Investigation of bacterial predators for the prevention of membrane biofouling

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Graduate school of UNIST

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Abstract

A flux decrease because of membrane biofouling is a crucial problem restricting membrane applications in conventional water treatment. Several investigations have been investigated to alleviate the biofouling problems; improving feed water qualities through coagulation, chlorine treatment, etc. Even though these methods improve the membrane performance, they are not sustainable due to the dosed chemicals. This is because the chemicals lead to the production of harmful disinfection by-products such as trihalomethanes, aldehydes, etc.

In this study, the bacterial predators, BALOs (*Bdellovibrio*-and-like-organisms), were investigated, as an alternative treatment to alleviate biofouling and its consequent performance decrease. Dead-end microfiltration (MF) tests were conducted on *Escherichia coli* (*E.coli*) and BALOs co-culture feed solutions. Predation of *E.coli* was represented by the multiplicity of infection (MOI), which is explained as the proportion of predator to prey cell. The tested conditions of predation were both high MOI (high predator, HP) and low MOI (low predator, LP), and the total number of viable *E.coli* prey and predators were counted over 48 hr. The membrane performance of cultures such as NP (no predation), LP and HP was evaluated using a resistance-in-series model. In the performance experiments with a microbial solution containing predator bacterium, its total resistance became lower than the control (NP culture) over 48 hr. However, the LP culture showed an increase of irreversible fouling of the membranes. This was most likely due to prey cell debris produced by predation.

In contrast, previous investigations of other research groups showed that coagulation using alum can mitigate membrane biofouling. Additionally, several studies found that lysing the microorganisms utilizing ultrasonication can enhance the membrane performance with alum coagulation. It was hypothesized that the predation impact may be comparable with that of the ultrasonication. This is because both predation and ultrasonication have an effect on the lysis of the bacteria leading to membrane fouling. Thus, it was predicted that a combined pre-treatment of bacterial predation and alum coagulation could improve the membrane performance. From this hypothesis, the goal of another investigation was to evaluate a combined pre-treatment using both bacterial predation and alum coagulation in order to reduce membrane biofouling, specifically, the irreversible fouling from the LP culture. Dead-end microfiltration (MF) tests were conducted on co-culture feed solutions using *Escherichia coli* and *B. bacteriovorus* after coagulation with diverse concentrations of alum. The results represented that when 10 ppm of alum was utilized, the membrane fouling got worse for both NP and LP cultures, as compared to no alum addition, because the

irreversible resistance of the membrane was a lot higher. Conversely, using alum at 100 ppm reduced the total resistance similarly in both NP and LP cultures noticeably. In addition, for using 100ppm of alum, the LP culture led to both a lower total and irreversible resistance compared to the NP culture. This was because the LP culture with alum coagulation was well aggregated. These results indicate that combined treatments of both *B. bacteriovorus* predation and a suitable concentration of alum can be an effective pretreatment method for improving membrane performance.

Powdered activated carbon (PAC) was used as alum coagulation, for the NP and LP cultures in order to decrease membrane biofouling caused by prey cell debris of the LP culture. Dead-end microfiltration (MF) tests were conducted on both NP and LP cultures, after treatment with various concentrations of PAC. The results showed that when 10 ppm or 100ppm of PAC were added, the performance of the membrane was better for both cultures as compared to no PAC addition. This was because using PAC could have effects on the reduction of reversible resistance. This finding concurs with another previous study that the application of PAC was related to decrease of reversible resistance. Furthermore, for the LP culture, 100ppm of PAC led to a decrease of irreversible resistance compared to 0ppm of PAC. In addition, when 10 ppm or 100ppm of PAC were added, the LP culture caused less total resistance of the membrane compared to the NP culture. These results also show that combined pre-treatments of bacterial predation and PAC treatment can be an effective method for enhancing membrane performance.

In conclusion, this study showed that using bacterial predators at a suitably high concentration was useful at mitigating microbial fouling of the membrane. Also, even though using bacterial predators at a low concentration led to an increase of irreversible resistance of the membrane, either a proper alum concentration or PAC treatment, in combination with bacterial predation, can be a beneficial pretreatment method for reducing membrane biofouling.

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Chapter 1. Introduction

Ⅰ. Research background

The membrane is used as a semi-permeable layer in the middle of two phases and separation technology with the membrane for water treatment has grown notably in the industry, especially with the introduction of low pressure membranes, i.e. microfiltration (MF) and 'untied' ultrafiltration (UF) membranes (Huang et al., 2009). Figure 1 shows various pressure-driven membranes for processes of water treatment. In particular, the membrane for microfiltration process which has a pore size range between 0.1 and 10 μm has been widely utilized for various chemical and biochemical processes in order to remove particulate matter (Keller et al., 2001). The sieving effect is generally considered being the major mechanism of the microfiltration process. This effect indicates that target materials could be controlled by the membrane according to its pore size (Hwang and Sz, 2011).

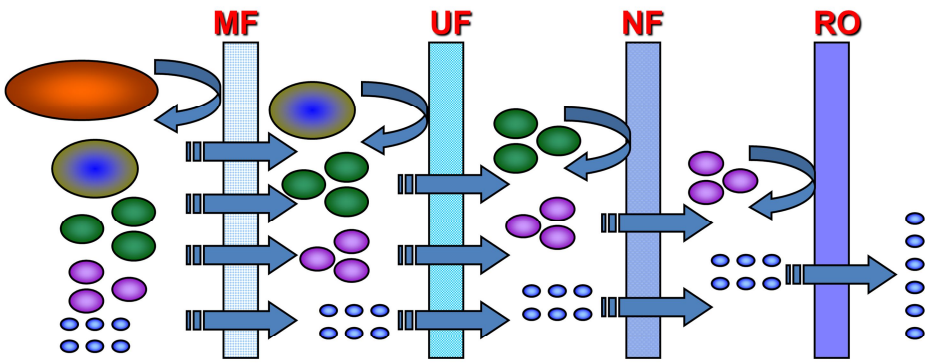

Figure 1. Pressure-Driven Membrane Processes

Nevertheless, one of the most critical disadvantages for membrane processes is membrane biofouling resulting in the deterioration of the membrane performance and increment of operation cost (Magara and Itoh, 1991). Furthermore, the adherence of microorganisms on the membrane surface generally causes membrane biofouling, and then the microorganisms begin to proliferate and grow a biofilm, including extracellular polymeric substance (EPS) (Kolari et al., 2001). The biofilms are communities of microbes related to a surface, generally enclosed in an extracellular matrix. It is hard to eliminate biofilms on the membrane surface because they are strongly attached. Figure 2 shows

formation steps of the biofilm.

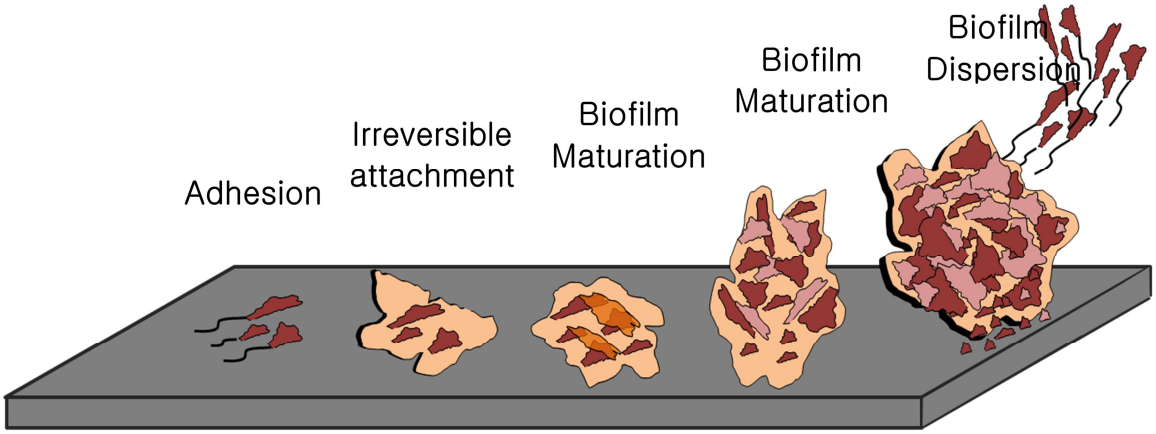

Figure 2. the formation step of biofilms

Various studies have been conducted for solving the biofouling problem through: (i) improving feed water qualities using coagulation (Gamage and Chellam, 2011), and preoxidation through ozone (Hwang et al., 2010) and chlorine (Rajagopal et al., 2003), etc. ; (ii) modifying the membrane surface utilizing diverse antimicrobial matters such as silver nanoparticles (Mollahosseini et al., 2012, Sawada et al., 2012). Even though these methods improve the performance of the membrane processes, in some cases, it is not environment-friendly because of the dosed chemicals. For instance, the chlorine has an adverse impact on the polyamide membrane because amide bonds (-CO-NH-) of the commercial membrane are extremely susceptible to chlorine (Kwon et al., 2011b). In addition, the oxidation treatments using ozone brought about destructive disinfection by-products (DBPs) such as aldehydes, bromated, carcinogenic haloacetic acids (HAAs), and trihalomethanes (THMs) (Boorman et al., 1999, Huang et al., 2005).

With the increasing interest in finding effective, novel, and environmentally safe tools to alleviate membrane biofouling, researchers started investigating the utilization of biological treatments like eukaryotic predators as alternatives (Derlon et al., 2012). However, those predators could not penetrate bacterial cell aggregations (microcolonies) inside biofilms; hence their capabilities were limited (Bohme et al., 2009, Derlon et al., 2012). Unlike eukaryotic predators, which had some limitations in penetrating the biofilms and attacking the bacteria, another class of predators, bacterial predators such as BALO (bdellovibrio and like organisms) were shown previously to be able to penetrate deeply inside biofilms and effectively eradicate them (Dashiff et al., 2011, Fratamico and Cooke, 1996, Kadouri and O'Toole, 2005). In addition, previous studies also showed that the thick capsuled prey, which is usually resistant to bacteriophages, can still be attacked and predated easily by

Bdellovibrio (Koval and Bayer, 1997).

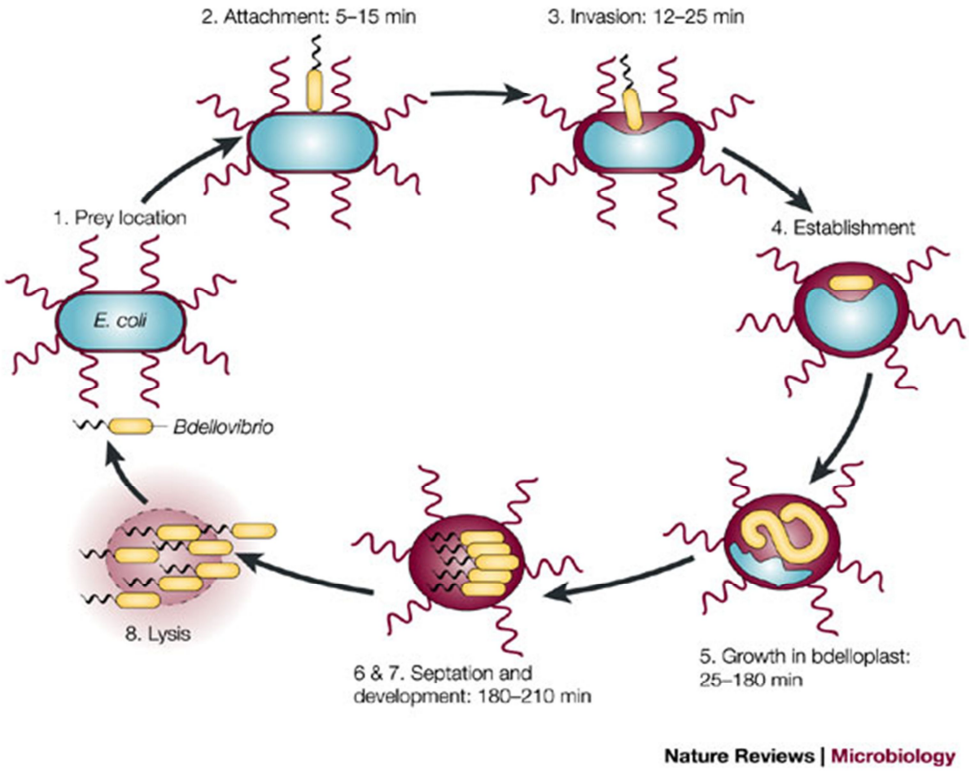

Figure 3. the life cycle of *Bdellovibrio bacteriovorus* HD 100

Bdellovibrio bacteriovorus HD 100 is a Gram negative predatory bacterium, which lives through attacking other Gram negative bacteria, penetrating into prey periplasm, where it multiplies and finally lyses the prey from inside to allow the progeny to eixt and attack other prey in the medium (Dwidar et al., 2012b, Sockett and Lambert, 2004), and it has a normal width of 0.3 μm (Stolp and Starr, 1963). Figure 3 represents its life cycle, which has two major phases; the assault phase in which the predatory bacterium swim in the medium utilizing its single polar flagellum finding for prey, and the intraperiplasmic phase in which the predatory bacterium begin to confront an appropriate prey cell(Sockett and Lambert, 2004). In this phase, the predatory bacterium mislay its flagellum and enter the prey periplasm, where it extends, lyses, and septates the prey from inside before the offspring finally emerge and assault other prey in the medium (Dwidar et al., 2012a, Sockett and Lambert, 2004). This intraperiplasmic phase typically takes 3–4 hr and makes 3–6 progeny cells from a one *E. coli* cell (Sockett and Lambert, 2004). Actually, *B. bacteriovorus* was found in diverse studies to be very useful for attacking its prey bacteria and reducing their populations in the liquid medium (Dashiff et al., 2011, Dwidar et al., 2012b).

Ⅱ. Objectives of the study

In this study, there were three specific objectives:

1. BALOs (*Bdellovibrio*-and-like-organisms), a gram-negative predatory bacterium, were investigated as an innovative way to alleviate membrane biofouling and its consequent flux decrease.

For this objective, Dead-end microfiltration (MF) tests were conducted on prey (*E. coli*) and BALOs co-culture medium; control (no predation, NP), low MOI (low predator, LP) and high MOI (high predator, HP). The flux performance after filtration of these cultures was evaluated by resistance-in-series model.

2. The objective of this study was to investigate combined pre-treatment methods such as alum and bacterial predation for reducing membrane biofouling.

For this objective, Dead-end microfiltration (MF) tests were carried out using prey (*E. coli*) and BALOs co-culture medium; control (no predation, NP), low MOI (low predator, LP). After coagulation using diverse dosages of alum, the flux performance of membrane filtered these cultures was analyzed by a resistance-in-series model.

3. The objective of this study was to investigate the combination of pre-treatments such as powdered activated carbon (PAC) and bacterial predation for reducing membrane biofouling.

In order to mitigate membrane biofouling triggered by prey cell debris of the LP culture, the PAC was used for both the NP and LP cultures. After treatment using diverse concentrations of PAC, Dead-end microfiltration (MF) tests were carried out using both for both the NP and LP cultures. The membrane performance was evaluated using a resistance-in-series model.

Chapter 2. Experimental Methods & Materials

Ⅰ. Bacterial strains, media and culture conditions

Culturing of the microorganisms was done by Mohammed Dwidar in Prof's Robert J. Mitchell's lab in UNIST. The detailed method for culturing was as described in the paper (Kim et al., 2013).

Ⅱ. Preparation of the experimental culture medium

2.1. Investigation of Pre-treatment using *Bdellovibrio*-and-like-organisms (BALOs) on the dead-end microfiltration of *Escherichia coli* (*E.coli*) solution

In this study, Diluted nutrient broth (DNB) (1:10 dilution of nutrient broth, Difco) was applied for the experimental culture medium. 1.25 L of DNB was contained by 2 L flasks. This broth was prepared in 2 L flasks; each containing 1.25 L of media. After sterilization, 2 mM $CaCl_2$ and 3 mM $MgCl_2$ was added to the broth. *E. coli* DH5α was then spiked into the broth at a beginning concentration of about 1×10^5 colony-forming units (CFU)/ml in all flasks. Comparably, the predator *B. bacteriovorus* HD 100 was inoculated at an initial concentration of about 2×10^5 plaque-forming units (PFU)/ml for the low predator (LP) culture, and 2×10^7 PFU/ml for the high predator (HP) culture. No predator was supplemented to the control sample. The cultures were then inoculated into 2 L flask containing 1.25 L of DNB medium at 25°C with 350 rpm of agitation during for 48 h. At diverse time intervals, 400 ml of cultures were taken for OD at a wavelength of 600 nm (OD_{600}) measurement, total organic carbon (TOC), and viable counts and flux analyses. Microbial solutions according to diverse samples (NP, LP, HP) were handled at 25℃ using a dead-end microfiltration (MF) system in order to estimate the permeability of the microbial solutions that were obtained after different growing time of 12, 24 and 48hr.

