博士論文（要約）

Bacterial leakage potential through microfiltration membranes with different nominal pore sizes under the normal operating conditions for water treatment

(水処理に用いられる通常運転条件下での異なる公称孔径を有する精密ろ過膜の細 菌リークポテンシャル)

THEEPHARAKSAPAN SUTHIDA

ティーパラクサパン　スティダー

One of the major advantages of membrane filtrations over more conventional processes in water treatment is their efficiency for the microorganism removal. Microfiltration (MF) is one of classical membrane processes. It acts as a physical separation process based on size-sieving mechanism, precluding the transfer of bacteria, and other microorganism from liquids. However, several reports are available for the harvesting of bacterial leakage cells to MF membranes. When used as a pre-treatment of water purification and water reclamation processes, leakage of microorganisms induces a potential risk of microbiological growth-associated problems in the downstream lines, such as biofouling in reverse osmosis, and contamination of drinking-water by microbial pathogens. To achieve basic information for appropriate selection of membrane pore size in terms of bacterial leakage event, the bacterial leakage potential through MF membranes with different nominal pore size under normal operation conditions is to be investigated.

The objectives of this study were *(i)* to evaluate the degree of bacterial leakage passing through MF membranes, characterized by nominal pore sizes smaller than cell size, considering the effect of the operating conditions for the microfiltration process, *(ii)* to evaluate bacterial leakage potential in microfiltration membrane based on membrane characterization (i.e., pore morphologies and pore size distribution), and bacterial cell size distribution, and (*iii*) to show evidence of bacterial deformation through micron channel in microfluidic device, which was similarly designed with a pore size of microfiltration membrane.

The *Escherichia coli* (*E. coli*) strain DH5α with green fluorescence protein (GFP) was used in this study. Approximately 20,000 of *E. coli* cells and membrane pores were measured for their sizes by scanning electron microscopy (SEM). The width of GFP *E. coli* cells was 0.56 μm in average, ranging from 0.13 μm to 0.81 μm. For membrane pore, the maximum hydraulic diameters of membrane pores (commercial track-etched membranes) with nominal pore sizes of 0.4 μm, 0.2 μm, and 0.1 μm were 0.67 μm, 0.37 μm, and 0.18 μm, respectively. These indicated that microfiltration membrane was not a complete barrier with a nominal pore size that allows the presence of large pores which were larger than the nominal pore size.

In addition, bacterial size was set in variation. The occurrence of small-size bacteria that was smaller than the pore size could provide high potential for leakage through pore membrane without cell disruption. Thus, from membrane pore and *E. coli* distributions, there was a significant leakage potential through MF membranes.

Degree of bacterial leakage was evaluated by batch filtration experiment under two applied pressure modes of suctioned and pressurized ones with a static condition and the four cycle times of fluctuating intermittent pressures of 0.5 sec, 1.0 sec, 2.0 sec, and 4.0 sec. Commercial track-etched membranes were selected for this study using

microfiltration membrane with three nominal pore sizes of 0.4 μm, 0.2 μm, and 0.1 μm and ultrafiltration membrane with nominal pore size of 0.02 μm. The two bacterial feed concentrations used in this chapter were 10^4 CFU/mL and 10^6 CFU/mL. These concentrations were chosen in order to observe the effect of membrane fouling on bacterial leakage through membrane.

The results showed significant leakage through membrane with nominal pore sizes of 0.4 μm, 0.2 μm and 0.1 μm that were smaller than their cell size. On the other hand, there was no bacterial permeation observed through ultrafiltration membrane with nominal pore size of 0.02 μm. Although the primary factor was the size-sieve mechanisms of membrane pore, but fouling on membrane surface enhance the rejection of leakage potential for *E.coli* leakage. For the effect of applied pressure modes, the bacterial filtration under the suction mode might enhance bacterial leakage potential compared with the filtrations under pressurized mode. However, they were the minor factor.

In order to further study the effect of membrane fouling on bacterial leakage, a method based on a simple pore flow concept was applied to develop empirical model that corresponds to two basic types of fouling: pore blocking and internal fouling for dead-end filtration under constant pressure. In the blocking model, the blocking phenomena was described by shading effect of *E. coli* cell on membrane based on cell size and pore size distributions. An *E. coli* cell was assumed to reaches on membrane surface, and completely blocked the entrance of a membrane pore by shading effect of rod-shape of *E. coli* cell, and cell never settle on another cell that has previously blocked a membrane pore.

In the internal fouling model, it was considered that molecules of dissolved organic matter were adsorbed to over the walls inside the membrane pore, because a high concentration of organic matters in *E. coli* suspension which was estimated around 180 mg/L was similar order of magnitude of municipal waste water. The volume of membrane pores was assumed to decrease proportionally of the volume of filtration. Thus, the radius of a pore was decreased during filtration.

