flow cell was constructed from polyacrylic plate and the glass window. It was mounted on a microscope stage to allow direct visual observation of

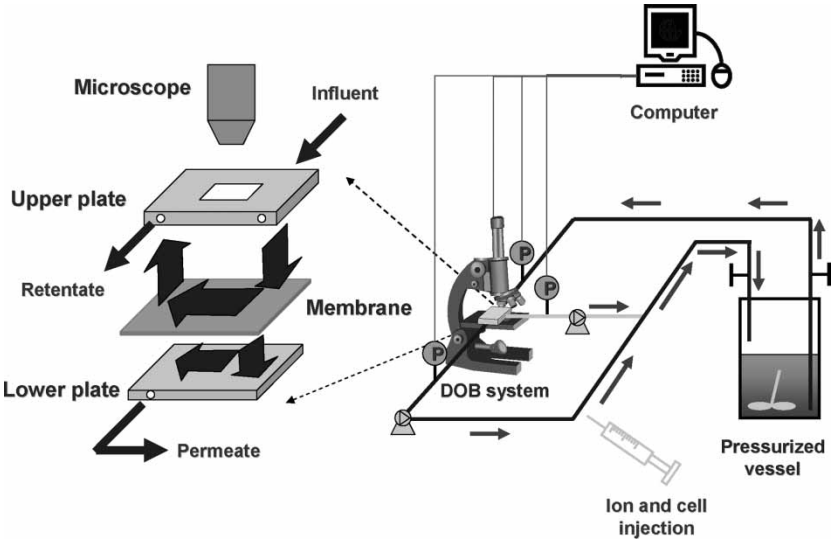

Figure 1. Schematic diagrams of direct observation system.

microbial deposition via light and fluorescence microscopy as shown in Fig. 1. The dimensions of the flow channel are 1 mm (height) by 20 mm (width) by 75 mm (length). A detailed explanation about experimental set-up and procedures can be found elsewhere (6). Briefly, the rate of cell adhesion was monitored visually through the top plate using a microscope, with images taken at the center of the flow channel by a Nikon Digital Sight DS-U1 employing the ACT-2U image analyzing program. In this setup, one image covered a surface area of 0.6×0.45 mm, i.e. 0.27 mm^2. The monitoring of various points of membrane surface confirmed that the observed small area was representative of the entire surface. Images were transferred to the public-domain NIH image J program and processed to determine the microbial surface coverage. All experiments were done in cross-flow velocity of 2.5 cm/s, permeation velocity of 20 μm, ionic strength of 10 mM as NaCl, and cell concentration of 5×10^6/ml with pH of 5.6 ± 0.1 at the room temperature of 22°C.

RESULTS

Surface Properties of Cells

The cell diameter, zeta potential, and MATS results of the *S. cerevisiae* are summarized in Table 1. The hydrodynamic diameter of *S. cerevisiae* was found to be 4.8 μm. The zeta potential of the yeast cells was −8.7 mV in

Table 1. Summary of the surface characteristics of cells in 10 mM NaCl at pH 5.6

Diameter (μm)	Zeta potential (mV)	MATS			
		Chloroform (%)	Hexadecane (%)	Ethyl acetate (%)	Decane (%)
4.8	−8.7 ± 1.5	84 ± 7	81 ± 5	26 ± 4	78 ± 3

10 mM NaCl at pH 5.6, with a point of zero-charge around pH 3 (not shown), which was in accordance with our previous result on the same cell strain (6).

In the MATS results, the relative affinity of chloroform (solvent for electron acceptor) was higher than that of ethyl acetate (solvent for electron donor), and those of hexadecane and decane (nonpolar solvent) were also high. These results implied the hydrophobic nature of cell surfaces with a moderate electron accepting nature compared with other strains reported (25).