2.2. Combined pre-treatments of bacterial predation and alum coagulation to reduce membrane biofouling & Combined pre-treatments of bacterial predation and powdered activated carbon (PAC) to reduce membrane biofouling

In this study, DNB broth was prepared in 5 L flasks; each containing 1.5 L of media. In order to prepare the low predator (LP) culture, *E. coli* DH5α and *B. bacteriovorus* HD 100 were spiked into the broth at an initial concentration of about 1×10^5 CFU/ml and 2×10^5 PFU/ml respectively. Comparably, *E. coli* DH5α was inoculated at an initial concentration of about 1×10^5 CFU/ml for the no predation (NP) culture. The NP and LP cultures were then inoculated into 5 L flask containing 1.5 L of DNB medium at 25°C with 350 rpm of agitation during for 48 h. All cultures were taken for coagulation and activated carbon experiments.

Ⅲ. Pretreatments

3.1. Combined pre-treatments of bacterial predation and alum coagulation to reduce membrane biofouling

Coagulation experiments were conducted with 400mL of microbial samples in a glass beaker. The samples were stirred magnetically (200rpm). Aluminum sulfate hydrate ($Al_2(SO_4)_3 \cdot 18H_2O$; Bodi, China, >99%) 'alum' was utilized as a coagulant in this study. Three dosages of coagulants, 0, 10 and 100ppm of alum were used. For coagulation treatment, samples were mixed at 200rpm for 1min followed by 15min of slow agitation at 30rpm. After 30min of settling, supernatant samples were taken for OD_{600} determination, measurement of size and zeta potential, living cell counts, and flux analyses.

3.2. Combined pre-treatments of bacterial predation and powdered activated carbon (PAC) to reduce membrane biofouling

Samples were contacted with powdered activated carbon (PAC). The average size of the PAC was in the range of 250 μm passing 50-mesh sieve. Three dosages of coagulants, 0, 10 and 100ppm of PAC were utilized. For PAC treatment, samples were stirred at 100rpm for 30min. The treated samples were then filtered through a 10 μm pore size filter to remove remaining PAC. After that, samples were taken for OD_{600} determination, measurement of size and zeta potential, living cell counts, and flux analyses.

Ⅳ. Membrane filtration system (Experimental set-up & procedure)

Figure 4 shows the experimental setup in this study. Cellulose mixed ester (CM) membranes was used (Macherey-Nagel, Bethlehem, PA, USA). The average pore size of the membrane was 0.45 μm and its geometric (flat surface) area was 41.8 cm^2. The membranes were fully soaked for preparation during more than 24hours in deionized (DI) water. The MF experiments were carried out in Amicon cells with a capacity of 350mL which were pressurized at 50 kPa (0.5 bar) through laboratory N2 gas, and were stirred at 100rpm. The filtrate was accumulated in a plastic beaker located on an electronic mass balance, and its load was monitored at the time interval of 5s and 1min in the course of microfiltration.

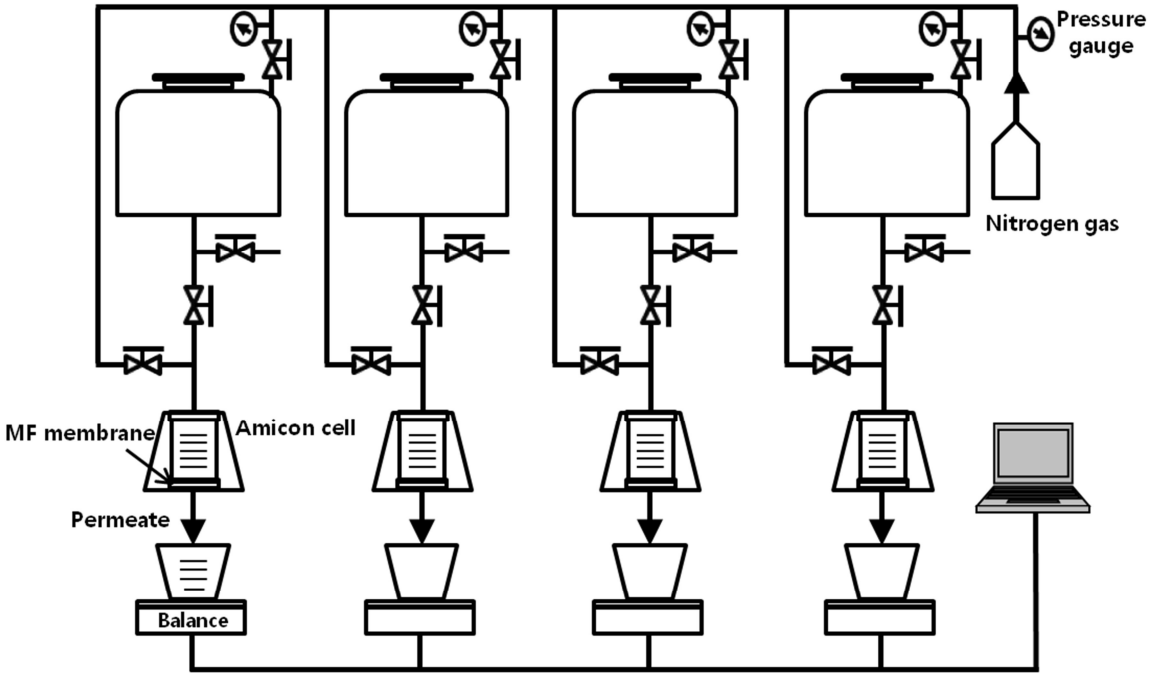

Figure 4. Schematic of the MF (microfiltration) dead-end system

Ⅴ. Microbial solution analysis and Membrane morphology

OD_{600} was measured using an S-3100 UV/VIS spectrophotometer (Scinco Co, Seoul, Korea). In order to determine the concentration of DNB broth depends on the time, total organic carbon (TOC) was measured by a Shimadzu TOC analyzer (Shimadzu, Tokyo, Japan) after filtration using a 0.22 μm pore size filter to remove microorganisms such as E.coli and BALOs. The morphology of the membrane surface was detected by a scanning electron microscope (SEM) (Cold FE-SEM s-4800, Hitachi, Japan). For SEM analyses, the membranes were dried at 30℃ in an oven during 7 days before measuring and then coated by platinum at 20mA for 30s through sputter coater (K575X, TESCAN, Seoul, Korea). Also, S8000 digital camera (Nikon, Tokyo, Japan) was used for determination of top views of filtered membranes. Fluorescent images of the membranes were photographed utilizing an SZX16 stereoscope linked to a DP72 CCD camera, and managed by DP2-BSW imaging software (Olympus, Center Valley, PA, USA).

Ⅵ. Analysis of membrane performance

6.1. Investigation of Pre-treatment using *Bdellovibrio*-and-like-organisms (BALOs) on the dead-end microfiltration of *Escherichia coli* (*E.coli*) solution

The permeate flux (J) had a unit of $Lm^{-2}h^{-1}$, and it was calculated as

$$J = (\Delta M / \rho) / (A \cdot t) \qquad (1)$$

where ΔM is filtrate mass(g) collected at a particular time (h^{-1}); ρ is the density of filtrate (g/L) and A is the effective membrane area(m^2) in contact with the solution.

Flux decline in dead-end filtration system can be induced by diverse factors such as concentration polarization, cake formation and plugging of the pores (Mulder, 1991). Based on these factors, the resistance of fouled membrane was measured by a resistance-in-series model:

$$J = \Delta P / (\mu \cdot R) \qquad (2)$$

where R is filtration resistance (m^{-1}), ΔP is applied pressure (Pa), μ is solution viscosity (Pa·s). Through the resistance-in-series, the total filtration resistance (R_t) illustrates the sum of specific resistances:

$$R_t = R_m + R_{cp} + R_c + R_p \qquad (3)$$

where R_m is the intrinsic membrane resistance, R_{cp} is the resistance due to concentration polarization, R_c is the resistance caused by the cake layer, and R_p is the resistance due to pore blocking and is further defined by an irreversible resistance (R_{ir}) .

The R_m was calculated from Eq. 2 by measuring the flux of virgin membrane using pure water, while R_t was determined from pure water flux after filtration of 350mL microbial solution. After the Amicon cell was evacuated and the fouled membranes were gently wiped away with 150mL of pure water through stirring at 100rpm for 5min using a shaker. The value of ($R_c + R_p$) was obtained from pure water flux by deducting ($R_m + R_{cp}$). Lastly, the irreversible resistance (R_{ir}) was determined from Eq. 3 after tough washing with 150mL of pure water through stirring at 200rpm for 15min, and then pure water flux was estimated once more.

6.2. Combined pre-treatments of bacterial predation and alum coagulation to reduce membrane biofouling & Combined pre-treatments of bacterial predation and powdered activated carbon (PAC) to reduce membrane biofouling

In order to analyze membrane performance, a resistance-in-series model was used for measurement of intrinsic membrane resistance, cake layer resistance, and pore blocking resistance.

$$J = (\Delta M / \rho) / (A \cdot t) \qquad (1)$$

where J is permeate flux ($Lm^{-2}h^{-1}$); ΔM is filtrate mass(g) collected at a particular time (h^{-1}); ρ is the density of filtrate (g/L) and A is the effective membrane area(m^2) in contact with the solution.

$$J = \Delta P / (\mu \cdot R) \qquad (2)$$

where R is filtration resistance (m^{-1}), ΔP is applied pressure (Pa), μ is solution viscosity (Pa·s). Based on the resistance-in-series model, the total filtration resistance (R_t) shows the sum of specific resistances:

$$R_t = R_m + R_c + R_p \qquad (4)$$

where R_m is the intrinsic membrane resistance, R_c is the cake layer resistance which is defined by hydraulically reversible resistance (R_{rev}), and R_p is the resistance due to pore blocking and is further defined by an irreversible resistance (R_{ir}) .

The R_m was determined from Eq. 2 by measuring the pure water flux of clean membrane, while R_t was estimated from pure water flux after filtration of pre-treated and non pre-treated samples. After the Amicon cell was evacuated and the fouled membranes were toughly washed with 150mL of pure water through stirring at 200rpm for 15min using a shaker, the value of ($R_m + R_c$) was obtained from pure water flux through washed membranes, thus R_p can be calculated. After calculating R_t, R_m and R_p, R_c can be estimated from Eq. 2

Chapter 3. Results and discussion

Ⅰ. Investigation of Pre-treatment using *Bdellovibrio*-and-like-organisms (BALOs) on the dead-end microfiltration of *Escherichia coli* (*E.coli*) solution

1.1. Microbial solution quality

The viability of *E. coli* within DNB media was observed over 48 h when *E. coli* alone or in the existence of the bacterial predation from *B. bacteriovorus* HD 100. Predation of *E. coli* was achieved at either a low or high multiplicity of infection (MOI), *i.e.*, 2 and 200. The MOI is defined as the predator to prey cell ratio. Concretely, the predatory bacterium was either not supplemented, *i.e.*, no predation (NP), or at a low predator (LP) or high predator (HP) concentration in accordance with MOIs of 2 and 200, particularly. Figure 5 represents the evaluated OD of these cultures.

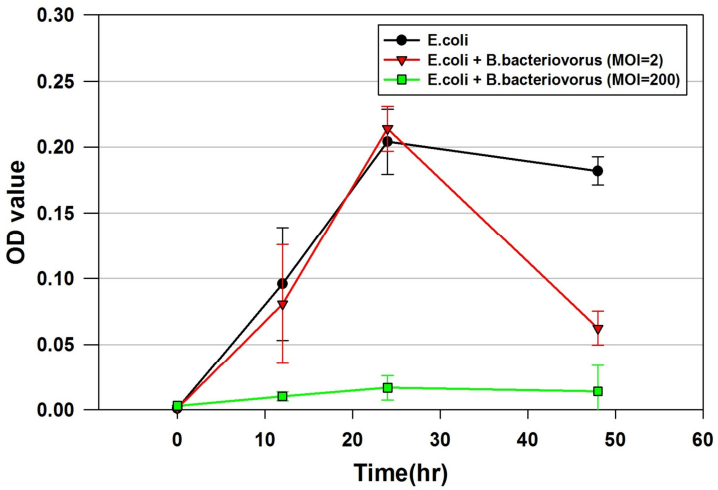

Figure 5. Optical density at 600nm

According to spiking various initial concentrations of the predatory bacterium (*B. bacteriovorus* HD 100), microbial growth could be different over the time. In the case of LP sample, it had a similar pattern with NP sample during the first 24 hr in Figure 5. However, its OD value decreased significantly after 48hr by comparing with NP case. Likewise, viable *E. coli* cell counting

values from CFU method showed a resembling tendency with OD values in Figure 6a. The similar patterns of OD values and living *E. coli* cells in NP and LP until initial 24hr are because of the longer doubling time of *B. bacteriovorus* in comparison with that of *E. coli*. In addition, the decrease of OD and viable *E. coli* cells in LP sample after 24hr shows predation of *E. coli* prey because the OD and living *E. coli* cells of NP sample was not adequately changed from 24 to 48 hr. That was why predatory activities of *B. bacteriovorus* started vigorously following complete *E. coli* propagation. In contrast, in the case of HP sample (an initial MOI of 200), its values of OD and living *E. coli* cells were lower than other samples such as NP and LP from start to finish (Figure 5 and Figure 6a). This was due to continuous predation of *B. bacteriovorus* to begin with.

(a) (b)

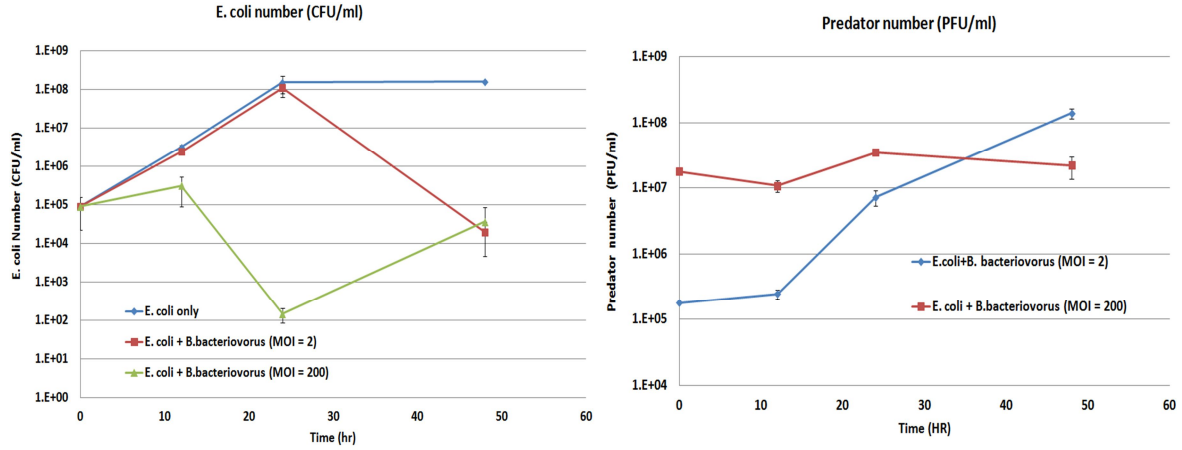

Figure 6. Viable cell counts; (a) *E. coli*, (b) *B. bacteriovorus*

Figure 6b shows the viable predator population over time from plaque-forming units (PFUs). The PFUs of LP cultures were constantly escalating over time. Also, its value after 48hr was larger than that of HP cultures. The difference of PFUs in LP and HP cultures can be contributed to the number of *E. coli* prey available. In the case of LP cultures, the *E. coli* cells could grow initially during 24 hr and, thus, could also devour more of the soluble organics in the DNB media shown as Figure 7. The higher viable *E. coli* number suggested that predation of *B. bacteriovorus* showed more active, which also triggered a much higher predator number later. On the other hand, the number of predators was inversely proportional to that of living *E. coli* cells in the HP culture. This was in that remaining DNB media after microbial consumption was used continuously in the Figure 7. Therefore, the number of *E. coli* cells which were not completely removed by predatory bacterium showed a repeat performance of the increase and the decline depends on the growth of *B. bacteriovorus*.

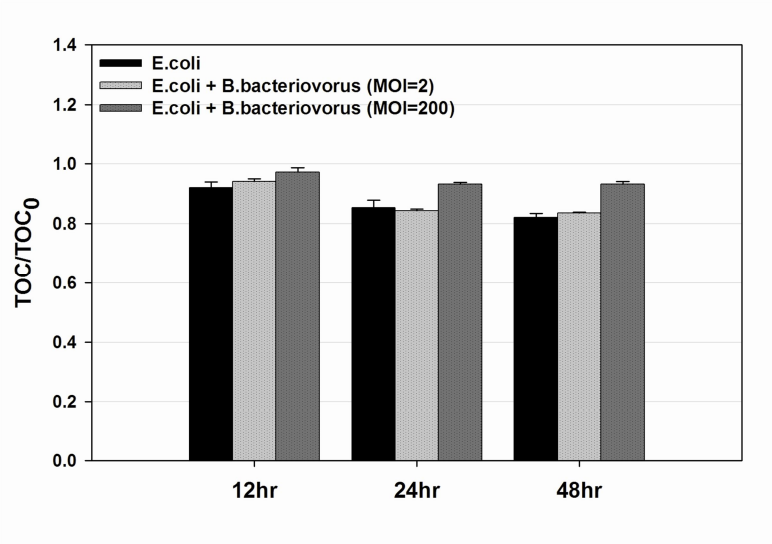

Figure 7. TOC analysis of microbial solutions based on the initial TOC of DNB broth; TOC indicates the organic concentrations following filtration (0.22μm) of the cultures of microorganisms. TOC_0 shows the organic concentrations following filtration of DNB broth only.

