Study of copper-charged membranes for control of fouling due to bacteria and algae organic matter

Sunitha Asapu, Santosh Pant, Peyman Majid, Isabel C. Escobar and Cyndee L. Gruden

ABSTRACT

The accumulation of micro-organisms, along with the presence of nutrients, forms biofilms. Biofoulants that are typically encountered in desalination systems include cellular organisms (e.g., bacteria or algae) and organic debris, including algae organic matter. The accumulation of these micro-organisms is problematic to membranes by causing irreversible fouling. The most adverse effects due to biofouling include declines in permeate flux and salt rejection. In addition, biofilm formation necessitates frequent membrane cleaning, increasing operating costs and decreasing membrane life. The goal of this research was to investigate the performance of low-fouling copper-charged membranes for microbial resistance. The extent of fouling on the microbial resistant membranes was characterized by assessing surface area coverage by image analysis. Fluorescent microscopy was used to determine activity of biofilm cells on the surface. The presence of extracellular polymeric substance was verified using Fourier transform infrared spectroscopy. The permeate flux values were compared for both unmodified and copper-charged membranes by conducting dead-end filtration experiments using synthetic brackish water.

Key words | algae, bacteria, cellulose acetate, copper, fouling

INTRODUCTION

Water is essential for the survival of life on Earth, but pollutants in water can cause dangerous diseases and fatalities. Over the years, natural sources of water have been falling short of providing enough drinkable water, so the need for purified water has been increasing with the increasing population. During membrane filtration, materials that are rejected by the membrane may accumulate on the surface of the membrane to foul it. Such materials include organic matter, colloids and micro-organisms. The former two can be controlled via pretreatment; however, the accumulation of micro-organisms is more problematic to membranes. Accumulation and development of the micro-organisms on the polymeric membrane surfaces and on the surface of the feed spacers is known as biofouling (Hausman et al. 2010b). A substance called extracellular polymeric substance (EPS) is emitted by these micro-organisms and forms around the organism’s outer layer as biofilm (Herzberg & Elimelech 2007). These biofilms are detrimental and result in irreversible membrane fouling. Biofoulants that are typically encountered in desalination systems include cellular organisms (e.g., bacteria or algae) and cellular debris including algae skeletons (Farooque et al. 2004; Brant et al. 2010). Biofouling is inherently more complicated than other types of fouling. This is a formidable challenge for membrane plants that obtain feedwater from surface water, due to the presence of a variety of biotic life available. Cellular organisms have the opportunity to proliferate in saline environments since there is adequate organic material (up to 5 mg/L) available as a nutrient source (Brant 2010). These micro-organisms, particularly algae, seasonally produce high concentrations of biomass (thousands to millions of cells/mL) and organic substances, which can adversely affect the operation of membrane-based water treatment plants, mainly because of fouling.
(Passow 2002; Fatibello et al. 2004; Bar-Zeev et al. 2009; Berman et al. 2011). However, the types and quantities of algae vary dramatically depending on the season, the weather, and the geographical location (Caron et al. 2010).

The conventional approach to coping with biofouling challenges has been to set up a cleaning schedule for the membrane, limiting the influx of nutrients to the system in the pretreatment process, applying a biocide, or maximizing shear forces at membrane surfaces in order to control the build-up of biofilm. However, chemical pretreatment, common to membrane systems, renders the organic matter easily assimilable for biofouling organisms. Although 99.9% of micro-organisms may be removed using these approaches, the remaining micro-organisms proliferate whenever trace amounts of nutrients are present in water and can cause irreversible biofouling.

In this study, we evaluated the impact of a model microalgae expected to be present in seawater and a model bacterium expected to be present in brackish water. Fouling due to these biofoulants was tested on copper-charged cellulose acetate (CA) membranes and compared with pure CA membranes. The modification of the CA membranes involved a three-step procedure, as previously described (Asapu et al. 2014). In the first step, glycidyl methacrylate (GMA) was homopolymerized separately and added as a blend to the CA polymer dope. The flat sheet membranes were then cast using the phase inversion method. In the second step, a metal chelating ligand, iminodiacetate dibasic (IDA), was attached to the membrane surface via the spacer arm of GMA. Lastly, the membrane was charged with copper ions by covalently binding the copper ions to the chelation group of IDA, and attached to the surface of the membrane. Characteristic Fourier transform infrared spectroscopy (FTIR) absorption bands were observed at 860, 955 and 2,990 cm$^{-1}$ for the modified dope (i.e. CA/GMA/NMP dope) and IDA treated membranes. These bands were absent from the CA sample, which confirmed the presence of GMA in the modified dope polymer solution. IDA addition was verified by peaks for $-\text{C-H}$ bend and $-\text{O-H}$ stretch, associated with the presence of alkenes and carboxylic acid due to the presence of the carboxylic acid from IDA (Asapu et al. 2014).

