Control and cleaning of membrane biofouling by biogenic silver nanoparticles

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Abstract. Membrane biofouling as the main obstacle strongly hinders the wider application of membrane technology. The control of biofouling development and the fouled membrane cleaning still are the big challenge in membrane industry. The present study investigated the control of initial bacterial attachment, biofilm development and finally the membrane cleaning by biogenic silver nanoparticles (Bio-Ag⁰-⁶) using commercial microfiltration membranes as the substrate. The results revealed that the Bio-Ag⁰-⁶ could inhibit the development of biofilm by decreasing the attachment of cells and the production of extracellular polymeric substances (EPS). Significant improvements of flux recovery ratio (FRR), the distribution of fouling resistances and the decrease of total fouling resistance (R_f) were achieved when introducing Bio-Ag⁰-⁶ to dead-end microfiltration systems. The membrane cleaning experiment verified the enhanced biofilm detachment from membrane surface by Bio-Ag⁰-⁶. It shows that Bio-Ag⁰-⁶ would be a good alternative to relieve membrane biofouling.

1. Introduction
Membrane biofouling is a major challenging issue of membrane application in water industry[1,2]. Biofouling, which is considered more problematic than organic fouling forms through the initial deposition of bacterial cells on the substrate, accumulation of organic macromolecules in EPS, colony development and finally maturation of biofilm [3]. Biofouling is difficult to eliminate because microbes could fully utilize the nutrient in the feed to multiply rapidly. As the products of microbial aggregates, EPS could bridge cells together [4]. EPS played an important part in biofilm formation, and was considered to be the main reason of membrane biofouling [5]. Besides, it is considered that the initial irreversible attachment, the activity and proliferation of microorganisms all have great influence on the process of biofouling [6,7]. Biofouling results in the flux decline and increased operation and maintenance costs [8].

Common treatments are used to prevent the development of biofouling. Disinfectants such as ozone and chlorine or UV irradiation as pretreatment, reducing nutrients in the permeate or utilizing antifouling membrane materials to control the formation of biofilm. However, the continuous application of oxidative disinfectants may form disinfection byproducts and are detrimental to membrane[9].

Besides promoted biofilm detachment is another method to alleviate membrane fouling[10]. Many physical-chemical ways such as sonication, back-washing and chemical cleaning have been used to remove biofilm. However, microorganisms may develop resistance to the traditional processes and the mature biofilm is often hard to remove totally [11,12]. Moreover, conventional approaches to deal...
with membrane biofouling may be costly and time-consuming. Hence, the research of effective new methods to relieve biofouling has attracted tremendous interest. Silver nanoparticles (AgNPs) as a broad spectrum of anti-microbial agent have been widely used in healthcare products, water purification and membrane filtration systems. Silver nanoparticles have been incorporated into nanofiltration (NF), reverse osmosis (RO) and ultrafiltration (UF) membranes [13-16] and other materials. The fabrication of silver nanocomposite membranes significantly improved the antifouling and filtration performances.

The chemical reduction methods are the most commonly used method to synthesize silver nanoparticles. However, the chemical silver nanoparticles often have poor particle stability and tend to aggregated easily [17]. Recently, biosynthesis of silver nanoparticles has attracted much attention. As reported before, we fabricated small sized (≈6 nm) novel biogenic silver nanoparticles (Bio-Ag6). The results demonstrated that the small sized and highly dispersed Bio-Ag6 were efficient to control bacterial and biofilm growth[18].

In this study, the small sized and highly dispersed Bio-Ag6 nanoparticles were firstly introduced into the commercial microfiltration membrane system which was quite different from our previous reports. This study further survey the effect of Bio-Ag6 on the microbial attachment and the biofilm formation on commercial membrane surfaces. Changes of the filtration flux and the distribution of membrane fouling resistances were both evaluated by dead-end filtration test. Furthermore, we studied the influences of biogenic silver on the detachment of established biofilms from membrane surfaces and compared the membrane cleaning performance with traditional chemical agents. Thus, it is expected that the small sized Bio-Ag6 will be an efficient alternative to alleviate biofouling in commercial membrane systems.