1.2. Filtration of microbial solution with 350ml

As aforementioned in the introduction, the 'Sieving effect' is a major mechanism in the MF membrane system. Based on this effect, the MF membrane of 0.45 μm pore size was investigated in the experiment. Particles of DNB medium and *B. bacteriovorus* HD 100 mostly permeated through the MF membrane. In addition, because *E. coli* had a size of 2~3μm, it was filtrated by the MF membrane of 0.45 μm pore size. Therefore, it could be observed whether *B. bacteriovorus* had abilities to remove biofouling from *E. coli* or not.

The MF membrane was used to conduct the experiment to permeate 350mL of different microbial solutions according to spiking various initial concentrations of predated bacteria. Its results of normalized & original flux are shown in Figure 8 and Figure 9 below. For HP cultures, its flux values were significantly higher than other cultures, such as NP and LP over the experimental time, for the reason that its total number of living *E. coli* cells was reduced as shown in Figure 6a. Also, the LP cultures represented lower decrease of flux when compared with the NP cultures. On the contrary, in the case of NP cultures, they had reduced flux values compared to that of LP and HP cultures throughout the whole time. These results indicated that viable *E. coli* cells had an important effect on the MF membrane performance due to the sieving mechanism, and membrane biofouling caused by viable *E. coli* cells was able to be mitigated because of bacterial predation.

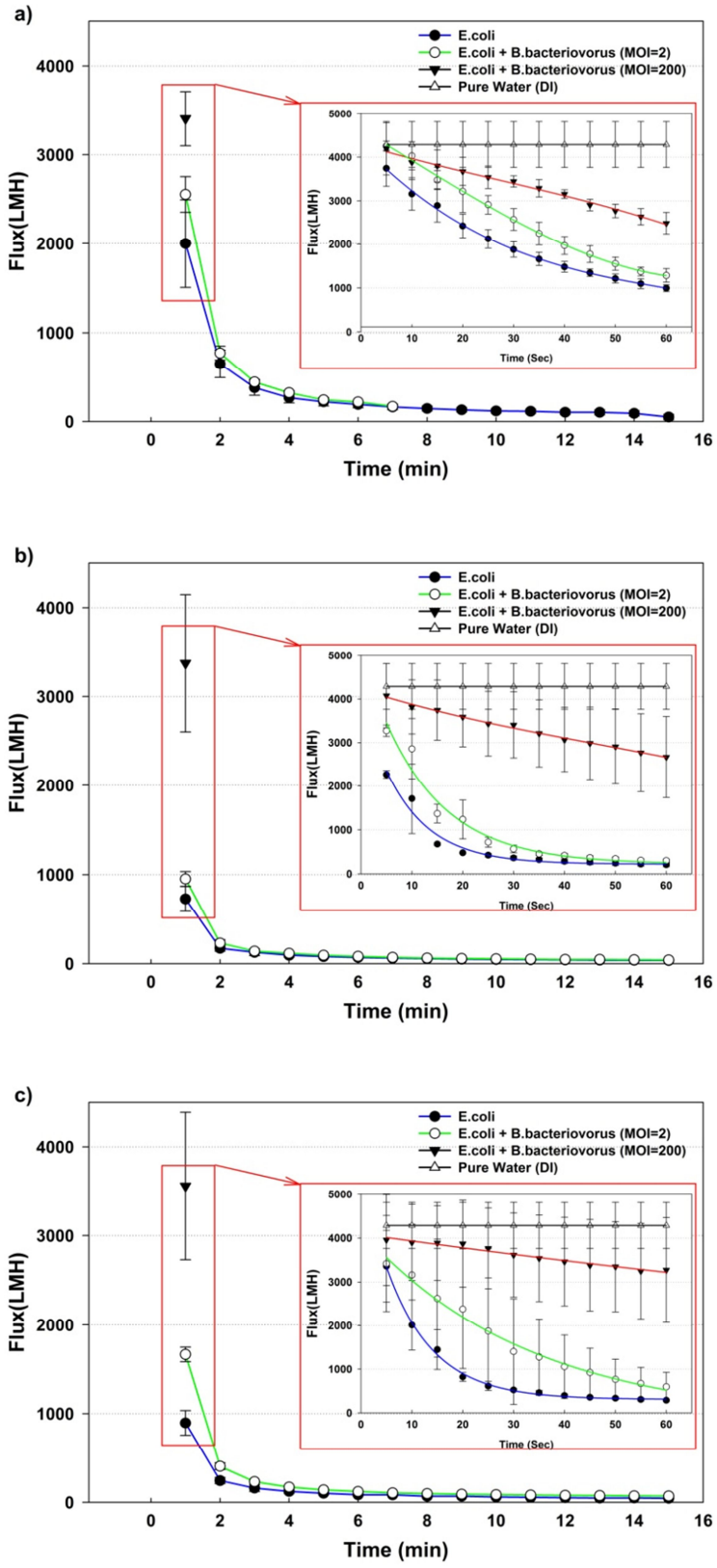

Figure 8. Original flux of membranes subsequent to (a) 12, (b) 24, and (c) 48hr of cultivation

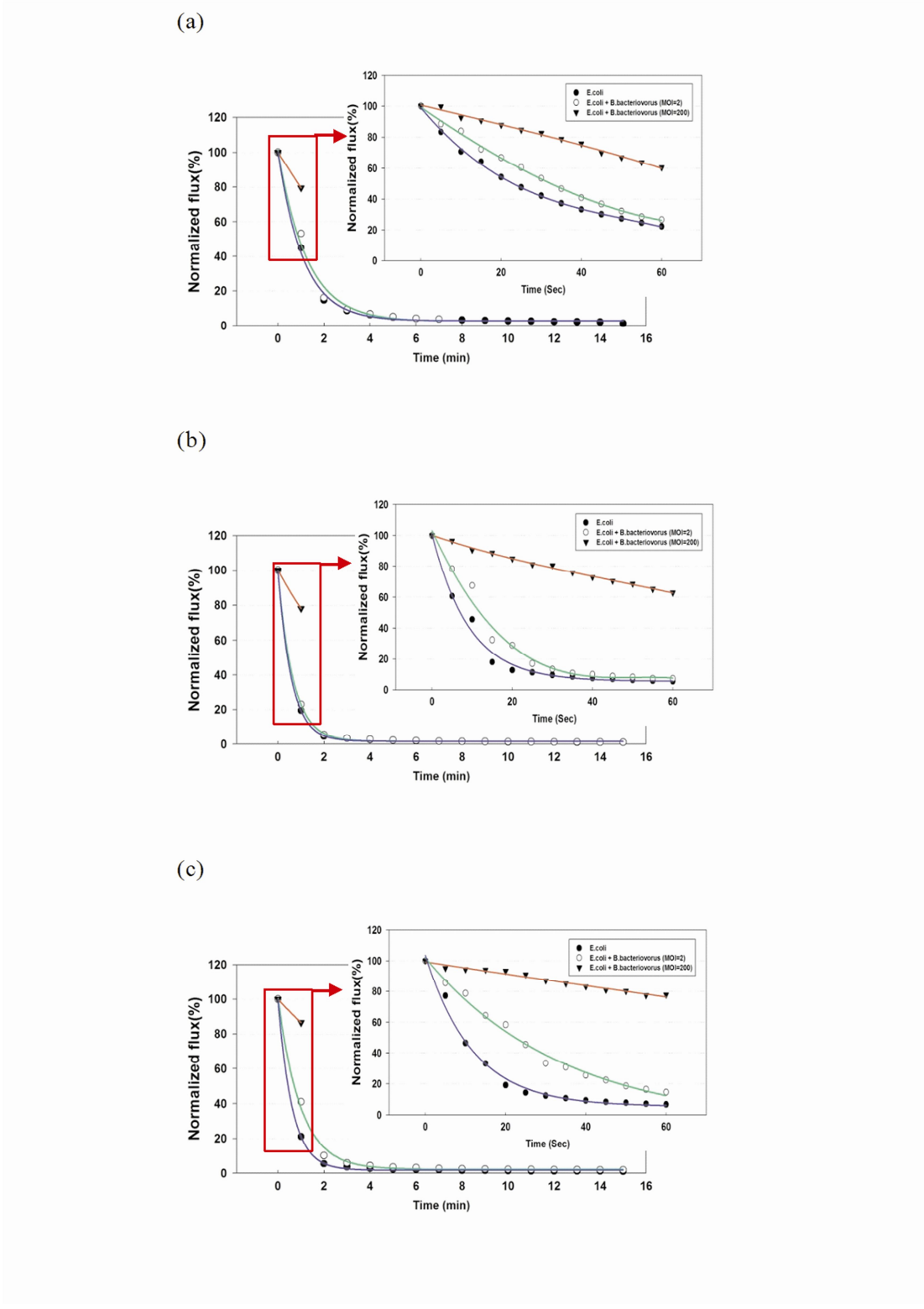

Figure 9. Normalized flux of membranes subsequent to (a) 12, (b) 24, and (c) 48hr of cultivation
The normalized flux is ((LMH value of microbial solution * 100) / (LMH value of prue water))

There are more detailed flux results in Table 1. The flux values of the HP sample were much higher than other two samples of NP and LP over the experimental time. Furthermore, when comparing the NP sample with the LP sample, these values at 12hr & 24hr were analogous. However, the values of flux were totally dissimilar at 48hr because the living cells of *E. coli* decreased after 48hr in the case of the LP sample. Even though the *E. coli* populations of the LP and HP samples were comparable at 48hr (Figure 6a), their flux values were completely different (40.9% and 86.5% respectively). Also, Figure 5 shows the solution of LP cultures after 48hr was quiet transparent. Nevertheless, Table 1 points out that the MF membrane of 0.45 μm pore-size could filtrate *E. coli* debris caused by bacterial predation. Therefore, the *E. coli* debris as well as living *E. coli* cells caused the MF membrane performance to be worse. In addition, Figure 9 and Table 1 show that normalized flux values of the 48hr LP cultures after 1min and 2min were 40.9% and 10.1% correspondingly. On the other hand, the values were 21.1% and 5.4% in the case of NP cultures. That was why the living *E. coli* cells more adversely influenced the membrane performance in comparison with the *E. coli* debris.

Table 1. Normalized flux point at 1min for NP, LP, and HP cultures

	12hr		24hr		48hr	
	Average	SD	Average	SD	Average	SD
NP (*E. coli* only)	44.8%	6.52	19.4%	3.57	21.1%	1.28
LP (MOI = 2)	53.1%	4.07	23.0%	4.73	40.9%	13.96
HP (MOI = 200)	79.5%	7.62	78.1%	9.63	86.5%	7.64

* The normalized flux is ((LMH value of microbial solution * 100) / (LMH value of prue water)). The SD is a standard deviation.

Fluorescent images of the membranes by stereomicroscopy directly after filtration, but before washing, represented that the number of *E. coli* cells filtered by membranes at 48 hr was considerably decreased in the samples of LP and HP (Figure 10b). A fluorescent signal was still observed in membrane filtering the LP cultures (Figure 10b), whereas membrane filtering the HP cultures was optically comparable to that of the virgin membrane. Figure 10b shows membrane filtering the NP solution was green because a lot of the *E. coli* was still alive. However, the membranes filtering the LP and HP solutions became dark since *B. bacteriovorus* preyed upon a large amount of *E. coli*. In addition, the membrane filtering of an initial MOI of 200 (HP) solution was a white color in the same way as the DNB solution filtration, whereas the LP solution case became yellow (Figure 10a). This implies *E. coli* debris on the membranes which was filtered by the MF membrane of 0.45 μm pore size (Figure 10a). In the case of LP cultures, its *E. coli* growth increased until 24hr, and then this

growth declined after 48hr because of bacterial predation (Figure 6a). This difference of *E. coli* populations between 24hr and 48hr was comparable to the amount of the *E. coli* debris produced by bacterial predation. Even if the OD and prey viability of LP cultures were basically comparable to that of HP cultures, the difference in the visualization of membrane filtering the HP and LP cultures after 48 hr suggests that an initial MOI of two (LP) conditions led to notably higher quantities of *E. coli* cell debris.

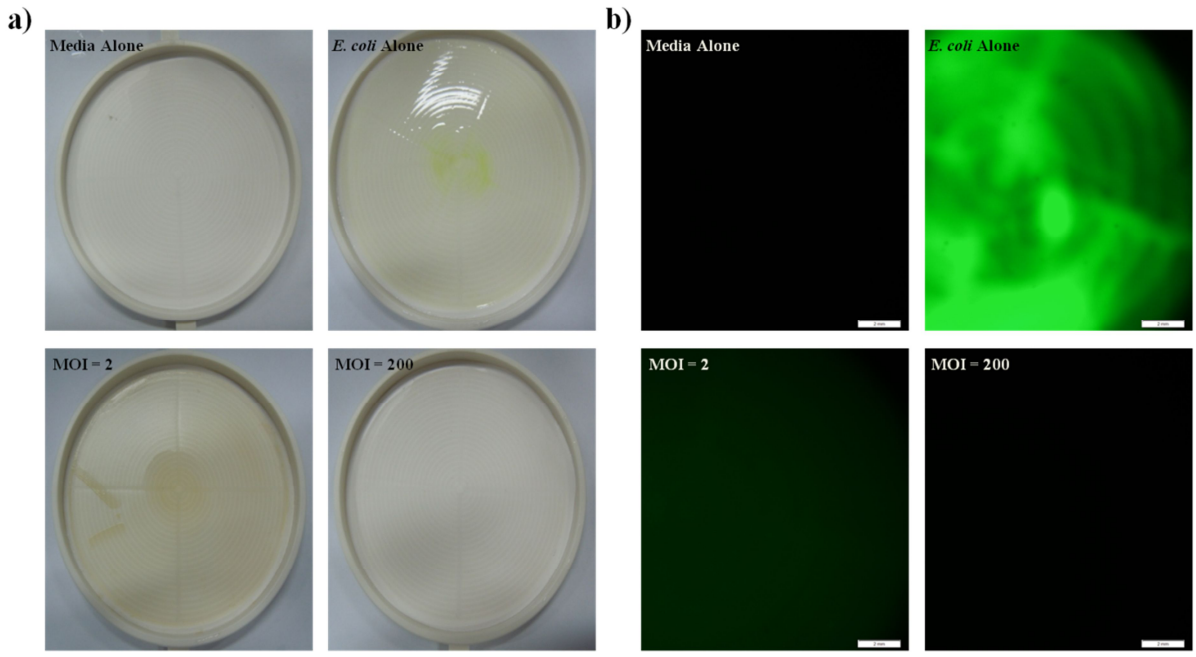

Figure 10. Digital (a) and Fluorescent (b) membrane images following filtration of microbial solutions during 48hr of culturvation

1.3. Resistance-in-series model

In order to measure which elements of membrane fouling attributed most to the problem, a resistance-in-series model was applied. As shown in Figure 11, there are results of various membrane resistances after filtration for three kinds of microbial solutions such as NP, LP and HP cultures. From eq. (2) and (3), the value of R_m was 0.4×10^{11} m^{-1}. The total resistance is defined by the sum of R_m, R_{cp}, R_c, and R_p (Figure 11a). Also, this resistance tendency was much similar to the trends observed in Figure 5 and Figure 6a. Concretely, the total resistance value of HP cultures was lower than that of NP and LP cultures over the experimental time. This even showed a 35 times reduced value when comparing the HP cultures with the LP cultures at 24hr. This was due to continuous bacterial predation from *B. bacteriovorus* HD 100 to begin with, resulting in a decrease of overall *E. coli*

propagation (Figure 6a).

In contrast, in the case of the LP cultures, total resistance values of the LP cultures were similar to that of NP cultures at both 12hr and 24hr, whereas its value decreased after 48hr. This was because the predatory activities of *B. bacteriovorus* started vigorously depending on the experimental time (Figure 6b), and then bacterial predation produced a lot of *E. coli* cell debris at 48hr, which caused a lower total resistance. In addition, this cell debris amount almost contained the difference between the viable *E. coli* cells at 24hr and at 48hr from Figure 6a.