Potential leaching of copper ions was also reported in previous studies, and in summary copper leaching studies showed that very little copper leached from the surface of the membranes. After 24 hours of continuous contact, the average copper that leached into a 35 g/L NaCl solution was 0.10 wt.%, into a 5 mM EDTA solution of pH 11 was 0.11 wt.%, and into a 10 mg/L Fe(II) solution was 0.22 wt.% (Asapu et al. 2014). The copper that leached from the surface was slightly higher in the Fe(II) solution compared to the NaCl and EDTA solutions, which is hypothesized to be due to the higher affinity of the iron for the IDA chelation causing displacement of copper from the membrane surface. It is important to note that leached copper ions would be rejected by membranes, so would not be in the permeate. Therefore, the focus of this study was on the fouling potential using the copper-charged membranes as compared to pure CA membranes when filtering bacterial and algal organic matter.

MATERIALS AND METHODS

Materials

The polymer dope used to cast flat sheet membranes was made of CA (average $M_N$ 30,000) with an acetyl content of 39.8 wt.% purchased from Sigma–Aldrich and GMA (average $M_N$ 142.16) purchased from Alfa Aesar and vacuum distilled before use, N-methyl-2-pyrrolidinone (NMP, >99%, Alfa Aesar) solvent. GMA was polymerized with toluene (99.9%, 92.14) purchased from Fisher Scientific and benzoyl peroxide (97%) purchased from Sigma–Aldrich. IDA hydrate 98% was purchased from Aldrich Chemistry (St Louis, Missouri). Dimethyl sulfoxide (DMSO) 99%, copper sulfate, acetone and hydrochloric acid were purchased from Fisher Scientific (Hampton, New Hampshire).

Methods

Membrane preparation

The preparation of copper-charged membranes is explained in detail elsewhere (Asapu et al. 2014), and will be summarized here. In the first step, the GMA was homopolymerized separately and added as a blend to the polymer dope. The flat sheet membranes were then cast using the phase
inversion method. The phase inversion process induced by immersion precipitation is a well-known technique for preparing asymmetric polymer membranes (Loeb & Sourirajan 1962; Hausman et al. 2000a; Hausman & Escobar 2012; Flanagan & Escobar 2013). Flat sheet membranes were cast with the polymer dope solution of 21/77/2 weight% ratio of CA/NMP/GMA using the phase inversion method. The polymeric dope solution was poured on a glass mirror and the flat sheets were cast using a doctor’s blade at a thickness of 130 microns (µm). These were then immersed in a water bath to allow interaction of the solvent in the casting solution film with the nonsolvent in the precipitation media. This process resulted in an asymmetric membrane with a dense top layer and a porous sublayer.

The metal chelating ligand IDA was attached to the epoxy group of GMA by treating the flat sheet membranes with 0.5 M IDA dissolved in a 50/50% water/DMSO mixture for approximately 2–4 hours at a constant temperature between 50 and 55 °C. Then, the membranes were washed with distilled (DI) water and placed in 0.6 M copper sulfate solution for 24 hours.

The CA membranes made here had an effective mean pore radius of 0.43 nm and the copper-charged membrane had a mean pore radius of 0.525 nm, which confirmed they were in the nanofiltration range (0.5–2 nm) (Asapu et al. 2014).

**Pseudomonas fluorescens migula (ATCC #12842)**

Membranes were tested with a Gram-negative aerobic bacterium, *Pseudomonas fluorescens migula* ATCC # 12842). This rod-shaped bacterium has the optimal growth temperature of 27 °C, pH 7.0, and uses glucose as its carbon source. Members of the Pseudomonas genus are one of the most ubiquitous bacterial species in the environment and water systems, and *Pseudomonas fluorescens* is well known to be good as a biofilm producer due to its short generation time and resistance to temperature fluctuations (Passow & Alldredge 1995; Claquin et al. 2008). The bacterial growth curve (cell counts vs. incubation time) was established to determine the lag, exponential and stationary growth phases of the organism. PicoGreen, purchased from Invitrogen, was used to non-specifically stain all organisms (green), and the stained sample was mounted on a slide using immersion oil. Slides were counted using a fluorescent microscope (1,000×). Bacteria were collected at the late exponential growth phase (27 hours) for filtration studies (Figure 2). The *P. fluorescens* were diluted in synthetic brackish feedwater to a final concentration of 10⁴ cells/mL.

