Pretreatment with alum or powdered activated carbon reduces bacterial predation-associated irreversible fouling of membranes

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This study evaluated the co-application of bacterial predation by \textit{Bdellovibrio bacteriovorus} and either alum coagulation or powdered activated carbon adsorption to reduce fouling caused by \textit{Escherichia coli} rich feed solutions in dead-end microfiltration tests. The flux increased when the samples were predated upon or treated with 100 ppm alum or PAC, but co-treatment with alum and predation gave the best flux results. The total membrane resistance caused by the predated sample was reduced six-fold when treated with 100 ppm PAC, from 11.8 to $1.98 \times 10^{13}$ m$^{-1}$, while irreversible fouling ($R_p$) was 2.7-fold lower. Treatment with 100 ppm alum reduced the total resistance 14.9-fold (11.8 to $0.79 \times 10^{13}$ m$^{-1}$) while the $R_p$ decreased 4.25-fold. SEM imaging confirmed this, with less obvious fouling of the membrane after the combined process. This study illustrates that the combination of bacterial predation and the subsequent removal of debris using coagulation or adsorption mitigates membrane biofouling and improves membrane performance.

\textbf{Keywords:} \textit{Bdellovibrio bacteriovorus}; membrane filtration; irreversible fouling; alum; powdered activated carbon

Introduction

Membrane biofouling is one of the most important issues limiting membrane applications in water treatment and separation processes. The biofouling usually occurs through the deposition of bacteria and other microorganisms onto the membrane surface and their subsequent growth and excretion of sticky extracellular polymeric substances (EPS), both of which contribute to the formation of biofouling cake layers. These cake layers cause significant decreases in membrane performance (Le-Clech et al. 2006), increase the operating costs (Magara & Itoh 1991) and abbreviate the life span of the membranes (Chang et al. 2002). To date, many research groups have carried out a range of investigations to alleviate biofouling issues, including modifying the membrane surface using antimicrobial agents (Cloete & Jacobs 2001) and enhancing the quality of the feed solutions through ozone and chlorine treatments (Rajagopal et al. 2003; Kim et al. 2008). Although these treatments using chemicals can improve the filtration performance, they are not environment-friendly solutions as they lead to the formation of deleterious disinfection byproducts, such as trihalomethanes and aldehydes (Huang et al. 2005; Cordier 2013). In a previous study, it was demonstrated that bacterial predation by \textit{Bdellovibrio bacteriovorus} HD100 mitigates membrane biofouling and the subsequent decrease in flux performance (Kim et al. 2013). The obligate predator \textit{B. bacteriovorus} predates Gram-negative bacteria, such as \textit{Escherichia coli}, by penetrating into the prey periplasm (Dwidar & Monnappa, et al. 2012) and rounding up the infected bacterium to form a structure called a bdelloplast. Inside this bdelloplast, the predator secretes hydrolytic enzymes to degrade the cytoplasmic contents and the inner membrane of its host bacterium into small molecules which can then be utilized by the predator to grow and septate (Capeness et al. 2013; Monnappa et al. 2013). At the end of this predation cycle (which takes about 3–4 h on average; Sockett & Lambert 2004), the predator progeny finally lyse the host cell and start a new cycle (Dwidar & Hong et al. 2012).

As noted above, predation of an \textit{E. coli} rich solution by \textit{B. bacteriovorus} enhanced the membrane performance and led to a much lower resistance during microfiltration (MF) (Kim et al. 2013). This was true when the predator was initially added to the \textit{E. coli} culture at both a high and low multiplicity of infection (MOI) of 200 and 2, respectively. However, the addition of the predator at the low MOI caused an increase in the irreversible resistance of the membranes during the initial filtration stages. This enhanced irreversible resistance at a low MOI was attributed to the lysis of the \textit{E. coli} prey cells by the predator, which resulted in the formation of bacterial debris that is smaller and more compact than the \textit{E. coli} cells themselves and, hence, can enter the membrane pores and cause permanent blockage.

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Some studies focusing on alternative strategies for mitigating membrane biofouling found that powdered activated carbon (PAC) can effectively reduce foulant attachment on surfaces and pores of the membrane, thereby enhancing the permeability of the membrane (Li et al. 2005; Williams & Pirbazari 2007). In addition, several previous studies showed that coagulation using aluminum sulfate (alum) can also delay membrane fouling and the irreversible blockage of the membrane pores (Holbrook et al. 2004; Nguyen et al. 2010). Consequently, the materials produced during predation that lead to irreversible fouling might be reduced by these coagulation or adsorption processes.