2. Materials and methods

2.1. Model bacterial strain and media
Brevundimonas diminuta (BD) was grown in LB medium at 37℃ and180rpm. The strain was chosen as the model bacterial because it is often found in biofilms and has been widely used to assess biofouling in membrane systems[19-21].

2.2. Fabrication and characterization of Bio-Ag6
The detailed fabrication and characterization of Bio-Ag6 can be found in our previous study [21].

2.3. Bacteria attachment assay
To investigate the influence of Bio-Ag6 in controlling biofilm formation, the bacteria attachment was conducted in microtiter plates (Nunc, Demark) [22,23]. Mixed cellulose ester (MEC) membranes ( 0.1μm, VCWP02500 ; Millipore, USA ) were the substrates for biofilm growth. BD was grown overnight and then diluted to get the working solution. The final silver concentrations were 0, 5, 10, 15mg/L. Finally the plate was cultivated at 37℃ for 72 h.

The membranes were fully immersed into phosphate buffer saline (PBS) solution to remove loosely adherent cells and then sonicated for 5min. The number of attached living bacteria on membrane samples was confirmed by gradient dilution and spread plate methods.

2.4. The analysis of EPS
EPS is mainly composed of proteins (PN) and polysaccharides (PS). After the 72-h-aged biofilms on MEC membranes were performed as described in the section headed ‘Biofouling formation assay’ above, EPS was extracted using methods similar to those reported before [24-25]. PN were assayed by BCA Protein Assay Kits (23227; Thermo, USA). PS were analyzed by anthrone-sulfuric acid method. BSA and glucose were used as the protein and polysaccharide standards.
2.5. SEM characterization
The morphologies of biofilms distributed on MEC membranes under treatment of Bio-Ag\textsuperscript{0}-6 were observed by scanning electron microscopy (SEM). The 72-h-aged biofilms were washed with PBS and treated with glutaraldehyde solution (2.5%) at 4 °C for 6 h. Before SEM observation, the samples were steply dehydrated and dried in an oven at 60 °C over 24 h [26].

2.6. Staining and CLSM imaging
The influence of Bio-Ag\textsuperscript{0}-6 pretreatment on the viability of attached cells and the PS distributions in biofouling layers were characterized by CLSM (LSM710, Germany). Propidium iodide (PI) and nucleic acid stains SYTO 9 (Ex=485nm, Em= 630/530 nm) was used to stained the biofilm [27]. PS was binded with Alexa Fluor conjugated Con A (Ex=590 nm, Em=617 nm) [28]. The membranes were stained in the dark for 30 min. Finally the membranes were washed with PBS and observed by CLSM.

2.7. Dead-end microfiltration test
The dead-end microfiltration test was conducted using filtration cell (Amicon 8010; Millipore) at 0.05MPa. The flux \( J \) was obtained with Equation (1):

\[
J = \frac{V}{A \Delta t}
\]

(1)

Where \( V \) (L) is the volume of permeated water, \( A \) (m\(^2\)) is the effective area and \( \Delta t \) (h) is the filtration time.

Then the biofouling experiment was performed. Following the 72 h biofouling, membrane sample was cultivated on LB agar to facilitate bacteria reproduction. The water flux (J\textsubscript{p}) was measured again. In order to compare the antifouling performance of the membrane, the relative flux reduction (RFR) was calculated as [28,29]:

\[
RFR = \left( \frac{J_{w1} - J_{p}}{J_{w1}} \right) \times 100\%
\]

(2)

Furthermore, the fouling resistance \( R_f \) and the intrinsic membrane resistance \( R_m \) were determined by the resistance-in-series model [21,29]:

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

(3)

where \( J \) is the water flux (m\(^3\) m\(^{-2}\) s\(^{-1}\)); \( \mu \) is the solution viscosity (Pa s) and \( \Delta P \) is the filtration pressure (Pa).

\[
R_t = R_m + R_f
\]

(4)

The \( R_m \) can be estimated from Equation (3) by measuring the flux of clean membrane, and \( R_t \) is obtainted from the fouled membrane water flux, thus \( R_f \) can be obtained.