**The marine algae, Chaetoceros affinis (CCMP 158)**

The presence of marine algae in feedwater can drastically change the behavior in the formation of biofilm on the membrane (Meng et al. 2013). For this analysis, *Chaetoceros affinis* (CCMP 158) was purchased from the National Center for Marine Algae and Microbiota. The Marine Algae and *Chaetoceros affinis* were grown on f/2-si medium in a growth chamber at the University of Toledo. The algae cultures were incubated at 20 ± 2 °C room temperature under an artificial light source (fluorescent lamp) at 12 hours light/dark regime and continuous slow mixing condition. Light intensity was set at 40–50 µmol/m²s. The cell concentration of algae was measured every 2–4 days with the help of a counting chamber and light microscope to develop a growth curve. The counting chamber was rotated between transects to randomly chosen positions (Brierley et al. 2007). For the counting procedure, an Olympus BX51 microscope (made in Japan) at 20× was used, and the organisms were counted. Samples for these studies were collected from the stationary growth phase. To test the impact of algae organic matter (AOM) on membrane fouling, *c. affinis* cells were centrifuged at 4,000 rpm for 20 minutes (Eppendorf Centrifuge 5804 R; Hamburg, Germany), and the supernatant was discarded using a

![Figure 1](https://iwaponline.com/jwrd/article-pdf/5/4/516/377850/jwrd0050516.pdf)  
_Figure 1_ Bacteria growth observed with fluorescent microscopy (1,000×) following 22 hours of incubation in 1:400 dilutions.
pipet. The pellet, which included AOM, was suspended in laboratory grade water.

**Collecting and characterizing AOM**

During algal blooms, increased turbidity, total suspended solids and total organic content resulting from algal biomass and growth challenge the operations of desalination facilities. The organic content is composed of polysaccharides and proteins. From the membrane perspective, polysaccharides absorb on the membrane with six to eight times the efficiency of proteins due to their sticking characteristics. Specifically transparent exopolymer substances (TEP) are included in the total organic content released by algae and can be present at significant concentrations. In this project, we used TEP as a surrogate for AOM. Five mL of sample of *C. affinis* was centrifuged at 4,000 rpm for 20 minutes using an Eppendorf Centrifuge 5804 R (made in Hamburg, Germany). The supernatant was discarded, and the remaining pellet was AOM containing TEP.

In an effort to quantify the AOM, we measured the TEP concentration in the samples using Alcian Blue (AB) solution (11). AB is a hydrophilic cationic dye that complexes with anionic carboxyl or half ester-sulfate groups of acidic polysaccharides, causing the substance to be stained blue. Two mL of pre-filtered AB solution (Passow & Alldredge 1995) was added to the pellets and was centrifuged for 20 minutes at 4,000 rpm (Claquin et al. 2008). One mL of DI-water was transferred to the pellets to wash the excess dye and was centrifuged several times at the 4,000 rpm rate for a few minutes until the excess dye was removed. Then, 4 mL of 80% (v/v) sulfuric acid was added to the pellets and left for 2 hours for the reaction. The absorbance of the supernatant was measured at 787 nm using a Shimadzu UV-1800 (UV_spectrophotometer, Addison, Ill).

**Figure 3(a)** is a microscopic image of *C. affinis* collecting during enumeration. After *C. affinis* was quantified and pooled, AOM was extracted. In an effort to quantify AOM, we measured the TEP in the samples (Figure 3(b)). Figure 4 demonstrates the growth curve for *C. affinis* and the corresponding concentration of TEP. The data were similar to the study carried out by previous investigators (Villacorte et al. 2013). The maximum population reached was $2 \times 10^6$ cells per mL (Figure 4). After the maximum count was reached, a sharp decline in cell count was observed at about 8 days. This is considered the cell death phase, which is associated with depletion of nutrients in the medium. The TEP concentration corresponds to cell growth, increasing with incubation time, until the stationary/death phase. Then, the TEP concentration appears to increase despite cell death. More than 50% of the total accumulated TEP were produced during the stationary/death phase. This can be attributed to the algal cells’ mechanism for survival under nutrient stress (Villacorte et al. 2013).