The objective of this study, therefore, was to evaluate the combined processes, ie inactivation of biofouling-causing microorganisms by predation and the subsequent removal of the debris produced during this process, to control membrane biofouling. Both predated and unpredated E. coli cultures were tested for their susceptibility for coagulation using alum and adsorption using PAC. The supernatant after each treatment was then taken and filtered through MF membranes to test their effects on membrane performance. The effect of the concentration of either alum or PAC, combined with bacterial predation, was evaluated on the prevention of membrane biofouling.

Materials and methods

Microorganisms and culturing conditions

The strains used in the current study were Escherichia coli DH5α and Bdellovibrio bacteriovorus HD100. Storing of these two strains and culturing procedures were described previously (Kim et al. 2013). Diluted nutrient broth (DNB) (1:10 dilution of nutrient broth, Difco, Franklin Lakes, NJ, USA) was used as the experimental culture medium. The broth was prepared in 5 l flasks; each containing 1.5 l of medium. After autoclaving, 2 mM CaCl_2 and 3 mM MgCl_2 were added to the broth. A total of six flasks was prepared for each test, three for the control cultures and three more for the predatory cultures. E. coli DH5α (from an overnight culture) was then inoculated in each flask at a concentration of 1 × 10^5 colony-forming units (CFU) ml⁻¹. Additionally, for the predatory cultures only, B. bacteriovorus was added at a concentration of 2 × 10^5 plaque-forming units (PFU) ml⁻¹. All the flasks were then incubated for 48 h at 25°C with continuous stirring at 350 rpm.

Treatment of the samples with alum or powdered activated carbon

For coagulation, aluminum sulfate hydrate (alum) (Al_2(SO_4)₃·18H₂O; Sigma-Aldrich, St Louis, MO, USA) was used as coagulant. Coagulation experiments were carried out in glass beakers using 400 ml of cultures. Alum was used at concentrations of 0, 10 and 100 ppm. The pH of both the predated and unpredated cultures was ~ 7. The samples were magnetically agitated using a stirrer before the coagulant was added. Stirring was continued for 1 min at 200 rpm, and then it was slowed down to 30 rpm for 15 min. The produced flocs were permitted to settle for 30 min, and the supernatants were then collected for measuring their OD600 and viable cell counts. In addition, the supernatants were tested for flux performance.

For experiments with powdered activated carbon (PAC), 100 particle mesh size (Sigma-Aldrich) was used. Similarly, three dosages of the PAC (0, 10 and 100 ppm) were tested. The microbial samples were mixed with the PAC and stirred at 100 rpm for 30 min. The treated samples were then filtered using a paper filter (Grade 5A, ADVANTEC, Tokyo, Japan) to remove the remaining PAC particles. Afterwards, the filtered samples were analyzed for optical density (OD600), viable cell counts, and flux performance.

Analytical methods and microscope analysis

For counting live E. coli and B. bacteriovorus cells in culture solutions and supernatants, samples were diluted and serially plated on agar plates. For counting E. coli cells, Lauria-Bertani (LB, Difco) agar plates were used. For B. bacteriovorus cells, DNB top agar plates were prepared using E. coli as a prey. The OD600 of the samples was measured using a S-3100 UV/vis spectrophotometer (Scinco Co., Seoul, Korea). The zeta potential was measured using Zeta-Sizer Nano ZS (Malvern Instruments Ltd, Malvern, UK). Optical microscope imaging for the unpredated and predated cultures was done using an inverted microscope (IX81, Olympus, Center Valley, PA, USA) equipped with an Andor iXon EM² camera and operated by Xcellence software. In addition, scanning electron microscopy (SEM) of the membranes after filtration was done to analyze the effect of each solution/supernatant on membrane morphology. The SEM analysis was done as described previously (Kim et al. 2013).

Microfiltration experiments

Cellulose mixed ester (CM) membranes having an average pore size of 0.45 μm were used in this study (Macherey-Nagel, Bethlehem, PA, USA). The effective area of these membranes for filtration was 41.8 cm². The membranes were soaked in deionized (DI) water for 24 h before performing the filtration tests. The MF experiments were conducted in Amicon cells having a capacity of 350 ml. The solutions inside the cells were pressurized at 50 kPa (0.5 bar) using laboratory N₂ gas and stirred at 100 rpm. The filtrate was collected in a plastic beaker placed on an electronic mass balance. The load
of the filtrate was determined at time intervals of 5 s and 1 min in order to investigate the initial flux behavior during the microfiltration experiments. The detailed flow of the MF dead-end system was described in a previous study (Kim et al. 2013).