2.8. Membrane cleaning
In order to compare the cleaning effect of different agents, the bacterial suspension filtration experiment was conducted by dead-end filtration. After the initial pure water flux (J\textsubscript{w1}) was obtained, 2 mL bacterial suspension was filtered without stirring to facilitate the deposition and adhesion of cells on membrane. Following filtration of BD suspension, each sample was placed on LB agar to enhance the bacteria multiplying. The water flux (J\textsubscript{p}) after 24 h biofouling was determined again. Finally, the
samples were cleaned with Bio-Ag\textsuperscript{0-6} (30 mg/L), sodium hypochlorite (NaClO, 200 mg/L) and PBS (0.1 M, pH 7.2) by shaking for 30 min, respectively. Then the flux ($J_{w2}$) was tested again. The flux recovery ratio (FRR) can be got using Equation (5):

$$FRR = \left( \frac{J_{w2}}{J_{w1}} \right) \times 100\%$$

(5)

Moreover, the fouling resistance parameters were analyzed to study the fouling process [24]. The irreversible ($R_{ir}$), reversible ($R_r$) and total fouling ratio ($R_t$) were calculated as:

$$R_{ir} = \left( 1 - \frac{J_p}{J_{w1}} \right) \times 100$$

(6)

$$R_r = \left( \frac{J_{w2} - J_p}{J_{w1}} \right) \times 100$$

(7)

$$R_t = \left( \frac{J_{w1} - J_{w2}}{J_{w1}} \right) \times 100$$

(8)

3. Results and discussion

3.1. Effect of Bio-Ag\textsuperscript{0-6} on BD attachment

The cell numbers during 72 h incubation are plotted as a function of different Bio-Ag\textsuperscript{0-6} concentrations in Figure 1. After 72 h of cultivation, the strain BD displayed excellent biofilm formation property. As shown in Figure 1, without Bio-Ag\textsuperscript{0-6} pretreatment, BD showed significant growth after 72 h incubation with the highest log CFU of about 7.0. Bio-Ag\textsuperscript{0-6} of 5 mg/L slightly decreased the number of live bacterial. The more Bio-Ag\textsuperscript{0-6} content increased, the more obvious the decrease effect became. As for 10 mg/L and 15 mg/L of silver, there were over four and five orders of magnitude decrease for BD, respectively. The figure showed that the Bio-Ag\textsuperscript{0-6} significantly affected the multiplying and attachment of BD on MEC membranes, which was probably due to the excellent antibacterial and antifouling effect of Bio-Ag\textsuperscript{0-6} as reported before [30].

![Figure 1](image.png)

**Figure 1.** The cell numbers of BD biofilms on MEC membranes after 72 h of cultivation.

3.2. EPS analysis

EPS is considered as the source of organic fouling and play an important part in membrane fouling process. EPS are composed of polymers produced by bacteria, such as humic substances, proteins and carbohydrates [22]. The concentrations of proteins (PN) and polysaccharides (PS), as the most important components in biofilms are shown in Table 1. As for the control sample (0 mg/L), the concentrations of PS and PN were 431.12µg/cm\textsuperscript{2} and 250.07µg/cm\textsuperscript{2}, respectively. The PS contents were generally higher than PN which was consistent with reports before [22,31]. For all the samples,
the productions of PS and PN were inversely proportional with the Bio-Ag\textsuperscript{0-6} content. As for 15 mg/L of silver, the concentrations of PS and PN dropped dramatically to 57.75µg/cm\textsuperscript{2} and 34.72µg/cm\textsuperscript{2}, respectively. The results indicated that lower concentration of EPS may be an useful method to relieve membrane biofouling [31].

### Table 1. The concentrations of polymers in EPS in 72h aged biofilms.

| Bio-Ag\textsuperscript{0-6} (mg/L) | polysaccharides concentration (µg/cm\textsuperscript{2}) | proteins concentration (µg/cm\textsuperscript{2}) |
|----------------------------------|-------------------------------------------------------|-----------------------------------------------|
| 0                                | 431.12±6.52                                          | 250.07±3.22                                   |
| 5                                | 284.10±4.38                                          | 205.97±2.75                                   |
| 10                               | 201.99±2.96                                          | 162.11±2.28                                   |
| 15                               | 57.75±1.63                                           | 34.72±0.76                                    |

3.3. SEM and CLSM images of biofouling layer

To further monitor the biofilm formation under different concentration of Bio-Ag\textsuperscript{0-6}, the SEM characterization was carried out. As for the control sample in Figure 2, BD displayed strong biofilm formation ability. The membrane surface was fully covered by bacterial clusters and completely fouled. Quite different from the control sample, much fewer cells were observed on the membrane even when the Bio-Ag\textsuperscript{0-6} concentration was only 10 mg/L. Most of the foulants including EPS had disappeared on the membrane surfaces.