The AOM obtained for the filtration experiment were extracted during the death phase of the algal sample because a previous study indicated that the AOM in stationary/death phase contained 57% polysaccharides compared to its precedent phase,
which contained 50% polysaccharides. Polysaccharides are significant components of TEP with good surface adhering capacity (Bar-Zeev et al. 2009; Meng et al. 2013; Villacorte et al. 2013). In addition, it is likely that algae would be in the stationary/death phase prior to reaching filtration systems.

Permeation experiments

Dead-end filtration

Filtration experiments were conducted using an Amicon dead-end filtration cell with an effective cross sectional area of 4.1 cm² at an operating pressure of 483 kPa. The membranes were tested by precompacting with DI water for the first 8 hours. The flux was determined by measuring the amount of time taken for 2 mL of the permeate solution to pass through the membrane in repeated processes (up to 8 mL total). Dead-end filtration experiments were performed using synthetic brackish feedwater (Table 1) containing P. fluorescens to evaluate the low-fouling abilities of the membranes. Bacteria were harvested at the late exponential growth phase, since maximum EPS formation occurs at this stage, and were added to the feedwater at a concentration of 10⁴ cells/mL (Asapu et al. 2014).

Dead-end filtration experiments were also carried out with saline feedwater (salt content of 35 g/L) and salt and AOM (salt content 35 g/L and 1.4 abs/cm/mL TEP content). It is important to note that dead-end filtration is not the most ideal filtration method for biofouling assessment, since biofouling needs time to develop. The dead-end filtration experiments can only provide information regarding the physical interaction between bacteria/algae and

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺</td>
<td>849</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>1,330</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>514</td>
</tr>
<tr>
<td>Ba²⁺</td>
<td>2.0</td>
</tr>
<tr>
<td>Fe³⁺</td>
<td>2.3</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>3,933</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>991</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>89</td>
</tr>
<tr>
<td>Alkalinity (as CaCO₃)</td>
<td>780</td>
</tr>
<tr>
<td>Total dissolved solids</td>
<td>8,790</td>
</tr>
</tbody>
</table>
the membrane surface. Dead-end filtration experiments were performed since the work focuses on material development, hence membrane sample sizes are small.

Characterization

Surface area coverage

Surface area coverage of the biofoulants from *P. fluorescens* on both modified and unmodified membranes was calculated using the software Image J 1.41 (Zaky et al. 2012, 2013). The commercially available software was downloaded (http://rsbweb.nih.gov/ij/) to use as a direct observation method to detect foulant coverage (area) on membranes. The fouling layer formed from the algae organic material was not able to be captured, since most AOM is invisible.

SEM imaging of fouled membranes

After biofouling filtration experiments, samples of CA and copper-charged membranes were taken from similar locations on the membrane surface. These membrane samples were dried at ambient temperature to remove all water and then coated using a gold-palladium target for 30 seconds to aid in electron imaging and prevent charging. No additional steps were taken for the fixation of foulants onto the membrane for scanning. Scanning electron microscopy (SEM) imaging (Hitachi S-4800 High Resolution Scanning Electron Microscope, Japan) of membrane samples was performed.

FTIR spectroscopy of fouled membranes

FTIR analysis using an attenuated total reflection Fourier transform infrared spectrometer (ATR-FTIR, Digilab UMA 600 FT-IT, Holliston, MA) microscope with a Pike HATR adapter and an Excalibur FTS 400 spectrometer, Ge crystal with a refractive index of 4.0 was performed on a clean CA membrane, as well as biofouled CA and copper-charged membranes in three locations on each membrane and averaged. The FTIR spectra of *P. fluorescens* biofilms have been studied (Pink et al. 2004), and the locations of peaks for different components are known.