Membrane resistance

To assess the membrane performance, a resistance-in-series model was utilized for determining the intrinsic membrane resistance, cake layer resistance, and pore blocking resistance.

\[
J = \frac{\Delta M/\rho}{(\Delta \cdot t)}
\]

where \( J \) is the permeate flux (Lm\(^{-2}\) h\(^{-1}\)), \( \Delta M \) is the mass of the filtrate (g) accumulated at a specific \( t \) (h\(^{-1}\)), \( \rho \) is the filtrate density (g l\(^{-1}\)) and \( A \) is the effective area of the membrane for filtration (m\(^2\)).

\[
J = \frac{\Delta P}{(\mu \cdot R_t)}
\]

where \( R_t \) is the resistance to filtration (m\(^{-1}\)), \( \Delta P \) is the used pressure (Pa) and \( \mu \) is the viscosity of the solution (Pa s).

The total resistance of the membrane \( (R_t) \) describes the sum of particular resistances:

\[
R_t = R_m + R_c + R_p
\]

where \( R_m \) is the intrinsic membrane resistance, \( R_c \) represents the resistance of the cake layer which is hydraulically reversible resistance \( (R_{rev}) \), and \( R_p \) is the membrane resistance because of the pore blocking and is considered as irreversible resistance \( (R_{ir}) \).

The \( R_m \) was measured from Equation 2 by evaluating the flux of pure water through a clean virgin membrane. \( R_t \) was determined from pure water flux after filtration of 200 ml of the microbial samples. To determine the \( R_p \) values, the Amicon cells were evacuated, and the fouled membranes were placed in glass bottles containing 150 ml of pure water. Subsequently, the fouled membranes were vigorously rinsed with pure water through shaking at 200 rpm for 15 min. The value \( (R_m + R_p) \) was evaluated from the pure water flux of the vigorously washed membranes, and the value of \( R_p \) was then calculated from the discrepancy between the previously determined \( R_m \) and \( (R_m + R_p) \) values.

Results and discussion

Alum coagulation is more effective than PAC adsorption for removing E. coli

In a previous study it was found that predation of \( E. \) coli by the predator \( B. \) bacteriovorus at a predator-to-prey ratio of two led to a marked decrease in the optical density (OD\(_{600}\)) of the solution and more than a 3-log reduction in \( E. \) coli numbers over 48 h (Kim et al. 2013). Similar results were obtained again here when \( B. \) bacteriovorus was added, as the number of viable \( E. \) coli cells decreased from an average of \( 1.16 \pm 0.03 \times 10^8 \) CFU ml\(^{-1}\) in the control cultures to only \( 8.3 \pm 2.5 \times 10^4 \) CFU ml\(^{-1}\) (Figure 1), a decrease of 1,390-fold. Examining these two solutions (unpredated and predated) microscopically further confirmed this as intact \( E. \) coli cells were abundant in the unpredated cultures but were rarely detected in the predated samples (Figure S1). [Supplementary information is available via a multimedia link on the online article webpage.]

When portions of unpredated and predated cultures were treated with alum (10 or 100 ppm), both the OD\(_{600}\) and the final \( E. \) coli concentrations decreased in a dose-dependent manner, with greater alum concentrations leading to better removals (Figure 1). This was expected since alum is known to coagulate suspended particles, which in this case would include both bacterial cells and large debris such as the bacterial ghosts that are formed through predation (Barel et al. 2005). Images of the flocs resulting from each treatment are provided in Figure S2. With 0 and 10 ppm alum addition, precipitation was not obvious. This suggests that 10 ppm alum was not a suitable dosage to treat these samples or to remove the bacteria and debris as the aggregates formed at this concentration were primarily active flocs suspended in the solution. In contrast, when 100 ppm of alum was used, a significantly higher degree of precipitation was seen, especially with the predated samples. This is in agreement with the results shown in Figure 1, where the 10 ppm alum treated samples showed only a slight reduction in both their OD\(_{600}\) and \( E. \) coli numbers after treatment with 100 ppm alum, while the viable \( E. \) coli numbers were 37-fold and five-fold lower in the unpredated and the predated samples, respectively. Within the predated sample, the \( B. \) bacteriovorus population was also clearly impacted by alum coagulation, as the number of predatory bacterial cells in the sample decreased 21-fold when 100 ppm was used. It should be noted that while the effect of alum on the viable \( E. \) coli concentration in the medium was significant (a 37-fold reduction), it was only modest when compared to that incurred by bacterial predation (1,390-fold).