Surface Properties of Membranes

The membranes which were used in this study had a wide range of surface potentials, surface roughness, and contact angles (Table 2). Generally, hydrophobic membranes which had bigger contact angles (C, E, and F) showed lower surface potentials because they had less ionic or polar molecules on the exposed surface of membranes. Results also showed that E and F membranes had extremely rough surfaces than other membranes, due to the stretching (E) and fabrication (F) during the manufacturing process.

Table 2. Summary of membrane surface properties

Types		Materials	Surface potentials[a] (mV)	Relative roughness[b] (nm)	Contact angle (°)
A	UF	Polyacrylonitrile	−19.7 ± 3.2	0.4 ± 0.1	35.6 ± 5.1
B	MF	Polyacrylonitrile	−18.5 ± 2.4	0.9 ± 0.3	37.7 ± 4.3
C	UF	Polysulfone	−3.4 ± 2.7	10.6 ± 3.1	64.4 ± 4.7
D	UF	Modified polysulfone[c]	−10.4 ± 3.0	17.8 ± 2.3	43.6 ± 3.2
E	MF	Polypropylene	−8.6 ± 1.4	47.9 ± 8.1	60.6 ± 2.7
F	MF	Teflon	−5.5 ± 2.9	103.3 ± 10.7	57.5 ± 7.1

[a]measured at ionic strength of 10 mM as NaCl, pH of 5.6, and temperature of 22 ± 1°C.

[b]RMS roughness measured by atomic force microscopy from 5 different lines in 5 × 5 or 10 × 10 μm^2 area.

[c]hydrophilic surface modification.

Determination of Cell Adhesion Rate

Direct microscopic images from representative experiments during 60 minutes of filtration time are provide in Fig. 2 at (a) 10 minutes, (b) 30 minutes, and (c) 60 minutes. It showed increasing extents of surface coverage with time. Cells appeared to deposit randomly and the number of cell attached on the membrane surface increased in proportion to time as shown in Fig. 2(d). The number of cells in the observed membrane area increased linearly up to a certain point, and then tapered off with continued cell deposition due to blocking new cell deposition by already deposited cells as explained in previous study (6).

The rate of cell adhesion, k_d, onto various membrane surfaces was then calculated by taking the slope of the linear region of the curve normalized by the number concentration of cells fed into the flow cell via

$$k_d = \frac{\theta(t)}{dt} \times \frac{1}{C_0} \tag{1}$$

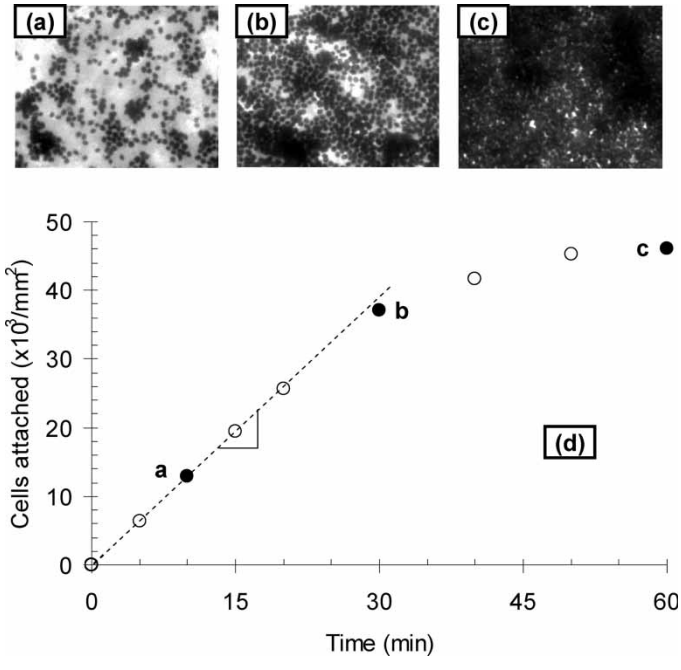

Figure 2. Direct microscopic images of cells deposited on membrane A after (a) 10 minutes, (b) 30 minutes, and (c) 60 minutes. The number of cells deposited on unit area with time is plotted in (d). Constant experimental conditions employed were permeation velocity 20 μm/s, cross-flow velocity 2.5 cm/s, 10 mM NaCl, pH 5.6 (unadjusted), feed cell concentration 5×10^6/ml, and temperature $22 \pm 1^\circ$C.

where $\theta(t)$ is the number of cells attached on the unit area at each time and C_0 is the bulk feed cell concentration.