RESULTS AND DISCUSSION

Dead-end filtration with bacteria

Dead-end filtration experiments were performed with synthetic brackish water containing the biofoulant *P. fluorescens* Migula to test CA and copper-charged membranes (Figure 5). The initial flux of the copper-charged membranes was 13.8 L/m²-hour, while the CA membrane flux was 6.3 L/m²-hour. It is important to discuss how the initial flux was measured in order to discuss differences between initial flux values. Flux was measured by determining the time to collect 2 mL of permeate, which took at least 20 minutes. Therefore, the initial flux was not instantaneous but rather after a certain period of time, during which it is hypothesized that copper-charged membranes might have provided some prevention of initial bacterial accumulation on the surface (i.e. prevented instantaneous biofouling (Peng & Escobar 2005)). This hypothesis is supported by the pure water flux values previously reported during protein filtration experiments (Asapu et al. 2014). The flux of the copper-charged membrane was lower than the CA membranes during the filtration of DI water; this was likely due to an increase in resistance from the blending of GMA in the CA polymer solution and IDA treatment causing fewer pores in the membrane. On the other hand, the filtration of proteins showed slightly higher fluxes for copper-charged membranes compared to CA membranes (Asapu et al. 2014). Copper-charged membranes and CA

![Figure 5](https://iwaponline.com/jwrd/article-pdf/5/4/516/377850/jwrd0050516.pdf)
membranes resulted in a flux decline of 20% and 12%, respectively; however, the final flux of copper-charged membranes was still significantly higher than that of CA membranes. The flux recovered after backwashing was higher for the copper-charged membranes (92%) than for the CA membrane (84%).

**Dead-end filtration experiments with AOM**

To investigate the short-term effects of AOM on membrane performance, filtration experiments were conducted. Figure 6 illustrates the comparison in performance of copper-charged and CA membranes. To calculate the initial flux, 2 mL of the feedwater was filtered, and that value was used to calculate the normalized flux for 2–4 mL and then 4–6 mL. The normalized flux value was calculated by dividing the preceding flux values by its initial flux value ($J_0$), and is represented in percentage form ($J_f/J_0$). The initial average flux was 4.26 ± 0.57 L/m²-hour for copper-charged membranes and 3.0 ± 0.58 L/m²-hour for CA membranes. The average flux decline for copper-charged membranes was 0.30 ± 0.12 L/m²-hour and for CA was 0.36 ± 0.07 L/m²-hour, and the average normalized flux decline was 6 ± 2.6% and 12.5 ± 3.9%, respectively. However, these average values include experiments using membranes that ranged from a low thickness of 93.5 μm to a high thickness of 188.5 μm, so these differences in thickness could be responsible for differences in flux values and flux declines. Therefore, only experiments made using membranes of approximately the same thickness were compared (i.e. 115 μm for copper-charged membranes and 106 μm for CA membranes). Under similar membrane thickness experiments of filtering AOM, copper-charged membranes displayed an initial flux of 4.8 L/m²-hour with a flux decline of 6.5%, while CA membranes displayed an initial flux of 2.8 L/m²-hour with a flux decline of 10.1%. Based on such a minor difference, it is not possible to determine if the copper-charged membranes were more effective at minimizing the effect of AOM during filtration. However, copper-charged membranes showed a trend toward being more effective at minimizing the effects of AOM during filtration.

Normalized flux decline values during AOM filtration experiments are presented in Figure 6 with filled squares for CA membrane data points and filled circles for copper-charged membrane data points. A linear trend line for each data set is shown in dashed lines for CA membranes and solid lines for copper-charged membranes.

The slopes of the normalized flux decline curves of both copper-charged and CA membranes, along with their respective $R^2$ value, are given in Table 2. The slopes were used for comparison since membrane thickness values were different, as previously described. As shown in Figure 6, the copper-charged membrane trend lines showed flatter slopes compared to the CA trend lines. The slope values are compared in Table 2, and the average values with standard deviation of the data confirm the general flatter slope values of the modified membranes’ flux decline. This data suggests that CA membranes show less resistance to AOM fouling than copper-charged membranes, but to determine the significance of the data a t-test was carried out on slope values and flux decline values to test the hypothesis that the two membranes perform significantly differently. The result of the t-test with 5% risk level ($\alpha = 5\%$) showed that two data

![Figure 6](https://iwaponline.com/jwrd/article-pdf/5/4/516/377850/jwrd0050516.pdf)

**Figure 6** Comparison of flux decline between modified and unmodified membranes and 35 mg/L NaCl salt using an unmodified membrane.

<table>
<thead>
<tr>
<th>Copper-charged membrane slope</th>
<th>CA membrane slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>−0.0085</td>
<td>−0.015</td>
</tr>
<tr>
<td>−0.0194</td>
<td>−0.0225</td>
</tr>
<tr>
<td>−0.0199</td>
<td>−0.0345</td>
</tr>
<tr>
<td>Average −0.0159</td>
<td>Average −0.024</td>
</tr>
<tr>
<td>Standard dev. 0.0064</td>
<td>Standard dev. 0.0098</td>
</tr>
</tbody>
</table>
sets (both flux decline and slope values) were not significantly different. The difference was statistically significant at a confidence rate of 85% ($\alpha = 15\%$) for flux decline.

