Electrokinetic and Electrochemical Methods for Microbial and Organic Fouling Mitigation at Liquid-Solid Interfaces

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Electrokinetic and Electrochemical Methods for Microbial and Organic Fouling Mitigation at Liquid-Solid Interfaces

A dissertation presented

by

Qiaoying Zhang

to

The John A. Paulson School of Engineering and Applied Sciences

in partial fulfillment of the requirements

for the degree of

Doctor of Philosophy

In the subject of Engineering Sciences

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Electrokinetic and Electrochemical Methods for Microbial and Organic Fouling Mitigation at Liquid-Solid Interfaces

ABSTRACT

Organic and microbial fouling are the initial steps for biofilm formation, resulting in severe fouling problems in many environmental and engineered applications including membrane water filtration. Electro-active methods are proposed to mitigate microbial as well as organic fouling via electrokinetic and/or electrochemical mechanisms. In the first part of this thesis, a parallel-electrode configuration was adopted and the cathode antifouling was evaluated. A carbon nanotube-polyvinylidene fluoride (CNT-PVDF) porous non-Faradaic cathode was first fabricated on top of an ultrafiltration (UF) membrane to produce negative surface charges via capacitive charging at 2 V, which reduced energy requirements (up to 2-fold) in comparison to the unmodified control. A semi-quantitative study was then completed on cathode coatings of different nanomaterials (CNM) to reduce microbial fouling. The bacterial attachment and inactivation were correlated to the electric potential and cathodic H$_2$O$_2$ generation, respectively. Next, a CNT and carbon black (CB) composite cathode was made on a forward osmosis (FO) membrane surface and challenged with synthetic and actual wastewater, which reduced fouling in regard to initial flux loss (~60%) for the actual wastewater and fouling rate (~50%) for both solutions at 2 V in 84 h. In the other part of the thesis, the electrode configuration was improved by fabricating interlaced surface electrodes on a substrate or membrane surface instead of only using a cathode. Insulated interlaced Ag electrodes resulted in optimal bacterial inactivation (84%) and detachment (94% after 15 h biofilm growth) with 2 min treatment at 50 V AC (10 kHz). Interlaced CNT electrodes
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CHAPTER 1
INTRODUCTION AND OVERVIEW

1.1 ORGANIC AND MICROBIAL FOULING

Fouling is the undesired deposition of contaminates on a substrate surface and can be classified dependent upon the foulant species: 1) physical fouling by particles and colloids, 2) chemical fouling by inorganic and organic solutes, and 3) biofouling by organisms. Biofouling i.e., when biofilm results in unacceptable losses in process performance or product quality/quantity [1, 2], occurs in many aqueous environments. Due to the protective biofilm structure, biofouling is resistant against external physical and chemical stimuli, being a most severe and complicated fouling problem in many environmental and engineering applications. For example, marine biofouling on ship hulls significantly increases the fuel consumption due to increased frictional drag [3] and biofouling of pipeline causes potential water contamination in water distribution systems [4, 5].

In the area of environmental engineering, biofouling is a major obstacle for membrane water purification systems [6]. Membrane filtration separates water molecules from other contaminates with a semi-permeable film by different driving forces, such as hydraulic and osmotic pressure. Depending on membrane pore size, the pressure-driven membranes include microfiltration (MF, 0.1-10 µm), ultrafiltration (UF, 2-100 nm), nanofiltration (NF, 0.5-2 nm), and reverse osmosis (RO, <0.5 nm), rejecting suspend particles, macromolecules, small organics and multivalent ions, and monovalent ions, respectively [7]. Forward osmosis (FO) is driven by the osmotic pressure gradient to transport water molecules from a solution of low osmotic pressure to that of high osmotic pressure and is as highly selective as NF and RO processes [8]. Biofouling
occurs in almost all membrane processes, increases the operation and maintenance (O&M) cost due to increased filtration resistance and decreased cleaning efficacy, and reduces membrane longevity [9, 10].