Parallel tests were also performed using PAC at the same test concentrations. PAC was tested since it is known to adsorb organic compounds and particulate debris that may contribute to fouling of the water filtration membrane (Wickramasinghe & Grzenia 2008). The addition of 10 ppm PAC led to mild decreases in the OD\(_{600}\), as was seen with alum, but the \( E. \) coli numbers were generally unaffected and remained similar to the 0 ppm samples. Increasing the PAC addition to 100 ppm caused the OD\(_{600}\) of the unpredated sample to drop by just over 50% but had virtually no effect on the sample that was predated. The viable \( E. \) coli count was reduced by just half in...
both of these samples (unpredated and predated) by the PAC 100 ppm treatment. Although the viable *E. coli* numbers in the PAC-treated predated samples were lower compared with their alum counterparts, these differences can be attributed to variations in the predator activity that are inherent between independent preparations. The similar trend between the two PAC-treated samples, however, suggests that differences in the viable *E. coli* numbers have little or no impact on the PAC activity.

**Combining predation with coagulation or adsorption improves the membrane flux**

After removing the precipitated flocs, the supernatants from each of these samples were used to determine their effects on the initial flux of the membrane (Table 1). For the flux analyses, a microfiltration membrane with a pore size of 0.45 μm was used. As mentioned previously (Kim et al. 2013), this pore size is large enough to allow both the DNB medium components and the majority of predator cells to pass through while retaining the cells of the larger prey *E. coli*.

Table 1. Original and normalized initial flux for both unpredated and predated cultures after coagulation with different concentrations of alum or PAC.

| Concentration | Unpredated LMH* | Unpredated % | Predated LMH* | Predated % |
|---------------|-----------------|--------------|---------------|------------|
| 0 ppm         | 1,225           | 29           | 1,712         | 40         |
| 10 ppm alum   | 1,393           | 33           | 1,680         | 39         |
| 100 ppm alum  | 2,648           | 62           | 3,053         | 71         |
| 10 ppm PAC    | 1,406           | 33           | 1,768         | 41         |
| 100 ppm PAC   | 1,495           | 35           | 2,352         | 55         |

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

As in the previous study, the initial flux was enhanced when *E. coli* was predated upon by *B. bacteriovorus* (Kim et al. 2013). Likewise, coagulation by alum is an established technique that is known to enhance the flux of waste water through filtration membranes (Rossi et al. 2004). As presented above, the addition of 100 ppm alum
led to a substantial loss in the OD_{600} of the solution and viable E. coli cell numbers. Consequently, a combination of these two treatments was evaluated to investigate the effect on the flux results (Table 1). As with the OD_{600} and cell counts, the use of 10 ppm alum was fairly ineffective and the resulting fluxes were basically indistinguishable from those of the untreated samples, regardless of whether predation was performed or not. When 100 ppm alum was used, however, the fluxes increased substantially, confirming the results in Figures 1 and S2, which suggests that fewer bacteria and less cell debris would be present in these samples to cause fouling. In all the tests, the combined application of predation and alum led to the highest fluxes observed, indicating that these two treatments can be used in combination to improve membrane function and lifetime.

This observation is further illustrated in Table 2, which shows the total resistance within the membrane based upon the resistance-in-series model. Treatment of the unpredated samples with alum led to a dose-dependent drop in the total resistance, while this was improved if the sample was predated upon. Consequently, the enhanced flux brought on by combining the predation and alum coagulation is clearly associated with a reduced membrane resistance and, hence, less biofouling. This can be attributed to the fact that predation caused lysis of the E. coli cells, which resulted in a smaller number of cells being deposited on the membrane. Furthermore, alum coagulation removed a significant proportion of the remaining live E. coli cells together with E. coli debris which remained after predation.