**Detection of biofouling using SEM**

To image the microbial attachment, SEM images of fouled CA and copper-charged membranes were taken. The membranes, which were fouled during 5- and 7-hour dead-end filtration experiments, were analyzed and the resulting images are seen in Figure 7, where the right side corresponds to the 5-hour filtration and the left side to the 7-hour filtration. Because AOM was mostly clear, SEM images of those experiments were inconclusive.

Though bacterial cells were present on all the three membrane surfaces, it appears that the fouled CA membrane had substantially higher amounts of cells on the surface. On the other hand, copper-charged membranes appeared to have only a few cells on their surface. This observation supports filtration experiments that showed lower flux declines and higher flux recoveries during biofoulant filtration by copper-charged membranes.

**Surface area coverage**

Image J analysis was used to compare the surface coverage of foulants on CA and copper-charged membranes exposed to *P. aeruginosa* during filtration experiments. Copper-charged membrane surfaces showed that the foulant surface area was significantly less than the biofilm surface area formed on unmodified membrane (Figure 8 and Table 3).

**Detection of EPS using FTIR**

FTIR analysis was performed to attempt to determine the differences between the mechanisms of cell attachment and biofouling occurring on CA and copper-charged membranes. During AOM filtration, no peaks related to proteins or polysaccharides were observed, likely due to the small filtration volume (4 mL). Figure 9 shows FTIR spectra of the membranes fouled during the 5-hour dead-end filtration experiments as well as for an unfouled CA membrane. The FTIR spectrum of the clean copper-charged membrane was identical to that of the clean CA membrane.

![Figure 7](https://iwaponline.com/jwrd/article-pdf/5/4/516/377850/jwrd0050516.pdf)

**Figure 7** | SEM images of the fouled membranes after 5 hours' filtration (left) and fouled and back flushed membranes after 7 hours' filtration (right).
The FTIR analysis supported the fact the fouled CA membranes had higher levels of polysaccharides compared to fouled copper-charged membranes. Polysaccharides are known to make up a significant portion of EPS, and are related to cell adhesion during the initial stages of cell adhesion and biofilm formation (Tsuneda et al. 2003). It is important to note that EPS composition is to be variable depending upon the circumstances and the exposed surface. The fouled copper-charged membranes had fewer polysaccharides on their surfaces. This, along with the presence of proteins, is an indication that polysaccharides did not control biofouling (Hausman & Escobar 2012), as they did with the CA membrane.

The spectrum of the fouled CA membrane showed considerable differences from the fouled copper-charged membranes. The most prominent of these differences were the additional peaks at wavenumbers 1,020, 1,385 and 1,743 cm\(^{-1}\), which are indicative of mainly polysaccharides, carboxylate ion and also lipopolysaccharides, fatty acids and phospholipids (Ivnitsky et al. 2005; Kong & Yu 2007; Serra et al. 2007; Xu et al. 2012). When analyzing the fouled CA membrane.

**Figure 8** | Foulant surface area coverage assessment using Image J. (a) Clean membrane; (b) copper-charged fouled membrane; and (c) CA fouled membrane.

**Table 3** | Biofoulant surface area coverage using Image J

<table>
<thead>
<tr>
<th>Samples</th>
<th>CA membrane (Biofilm surface area (% coverage))</th>
<th>Copper-charged surface (Biofilm surface area (% coverage))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>91.7</td>
<td>65.8</td>
</tr>
<tr>
<td>2</td>
<td>94.5</td>
<td>67.8</td>
</tr>
<tr>
<td>3</td>
<td>93.4</td>
<td>72.3</td>
</tr>
<tr>
<td>Average</td>
<td>93.2</td>
<td>67.9</td>
</tr>
</tbody>
</table>

**Figure 9** | FTIR spectra of CA and copper-charged membranes fouled after 5-hour filtration using synthetic brackish water and 10^5 cells/L.
and copper-charged membranes, three peaks appeared on all three membranes. These peaks were at wavenumbers 940, 1,530 and 1,635 cm⁻¹, which corresponded to polysaccharide peaks and mainly with proteins of Amide I and Amide II bonds (Kong & Yu 2007; Xu et al. 2012). A listing of these peaks, their band assignments and associated biomolecules can be seen in Table 4.