Biofouling occurs in the sequences of 1) organic matter adsorption as a conditioning film within minutes, 2) bacterial attachment and assembly into a slime biofilm layer within hours, and 3) macrofouling community formation comprising algae and invertebrates over days or even longer timescale [11, 12]. Thus proper control of the initial organic and microbial fouling can reduce biofouling.

1.2 FOULING MITIGATION TECHNIQUES

Adhesion is a primary process for both organic and microbial fouling. Hydraulic conditions affect the adhesion process via mass transport. For instance, improvement of boundary layer conditions with increased cross-flow velocity [13] and the introduction of spacers [14] will effectively reduce fouling. The thermodynamics of adhesion is determined by the interactions between the substrate surface and the foulants. Apart from the relatively weak van der Waals forces, significant electrostatic interaction is present between charged surfaces. Most bacteria [15, 16] and natural organic matter (NOM) [17] are negatively charged under natural conditions, thus a negatively charged surface is preferred in reducing organic and bacterial fouling. Hydrophobic interaction plays an important role in organic and bacterial adhesion as well, as a consequence of minimizing the overall hydrogen-bonding energy of apolar molecules (or apolar molecular segments) and the cohesion water molecules [18]. A hydrophilic surface is thereby generally favorable to sterically hinder organic and microbial adhesion.

After the adhesion process, biofouling can be mitigated via bacterial inactivation to prevent biofilm development [19] and bacterial detachment to regenerate the fouled surface.
Fouling mitigation in regard to all the above mechanisms can be divided into two categories—passive and active methods, dependent upon whether external chemical/energy inputs are introduced to the system during operation. A typical passive fouling control method is the permanent modification of a substrate surface. However, due to the complex and species-dependent microbial surface chemistry, a specific surface will not reduce biofouling for all microbes, and decreased antifouling performance is often observed in complex environments. Active fouling reduction methods by in situ tuning the surface properties and interfacial interactions provide a possible solution to this problem, among which electro-active i.e., electrokinetic and electrochemical fouling mitigation has the advantages of easy-automation and low environmental impacts [20, 21].

1.3 ELECTROKINETICS IN FOULING MITIGATION

By definition, electrokinetics is “the liquid flow that occurs along a solid/liquid interface as a result of an applied potential gradient, or conversely to the potential developed when a liquid is made to flow along an interface” [22]. Electrophoresis, dielectrophoresis (DEP), and electroosmosis are common phenomena associated with electrokinetics.

When an electric potential is applied to an electrical cell, the electrodes in an aqueous solution will be charged and develop an electric double layer (EDL) at the solid-liquid interface (Figure 1.1).
Figure 1.1: Models of the electric double layer at a positively charged surface: (a) the Helmholtz model, (b) the Gouy-Chapman model, and (c) the Stern model, showing the inner Helmholtz plane (IHP) and outer Helmholtz plane (OHP). The IHP refers to the distance of closest approach of specifically adsorbed ions (generally anions) and OHP refers to that of the non-specifically adsorbed ions. The OHP is also the plane where the diffuse layer begins. $d$ is the double layer distance described by the Helmholtz model. $\psi_0$ and $\psi$ are the potentials at the electrode surface and the electrode/electrolyte interface, respectively. [23]

In a non-Faradaic electrical system, the electrodes function as a capacitor and the electrode capacitance, cell capacitance, surface charge, and potentials are related as described by Eq 1.1 and 1.2:

$$Q = C_{cell} \times V_{cell} = C_{cat} \times V_{cat} = C_{ano} \times V_{ano}$$

(1.1)

$$V_{cell} = V_{cat} + V_{ano}$$

(1.2)

where $Q$ (C) is the capacitor charge accumulation; $C_{cell}$, $C_{cat}$, and $C_{ano}$ (F) are the capacitance of the overall cell, cathode, and anode; $V_{cell}$, $V_{cat}$, and $V_{ano}$ (V) are the potentials of the cell, cathode, and anode, respectively. An electric field is found only near the electrode surface as a result of EDL screening when the electrode is stably charged.
In a Faradaic system, the electric field also exists in the bulk solution between the anode and the cathode and the electric field strength is defined by Ohm’s Law [24]:

\[ j = \sigma E \]  

(1.3)

where \( j \) (A m\(^{-2}\)) is the current density, \( \sigma \) (S m\(^{-1}\)) is the solution conductivity, and \( E \) (V m\(^{-1}\)) is the electric field strength.