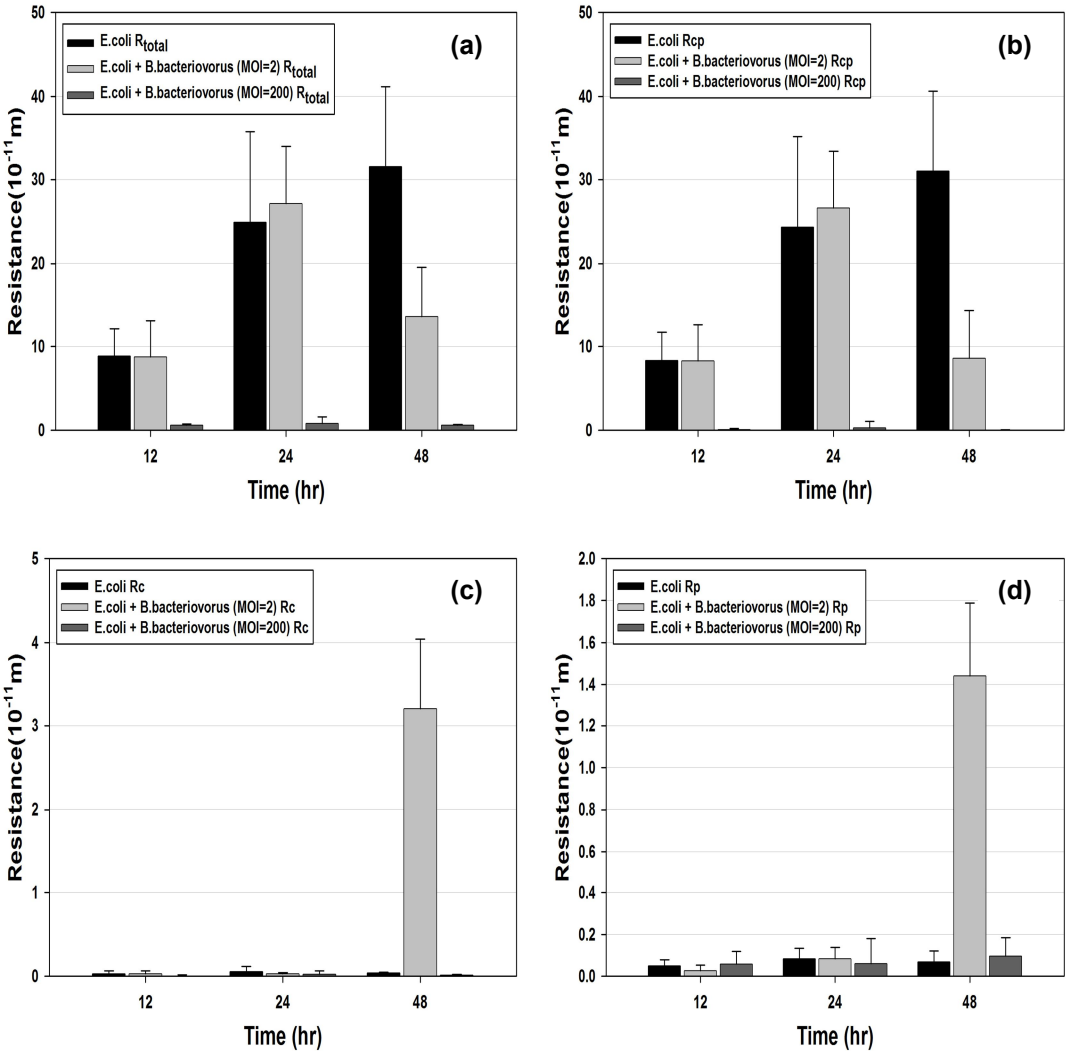

Figure 11. Membrane resistances using the Resistance-in-series model at diverse time intervals such as 12, 24, and 48hr; (a) Total resistance (R_t), (b) Resistance of concentration polarization (R_{cp}), (c) Resistance of cake layer(R_c), (d) Resistance of pore block(R_p)

Since MF membrane filtered the *E. coli* cell debris, even though the total living *E. coli* cell number of LP and HP cultures was comparable (Figure 6a) at 48hr, its total resistance value of LP cultures was 23 times that of HP cultures (13.67 and 0.59 respectively). In the case of NP cultures, the main influence resulting in membrane fouling was concentration polarization which contained a ratio of more than 98% of the total resistance. The resistances caused by cake layer and pore blockage became minor. Since viable *E. coli* cells triggered deposition on the membrane surface, they formed a reversible cake layer. Interestingly, its irreversible resistances for LP cultures, such as Rp, remained greater than other solutions of NP and HP cultures (Figure 11d). This increasing membrane resistance was caused by pore blockage through smaller particles and debris from the LP cultures.

In a study performed by V. Naddeo (Naddeo et al., 2007), small molecules broken by ultrasonic treatment could severely block the pores of the microfiltration membrane. The bacterial predation enables cell structures of viable *E. coli* to split biologically. This tendency of increasing irreversible resistance was in agreement with previous studies (Bai and Leow, 2002, Song, 1998) which suggested that smaller particles result in much rougher fouling than larger particles in cases of microfiltration. That was why irreversible resistances of LP cultures were higher than the other two cases, such as NP and HP cultures, because the debris blocked the pores of the MF membrane more severely even after tough washing of the membrane as shown in Figure 12, SEM images.

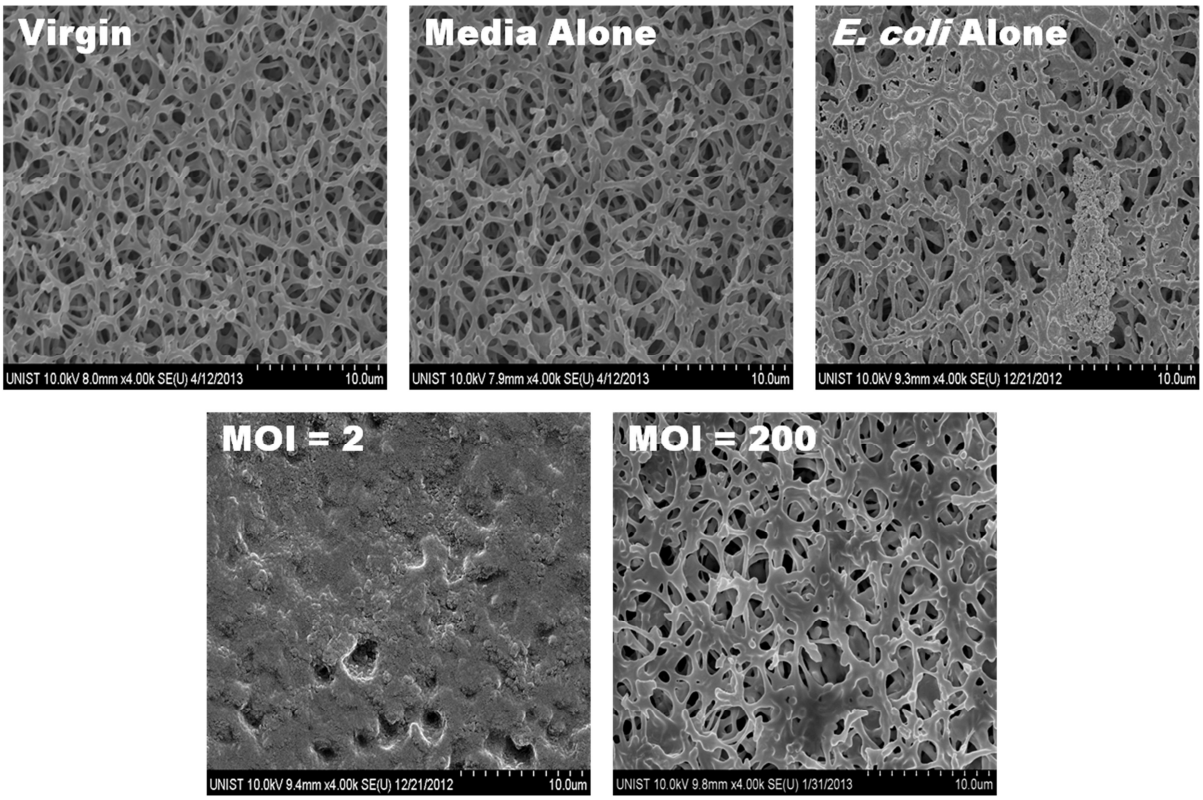

Figure 12. Scanning electron microscopy (SEM) images of the MF membranes following filtration of microbial solution during 48hr of cultivation

The table 2 illustrates the overall percent of membrane resistances depends on the time with three cases; the NP, LP and HP cultures. For NP cultures, the reversible resistances such as R_{cp} and R_c were mainly involved over the experimental time. This could lead to Biofilm formation on the membranes after a long period of time. In order to prevent the formation, suitable contents of predatory bacterium can be an alternative pre-treatment method, as shown in this study due to reduction in the total microbial individuals. The intrinsic membranes resistances of HP cultures, not biofouling resistances caused by microorganisms, was over 50% in the total resistance. Even though overall resistance tendencies of LP cultures were similar to that of NP cultures after 12hr & 24hr, its irreversible resistances of LP cultures increased at 48hr. The percents values between 24 and 48hr were about from 0% to 40% in the total resistance of LP cultures.

In conclusion, Figure 11 and Figure 12 show that the HP cultures result in a decrease of the membrane fouling. The HP condition provides the supplementary benefit of a reduced number of bacteria that caused membrane biofouling (Figure 6a) and, as a result, lower membrane resistances (Figure 11). This condition could lead to decreasing the times given to membrane washing and unnecessary treatment.

Table 2. Fouling resistance of microfiltration after 48hr

	NP (*E. coli* only)	LP (MOI = 2)	HP (MOI = 200)
Total Resistance	31.5 ± 9.64	13.67 ± 5.89	0.59 ± 0.12
R_m	1.3% (**0.41**)	3.0% (**0.41**)	76.3% (**0.45**)
R_{cp}	98.3% (**30.98**)	63.1% (**8.62**)	5.1% (**0.03**)
R_p	0.2% (**0.07**)	10.5% (**1.44**)	16.9% (**0.1**)
R_c	0.1% (**0.04**)	23.4% (**3.2**)	1.7% (**0.01**)

* The values in parentheses are the average determined resistances for each (unit: $10^{11}m^{-1}$).

1.4. Discussion

From Figure 5 & Figure 6a, in the case of MOI=200, the total number of *E.coli* cells was a lower value than both the NP and LP cultures throughout the entire time. There were other researches about microbial control using chemicals in the biological targets that were a major cause of membrane biofouling. Using chemicals leaded to inhibit microbial growth by producing less its ATP energy (Xu and Liu, 2011) or handling quorum sensing of Gram-negative bacteria (Dobretsov et al., 2009). Traditionally, it has been known that the growth of microorganisms can be controlled by the chemical treatments using chlorine and ozone. In the same way, this study suggested that it could be an alternative pre-treatment by utilizing suitable content of BALOs. Moreover, in the case of MOI=200, population of *E.coli* was similar to the initial dose or decreased during 48hr (Figure 6a).

The experimental results of indicate that flux was enhanced, and membrane fouling was decreased when MF was used with an adequate number of bacterial predators, like *B. bacteriovorus* HD 100. This is clearly useful to water/wastewater treatment plants using membrane processes. Notably, BALOs will not be possible to mitigate the membrane fouling triggered by gram-positive bacterial strains. Even if this is a restriction, it does not prevent bacterial predation from reducing the number of prey bacteria and their contrary influences on membrane processes. Actually, diverse studies have represented that in the existence of gram positive bacterial strains, such as *Bacillus* sp. (Hobley et al., 2006), *B. bacteriovorus* HD 100 was still efficient to preying upon and decreasing viable prey inhabitants. These results can help to overcome the limitation of this study and recommend that predation will still be useful under non-prey conditions, but the study of this condition is necessary. Another benefit of using bacterial predators is their wide prey spectrum. At least one BALO strain attacked a broad number of gram negative bacteria, including abundant human pathogens (Dashiff et al., 2011). Since human pathogens often inhabit wastewater streams and can be fouled on membrane filters (Jong et al., 2010, Kwon et al., 2011a), predation ability of BALOs is a supplementary advantage to these processes.

Ⅱ. Combined pre-treatments of bacterial predation and alum coagulation to reduce membrane biofouling

2.1. Microbial solution quality

2.1.1. Microbial solution characterization before coagulation using alum

Particle size distribution (PSD) and zeta potential of both the NP and LP cultures were investigated before alum coagulation. This was because these characteristics of bacteria can explain bacterial aggregation through the coagulation. The particle size distribution (PSD) was shown in Figure 13. PSD of NP and LP cultures were mainly between 200 and 2000 μm. The figure 13 shows that the intensity of particle size within the range 500-1000 nm was higher in the NP cultures by comparison with that of the LP cultures. Also, the average particle sizes were measured to be 2078 ± 147, 845 ± 149 nm in the NP and LP respectively. Due to the predation of BALOs, living *E. coli* cells were lysed and produced by a lot of cell debris. The cell debris from bacterial predation was attributed to a decrease of average particle size in the LP cultures.

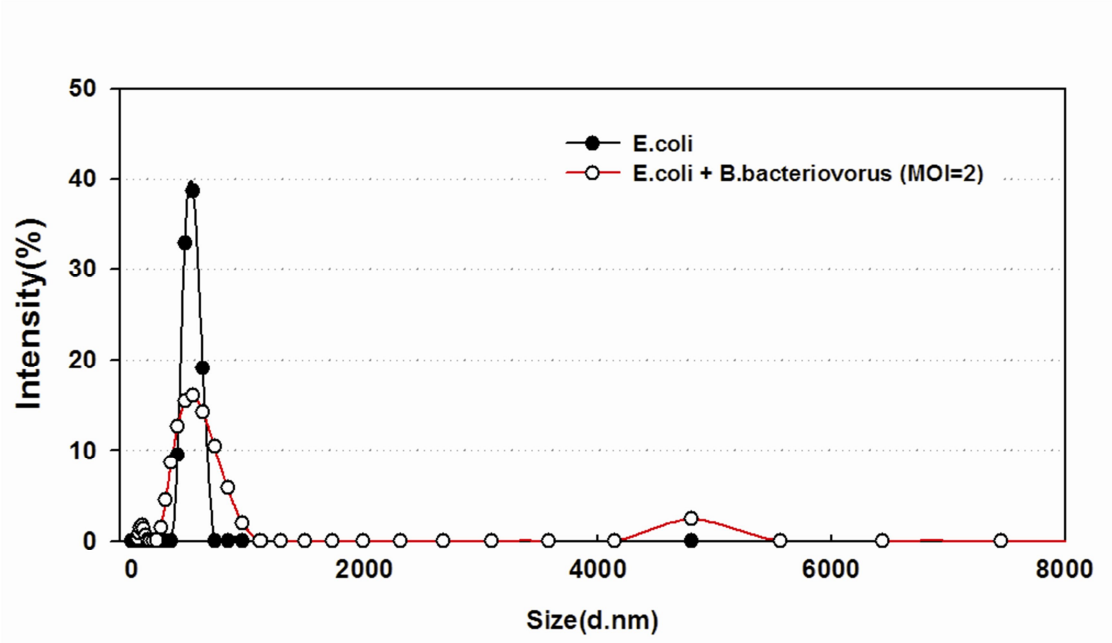

Figure 13. The particle size distribution (PSD) of both the NP and LP cultures

In addition, measurements of the zeta potential were performed for the NP and LP cultures. Actually, it was found that Gram-negative bacteria show a more negative charge as compared to

Gram-positive bacteria. Figure 14 represents distribution of zeta potential for both the NP and LP cultures. The distributions of both the NP and LP cultures were between -50 and 0 mV. The figure 14 describes that the total counts of zeta potential from -50mV to -30 mV was higher in the NP cultures by comparison with that of the LP cultures. Also, the average values of zeta potential were measured to be -33.3 ± 1 mV, -20.9 ± 2 mV in the NP and LP respectively. The zeta potential of the LP cultures showed low absolute value in comparison with that of the NP cultures. This result concur with finding of the work (Klodzinska et al., 2010), which found that dead bacterial cells showed lower zeta potential. This was because there was a lot of cell debris, dead bacterial cells in LP cultures caused by bacterial predation.