Cell Adhesion Rates of Membranes

Figure 3 shows the rate of cell adhesion (k_d) performed using various membranes at the same solution chemistry and operating conditions. The k_d of membrane A was 1.56 cm/hr and that of membrane B was not statistically different with membrane A considering the standard experimental error during the measurement. The k_d of E and F membranes ranged 2.35 to 2.57 cm/hr, which were almost twice than those of A and B membranes. Ideally, it implied that the surface of E and F membranes would be completely fouled by cells while only 50% of A and B membranes were covered by cells.

DISCUSSIONS

The initial adhesion rate of cells showed that 6 membranes can be categorized into two groups, low (A and B) and high (C through F), with respect to the biofouling potential. A and B membranes had more negatively charged, flat, and hydrophilic surfaces with smaller adhesion rates than other membranes. From the statistical analysis using SPSS (Ver. 10, SPSS Inc.) between k_d and various properties of membrane surface, the k_d appeared linearly correlated with

1. zeta potentials,
2. surface roughness, and
3. hydrophobicity denoted by contact angles as shown in Table 3.

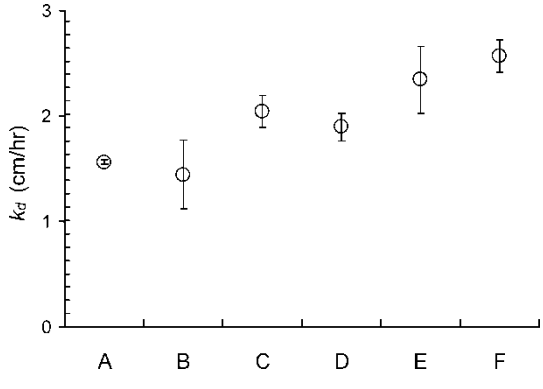

Figure 3. The initial cell adhesion rate, k_d, for various membranes.

Table 3. Summary of linear correlation factors between cell adhesion rates and surface properties of membranes

	Zeta potentials	RMS roughness	Contact angles
r[a]	0.69	0.79	0.68

[a]linear correlation factor significant at 95% confidence level.

Effect of Surface Potentials

The surface charge of both cells and membranes is one of the important physical factors to mediate the initial adhesion. Most membranes acquire a negative surface charge in water due to the protonation and polarization of their surface groups. At the higher negative charge of membrane surfaces, long-range electrostatic forces may influence the initial phase of cell adhesion onto membrane surfaces.

In order to explain the deposition profiles due to the surface potentials, DLVO interaction energy were calculated (26) and summarized in Table 4. Zeta potentials of the cells and membranes were used as surface potentials for the calculations.

The results of DLVO theory showed that all membranes were favorable for cell adhesion. Especially, energy profiles of membrane C, E, and F had no secondary minima, which implied strong and irreversible adhesion of cells. Cells on membrane A and B seemed to have stayed mostly in secondary minima, which could be removed by external forces such as the high cross-flow velocity as shown in previous study (6). But, some investigation did not follow DLVO predictions in this study. First, the adhesion rate of *S. cerevisiae* onto membrane D was not significantly affected by the relative surface charge. It had a comparable secondary minimum energy with that of membrane A and B, while had much higher initial adhesion rate constant. Moreover, the membrane C showed the less negative surface

Table 4. Summary of DLVO interaction energy calculations

Membranes	A	B	C	D	E	F
Secondary minimum depth (kT)[a]	−7.7	−7.9	N.B.[b]	−10.8	N.B.	N.B.
Distance of secondary minimum (nm)	17	17	N.B.	12	N.B.	N.B.
Height of energy barrier (kT)	106.9	94.7	N.B.	8.9	N.B.	N.B.