### 1.3.1 ELECTROPHORESIS

Electrophoresis refers to the movement of a charged particle or macromolecule in solutions with an external electric field and the velocity is given by:

\[ v_{ep} = \mu_{ep} E \]  

(1.4)

where \( v_{ep} \) (m s\(^{-1}\)) is the electrophoretic velocity and \( \mu_{ep} \) (m\(^2\) V\(^{-1}\) s\(^{-1}\)) is the electrophoretic mobility. Due to the dependence on electric field, the electrophoresis is mostly studied at DC potentials. As mentioned above, in a non-Faradaic system, the electrodes are capacitively charged and electrostatic force exerted by electric field within the EDL will be the governing mechanism for the surface-foulant interactions. Whereas in a Faradaic system, the electric field in the bulk solution may also affect the charged particle migration.

The application of electrophoresis to colloidal membrane fouling control is well established [24]. Electrophoretic bacterial fouling reduction has been investigated on other surfaces like conductive glass [25] and yarns with conductive graphite and carbon black paint [26], but most previous studies are empirical without quantitative underlying mechanism analysis. The application of electrophoresis in membrane fouling reduction as well as the combination with other electro-active effects also needs investigation.
### 1.3.2 DIELECTROPHORESIS

DEP is the movement of polarizable particles in a non-uniform electric field and can occur for cell components, cells, and cell aggregates with dimensions of <1 to 100 µm [27-29]. The expression for the DEP force on a dielectric sphere in a dielectric medium is [30, 31]:

\[ F = 2\pi R^3 \varepsilon_0 \varepsilon_m Re[K] \nabla E^2 \]  \hspace{1cm} (1.5)

where \( R \) (m) is the radius of the sphere, \( \varepsilon_m \) is the relative permittivity of the electrolyte medium, \( \varepsilon_0 \) is the permittivity of free space, and \( Re[K] \) is the Clausius-Mossoti function determining the effective polarizability of the particle. In contrast to electrophoresis, DEP is independent of the direction of the electric field due to the term \( \nabla E^2 \). Therefore, DEP is effective at both DC and AC potentials.

DEP is typically used in microelectromechanical systems (MEMS) for bacterial cell trapping or separation [32, 33] and has recently been investigated in clay particle fouling reduction in MF with interlaced metal wires [34]. Typically, DEP electrodes are fabricated at intensive material and energy cost and not feasible for scale-up; therefore, the application of DEP with easy fabrication methods to reduce microbial fouling is an interesting research area.

### 1.3.3 ELECTROOSMOSIS

Apart from electrophoresis and DEP, there might be other electrokinetic effects that help reduce fouling as well, such as electroosmosis. In contrast to electrophoresis where charged particles move in the solution, electroosmosis is the migration of solution on a stationary charged surface in the electric field. The velocity of migration is given by:

\[ v_{eo} = \mu_{eo} E \]  \hspace{1cm} (1.6)
where \( v_{eo} \) (m s\(^{-1}\)) is the electroosmotic flow velocity and \( \mu_{eo} \) (m\(^2\) V\(^{-1}\) s\(^{-1}\)) is the coefficient for electroosmotic flow.

Electroosmosis is frequently demonstrated as a possible mechanism for electric-enhanced filtration [24]. For instance, electroosmotic flow was suggested to occur on inorganic membrane surfaces using 500 to 4,500 V m\(^{-1}\) electric field to intensify the microfiltration of synthetic activated sludge effluent and the effect should increase with electric field strength [35]. Thus, it is another possible mechanism for microbial fouling mitigation on charged surfaces in the presence of sufficient electric field.