![SEM images of fouling layers after 72h growth at different Bio-Ag\textsuperscript{0-6} concentrations.](image)

Figure 2. SEM images of fouling layers after 72h growth at different Bio-Ag\textsuperscript{0-6} concentrations.

Multicolor fluorescence labeling and CLSM observation were used to characterize the composition and structure of biofouling layers. Figure 3 showed the fluorescent labeling graphics of control (without Bio-Ag\textsuperscript{0-6}) and biofouling samples grown in 10 mg/L of Bio-Ag\textsuperscript{0-6} as an example. As shown
in Figure 3a1, numerous green clusters evenly dispersed on the MEC membrane surface, indicating that almost all the bacteria in the control biofilm were alive. As for the treated samples (Figure 3a2), the total coverage of cells decreased dramatically. Very few cells, most of which showed as small red dots were present, indicating that most cells were dead. Furthermore, the concentration and distribution of organic macromolecules such as polysaccharides (PS) in EPS became visible. A large number of polysaccharides clusters were observed (in Figure 3b1) in control biofouling layer, whereas a significant decrease in the production of polysaccharides (in Figure 3b2) was found on the membrane samples cultivated in 10 mg/L of Bio-Ag⁰-6. This is in agreement with the SEM results in Figure 2. This is also consistent with the above EPS analysis in Table 1. EPS which is defined as a bio-glue to maintain the 3D structure of a biofilm is the main contributors to biofouling [28]. All the findings above implied that the pretreatment of Bio-Ag⁰-6 could remarkably reduce the initial attachment of biofilm on the membrane surfaces and the production of EPS, thus may have a positive impact on the membrane biofouling control.

![Figure 3. CLSM images for 72-h old biofouling layers of control (a1-b1) and samples cultivated in 10 mg/L of Bio-Ag⁰-6 (a2-b2). (a) Adherent biomass (live cells in green; dead cells in red); (b) PS (polysaccharides in magenta).](image)

3.4. Membrane flux

The membrane biofouling tests were conducted using dead-end filtration cell to compare the flux differences that caused by the application of Bio-Ag⁰-6. As shown in Figure 4, the original flux (J_{w1}) of all samples were over 1000 L m⁻² h⁻¹. After the bacteria attachment and 24 h cultivation, all the flux (J_p) decreased. The flux of MEC membrane without Bio-Ag⁰-6 addition declined almost 73% compared with its original pure water flux. With the concentration increase of Bio-Ag⁰-6, the flux decline was only 52%, 29% and 8% for 5 mg/L, 10 mg/L and 15 mg/L of Bio-Ag⁰-6 addition, which was relatively lower than the control MEC sample. This may due to the bacteria multiplying and biofilm sedimentation on membrane surfaces.
Figure 4. The water flux of membranes with Bio-Ag\textsuperscript{0}-6 pretreatment before and after biofouling (bars) also the flux decline of samples (points).

To further prove the superiority of the Bio-Ag\textsuperscript{0}-6 addition to the fouling control, the resistances after biofilm formation were calculated as described in Equation 3. As shown in Figure 5, all the MEC membranes have the similar intrinsic membrane resistance (\(R_m\)) of about 15 (10\textsuperscript{10} m\textsuperscript{-1}). The small errors were caused by the nominal inherent differences of all the MEC membranes. The values of \(R_f\) caused by the fouling increased a lot with the decrease of Bio-Ag\textsuperscript{0}-6 concentration, which may result from the biofouling and the membrane pore entrapped by bacteria [30]. Figure 5 also showed that the total filtration resistance (\(R_t\)), which is the sum of \(R_m\) and \(R_f\) was negatively correlated to the content of Bio-Ag\textsuperscript{0}-6. This is consistent with the change of \(R_f\) and the permeate flux in Figure 4.