A similar trend was seen with PAC, where greater additions led to lower total resistances for both the unpredated and predated samples (Table 3). Interestingly, the use of 100 ppm PAC in the unpredated samples reduced the resistance by just over half, a result that correlates well with the near identical reductions seen in both the OD_{600} and viable E. coli numbers (Figure 1). As with alum, the effects of PAC were enhanced by predation. This effect was consistent as the total resistance in the sample undergoing PAC treatment was further reduced by \(-7 \times 10^{11}\) m\(^{-1}\) when performed using the predated samples, suggesting that these two effects are probably independent of each other and additive.

### Treatment with alum significantly reduces predation-associated irreversible fouling

Despite finding that predation led to a significant improvement in membrane flux as well as reduced total membrane biofouling, it also resulted in a higher irreversible biofouling of the membrane (R\(_p\)), Table 2. A previous study by Bai and Leow (2002) with activated sludge found that smaller particles caused more biofouling due to their stacking on the membrane. Being smaller and more pliable than intact E. coli cells, the debris resulting from predation can be compacted tightly inside the pores where it causes blockage. On the other hand, studies also reported that coagulation using alum can mitigate membrane fouling (Nguyen et al. 2010). Therefore, a combined application using both alum and predation was evaluated to investigate whether a combined treatment overcomes the irreversible fouling problem.

As shown in Table 2, the irreversible membrane resistance (R\(_p\)) was reduced from 1.36 to only 0.32 (equivalent to a 4.3-fold reduction) in the predated cultures after treatment with 100 ppm alum. It was also observed that treatment of the unpredated samples with alum led to increases in the irreversible resistances (Table 2). This was intriguing since treatment with 100 ppm alum successfully reduced the total resistance of the unpredated cultures by \(-6.6\)-fold. However, this result is similar to that reported previously by Goh et al. (2010) in that alum coagulation increases the irreversible resistance. The increased resistance is attributed to the fine alum particles that remain suspended after precipitation of the flocs and their subsequent clogging of the membrane pores during the filtration steps, leading to the observed R\(_p\) value of 2.19 in this study. When the 100 ppm alum treatment was performed using the predated samples, however, the R\(_p\) was nearly seven-fold lower, i.e. a value of only 0.32. Thus, as alum helped to reduce the R\(_p\) associated with predation, predation also benefits alum treatments in the same way.

### Table 2. Membrane fouling resistances after filtration of unpredated and predated cultures treated with different concentrations of alum.

| Total resistance | 0 ppm          | 10 ppm         | 100 ppm        |
|------------------|----------------|----------------|----------------|
|                  | Unpredated     | Predated       | Unpredated     | Predated       | Unpredated     | Predated       |
| R\(_m\)          | 18.5 ± 0.42    | 11.8 ± 2.77    | 10.5 ± 3.04    | 10.1 ± 1.91    | 2.79 ± 0.31    | 0.79 ± 0.2     |
|                   | 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} \text{m}^{-1}).
These findings clearly demonstrate that alum coagulation can be a promising solution for irreversible fouling associated with bacterial predation. Indeed, previous studies by other groups found that alum coagulation is enhanced upon ultra-sonicating the feed solution before its addition (Hakata et al. 2011), which is not dissimilar to the lysis occurring during predation. The reason for this enhanced activity is that alum coagulation is sensitive to many physical and chemical factors, including the zeta potential of the particles suspended in the medium. Studies done by different groups have found that minimizing the zeta potential of the particles in the solution triggered production of intense flocs (Sharp et al. 2006; Liu & Sun 2010). In addition, a previous study by Klodzinska et al. (2010) showed that dead E. coli cells have lower absolute zeta potential values when compared to live intact cells. As bacterial predation results in killing and lysis of the E. coli cells, it was expected that it may cause a reduction in the zeta potential value. Furthermore, although B. bacteriovorus HD 100 is itself a Gram-negative bacterium, its outer membrane has a different structure from other Gram-negative strains in that the phosphate groups in its outer lipopolysaccharide layer are replaced with mannose groups (Schwudke et al. 2003), leading to a lower negative charge associated with its outer membrane when compared to other Gram-negative bacteria, such as E. coli.

Consequently, to assess the effect of predation on the zeta potential of the culture, this value was measured for both unpredated and predated E. coli cultures. As shown in Figure 2, predation of the E. coli culture by B. bacteriovorus HD 100 shifted the zeta potential from an average of −35 mV in the unpredated solution to −23 mV for the predated solution. This zeta potential change helps to explain the better coagulation by alum, particularly at 100 ppm as evidenced by the large amount of precipitate formed (Figure S2).