### 1.4 ELECTROCHEMISTRY IN FOULING MITIGATION

Electrochemical reactions occur on the electrode surface in a Faradaic system. For example, water splitting reactions on the cathode and anode are governed by Eq 1.7 and 1.8 [36]:

\[
2H^+(aq) + 2e^- \rightarrow H_2(g), E_{red}^0 = 0.00 \text{ V} \quad (1.7)
\]

\[
2H_2O(l) \rightarrow 2H^+(aq) + \frac{1}{2}O_2(g) + 2e^-, E_{ox}^0 = -1.23 \text{ V} \quad (1.8)
\]

Electrochemical bacterial inactivation will contribute to biofouling reduction via different mechanisms. For example, numerous studies have observed bacterial disinfection when an anodic current is applied [19, 37]. Two possible bacterial inactivation pathways have been identified—direct inactivation by the electrode surface and indirect inactivation by electrochemical production of Cl\(^-\) or HO\(^-\) [38]. When a cathodic current is applied, hydrogen peroxide is a common cathodic electrolysis product via the reduction of dissolved oxygen [39, 40] and its presence can be an evidence for the production of reactive oxygen species (ROS), such as HO\(^-\) [41] and HO\(_2^\cdot\) [40], which can cause bacteria inactivation as well.
The application of electrochemistry to biofouling mitigation is mainly focused on the anodic oxidation; for example, a conductive carbon-chloroprene sheet coating on the inner surface of pipeline significantly reduced biofouling in comparison to the control after ~1 year operation in seawater at 1.2 V [19]. However, since a cathode surface is beneficial for reducing bacterial and organic adhesion due to the negative surface charge, cathodic inactivation may further reduce biofouling, on which exclusive and quantitative studies are limited.

Other than inactivation, microbubbles may form on the electrodes as a result of electrochemical reactions as shown in Eq 1.7 and 1.8, which remove organics [42] and microbial [43, 44] fouling on the electrode surface via physical cleaning.

1.5 ELECTRODE MATERIAL AND CONFIGURATION

The electrode material is a key factor determining the success of electro-active microbial and organic fouling control. Recent development of CNT electrodes [45] provides an excellent option for electro-active fouling control since the CNT has superb chemical stability and electrical conductivity. The nano size is beneficial for the fabrication of CNT-polymer composites of uniform structure, low material cost, and tunable Faradaic properties. Besides, CNT forms a network with a pore size of ~100 nm upon dispersion and vacuum filtration and can act as a filter and an electrode for simultaneous bacterial removal and inactivation [38], therefore, it is suitable to make permeable membrane surface electrodes. The industrial price of CNT has decreased to 100 US$ kg⁻¹ [46], further promoting its rapid development and wide application.

The electrode configuration is another critical factor in electrokinetics and electrochemical systems. In a conventional electrochemical membrane system, the two electrodes are mostly placed in parallel, on both sides [24, 47, 48] or both on the feed side [49, 50] of the membrane to generate a relatively uniform electric field. In such scenarios, only one electrode is functional
whereas the other acts as a counter electrode, complicating the system design. Additionally, this configuration is not necessary based on the electrokinetic and electrochemical mechanisms e.g., DEP occurs only in non-uniform electric field and both anode and cathode can inactivate bacteria on the surface. Interlaced surface electrodes, modified from interlaced electrodes in MEMSs [32, 33] and other filtration systems [34], may address the above issues. Two methods i.e., direct ink write (DIW) 3D printing [51] and vacuum filtration through a laser-cut stencil, will be investigated for potential antifouling applications such as pipeline internal surfaces and membrane filtration systems.

1.6 OVERVIEW OF THE THESIS STRUCTURE

The work presented in this thesis focuses on the mechanism study of electro-active methods for microbial and organic fouling control, with an emphasis on the relevant application in various membrane filtration systems.