The FTIR analysis of the membrane fouled after 7 hours of filtration and backwashed can be seen in Figure 10, and it looked almost identical to the spectra of the membranes biofouled after 5 hours (Figure 9) showing the presence of similar peaks.

The FTIR spectra from the fouled CA membrane after 7 hours of filtration (Figure 10) again showed peaks at wavenumbers 720, 1,100, 1,385 and 1,630 cm⁻¹, which are indicative of mainly lipopolysaccharides and polysaccharides (Ivnitsky et al. 2005; Kong & Yu 2007; Serra et al. 2007; Xu et al. 2012). When analyzing the fouled CA and

<table>
<thead>
<tr>
<th>Wavenumber (cm⁻¹)</th>
<th>Band assignment</th>
<th>Associated biomolecule</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>940</td>
<td>C-O stretch</td>
<td>Polysaccharides</td>
<td>Ivnitsky et al. (2005)</td>
</tr>
<tr>
<td>1,020</td>
<td>C-O-C and C-O ring vibrations</td>
<td>Polysaccharides</td>
<td>Xu et al. (2012)</td>
</tr>
<tr>
<td>1,385</td>
<td>Carboxylate or inorganic ion</td>
<td>Polysaccharides</td>
<td>Ivnitsky et al. (2005); Serra et al. (2007); Xu et al. (2012)</td>
</tr>
<tr>
<td>1,530</td>
<td>Stretching vibration of C-N and deformation vibration of N-H (amide II) bonds</td>
<td>Proteins</td>
<td>Kong &amp; Yu (2007)</td>
</tr>
<tr>
<td>1,635</td>
<td>Stretching vibration of C=O and C-N (amide I)</td>
<td>Proteins</td>
<td>Xu et al. (2012)</td>
</tr>
<tr>
<td>1,743</td>
<td>Stretching vibration of C=O</td>
<td>Polysaccharides, fatty acids, phospholipids, lipopolysaccharides</td>
<td>Kong &amp; Yu (2007); Serra et al. (2007)</td>
</tr>
</tbody>
</table>

Figure 10 | FTIR spectra of membranes fouled after 7-hour filtration and backwash using synthetic brackish water and 10^4 cells/L and back flushed.
copper-charged membranes, three peaks appeared commonly on all the three membranes. These peaks were at wavenumbers of 940, 1,440, and 1,530 cm$^{-1}$, which corresponded to polysaccharides, lipopolysaccharides and proteins. Additional peaks appeared for the copper-charged membrane at wavenumber 1,630 cm$^{-1}$ indicating proteins. A listing of these peaks, their band assignments and associated biomolecules can be seen in Table 5.

The FTIR spectra of the membrane surfaces after 5- and 7-hour filtration experiments indicated that there was an accumulation of cells on the CA membranes. Since the filtration was only conducted for a shorter period of time, there was not enough time to actually form biofilm; therefore, it is hypothesized that the presence of polysaccharides on the CA membranes was due to strained cells that will lead to biofilm in the long-term. The presence of protein peaks on the copper-charged membranes indicated the presence of bacteria on the surface. Therefore, FTIR evidence led to the conclusion that biofilm initiation and adhesion to the membrane surface was more significant on fouling of CA membranes. This observation supports the role of the copper-charged membranes in controlling biofouling by preventing the attachment of cells to the membrane surface.

**CONCLUSIONS**

During biofoulant filtration experiments, copper-charged membranes resulted in lower flux declines and higher flux recoveries after backwashing when compared to CA membranes. In addition, the adhesion of bacterial cells on the copper-charged membranes was less than on the CA membranes. The absence of traditional EPS-controlled biofouling on copper-charged membranes was supported by FTIR analysis, which displayed fewer peaks associated with polysaccharides. Therefore, the use of copper-charged membranes has the potential to increase membrane life and decrease chemical cleanings associated with detrimental biofouling of membranes. Additional long-term studies are, however, needed to verify results.

**ACKNOWLEDGEMENT**

We are obliged to the United States Bureau of Reclamation (agreement # R11 AC 81 536) for funding this project.

**REFERENCES**


Berman, T., Mizrahi, R. & Dosoretz, C. G. 2011 Transparent exopolymer particles (TEP): a critical factor in aquatic