**PAC treatment only mildly improves predation-associated irreversible fouling**

In contrast to alum, a combination of PAC treatment and predation produced only additive effects on the membrane resistance (Table 3). The total membrane resistance after filtration of the samples decreased as the PAC concentration increased, regardless of whether the sample was predated. As mentioned above, this decrease was consistent and additive to the impact seen with predation alone. Regarding the Rp values, they were basically consistent in both the unpredated samples and in the predated samples treated with 0 or 10 ppm PAC. Treatment of the predated samples with 100 ppm PAC led to an ~60% drop in the irreversible fouling (Table 3); a value that closely resembles the loss in the OD600 and E. coli numbers (Figure 1).

It is also notable that when a low concentration of alum was used (10 ppm), the membrane performance was poor for both the unpredated and predated cultures in that the Rp for both increased when compared with tests having no alum treatment (Table 2). In contrast, treatments using PAC did not increase the Rp in either culture. This could be because of the different mechanisms employed by alum flocculation and PAC adsorption.

**SEM analysis**

The results in Tables 2 and 3 were further confirmed through SEM imaging of the membrane surfaces. The
initial water flux of the predated cultures was higher than that of the unpredated cultures, as shown in Table 1. However, the SEM images obtained after thorough washing of both membranes show that predation led to a greater clogging and irreversible fouling of the membrane (Figure 3). Treatment with 10 ppm alum exacerbated the clogging, which is in agreement with Table 2 where $R_p$ increased by about four-fold in the predated samples. A similar result was seen with the unpredated E. coli sample, suggesting that alum is the major contributor to membrane fouling. The use of 100 ppm alum, however, reversed this effect and led to clear pore structures in the predated samples, which again agrees well with the results reported in Table 2.

In the tests with unpredated E. coli, the addition of PAC led to a slight increase in the $R_p$ values (Table 3). The images in Figure 3 for these samples are in agreement with this as the membrane fibers are still plainly visible but slightly coated, leading to smaller pores. With the predated samples, however, this was not the case. The images show the addition of 10 ppm PAC clearly reduces membrane fouling, with the pores and membrane fibers easily apparent. This was intriguing as the results in Table 3 show the $R_p$ for 0 and 10 ppm PAC were basically identical. These findings strongly imply that the association of the bacterial debris at the membrane surface was not responsible for irreversible fouling, but rather components that were deeply embedded within the membrane. This is further evidenced by the images obtained after 10 and 100 ppm PAC treatment. Although no visually definitive difference is apparent between these four samples (unpredated and predated samples at 10 and 100 ppm PAC), the $R_p$ values were always much higher if predation was performed.

Overall, as presented in this study, predation offers a benefit by substantially reducing the total resistance. Furthermore, several previous studies have shown that predation by B. bacteriovorus can reduce the population of many Gram-negative bacteria (Sockett & Lambert 2004; Dashiff et al. 2011; Dashiff & Kadouri 2011; Dwidar et al. 2013) which otherwise can secrete EPS and form biofilm layers on the membrane, as well as being effective in eradicating the biofilms established by these microorganisms (Kadouri & O’Toole 2005; Dwidar et al. 2012, 2014). In contrast, being an obligate predator, the wild-type B. bacteriovorus cannot grow outside its prey under normal conditions and consequently cannot form biofilms like those formed by other bacteria. In addition, although B. bacteriovorus is unable to predate Gram-positive bacteria, the hydrolytic enzymes secreted...
by this predator were found to be effective in preventing and mitigating the biofilms made by Gram-positive bacteria such as *Staphylococcus aureus* and *Staphylococcus epidermidis* (Monnappa et al. 2014). Also, as these Gram-positive bacteria can be predated upon by other predators in nature such as bacteriophages (Brnakova et al. 2005) and being lysed by the bacteriophage lysins (Gilmer et al. 2013), a possible combined application of *B. bacteriovorus* with other predators such as bacteriophages to effectively kill and lyse the majority of the microorganisms responsible for the fouling process can be envisaged. However, future work is needed to demonstrate this.

Based on this study and the previous study by Kim et al. (2013), the resulting increase in irreversible fouling due to lysis of the prey was the main drawback associated with the predation. This can, however, be overcome by the subsequent treatment of the predated samples with 100 ppm alum or to a lesser extent PAC as shown here (Tables 2 and 3, and Figure 3). The exact optimum dose of the coagulant (alum) or the adsorbent (PAC), however, may vary for each water treatment plant depending on the particular water chemistry and the types of particulates, and consequently, has to be determined by the jar test. The jar test, used widely in water treatment plants, consists of several beakers containing raw water into which various amounts of the dose are administered. After stirring the solutions, the nature and settling characteristics of the flocs are observed and the optimum amounts are determined (Sincero & Sincero 2003).