The first part of the work examines the cathode organic and microbial fouling mitigation in systems of parallel electrodes. A polyvinylidene fluoride (CNT-PVDF) composite cathode was evaluated for capacitive (non-Faradaic) organic fouling mitigation on an UF membrane surface in CHAPTER 2. Various carbon nanomaterials (CNM) including CNT and carbon black (CB) were then assessed as a cathode coating on a Ti substrate to reduce microbial fouling. A semi-quantitative mechanism analysis of the electrokinetic effects on bacterial attachment and electrochemical effects on bacterial inactivation is in CHAPTER 3. Based on the work above, a CNT+CB composite electrode was fabricated and utilized as a cathode on a FO membrane surface, which showed antifouling properties when challenged with both synthetic and actual wastewater as presented in CHAPTER 4.
Another part of the work examines the microbial fouling mitigation potential of interlaced surface electrodes. In the first step, interlaced surface Ag electrodes were fabricated on a glass substrate using a 3D printer and were insulated by a thin polymer coating to avoid electrode corrosion and to exclude the biotoxicity of Ag. The bacterial inactivation and biofilm detachment were evaluated in CHAPTER 5. In the following step, interlaced CNT surface electrodes were developed on a MF membrane surface by vacuum filtering CNT solution through a laser-cut stencil. The electrokinetic effects on bacterial density distribution and the electrochemical effects on bacterial morphology were evaluated and the MF operation and backwash conditions were optimized in CHAPTER 6.
CHAPTER 2
CONDUCTIVE CNT-PVDF MEMBRANE FOR CAPACITIVE ORGANIC FOULING REDUCTION

2.1 ABSTRACT

Organic fouling of ultrafiltration (UF) membranes results in decreased water flux and increased energy requirements. Modification of UF membrane surfaces is one possible method to mitigate natural organic matter (NOM) fouling, yet to date; most modifications have been passive. In this study, we investigate the use of a carbon nanotube-polyvinylidene fluoride (CNT-PVDF) porous non-Faradaic cathode on top of a UF membrane to actively produce negative surface charges via capacitive charging. The study is divided into three elements: 1) modification of the UF system with the capacitive CNT-PVDF electrodes and determination of the optimal electrode-membrane configuration, 2) analysis of the fouling mitigation mechanism, and 3) evaluation of the practical potential of capacitive fouling reduction. All experiments were completed in the cross-flow configuration. The optimal electrode-membrane configuration for organic fouling reduction was when the permeate first flowed through the porous anode, then the CNT-PVDF cathode, and finally the polyethersulfone (PES) UF membrane. The extent of capacitive fouling reduction was determined to be a function of anode material, ionic strength, and cathode potential. The primary fouling reduction mechanism is the potential-induced cathodic negative surface charges that increase the Derjaguin-Landau-Verwey-Overbeek (DLVO) energy barrier and decrease the collision efficiency of negatively-charged organic matter with the membrane surface. The
capacitive system has potential to reduce energy requirements by up to 2-fold as compared to the unmodified UF system when challenged with 10 ppm NOM solutions at low ionic strength.

2.2 INTRODUCTION

Ultrafiltration (UF) is a promising separation technology for drinking water treatment since it is effective and efficient towards turbidity and pathogen removal at low pressures. Direct ultrafiltration can simplify the conventional drinking water treatment process by eliminating multiple steps such as coagulation, sedimentation, and granular filtration from the process train. The advantages of UF include high product water quality, small footprint, compact modular design, and the ability to handle large fluctuations in feed water quality [52, 53] and low pressure UF membrane systems are utilized at drinking water treatment facilities across the world [54, 55]. However, a critical issue for all membrane applications is fouling [54] since it increases hydraulic resistance and decreases permeability resulting in increases in operation and maintenance (O&M) costs.