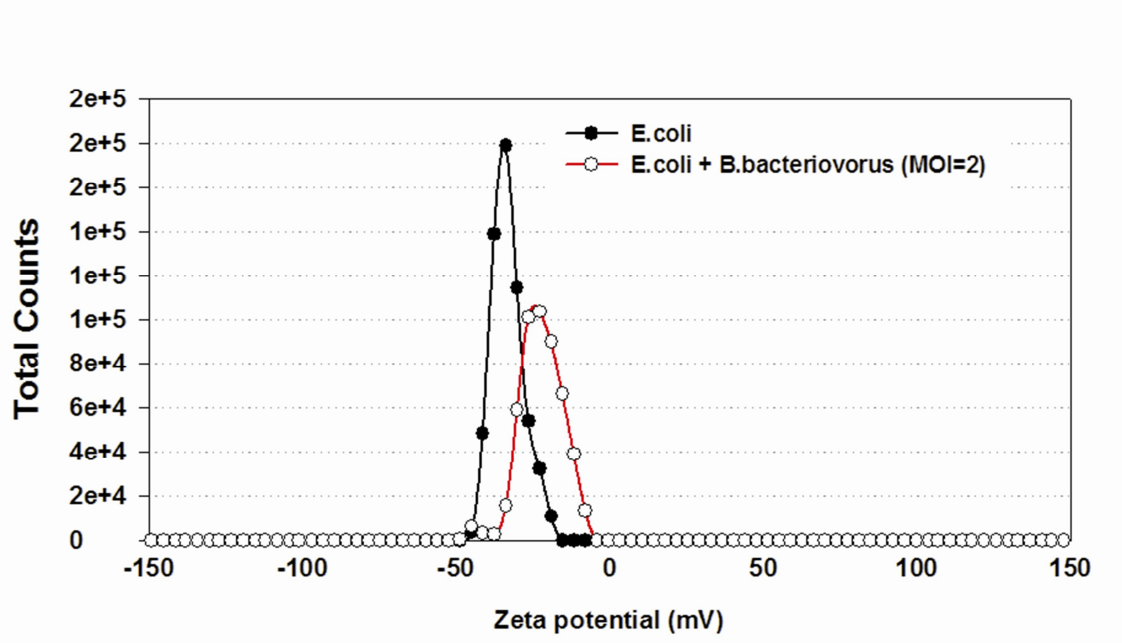

Figure 14. The distribution of zeta potential for both the NP and LP cultures

2.1.2. Microbial solution characterization after coagulation using alum

The survival of *E. coli* within DNB (0.1 x nutrient broth) media was observed over alum concentration when the NP and LP cultures after cultivation during 48hr. Figure 15 represents the observed OD of these cultures. The ODs of the NP and LP cultures showed a similar pattern which the values of OD were inversely proportional to alum contents. The decrease of OD in both the NP and the LP samples indicated that some particles in the samples were precipitated due to alum coagulation. Especially, in the case of 100ppm alum, the ODs showed the lowest values. This is further demonstrated by the count of viable *E. coli* cells (Figure 16).

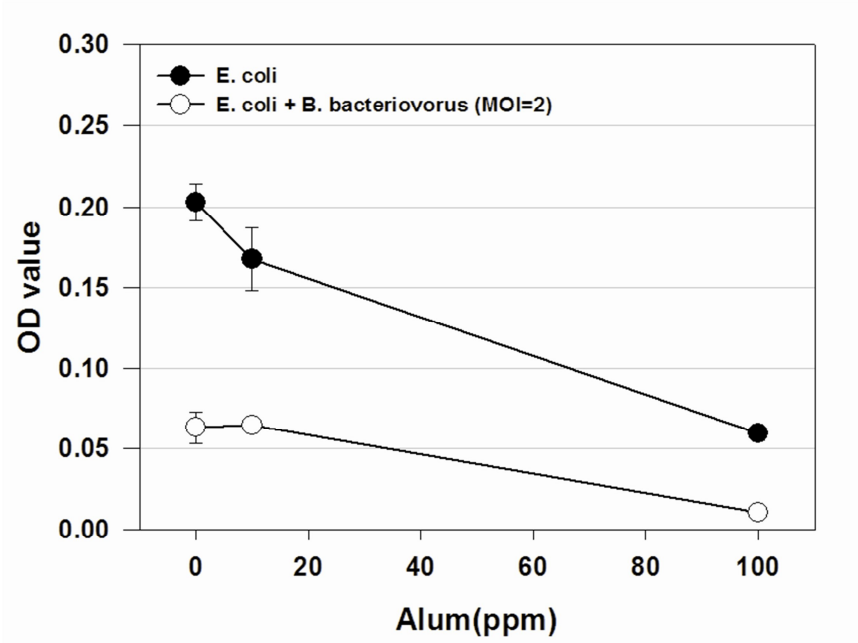

Figure 15. Optical density at 600nm for both the NP and LP cultures depending on the alum concentration

In addition, the count number of viable *E. coli* cells for the NP and LP cultures showed a similar pattern which the values of the count number were inversely proportional to alum concentrations, and the values of the count number for the LP cultures were always lower than that of NP cultures over all concentration of alum. For the NP cultures, the count number of viable *E. coli* cells dropped 37-fold from 1.16 x 10^8 CFU to 3.13 x 10^6 CFU under 100ppm alum, and the cell number of LP cultures decreased 5-fold from 8.33 x 10^4 CFU to 1.67 x 10^4 CFU under 100ppm alum. In addition, for LP cultures between 0ppm and 10ppm of alum, the count number of viable *E. coli* cells decreased 2.5-fold. When the initial MOI was 2, the difference of viable *E. coli* cell number over alum concentration was not much big as compared to the control sample. However, as the results of the study, Investigation of Pre-treatment using *Bdellovibrio*-and-like-organisms (BALOs) on the dead-end microfiltration of *Escherichia coli* (*E.coli*) solution, the *E. coli* debris of the LP cultures from bacterial predation should be considered, because MF membrane of 0.45 μm pore-size could filter the debris.

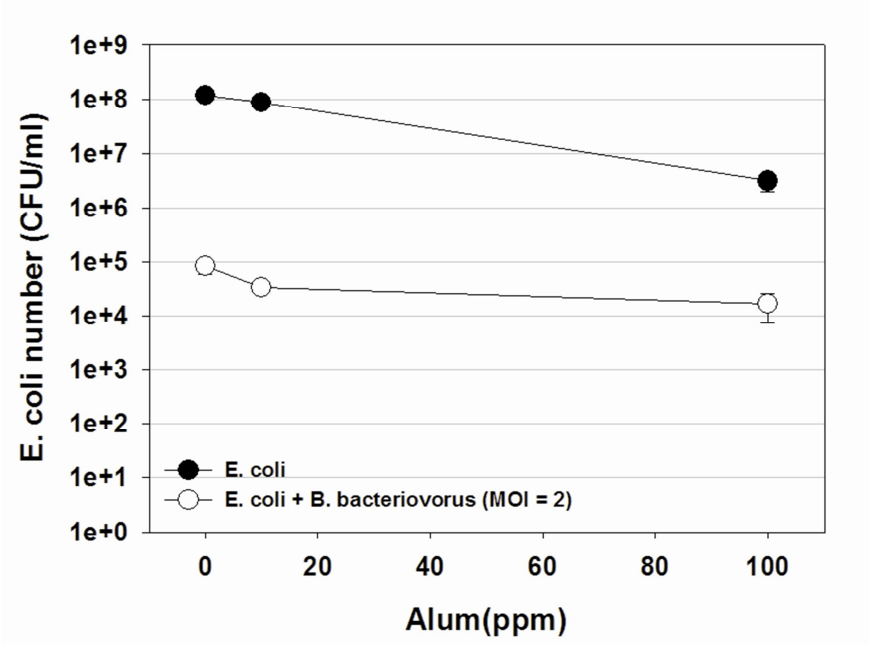

Figure 16. Viable *E. coli* cell counts for both the NP and LP cultures depending on the alum concentration

In the chemical-physical treatment, the sludge is produced because of the quantity of total solids and organic substance that are eliminated. The flocs are formed from the alum coagulation because approximately all of the material will produce a kind of the sludge solids. Typically, the characteristics and amount of the sludge formed from coagulation are related to the operating conditions and the type of coagulants (Ahmad et al., 2008). In addition, the settling of the flocs is crucial in the coagulation process because this will have a positive effect on the cost and effectiveness.

In order to investigate the settling characteristics and overall volume of the sludge formed, settled sludge volume (SSV) was detected. The results are shown in Table 3. The value of the SSV represented 0 when the concentration of alum was 0 and 10ppm in both the NP and the LP cultures. This indicated that 10ppm of alum was not a suitable dosage for both the NP and the LP cultures. That was why there was not precipitation, and almost all of the particles were present in active flocs in the suspension of both the NP and the LP cultures.

On the other hand, settlement was observed when 100ppm of alum was used. In Table 3, the SSV increased from 0 mL/L up to 22 mL/L when 100ppm of alum was used on the NP cultures, and the SSV increased from 0 mL/L up to 34 mL/L when 100ppm of alum was used on the LP cultures. In addition, the SSV of the LP cultures was higher than that of the NP cultures when 100ppm of alum was used. This pointed out that the particles of the LP cultures were well aggregated and more precipitated in comparison with that of the NP cultures when 100ppm of alum was used.

Table 3. Settled Sludge Volume (SSV) for both the NP and LP cultures depending on the alum concentration

Alum concentration	0ppm		10ppm		100ppm	
	Average	SD	Average	SD	Average	SD
NP (*E. coli* only)	0	0	0	0	22	1.41
LP (MOI = 2)	0	0	0	0	34.25	1.77

* The unit of the SSV is mL/L. The SD is a standard deviation.

From Figure 15, Figure 16, and Table 3, it was found that the number of viable *E. coli*, the ODs and the SSV were different according to alum concentration. When 100ppm of alum was used, precipitation was observed, and it caused the increase of SSV in both the NP and the LP cultures. Specifically, the SSV of the LP cultures was higher than that of the NP cultures in the case of 100ppm alum. Furthermore, in Figure 16, the difference of viable *E. coli* cell number in the LP cultures was not much big from 10ppm to 100ppm of alum. However, the SSV was completely different when 10ppm and 100ppm of alum. This was because the *E. coli* debris in LP cultures produced bacterial predation was well precipitated in the case of 100ppm alum as compared to the 10ppm alum, which led to production of active floc, not settlement of particles in LP cultures.

2.2. Filtration of 200ml microbial supernatant

The MF membrane was conducted to permeate 200ml of both the NP and the LP cultures after coagulation with various concentration of alum. The result of the original flux is represented in Figure 17 below. The flux of the NP and LP cultures represented comparable tendency which the values of flux were proportional to alum contents. The increase of flux in both the NP and the LP samples implied that some materials leading to the membrane fouling in the samples were precipitated and removed because of alum coagulation. In addition, for LP cultures, their flux values were always higher than NP cultures over all concentration of alum, because their total number of living *E. coli* cells was always lower than NP cultures, as shown in Figure 16. This result showed that viable *E. coli* cells had a crucial effect on the MF membrane performance because of the sieving mechanism, and membrane biofouling caused by viable *E. coli* cells was able to be alleviated by using alum coagulation and bacterial predation.

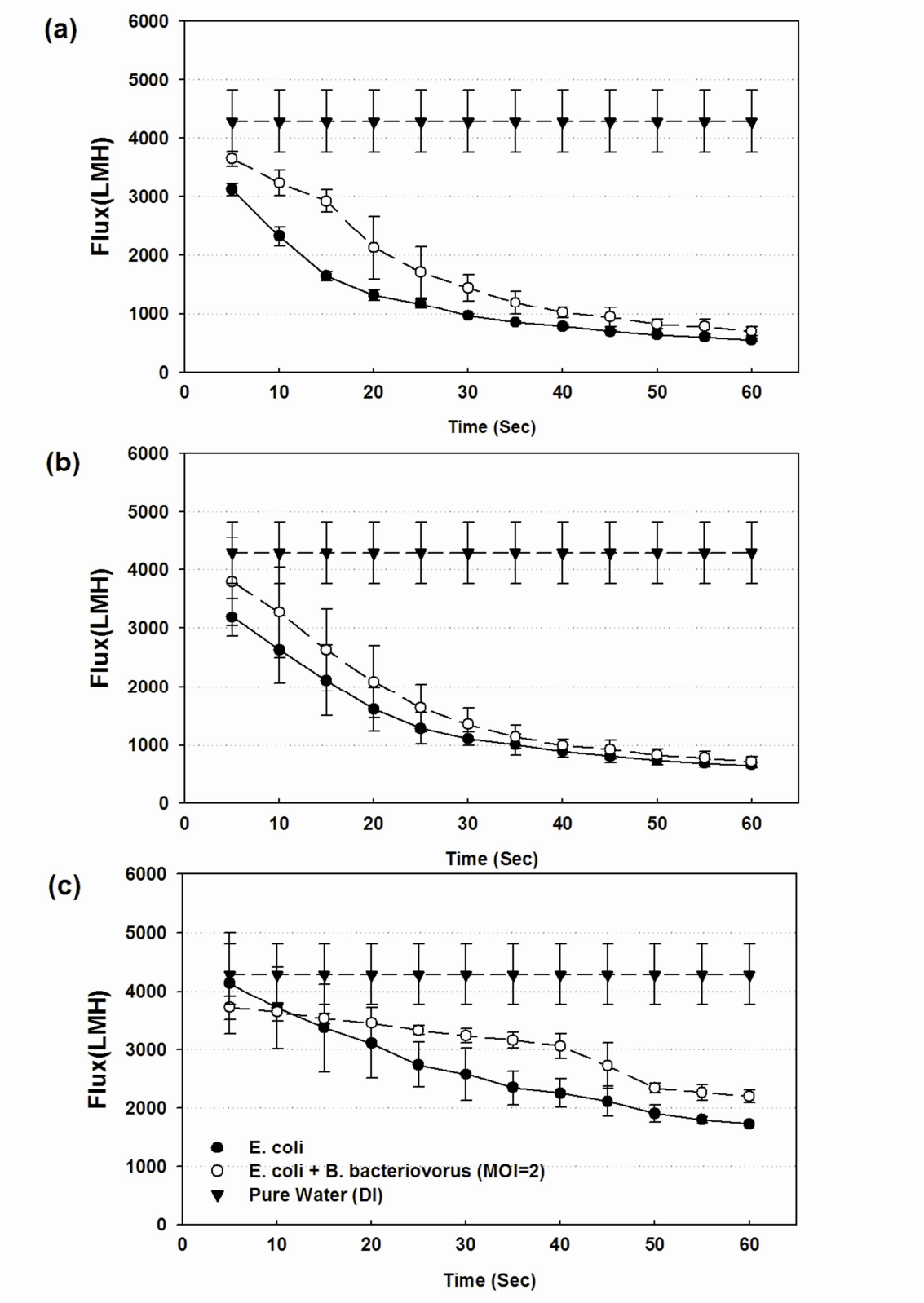

Figure 17. Original flux of membranes for both the NP and LP cultures after coagulation with (a) 0ppm, (b) 10ppm, and (c) 100ppm of alum

There are more detailed flux results in Table 4. The normalized flux of both the NP and LP cultures showed analogous trend which the normalized flux was proportional to alum concentration. Also, when comparing 0ppm of alum with 10ppm of alum, these flux values of the NP cultures were not much different (29% and 33% respectively). As the case of the NP cultures, the normalized flux of LP cultures represented comparable values for both 0ppm and 10ppm of alum (40% and 39% respectively). This result indicated that 10ppm of alum was not a suitable concentration of alum for the NP and LP samples. Thus, the value of the SSV represented 0 when the concentration of alum was 0 and 10ppm in both the NP and the LP cultures, as shown Table 3.

On the other hand, for the NP and LP cultures after coagulation with 100ppm of alum, their flux performance was enhanced in comparison to the flux using coagulation with 0, 10 ppm of alum. This was because using 100ppm of alum removed particles of both the NP and the LP cultures leading to membrane fouling. Specifically, the particles were precipitated, and removed when 100ppm of alum used, as shown in Table 3.

Table 4. Original and Normalized flux point at 1min for both the NP and LP cultures after coagulation with various concentration of alum

Dosage of alum	0ppm		10ppm		100ppm	
	LMH	%	LMH	%	LMH	%
NP (*E. coli* only)	1225	29	1393	33	2648	62
LP (MOI = 2)	1712	40	1680	39	3053	71

* LMH value is an average of original flux until 1 min. The % value, a normalized flux, is ((LMH value of microbial solution * 100) / (LMH value of prue water)).

Furthermore, for LP cultures, the normalized flux of the LP cultures became significantly different when comparing 10ppm of alum with 100ppm of alum (39% and 71% respectively). This was because 100ppm of alum led to aggregation of the *E. coli* debris produced through bacterial predation. For LP cultures, the SSV increased after coagulation with 100ppm of alum, as shown in Table 3. The increased SSV indicated that the *E. coli* debris was aggregated and precipitated by using 100ppm of alum, even though the difference of viable *E. coli* cell number for LP sample between 10ppm of alum and 100ppm of alum was not much large (Figure 16). Therefore, for LP cultures, the *E. coli* debris was removed, and the normalized flux showed the highest in all of alum concentration, following coagulation with 100ppm of alum, not 10ppm of alum.

2.3. Resistance-in-series model

In order to detect which components of membrane fouling contributed most to the fouling issue, a resistance-in-series model was used. As shown in Figure 18, there are consequences of diverse membrane resistances, following filtration of 200mL supernatant of the NP and LP cultures, with coagulation treatment using various concentration of alum such as 0, 10 and 100ppm. From eq. (2) and (4), the R_m value was obtained by 0.4×10^{11} m^{-1}. The total resistance of the membrane is described by the sum of R_m, R_c, and R_p (Figure 18a). In addition, the total resistance of both the NP and LP cultures became inversely proportional to alum concentration. In the concrete, in the case of 100ppm alum, the total resistance of both the NP and LP samples represented the lowest values. The total resistance of LP cultures even became a 14 times decreased value, and that of NP cultures showed a 6 times reduced value, when comparing 100ppm of alum with 10ppm of alum.