Figure 5. The distribution of intrinsic membrane resistance (\(R_m\)) and fouling resistance (\(R_f\)) in total resistance (\(R_t\)) of all the membranes with different Bio-Ag\textsuperscript{0}-6 dosages.

It has been known that the Bio-Ag\textsuperscript{0}-6 could damage the cells and showed great effectiveness in controlling the biofilm formation due to the release of biotoxic Ag\textsuperscript{+} to the surrounding [29]. The
results above suggest that the addition of small sized and highly dispersed Bio-Ag\textsuperscript{0-6} may play a positive role for membrane biofouling control.

3.5. Membrane cleaning effect
To investigate the membrane cleaning effect using different cleaning agents, the dilute BD solution permeation process was conducted. Figure 6 shows the flux recovery ratio (FRR) after treated by different reagents. A more significant FRR was obtained after the fouled MEC membrane was soaked in Bio-Ag\textsuperscript{0-6} solution (30 mg/L) than in PBS solution and NaClO (200 mg/L). For example, the FRR for PBS treated membrane was only 12%. Nevertheless, after soaking in NaClO solution, the FRR was increased to 47.7% and further to 53.2% after soaking in Bio-Ag\textsuperscript{0-6} solution under the same condition. This also indicates that Bio-Ag\textsuperscript{0-6} is more efficient to promote the detachment of biofilms from membrane surface, leading to the improved water flux.

To quantitatively assess the membrane cleaning effect of different agents, fouling resistance ratios were obtained as shown in Figure 6. Obviously, \(R_t\) was the sum of \(R_r\) and \(R_{ir}\). \(R_r\) represents the flux decline caused by the gel layer formation and reversible foulant attachment, which could be wiped off by membrane cleaning. While \(R_{ir}\) represents the flux decline induced by pore blocking and strong sedimentation of biofoulants in the pore or on the membrane surface [31]. Figure 6 showed that all the MEC membranes had the similar total fouling ratio after the biofouling was maximized. However, the proportion of \(R_r\) and \(R_{ir}\) in the total fouling ratio was quite different. When using PBS solution as the cleaning agent, the \(R_t\) of MEC membrane was made up of nearly 87.9% \(R_{ir}\) and 10.4% \(R_r\). As for 200 mg/L of NaClO solution, the \(R_{ir}\) significantly reduced and the \(R_r\) increased. The 30 mg/L of Bio-Ag\textsuperscript{0-6} treated MEC membrane has the highest reversible resistance ratio (\(R_r\)) of about 52% and the lowest irreversible resistance ratio (\(R_{ir}\)) of about 46.8%. Larger \(R_r\) indicated that more adherent foulants could be removed more easily by Bio-Ag\textsuperscript{0-6} washing. The reduction of \(R_{ir}\) also directly confirmed that the membrane cleaning effect was elevated a lot by Bio-Ag\textsuperscript{0-6} compared with the traditional cleaning agents.

![Figure 6](image_url)

**Figure 6.** The flux recovery ratio (points) and the fouling resistance ratio of MEC membranes (bars) under different membrane cleaning methods.

4. Conclusions
In the present work, the small sized Bio-Ag\textsuperscript{0-6} were introduced into commercial membrane systems. The biofouling formation assay and SEM results showed that the Bio-Ag\textsuperscript{0-6} could affect the growth and the initial attachment of BD on MEC membranes. The EPS analysis and the multicolor fluorescence labeling in CLSM observation were consistent, revealing that Bio-Ag\textsuperscript{0-6} showed excellent antibacterial activity, effectively reduced the EPS production and biofilm formation. The permeate flux changes and the fouling resistance calculation showed that the pretreatment of Bio-Ag\textsuperscript{0-6}
6 could control the development of biofouling and decrease the membrane fouling resistance caused by fouling ($R_f$) in commercial membrane system. Moreover, it was much more efficient in facilitating the detachment of rigid biofilm from commercial membrane surface. From the results above, it is considered that the highly dispersed Bio-Ag$_2$O nanoparticles could be used to relieve membrane biofouling, leading to the development of commercial membrane fouling control and effective membrane cleaning technology in membrane industry.

5. References

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