Many studies have identified aqueous natural organic matter (NOM) as one of the most detrimental UF surface water foulants [52, 56, 57]. The NOM fouling behavior is controlled by many factors including the chemical and physical properties of the organic matter, membrane surface and pore characteristics, and feed solution chemistry [58]. The increase in hydraulic resistance and subsequent permeability decline can be caused by concentration polarization, pore blocking, pore thinning, and cake layer formation on the membrane surface [59]. Mechanistically, organic fouling is attributed to the high interfacial energy of the hydrophobic UF membrane surface when in contact with water that is decreased upon sorption and deposition of organic matter [60, 61].
Based on known fouling mechanisms, continuous efforts have been made to reduce UF organic fouling through permanent membrane surface modification. For instance, hydrophilic inorganic Al₂O₃ [62] and TiO₂ [63] nanoparticles and amphiphilic organic copolymers such as polyacrylonitrile-g-poly(ethylene oxide) [64] and poly (2-dimethylaminoethyl methacrylate)-b-poly(methyl methacrylate)-b-poly(2-dimethyl aminoethyl methacrylate) [61] have been utilized as UF membrane additives to yield a more hydrophilic surface that tightly binds a thick water layer, which in turn sterically hinders organic matter attachment. Alternatively, UF membrane surfaces have been modified to increase negative surface charge density that reduces fouling via charge repulsion [65] since aqueous NOM is negatively charged [66]. For example, Wei et al. modified poly(ethersulfone) (PES) membranes by a electrophoresis-UV electrolyte monomer grafting process and observed that the grafted membranes exhibited less NOM fouling as compared to the unmodified membranes [67]. Bowen et al. added hydrophilic sulfonated poly(ether ether ketone) to polysulfone casting solutions and observed reduced humic acid fouling as compared to the unmodified membranes [68]. However, an increased membrane negative surface charge density will also result in a concomitant increase in membrane hydrophilicity, thus it is difficult to make a definite mechanistic conclusion on how an increased negative surface charge density reduces membrane fouling [65]. Surface modifications to reduce organic fouling are further complicated because certain functional groups such as carboxylates can form calcium ion bridges between the membrane surface and NOM resulting in lower permeability and less reversible fouling [69].

An alternative to passive modification of a membrane surface would be to introduce negative charge via an active process such as capacitance e.g., the deposition of carboxylated latex nanoparticles on a CNT filter was reduced when a potential of -2 V was applied [70]. The combination of UF and active electrical processes has been of research interest for the past few
decades with most previous studies focusing on the process of electro-filtration where an electric field controls the mass transfer of charged solutes by electrophoresis [24]. Nevertheless, the effect of surface charge densities has seldom been discussed even for systems utilizing a conductive electrode membrane. Classical materials utilized as porous conductive electrodes include metal alloys, metallic ceramics [71], and conductive polymers [72]. More recently, carbon nanotube (CNT)-polymer composites have been utilized as a novel class of conductive materials [73, 74], filters [75-78], and hybrid conductive filters [79, 80]. These CNT-polymer composites have been fabricated by methods such as surface deposition, covalent cross-linking, and phase inversion. The CNT-polymer composites exhibit tunable electrical conductivity, porosity, and mechanical properties that all may contribute to the development of novel permeable flow-through electrodes.

For example, a conductive non-Faradaic CNT-PVDF membrane produced by phase inversion [81] can produce capacitive surface charge with minimal energy requirements and toxic by-products e.g., chlorine or bromine, due to negligible Faradaic current. Thus, one application of the CNT-PVDF membrane would be to actively produce capacitive negative surface charge to reduce NOM fouling. However, due to the large CNT-PVDF pore size and permeability (Table 1), it cannot serve directly as the UF separation membrane, but could be used as a capacitive layer on top of a UF membrane.

Here, the potential of a conductive CNT-PVDF membrane for the capacitive reduction of organic fouling of a PES UF membrane is investigated. First, three different CNT-PVDF electrode-membrane configurations were evaluated to determine the optimal configuration for organic fouling reduction. Next, using the optimal configuration, the mechanism of NOM fouling reduction was investigated by completion of experiments with various anode materials, ionic strengths, and total applied potentials. Finally, the optimal configuration was challenged with
solutions mimicking surface water conditions over extended periods to evaluate the potential of capacitive fouling mitigation for practical application to drinking water treatment.