**Conclusions**

Predation of bacteria within the feed solution increased the irreversible fouling of MF membranes. This study, however, shows that, under the conditions described, the combined use of predation and coagulation with 100 ppm alum overcame this drawback and led to better results, ie lower Rp values with MF membranes than when either was performed independently. An additive rather than synergistic effect, however, was seen with PAC as the Rp values for the predated samples were always higher than their unpredated counterparts with the same PAC addition. This was true although a combined predation/PAC process improved the flux compared to predation alone.

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**References**

Bai RB, Leow HF. 2002. Microfiltration of activated sludge wastewater – the effect of system operation parameters. Sep Purif Technol. 29:189–198.

Barel G, Sirota A, Volpin H, Jurkevitch E. 2005. Fate of predator and prey proteins during growth of *Bdellovibrio bacteriovorus* on *Escherichia coli* and *Pseudomonas syringae* prey. J Bacteriol. 187:329–335.

Brnakova Z, Farkasovsky J, Godany A. 2005. The use of bacteriophages in eliminating polyresistant strains of *Staphylococcus aureus* and *Streptococcus agalactiae*. Folia Microbiol. 50:187–194.

Capeness MJ, Lambert C, Lovering AL, Till R, Uchida K, Chaudhuri R, Alderwick LJ, Lee DJ, Swarbreck D, Liddell S, et al. 2013. Activity of *Bdellovibrio* hit locus proteins, Bd0108 and Bd0109, links type IVa pilus extrusion/retraction status to prey-independent growth signalling. PloS one. 8:e79759.

Chang IS, Le Clech P, Jefferson B, Judd S. 2002. Membrane fouling in membrane bioreactors for wastewater treatment. J Environ Eng-ASCE. 128:1018–1029.

Cloete TE, Jacobs L. 2001. Surfactants and the attachment of *Pseudomonas aeruginosa* to 3CR12 stainless steel and glass. Water SA. 27:21–26.

Cordier S. 2013. Disinfection by-products in drinking water and reproductive health. Occup Environ Med. 70:753.

Dashiff A, Junka RA, Libera M, Kadouri DE. 2011. Predation of human pathogens by the predatory bacteria *Micavibrio aeruginosavorus* and *Bdellovibrio bacteriovorus*. J Appl Microbiol. 110:431–444.

Dashiff A, Kadouri DE. 2011. Predation of oral pathogens by *Bdellovibrio bacteriovorus* 109J. Mol Oral Microbiol. 26:19–34.

Dwidar M, Hong S, Cha M, Jang J, Mitchell RJ. 2012. Combined application of bacterial predation and carbon dioxide aerosols to effectively remove biofilms. Biofouling. 28:671–680.

Dwidar M, Leung BM, Yaguchi T, Takayama S, Mitchell RJ. 2013. Patterning bacterial communities on epithelial cells. PloS one. 13:e67165.

Dwidar M, Monnappa AK, Mitchell RJ. 2012. The dual pro- biotic and antibiotic nature of *Bdellovibrio bacteriovorus*. Bmb Rep. 29:71–78.

Dwidar M, Nam D, Mitchell RJ. 2014. Indole negatively impacts predation by *Bdellovibrio bacteriovorus* and its release from the bdelloplast. Environ Microbiol. Mar 27. Epub 2014/03/29. doi:10.1111/1462-2920.12463.

Gilmer DB, Schmitz JE, Euler CW, Fischetti VA. 2013. Novel bacteriophage lysin with broad lytic activity protects against mixed infection by *Streptococcus pyogenes* and methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Ch. 57:2743–2750.

Goh YT, Harris JL, Roddick FA. 2010. Reducing the effect of cyanobacteria in the microfiltration of secondary effluent. Water Sci Technol. 62:1682–1688.

Hakata Y, Roddick F, Fan LH. 2011. Impact of ultrasonic pre-treatment on the microfiltration of a biologically treated municipal effluent. Desalination. 283:75–79.

Holbrook RD, Higgins MJ, Murthy SN, Fonseca AD, Fleischer EJ, Daigger GT, Grizzard TJ, Love NG, Novak JT. 2004. Effect of alum addition on the performance of submerged membranes for wastewater treatment. Water Environ Res. 76:2699–2702.