This was because particles in the samples causing membrane fouling were precipitated and removed by the coagulation using 100ppm of alum, of which the SSV demonstrated settlement of the particles (Table 3). In addition, the total resistance of LP sample was lower than that of NP sample. This tendency was related to SSV results; Table 3 represents the SSV of LP sample is higher than that of NP sample, SSV results indicated that the particles of the LP cultures were well aggregated and more precipitated in comparison with particles of the NP cultures when 100ppm of alum was used.

For the LP cultures, coagulation with 100ppm of alum led to decrease of the irreversible resistance as well as the total resistance. Concretely, the irreversible resistance of the LP cultures became about a 4 times decreased value, when comparing 100ppm of alum with 0ppm of alum. This was because the E.coli debris, leading to increase of the irreversible resistance of the LP cultures, was precipitated and removed after coagulation with 100ppm of alum.

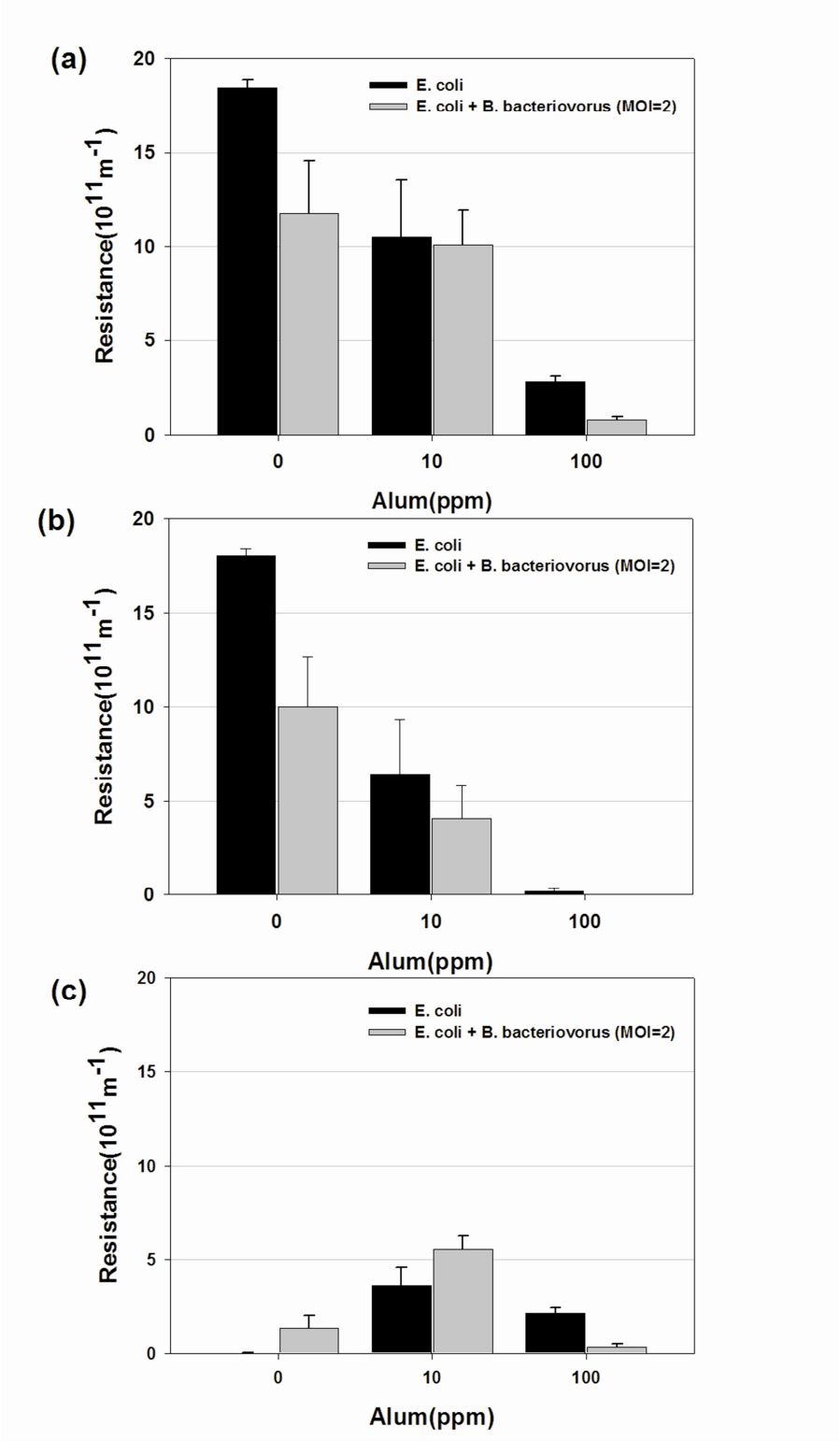

Figure 18. Membrane resistances for both the NP and LP cultures using the Resistance-in-series model at various concentration of alum such as 0, 10, and 100ppm; (a) Total resistance (R_t), (b) Resistance of cake layer(R_c), (c) Resistance of pore block(R_p)

As 100ppm of alum, the total resistance and reversible resistance of LP sample were lower than that of NP sample. Also, the total and reversible resistances were decreased for both the NP and LP samples, when comparing 10ppm of alum with 0ppm of alum. However, 10ppm of alum triggered the increase of the irreversible resistance of both the NP and LP samples. The difference of the irreversible resistance between the NP and the LP samples after coagulation with 10ppm alum was similar with that of the irreversible resistance for no coagulation.

As shown in Table 3, the value of the SSV showed 0 when the concentration of alum was 10ppm in both the NP and the LP cultures. Thus, there was not precipitation, and almost all of the particles were present in active flocs in the suspension of both the NP and the LP cultures. The active flocs caused an opposing effect on membrane performance of the NP and LP samples.

In other investigations, Howe and Clark represented that membrane performance consistently improved with doses for enhanced coagulation but that coagulation may improve or degrade membrane performance when low doses are used (Howe et al., 2006). Therefore, 10ppm alum was not a suitable dosage for both the NP and the LP cultures, and then the 10ppm of alum led to the MF membrane performance to be worse.

Since the irreversible resistance of both NP and LP cultures was increased after coagulation of 10ppm alum, the pores of the MF membrane were severely blocked even after tough washing of the membrane, as shown in Figure 19, SEM images. Furthermore, for LP cultures, the *E. coli* debris blocked the pores of the MF membrane with no coagulation treatment. However, in the case of coagulation with 100ppm alum, the pores of the MF membrane for LP cultures could be observed (Figure 19). This indicated that 100ppm alum led to decrease of irreversible resistance for LP cultures.

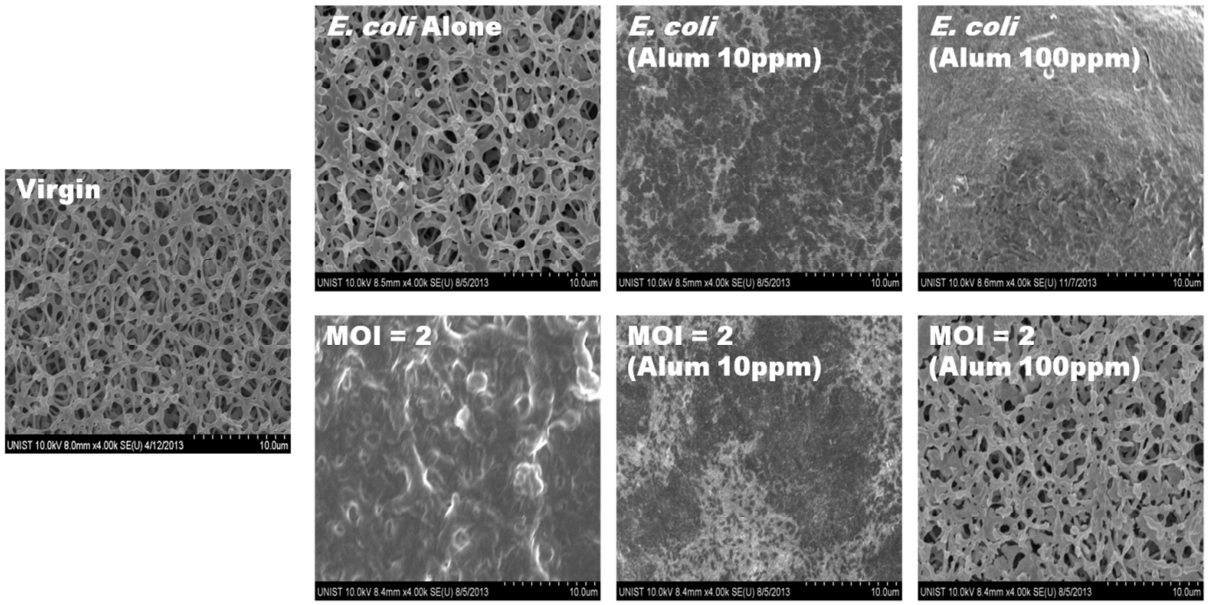

Figure 19. Scanning electron microscopy (SEM) images of the MF membranes filtered both the NP and LP cultures after coagulation with various concentration of alum

Table 5 represents this in detail. Table 5 illustrates overall percent of membrane resistances, depending on the alum concentration with two cases; *E. coli* and MOI = 2. In the case where alum was not used, the reversible resistance such as Rc was mainly involved for the NP cultures (97.4%). On the other hand, for the LP cultures, the irreversible resistance was higher compared to that of NP cultures. In addition, when 10ppm of alum was used, the ratio of the irreversible resistance to the total resistance was increased for both the NP and LP cultures (34.4% and 55.4% respectively).

Since 10ppm alum was not a suitable dosage for both the NP and the LP cultures, the 10ppm of alum triggered increase of the irreversible resistance for both cultures. However, both the NP and LP cultures represented greatly low total and reversible resistances, when 100ppm of alum was used. Also, the intrinsic membrane resistance (Rm) of LP cultures was the main portion to the total resistance, indicating the possible advantage of using both the alum and the predation for mitigating membrane biofouling.

Table 5. Fouling resistances of membranes filtered both the NP and LP cultures after coagulation with various concentration of alum

	0ppm		10ppm		100ppm	
	NP	LP	NP	LP	NP	LP
Total Resistance	18.5 ± 0.42	11.8 ± 2.77	10.5 ± 3.04	10.1 ± 1.91	2.79 ± 0.31	0.79 ± 0.2
R_m	2.5% (**0.46**)	3.8% (**0.45**)	4.1% (**0.43**)	4.3% (**0.44**)	15% (**0.42**)	58.3% (**0.46**)
R_p	0.1% (**0.01**)	11.5% (**1.36**)	34.4% (**3.61**)	55.4% (**5.57**)	78.6% (**2.19**)	39.8% (**0.32**)
R_c	97.4% (**18.0**)	84.7% (**10.0**)	61.5% (**6.47**)	40.3% (**4.06**)	6.4% (**0.18**)	1.9% (**0.02**)

* The values in parentheses are the average determined resistances for each (unit: $10^{11}m^{-1}$).

2.4. Discussion

From the results of resistance-in-series model, at low concentration of alum (10ppm), the membrane performance was poor for both the NP and LP cultures, in that the irreversible resistances of both cultures were increased in comparison with the cases of no alum treatment. However, at suitable dosage of alum (100ppm), it caused the considerable decrease of total and reversible resistances for both cultures. Furthermore, the total, reversible and irreversible resistances of LP cultures became less than that of NP cultures, and the SSV value of LP cultures was larger than that of LP cultures. This pointed out that the cultures of low MOI with 100ppm alum were well cohered and precipitated in comparison with the cultures of *E. coli*, and there were two factors for the phenomenon of coagulation, using 100ppm of alum.

At first, the size of particles has been considered to influence membrane process using coagulation. Figure 13 shows the particle size distribution (PSD), which represents the average particle size of LP cultures were lower than that of NP cultures. Because of the predation of BALOs, living *E. coli* cells were lysed and made by a lot of cell debris, which led to the improvement of membrane performance with alum coagulation.

Other investigations found that the combination of pre-treatment using ultrasonic and alum coagulation caused enhancing the permeate flux, by comparison with the case of utilizing coagulation alone. This was attributed to the disintegration of the particles (Hakata et al., 2011). The effects of predation seem to be similar with that of ultrasonic, because both predation and ultrasonic effects led to lysis of the fouling microorganisms. It was known that the predation resulted in the fragmentation of the particles, a lot of cell debris (as shown Figure 3). Thus, as the results of Hakata, a combined pre-treatment using both bacterial predation (low MOI) and alum coagulation (100ppm) led to enhance membrane performance.

Next, zeta potential is one of the important factors for the membrane performance using coagulation. Figure 14 represents the zeta potential, which shows the zeta potential of the LP cultures became a lower absolute value in comparison with that of the NP cultures. This result concurs with finding of Klodzinska work which found that dead bacterial cells showed lower zeta potential (Klodzinska et al., 2010). From bacterial predation, it was produced a lot of dead cells, debris in LP cultures.

The work of Sharp suggested that intense flocs and low residual contents could be produced by minimizing the zeta potential (Sharp et al., 2006). Clearly, decreased negative surface charge of particles could help the particles to aggregate strongly together, and further hinder membrane fouling, as shown in the study (Liu and Sun, 2010). Figure 14 shows that the particles of the LP cultures became a lower negative surface charge in comparison with that of the NP cultures. In addition, high negative surface charge of particles led to the harsh membrane fouling in MBR (Lee et al., 2003). Consequently, bacterial predation for LP cultures led to particles having a lower negative surface charge, which improved membrane performance after coagulation with 100ppm of alum.

Ⅲ. Combined pre-treatments of bacterial predation and powdered activated carbon (PAC) to reduce membrane biofouling

3.1. Microbial solution characterization after treatment using powdered activated carbon (PAC)

The survival of *E. coli* within DNB (0.1 x nutrient broth) media was observed over PAC concentration when the NP and LP cultures after cultivation during 48hr. Figure 20 represents the observed OD of these cultures. The ODs of the NP cultures showed a pattern which the values of OD were inversely proportional to PAC contents. However, The ODs of the LP cultures represented a different pattern that the values of OD were slightly proportional to PAC contents. This was because of particles of PAC which permeated by a 10 μm filter. The decrease of OD in the NP sample indicated that some particles in the sample were removed due to PAC treatment. Especially, in the case of 100ppm PAC, the ODs showed the lowest values. This is further demonstrated by the count of viable *E. coli* cells (Figure 21).

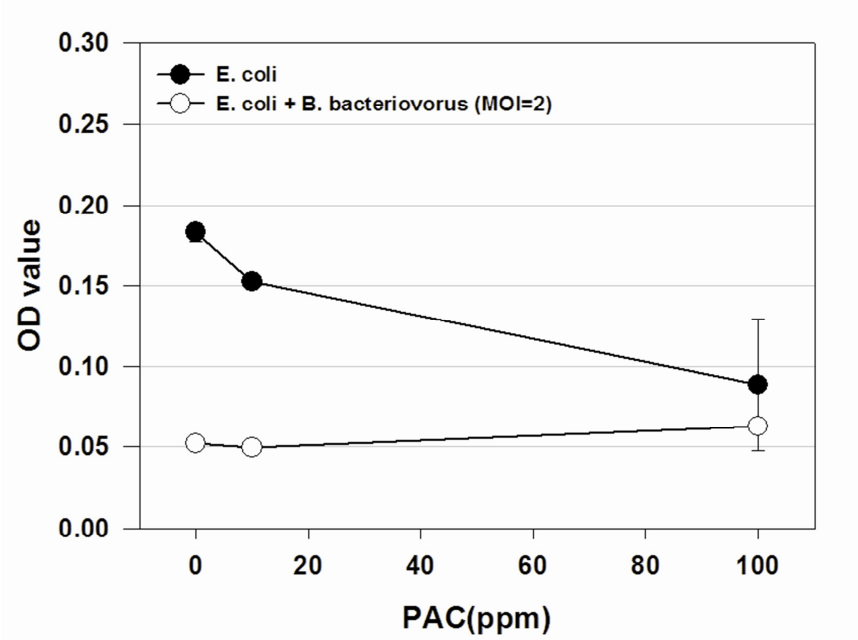

Figure 20. Optical density at 600nm for both the NP and LP cultures depending on the PAC concentration

In addition, the count number of viable *E. coli* cells for both the NP and LP cultures showed a similar pattern which the values of the count number were inversely proportional to PAC concentrations. However, the decrease of viable *E. coli* cells for both the NP and LP cultures was much low depending on the PAC concentrations. Also, the values of the count number for the LP

cultures were always lower than that of NP cultures over all concentration of PAC. For the NP cultures, the count number of viable *E. coli* cells dropped 2.1-fold from 1.31 x 10^8 CFU to 6.26 x 10^7 CFU under 100ppm PAC, and the cell number of LP cultures decreased 2.1-fold from 2.33 x 10^3 CFU to 1.09 x 10^3 CFU under 100ppm PAC. In addition, for LP cultures between 0ppm and 10ppm of PAC, the count number of viable *E. coli* cells decreased 1.2-fold. When PAC treatment was used, the difference of viable *E. coli* cell number for both the NP and LP cultures was not much big as compared to coagulation treatment using alum. However, as the results of the study, Investigation of Pre-treatment using *Bdellovibrio*-and-like-organisms (BALOs) on the dead-end microfiltration of *Escherichia coli* (*E.coli*) solution, the *E. coli* debris of the LP cultures from bacterial predation should be considered, because MF membrane of 0.45 μm pore-size could filter the debris.