[a]A value of 6.5×10^{-21} J was chosen as the Hamaker constant.
[b]No barrier to deposition and hence no secondary minimum.

potential and contact angle than the membrane E and F. But it exhibited significantly lower rate of cell adhesion, k_d, than the membrane E and F, which had 5 to 10 times larger surface roughness. It implied that the surface roughness might play some roles conjugated with surface potentials. Consequently, it can be concluded that zeta potential is one of important, but not a governing factor of cell adhesion onto membrane surfaces.

Effect of Surface Roughness

It has been reported that surface roughness influences colloidal particle (27, 28) and microbial adhesion (18, 29, 30). Although it is difficult to assess the effects of roughness on cell adhesion to membranes, mechanisms for the effect of surface roughness would depend on the dimension between cells and the "valley" or "hill" of membrane surfaces. For big irregularities of the membrane surface (i.e., E and F in this study), uneven flow distribution or channeling of flow over the surface of the membrane can be occurred. They may act as physical barriers and/or shear force reducers, and finally, entrap more bacteria or other particles than relatively smooth membranes (i.e. membrane C). Hoek et al. (28) showed that particles preferentially accumulate in the "valleys" of rough membranes, resulting in "valley clogging" which causes more severe flux decline than in smooth membranes.

A nanometer range of surface roughness, which is much smaller than the particle size, can also greatly reduce the energy barrier during adhesion of cells, and consequently, facilitate the biofouling. For membrane D, the secondary minimum is about $-10.8\,kT$ deep and exists at a distance of 12 nm from the membrane surface. The membrane D had over 17 nm of RMS surface roughness and it can by hypothesize that cells can reach through the primary energy barrier and attach more favorably on to the membrane surface. In our study, although we cannot conclude that surface roughness is a major factor affecting cell adhesion, at least it is one of the important factors promoting bacterial adhesion (highest correlation in Table 3). With this understanding, the management of surface roughness will be also important for reducing cell-membrane interaction during the development of new membranes and the membrane selection.

Effect of Hydrophobicity

Generally, it has been believed that hydrophobic membrane exhibited higher biofouling potentials than hydrophilic membranes (15, 17, 31). Other studies also confirmed that the increase of the hydrophobicity both in cells and membranes resulted in the increase of adhesion rate due to higher interaction energies between cells and membranes (15, 17, 18, 31–33). However, it can be the very opposite when particles or cells had a hydrophobic surface.

Brant and Childress (34) showed that hydrophobic polystyrene colloids adhered more weakly to three hydrophilic membrane compared to hydrophilic silica colloids. Hence, properties of colloidal foulants will also be important to assess fouling potentials. In this study, MATS analysis confirmed that majority of cell surfaces were mainly hydrophobic, and cells were expected to adhere more easily onto "hydrophobic" membranes than "hydrophilic" membranes by the hydrophobic-hydrophobic interactions between cells and membrane surfaces. But the interaction between hydrophilic cell surfaces groups with hydrophilic membrane also should be taken into account because cell surfaces had electron-accepting nature proven by MATS. Hence, the interaction between cells and membranes became more complicated, and therefore, gave low correlation value in Table 3. The origin of hydrophobicity of the cell surface is "hydrophobic" cellular materials such as glycans and mannoproteins, and the hydrophilic group may originate from amino acids and phosphates (35).

CONCLUSIONS

In this study, we examined the effect of membrane surface properties such as surface potentials, roughness, and hydrophobicity during the initial adhesion of cells using direct observation technique with parallel plate flow chamber. Despite the complexity of the affecting factors for cell adhesion onto various types of membranes, the results agreed that the membrane which had a hydrophilic, more negatively charged, and smooth surface, would be more effective in reducing the initial adhesion of microorganisms. The methods and results presented here can be directly applied to evaluate the biofouling potentials of new membranes, and to select the better membrane during water and wastewater treatments.

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