Huang WJ, Fang GC, Wang CC. 2005. The determination and fate of disinfection by-products from ozonation of polluted raw water. Sci Total Environ. 345:261–272.
Kadouri D, O'Toole GA. 2005. Susceptibility of biofilms to *Bdellovibrio bacteriovorus* attack. Appl Environ Microbiol. 71:4044–4051.

Kim EH, Dwidar M, Mitchell RJ, Kwon YN. 2013. Assessing the effects of bacterial predation on membrane biofouling. Water Res. 47:6024–6032.

Kim J, Davies SHR, Baumann MJ, Tarabara VV, Masten SJ. 2008. Effect of ozone dosage and hydrodynamic conditions on the permeate flux in a hybrid ozonation-ceramic ultrafiltration system treating natural waters. J Membr Sci. 311:165–172.

Klodzinska E, Szumski M, Dziubakiewicz E, Hrynkieiwicz K, Skwarek E, Janusz W, Buszewski B. 2010. Effect of zeta potential value on bacterial behavior during electrophoretic separation. Electrophoresis. 31:1590–1596.

Le-Clech P, Chen V, Fane TAG. 2006. Fouling in membrane bioreactors used in wastewater treatment. J Membr Sci. 284:17–53.

Li YZ, He YL, Liu YH, Yang SC, Zhang GJ. 2005. Comparison of the filtration characteristics between biological powdered activated carbon sludge and activated sludge in submerged membrane bioreactors. Desalination. 174:305–314.

Liu YJ, Sun DD. 2010. Comparison of membrane fouling in dead-end microfiltration of denitrifying granular sludge suspension and its supernatant. J Membr Sci. 352:100–106.

Magara Y, Itoh M. 1991. The effect of operational factors on solid liquid separation by ultra-membrane filtration in a biological denitrification system for collected human excreta treatment plants. Water Sci Technol. 23:1583–1590.

Monnappa AK, Dwidar M, Mitchell RJ. 2013. Application of bacterial predation to mitigate recombinant bacterial populations and their DNA. Soil Biol Biochem. 57:427–435.

Monnappa AK, Dwidar M, Seo JK, Hur JH, Mitchell RJ. 2014. *Bdellovibrio bacteriovorus* inhibits *Staphylococcus aureus* biofilm formation and invasion into human epithelial cells. Sci Rep-UK. 4:3811. doi:10.1038/srep03811.

Nguyen ST, Roddick FA, Harris JL. 2010. Membrane foulants and fouling mechanisms in microfiltration and ultrafiltration of an activated sludge effluent. Water Sci Technol. 62:1975–1983.

Rajagopal S, Van der Velde G, Van der Gaag M, Jenner HA. 2003. How effective is intermittent chlorination to control adult mussel fouling in cooling water systems? Water Res. 37:329–338.

Rossi N, Jaouen P, Legentilhomme P, Petit I. 2004. Harvesting of cyanobacterium *Arthrospira platensis* using organic filtration membranes. Food Bioprod Process. 82:244–250.

Schwudke D, Linscheid M, Strauch E, Appel B, Zahringer U, Moll H, Muller M, Brecker L, Gronow S, Lindner B. 2003. The obligate predatory *Bdellovibrio bacteriovorus* possesses a neutral lipid A containing alpha-D-mannoses that replace phosphate residues: similarities and differences between the lipid A and the lipopolysaccharides of the wild type strain *B. bacteriovorus* HD100 and its host-independent derivative HI100. J Biol Chem. 278:27502–27512.

Sharp EL, Jarvis P, Parsons SA, Jefferson B. 2006. The impact of zeta potential on the physical properties of ferric-NOM flocs. Environ Sci Technol. 40:3934–3940.

Sincero AP, Sincero GA. 2003. Physical–chemical treatment of water and wastewater. Boca Raton, FL: CRC Press.

Socquet RE, Lambert C. 2004. *Bdellovibrio* as therapeutic agents: a predatory renaissance? Nat Rev Microbiol. 2:669–675.

Wickramasinghe SR, Grzenia DL. 2008. Adsorptive membranes and resins for acetic acid removal from biomass hydrolysates. Desalination. 234:144–151.

Williams MD, Pirbazari M. 2007. Membrane bioreactor process for removing biodegradable organic matter from water. Water Res. 41:3880–3893.