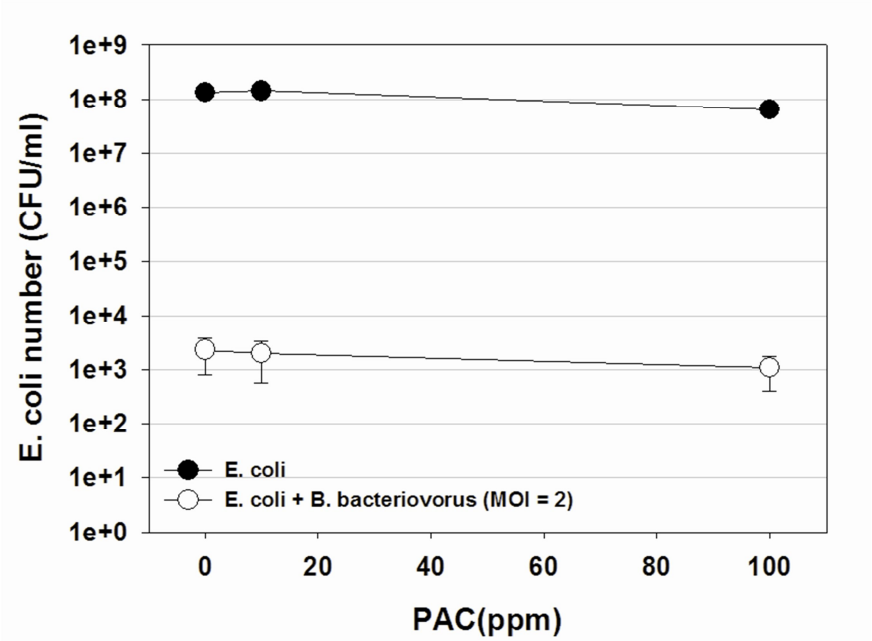

Figure 21. Viable *E. coli* cell counts for both the NP and LP cultures depending on the PAC concentration

From Figure 20 and Figure 21, it was found that the number of viable *E. coli* and the ODs were different according to PAC concentration. However, for both the NP and LP cultures after treatment using various concentrations of PAC, the extent of decrease of the viable *E. coli* cell showed a slight difference in comparison with coagulation treatment using alum. Even if same dosage of both the alum and PAC was used, the influence on a decrease of the viable *E. coli* cell was different for the NP and the LP cultures. This could be due to the dissimilar mechanism of treatment using the alum and the PAC. It has been known that coagulation treatment leads to change of surface charge for target

compounds, and this treatment cause the aggregation and the precipitation of the target compounds. On the other hand, the mechanism of the PAC treatment is the adsorption to the target compounds. These different mechanisms of both the coagulation and PAC treatments triggered different tendencies of both the viable *E. coli* cell and the ODs, when compared the results of the PAC treatment with that of coagulation treatment.

3.2. Filtration of 200ml microbial solution after treatment with the PAC

The MF membrane was conducted to permeate 200ml of both the NP and the LP cultures after treatments with various concentration of the PAC. The result of the original flux is shown in Figure 22 below. The flux of the NP and LP cultures represented comparable tendency which the values of flux were proportional to the PAC concentrations. The increase of flux in both the NP and the LP samples indicated that some particles causing the membrane fouling in the samples were adsorbed and removed due to the PAC treatment. Besides, for LP cultures, their flux values were always higher than NP cultures over all concentration of the PAC, because the total number of living *E. coli* cells of the LP cultures was always lower than that of the NP cultures, as shown in Figure 21. This result implied that viable *E. coli* cells had an important influence on the MF membrane performance because of the sieving mechanism, and membrane biofouling caused by viable *E. coli* cells was able to be mitigated by using the PAC treatment and bacterial predation.

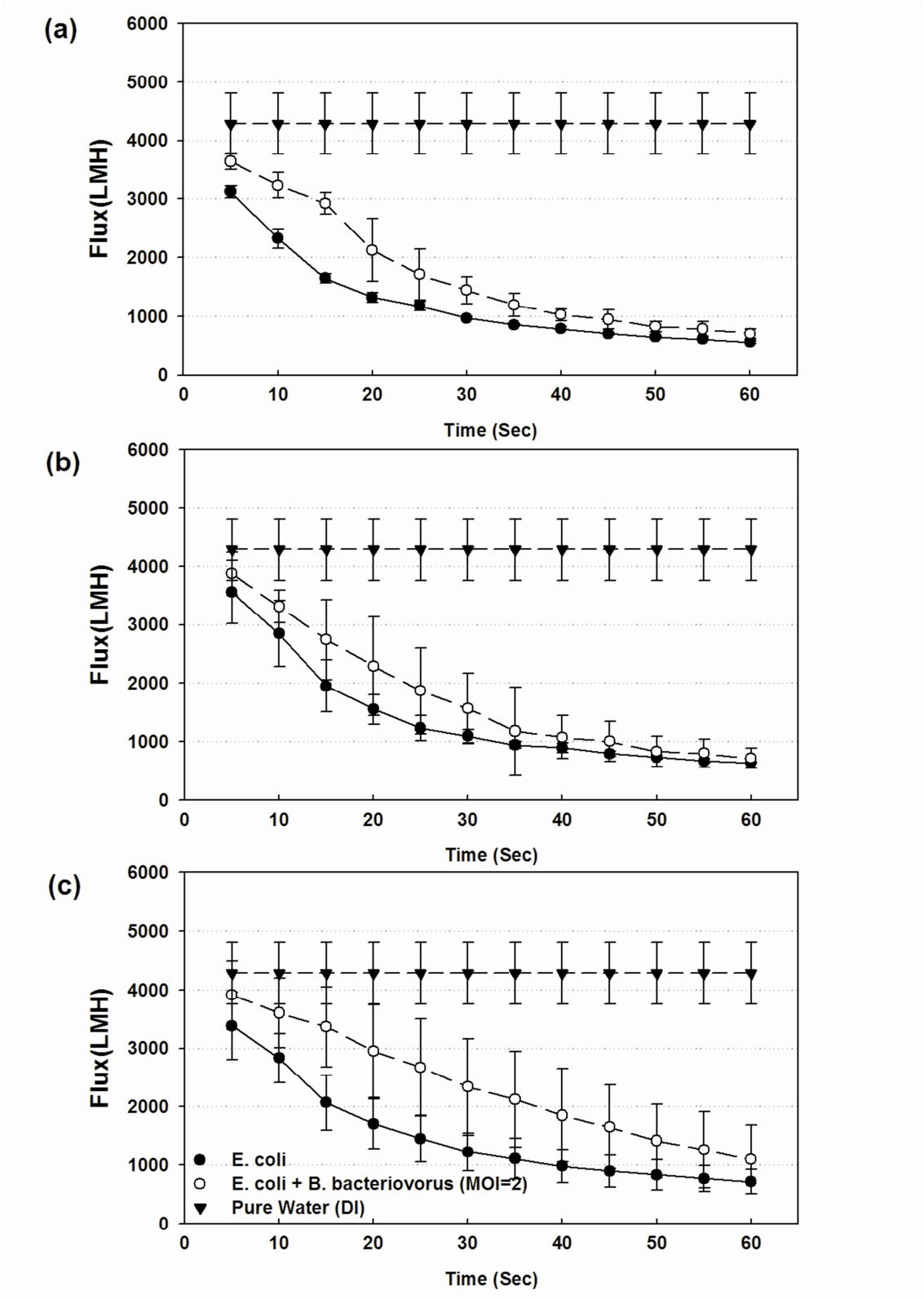

Figure 22. Original flux of membranes for both the NP and LP cultures after treatment with (a) 0ppm, (b) 10ppm, and (c) 100ppm of the PAC

There are more detailed flux results in Table 6. The normalized flux of both the NP and LP cultures represented a similar tendency which the normalized flux was proportional to the PAC concentration. In addition, when comparing 0ppm of the PAC with 10ppm of the PAC, these flux values of the NP cultures were not much different (29% and 33% respectively). As the case of the NP cultures, the normalized flux of LP cultures showed analogous values for both 0ppm and 10ppm of alum (40% and 41% respectively). This result implied that 10ppm of the PAC was not an appropriate concentration of the PAC for both the NP and LP cultures.

On the other hand, for the NP and LP cultures after treatment with 100ppm of the PAC, their tendencies of flux performance were totally different. For the LP cultures, the flux performance was improved in comparison to the flux using the treatment with 0, 10 ppm of the PAC. This was because using 100ppm of the PAC removed particles of the LP cultures causing to membrane fouling. Specifically, for the LP cultures, the normalized flux of the LP cultures became significantly different when comparing 10ppm of the PAC with 100ppm of the PAC (41% and 55% correspondingly). This was because 100ppm of the PAC caused the adsorption of the *E. coli* debris produced through bacterial predation.

When comparing 10ppm of the PAC with 100ppm of the PAC, the flux performance for the LP cultures was much different, even if the difference of viable *E. coli* cell number for LP sample between 10ppm of the PAC and 100ppm of the PAC was not much large (Figure 21). This indicated the treatment with 100ppm PAC led to removing a lot of the *E. coli* debris, not living *E. coli* cells. Thus, for LP cultures, the *E. coli* debris was removed, and the normalized flux showed the highest in all of the PAC concentration, after treatment with 100ppm of the PAC, not 10ppm of the PAC.

Table 6. Original and Normalized flux point at 1min for both the NP and LP cultures after treatment with various concentration of the PAC

Dosage of PAC	0ppm		10ppm		100ppm	
	LMH	%	LMH	%	LMH	%
NP (*E. coli* only)	1225	29	1406	33	1495	35
LP (MOI = 2)	1712	40	1768	41	2352	55

* LMH value is an average of original flux until 1 min. The % value, a normalized flux, is ((LMH value of microbial solution * 100) / (LMH value of prue water)).

In addition, when 100ppm of the PAC was even used, the flux performance for the NP cultures was not much enhanced in comparison to the flux using treatment with 0, 10 ppm of the PAC. Concretely, the flux of the NP cultures was slightly increased from 29% to 35%, when comparing

100ppm of the PAC with 0ppm of the PAC. This result represented a different trend in comparison with the case of using alum. When 100ppm of alum was used, the flux of the NP cultures was significantly enhanced from 29% to 62%, when comparing 100ppm of alum with 0ppm of alum (Table 4). This indicated that the coagulation using 100ppm of alum had a better effect on removing viable *E. coli* cells, compared to the treatment using 100ppm of the PAC.

In conclusion, it was found that the flux of the LP cultures was improved when 100ppm of the PAC was used. Thus, as using 100ppm alum for the LP cultures, the PAC treatment of 100ppm led to removing the *E. coli* debris and improving the flux performance. However, for the NP cultures, the treatment using 100ppm of the PAC did not have a considerable effect on removing viable *E. coli* cells and enhancing the flux performance, when comparing the coagulation using 100ppm of alum.

3.3. Resistance-in-series model

In order to detect which components of membrane fouling contributed most to the fouling issue, a resistance-in-series model was used. As shown in Figure 23, there are consequences of diverse membrane resistances, following filtration of 200mL supernatant of the NP and LP cultures, with the treatment using various concentration of the PAC such as 0, 10 and 100ppm. From eq. (2) and (4), the R_m value was obtained by 0.4×10^{11} m^{-1}. The total resistance of the membrane is described by the sum of R_m, R_c, and R_p (Figure 23a). In addition, the total resistance of both the NP and LP cultures became inversely proportional to the PAC concentration. In the concrete, in the case of 100ppm PAC, the total resistance of both the NP and LP samples showed the lowest values in the overall concentration of the PAC. The total resistance of LP cultures even became a 6 times decreased value, and that of NP cultures showed a 2 times reduced value, when comparing 100ppm of the PAC with 0ppm of the PAC.

This was because particles in the samples leading to membrane fouling were adsorbed and removed by the treatment using 100ppm of the PAC. Furthermore, the total resistance of the LP cultures was lower than that of the NP cultures. This tendency was related to flux results; Table 6 implied that the treatment using 100ppm of the PAC triggered removing the *E. coli* debris as well as enhancing the flux performance of the LP cultures, and the PAC treatment was not considerably efficient for both removing viable *E. coli* cells and improving the flux performance of the NP cultures.

For the LP cultures, the treatment with 100ppm of the PAC caused the decrease of the irreversible resistance as well as the total resistance. In the concrete, the irreversible resistance of the LP cultures became about a 2.7 times decreased value, when comparing 100ppm of the PAC with 0ppm of the PAC. This was because the E.coli debris, triggering the increase of the irreversible resistance of the LP cultures, was adsorbed and removed after the treatment with 100ppm of the PAC.

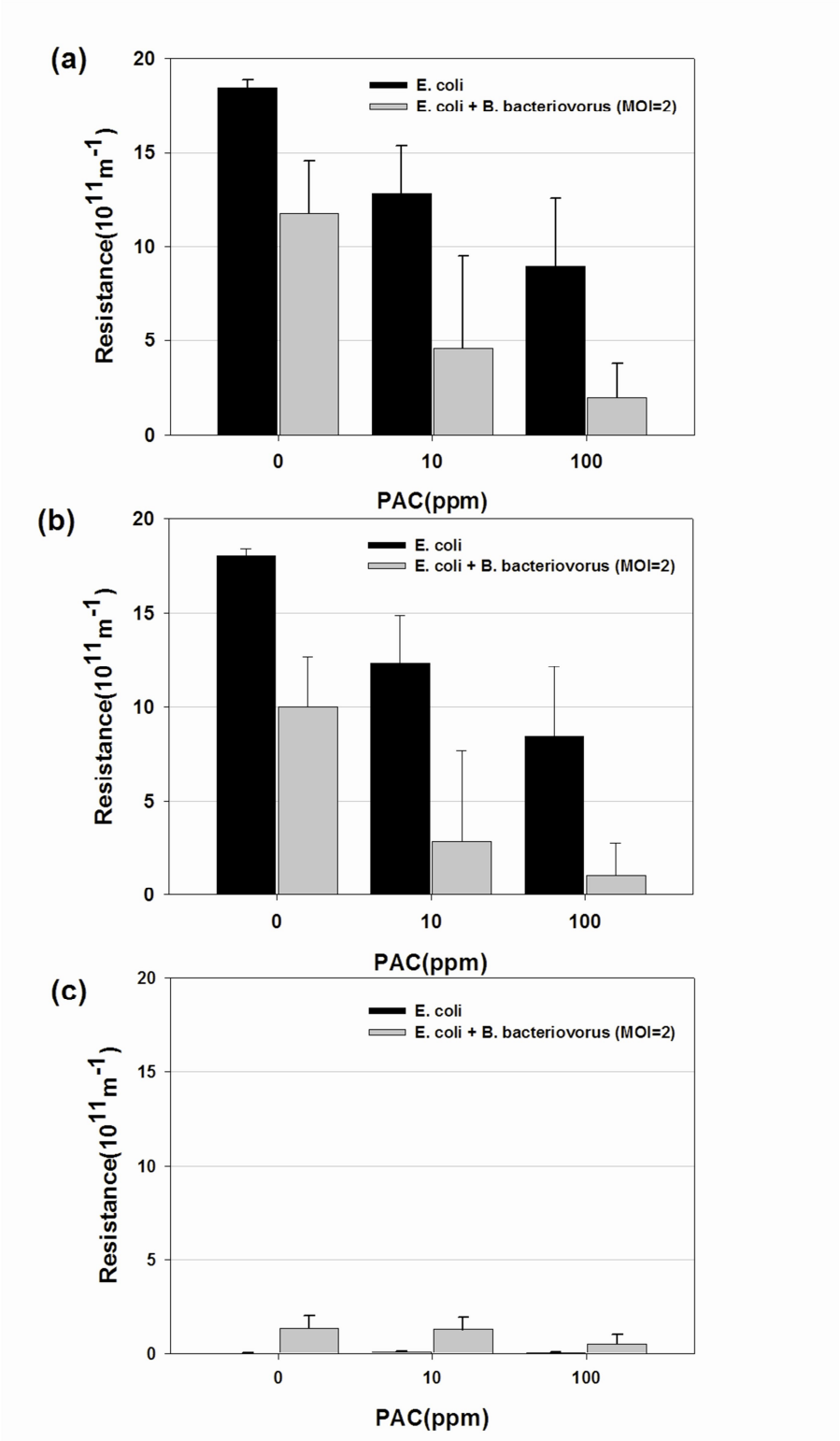

Figure 23. Membrane resistances for both the NP and LP cultures using the Resistance-in-series model at various concentration of the PAC such as 0, 10, and 100ppm; (a) Total resistance (R_t), (b) Resistance of cake layer(R_c), (c) Resistance of pore block(R_p)

Since the irreversible resistance of the NP cultures became significantly low over all concentration of the PAC, the pores of the MF membrane were observed after tough washing of the membrane, as shown in Figure 24, SEM images. In addition, for LP cultures, the *E. coli* debris blocked the pores of the MF membrane, when the treatment of PAC was not used. However, in the case of treatment with the PAC, the pores of the MF membrane for LP cultures could be observed (Figure 24). This implied that 100ppm PAC caused a decline of the irreversible resistance for LP cultures.