The result shows that flux decline from calculations were fitted well with the observed flux. This indicated that the experimental flux data could be explained by this fouling model that consists of pore blocking and internal fouling. And that model could be used to estimate *E. coli* leakage potential considering pore size and cell size distribution, and the experimental flux behavior.

The major cause of quick flux decline in the initial stage was adsorption of dissolved organic matter over the walls inside pores. These reduce the pore radius due to internal fouling. The results showed that at the end of filtration, the maximum hydraulic diameters of membrane pore with nominal pore sizes of 0.4 μm, 0.2 μm and 0.1 μm were

0.24 μm, 0.14 μm, and 0.09 μm respectively. Based on cell size distribution, *E. coli* cell was ranging from 0.13 μm to 0.81 μm, and there were two groups ranging from 0.13 to 0.30 μm. and from 0.31 to 0.81μm. This indicated that nominal pore size 0.4 μm could give potential for small *E. coli* leakage along filtration. For 0.2 μm, as pore sizes became similar size with 0.1 μm, so this could enhance the rejection of leakage potential for *E. coli* leakage due to the effect of blocking and internal fouling.

Based on cell size and pore size distributions, the potential of bacterial leakage were estimated. At the beginning, estimated log removal values (LRVs) for nominal pore sizes of 0.4 μm, 0.2 μm and 0.1 μm were 1.7, 2.7, and 5.8 respectively. The corresponding LRVs estimated at the end of filtration respectively were 2.7, 5.8, and 6.0. In bacterial filtration experiments, the LRVs of *E. coli* cells respectively were 3.8 ± 0.09, 4.1 ± 0.08, and 4.8 ± 0.09 for 0.4 μm, 0.2 μm, and 0.1 μm of nominal pore sizes at feed concentration $3.3x10^6$ CFU/ml.

The result shows that at the beginning, for nominal pore size 0.4 μm and 0.2 μm, the observed LRVs were higher than the estimates. On the other hand, the experimentally obtained LRV for nominal pore size 0.1 μm was lower than the estimated value. At the end of filtration, for nominal pore size 0.2 μm, the observed LRV was lower than the estimates. This was considered due to the dynamic of fouling during the filtrations, which decreased effective size-sieving and increased retention of *E. coli* cells. For nominal pore size 0.4 μm, the experimentally obtained LRV was still higher than the estimates.

Since the observed LRVs obtained from total volume in filtration through MF membranes, so the estimated LRVs in average also were considered. The LRVs in average values for nominal pore sizes of 0.4 μm, 0.2 μm, and 0.1 μm were 1.9, 3.0, and 5.9 respectively. The results showed that for nominal pore size 0.4 μm and 0.2 μm, the observed LRVs were higher than the estimates. This might considered due to incompletely blocking which provided several partially blocked pores on the surface of membrane. This might increase the rejection of cell leakage through membrane pore. For nominal pore size 0.1 μm, the observed LRV was lower than the estimates. This might be due to the deformation of *E. coli* cells through pore membrane.

Microscopic observation of GFP *E. coli* cells passing through MF membranes with pore sizes smaller than their cell size were performed by using transparent micron constrictors on a microfluidic device. Three cases of bacterial movement in 0.4 μm deep structures (i.e., slips and a narrow channel) of microfluidic device were presented. In the first case, the result showed that GFP *E. coli* was able to pass through a channel quickly. This could confirm the presence of small-size bacteria which could provide high potential for leakage through pore membrane without cell disruption. In the second case, the result showed that GFP *E. coli* could enter into the slip area and penetrated through the narrow

channel. However, the result clearly showed GFP *E. coli* had died due to the physical damage of cells which were too large in size compared with depth.

In the third case, time-laps movies show quick movement through the narrow channel. However, it can be observed that GFP *E. coli* stop a few moments at the entrance and exit of slips. Total fluorescence intensity values were determined directly from time-lapse movies of whole cell region. Although the resolution has bigger than the actual size of GFP *E. coli* cell which could not determine the actual shape, this is enough to identify the change of cell shape. The histograms of fluorescence intensity distributions positioned of cell along the channel, and showed elongation of GFP *E. coli* cell which pass through the narrow channel. Moreover, the relation of total fluorescence intensity during movement showed constant level of intensities between the entrance and exit of the 0.4 μm deep structures. This indicated that cells could pass through the narrow channel without the physical cell damage. And the elongation of GFP *E. coli* cell demonstrated the deformation of GFP *E. coli* through a structure that is smaller than cell size.

The obtained knowledge of GFP E. coli cell leakage potential in this study make an understanding how bacteria move and penetrate through microfiltration membranes with nominal pore sizes smaller than their cell size, and basic information for appropriate selection of membrane pore size in terms of bacterial leakage. Among of three nominal membrane pore size, it is recommended to use membrane nominal pore size 0.1 μm. For 0.2 μm, fresh membrane with 0.2 μm is not enough to be a barrier in bacterial leakage, whereas the used membrane is acceptable due to dynamic fouling during filtration.