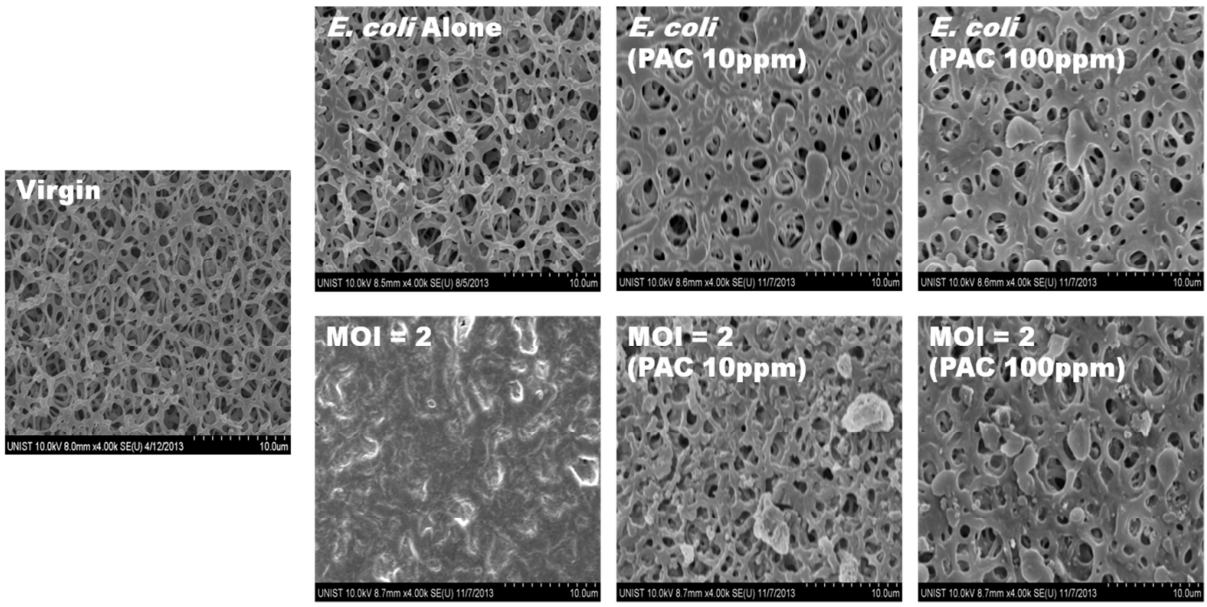

Figure 24. Scanning electron microscopy (SEM) images of the MF membranes filtered both the NP and LP cultures after treatment with various concentration of the PAC

Table 7 shows the fouling resistances of the MF membranes in detail. Table 7 represents overall percent of membrane resistances, according to the PAC concentration with two cases; *E. coli* and MOI = 2. For the NP cultures, the reversible resistance such as Rc was mainly involved over all concentration of the PAC. In concrete, it became more than 90% of the total resistance. Furthermore, when the PAC treatment was used, the total resistances of the NP cultures showed larger values in comparison with the cases of coagulation using alum. For example, the total resistance of the NP cultures was about a 2 times decreased value, when comparing 100ppm of the PAC with 0ppm of the PAC. On the other hand, the total resistance of the NP cultures became about a 23 times decreased value, when comparing 100ppm of alum with 0ppm of alum. This tendency was related to the number of viable *E. coli* cells.

Table 7. Fouling resistances of membranes filtered both the NP and LP cultures after the treatment using various concentration of the PAC

	0ppm		10ppm		100ppm	
	NP	LP	NP	LP	NP	LP
Total Resistance	18.5 ± 0.42	11.8 ± 2.77	12.9 ± 2.51	4.57 ± 4.93	8.95 ± 3.65	1.98 ± 1.80
R_m	2.5% (**0.46**)	3.8% (**0.45**)	3.1% (**0.40**)	9.2% (**0.42**)	4.7% (**0.42**)	20.6% (**0.41**)
R_p	0.1% (**0.01**)	11.5% (**1.36**)	0.6% (**0.08**)	28.7% (**1.31**)	0.6% (**0.05**)	25.6% (**0.51**)
R_c	97.4% (**18.0**)	84.7% (**10.0**)	96.3% (**12.4**)	62.1% (**2.84**)	94.7% (**8.48**)	53.8% (**1.07**)

* The values in parentheses are the average determined resistances for each (unit: $10^{11}m^{-1}$).

From Figure 21, it was found that the count number of viable *E. coli* cells for the NP cultures decreased 2.1-fold from 1.31 x 10^8 CFU to 6.26 x 10^7 CFU after the treatment with 100ppm PAC. However, when the coagulation with 100ppm of alum was used, the count number of viable *E. coli* cells for the NP cultures dropped 37-fold from 1.16 x 10^8 CFU to 3.13 x 10^6 CFU (Figure 16). This indicated that the decreased viable *E. coli* cells led to a decline of the total resistance. Thus, the coagulation using 100ppm of alum was more efficient than the treatment using the PAC, in that both the number of viable *E. coli* cells and the total resistance were reduced for the NP cultures.

On the other hand, for both the NP and LP cultures, the total resistance was decreased according to the concentration of the PAC. This tendency was mainly due to a decline of the reversible resistance, which represented more than half of the total resistance (Table 7). Hence, the PAC treatment had an effect on reducing the reversible resistance. These results concur with findings of Khan who found that the application of PAC was responsible for decrease of the cake layer resistance (Khan et al., 2012). Furthermore, for the LP cultures, the ratio of the reversible resistance to the total resistance was decreased according to the concentration of the PAC. In the case where 100ppm of the PAC was used, the ratio dropped from 84.7% to 53.8%. However, for the NP cultures, the ratio decreased from 97.4% to 94.7%, which the trend seemed to be comparable.

From Table 6, it was found that the treatment using 100ppm of the PAC did not have a considerable effect on removing viable *E. coli* cells and enhancing the flux performance for the NP cultures. However, Table 7 represents that the treatment using 100ppm PAC was in charge of the decrease of both the reversible and irrversible resistances for the LP cultures. This implied that the treatment with 100ppm PAC led to removing a lot of the *E. coli* debris, not most of vialbe *E. coli* cells. Therefore, both the PAC treatment and the bacterial predation contributed to alleviating membrane biofouling.

3.4. Discussion

From the results of resistance-in-series model, at appropriate concentration of the PAC (100ppm), it triggered the considerable decrease of total and reversible resistances for the LP cultures, while the decrease of both resistances was not much great for the NP cultures. Furthermore, the irreversible resistances of LP cultures became less than the case where the PAC was not used. This indicated that the cultures of low MOI were well adsorbed and removed by using the treatment with 100ppm PAC in comparison with the cultures of *E. coli*, and there were two factors for the phenomenon of the treatment, using 100ppm of the PAC.

At first, the size and shape of particles has been considered to influence membrane process using the treatment with the PAC. Figure 3 represents the observation of the bacterial predation process. The predator (*B. bacteriovorus*) approached the prey (*E. coli*), and then broke through the prey cell of which the shape was changed into a sphere. This sphere shape has been found in other investigations as a bdelloplast (Park et al., 2011, Varon and Shilo, 1968). From the result of 'Investigation of Pre-treatment using *Bdellovibrio*-and-like-organisms (BALOs) on the dead-end microfiltration of *Escherichia coli* (*E. coli*) solution', the debris produced by bacterial predation blocked the pores of the MF membrane more severely. This meant that the debris was attached to the pores of the MF membrane. As this trend, for the LP cultures, it is possible that the debris was well adsorbed after treatment using 100ppm of the PAC. This was because a shape of the *E. coli* debris was more appropriate to adsorbing the debris through the PAC particles, compared by the shape of the living *E. coli* cells.

Next, zeta potential is one of the crucial factors for the membrane performance using the PAC treatment. Figure 14 shows the zeta potential, which represents the zeta potential of the LP cultures became a lower absolute value in comparison with that of the NP cultures. The study of Matsushita found that a decreased repulsive force between the PAC particles and the microorganisms attributed considerably to removal of the microorganisms (Matsushita et al., 2013). Figure 14 represents that the particles of the LP cultures became a lower negative surface charge in comparison with that of the NP cultures. Thus, bacterial predation for LP cultures led to particles having a lower negative surface charge, which enhanced membrane performance after treatment with 100ppm of the PAC.

In addition, at low concentration of alum (10ppm), the membrane performance was poor for both the NP and LP cultures, in that the irreversible resistances of both the NP and LP cultures were increased in comparison with the cases of no alum treatment. This tendency was similar with the Goh work in the view of the increase of the irreversible resistance even after using alum coagulation (Goh et al., 2010). On the other hand, the treatment using the PAC did not have an effect on the increase of the irreversible resistances for both cultures. This could be because of the different mechanism of the

treatment using the alum and the PAC. It has been known that coagulation treatment causes a change of surface charge for target compounds, and this treatment trigger the aggregation and the precipitation of the target compounds. However, the mechanism of the PAC treatment is the adsorption to the target compounds. These different mechanisms of both the coagulation and PAC treatments caused different trends of membrane performance, when compared the results of the PAC treatment with that of coagulation treatment. Concretely, 10ppm of alum produced a lot of active flocs, which led to the increase of the irreversible resistances for both cultures. However, the treatment did not cause the increase of the irreversible resistances for both cultures. This was because the treatment using the PAC did not have an impact on the change of the surface charge of particles.

Chapter 4. Conclusions

In the first part of this investigation, Dead-end microfiltration (MF) tests were carried out on *E. coli* feed solutions, which were prepared by spiking various initial concentration of BALOs. The flux performance was analyzed by resistance-in-series model. Surface morphology was analyzed with Digital camera, Stereomicroscopy and SEM(Scanning Electron Microscopy). According to the initial concentration of spiked BALOs, microbial growth can be different over the time. In addition, when the feed solutions were initially contained by predatory bacteria in the DNB broth, the total resistance of the MF membrane became lower than the control over the experimental time. However, in the case where the initial MOI of 2 was used, there were still possibilities to induce a long term of the membrane fouling because its irreversible resistances were increased due to the *E. coli* debris from bacterial predation. Therefore, these results indicate that pre-treatment using an appropriate concentration of predatory bacteria such as BALOs is promising for an improvement of the membrane performance.

From the results of the first part, the low MOI triggered the irreversible fouling of the membrane due to the debris of prey cell. However, other research groups found that the ultrasonic treatment resulted in enhancing the coagulation performance. It was assumed that the effect of bacterial predation may be comparable with that of the ultrasonic treatment because both led to lysis of the microorganisms. Based on this assumption, the aim of the second study was to evaluate combined treatments of both the bacterial predation and the coagulation in order to decrease the membrane biofouling. The feed solutions were prepared by using *E. coli* and *B. bacteriovorus* after coagulation with diverse concentrations of alum. The results showed that when low concentration of alum (10ppm) was used, the membrane fouling got worse for both NP and LP cultures, as compared to no alum addition, in that the irreversible resistance of the membrane was much higher. On the contrary, using a suitable concentration of alum (100ppm) decreased the total and reversible resistances similarly in both NP and LP cultures considerably. Furthermore, at 100ppm of alum, the LP culture led to both a lower total and irreversible resistance compared to the NP culture. This was because the LP culture with alum coagulation was well aggregated. These results point out that combined treatments of both *B. bacteriovorus* predation and a suitable concentration of alum can be an efficient pretreatment method for improving membrane performance.

As the coagulation using alum, the feed solutions were prepared by using *E. coli* and *B. bacteriovorus* after treatment with various concentrations of the powdered activated carbon (PAC) in

order to mitigate membrane biofouling triggered by *E .coli* cell debris of the LP culture. The results represented that when 10ppm or 100ppm of PAC were used, the membrane performance became better for both cultures as compared to no PAC addition, in that the reversible resistance was decreased. In addition, for the LP culture, 100ppm of PAC caused reducing the irreversible resistance, as compared to 0ppm of PAC. In the case where 10ppm or 100ppm of the PAC was used, the LP culture led to less total membrane resistance as compared to the NP culture. These results also represent that an appropriate concentration of the PAC in combination with bacterial predation is an effective treatment for mitigating the membrane biofouling.

Consequently, this investigation represented that using bacterial predators at a suitable concentration was beneficial for reducing the membrane biofouling. In addition, even if using bacterial predators at an unsuitable concentration triggered an increase of the irreversible membrane resistance, combined treatments of both bacterial predation and an appropriate concentration of alum or the PAC, can be beneficial for enhancing the performance of MF membranes, which utilized for facilities of wastewater treatment.

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감사의 글

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다음으로 2년간 연구실 생활하며 도와준 연구실 멤버들, 처음에 아무것도 모를 때 기초를 잘 잡아준 정은 누나, 분리막에 관하여 실질적 조언주신 형규형, 배울 것이 많은 은태씨, 취업에 많은 도움주신 상우씨, 묻는 것에 정성을 다해 답해준 Nga, UNIST의 에이스 병문이, 모두에게 감사합니다. 혼자가 아니라 함께 할 수 있었기에 어려운 시기를 잘 보낼 수 있었답니다. 그리고 학부 학교선배이자 룸메이트인 요한이 형에게 많은 힘이 되었다고 말하고 싶습니다. 멀리 울산에서 만나 반가웠던 과 동기 요한이와 승희에게도 참 고마웠다 라고 말하고 싶습니다.

연고지가 아닌 울산에 생활하면서 적응하는데 주변 이들의 도움이 컸습니다. 먼저, 울산 TGIM 식구들. 직장에서의 마음을 터놓고 나누면서 IVF 마음의 고향에 온 듯했습니다. 지성의 대가이시고 따뜻한 아버지 같은 정한신 간사님, 동갑내기인 명락이, 현선이, 선화. 그 외의 식구들에게 감사하다고 말하고 싶습니다. 그리고 태화 교회에서 잘 적응토록 도와주신 윤미 누나. 누나의 삶을 통해 많은 도전을 받았습니다. 지금 교회 소그룹 구성원인 연희누나, 경민형, 도연 누나, 지연누나, 시영누나, 곧 결혼할 지성이. 많은 지체들에게 감사합니다. 그리고 태화 교회 UNIST 식구들인 성민, 재석, 구혁. 멀리 함께 예배드릴 수 있어 감사 했었습니다. 또한 군대 바로 후임인 순욱, 도연, 병탁. 울산에 와서 만나 참 반가웠습니다.

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싶습니다. 또한 간간히 찾아 뵐 때 마다 너무 잘 대해 주시고 많이 기도해 주시는 현지의 부모님께도 참 감사합니다. 20살 이후 함께 해온 IVF 10명의 05동기들. 너희는 존재자체로 내게 큰 힘이 된다고 말하고 싶습니다. 그리고 IVF 동아리 선후배들. 많은 인생 조언 주심에 변함없이 저를 맞아주심에 감사 드립니다. 그리고 간간히 방문할 때 마다 힘 주셨던 본 교회인 성광교회 지체들에게 감사의 표현을 하고 싶습니다. 오랜만에 봐도 늘 멋있는 대웅이 형, 창희 형. 멀리 있는 제게 결혼식 꼭 오라고 말해준 성광교회 누나들. 또한, 오랜만에 만나도 늘 편한 고등학교 친구들. 승수, 진우, 상수, 주석, 성식, 기태. 다 이름 언급 못하지만 저의 감사의 맘을 다 알 거라 생각됩니다. 그리고 하늘 나라에 있는 용준이. 네 덕에 수처리 분야 연구하게 되었다. 멀리서 응원하리라 믿는다.

수 많은 이들의 도움 덕에 학위기간을 보낼 수 있었습니다. 마지막으로 2년 학위기간 동안 수없이 포기하고 싶었지만, 지혜와 감당할 힘 주신 하나님께 감사 드립니다. 그리고 부족하고 자격 없는 제게 많은 은혜 주셔서 취업 가운데 선한 길로 이끄심에 감사 드립니다. 그리고 세상 끝날 까지 함께하실 주님께 참 감사 드립니다.