2.3 MATERIALS AND METHODS

2.3.1 CHEMICALS

Suwannee River fulvic acid (SRFA) standard II (2S101F) was purchased from the International Humic Substances Society (St. Paul, MN). Sodium alginate (ALG; medium viscosity) was acquired from MP Biomedicals (Solon, OH). Polyethylene glycols (PEGs) with different number average molecular weights (Mn) were purchased from Polymer Source (Canada). Polyvinyl pyrrolidone (PVP, Mn = 40,000 Da), polyacrylic acid (PAA, Mn = 8,000 Da) sodium salt (40% in water), sodium chloride (NaCl), and sodium hydroxide (NaOH) were acquired from Sigma-Aldrich (St. Louis, MO). PVDF (Kynar® 761) was kindly donated by Arkema (Philadelphia, PA). 1-Methyl-2-pyrrolidinone (NMP, ≥99%) was purchased from Alfa Aesar (Ward Hill, MA). Isopropyl alcohol (≥99.5%) was purchased from VWR International (West Chester, PA). Deionized (DI) water (>18 MΩ) produced by the Nanopure Infinity Ultrapure Water System (Barnstead/Thermolyne) was used to prepare all solutions and rinse all containers.

2.3.2 CONDUCTIVE NON-FARADAIC CNT-PVDF MEMBRANE SYNTHESIS

The conductive non-Faradaic CNT-PVDF membrane was produced via phase inversion using NMP as a solvent and water as a non-solvent following a previously developed procedure [81]. Briefly, 0.5 g CNTs and 0.1 g NaCl were dispersed in 100 g NMP by probe sonication (Branson, Sonifier S450D) for 15 min at an applied power of 400 W L⁻¹. After the solution had cooled to room temperature, 10 g PVDF (10% w/w NMP) and 1 g PVP (1% w/w NMP) were added to the CNT-NMP solution and mixed for 24 h using an overhead mixer (IKA RW16 Basic)
at 200 rpm. The resulting CNT/PVDF w/w ratio is 5%. Then, the casting solution was degased in an ultrasonic bath (Branson 2100) for 60 min and allowed to sit in the dark overnight. For the phase inversion process, the prepared casting solution was spread onto a glass plate with a bar coater of 100 µm thickness using an automated film applicator (Elcometer 4340 Automatic Film Applicator). Immediately after spreading, the glass plate with the casted solution was immersed in DI water at room temperature until the film detached from the plate. All the films were placed in a DI water bath (PolyScience Digital Temperature Controller) at 30 ºC for 24 h to remove any residual solvent and then stored in fresh DI water until use.

2.3.3 CROSS-FLOW ULTRAFILTRATION

CROSS-FLOW FILTRATION SETUP

Ultrafiltration was conducted with a bench scale system using a commercial flat-sheet cross-flow filtration cell (Sterlitech CF042) as previously described [82]. The feed solution was pumped (Micropump DJ604A) into the cross-flow filtration cell and the concentrate was recycled back to the feed reservoir. The temperature of the feed solution was in the range of 22-24 ºC. The feed flow rate and pressure were adjusted by a valve along the concentrate pipeline and recorded by a digital flow meter (Micro-Flow FTB321D) and pressure sensor (Omega PX482A-200GI). Permeate was collected to determine the flow rate and organic rejection. The remaining permeate (vast majority) was recycled back to the feed container to maintain a constant feed solution composition. The flow rate was measured with a graduated cylinder and timer. The permeate tubing was open to the atmosphere and the permeate pressure was considered to be constant at 1 bar.
SOLUTION PREPARATION AND CHARACTERIZATION

Four types of organic matter were used to evaluate membrane fouling. SRFA was chosen to represent molecular and ALG was chosen to represent colloidal organic matter found in surface waters [83]. In order to quantitatively analyze the effect of surface charge, two synthetic polymers: PAA and PEG, of well-known structure, surface charge density, and molecular weight were also utilized as organic foulants. The organic matter concentration of all feed solutions was 10 ppm (~4 ppm C), a moderate to high value for surface water organic matter content [56]. The ionic strength was adjusted with 0-20 mM NaCl. In the experiments to evaluate the effect of calcium, 0.067 mM CaCl$_2$ and 0.8 mM NaCl were added to the 10 ppm organic feed solution to maintain an ionic strength of 1 mM. All foulant solutions were prepared from a stock solution less than 2 days prior to UF tests. Aliquots of permeate, feed, and concentrate were sampled at the beginning and end of an experiment and the samples were evaluated for organic carbon content within 2 days of acquisition. Concentrations of PEG, PAA, and ALG were measured by a total organic carbon (TOC) analyzer (Shimadzu TOC-Vw) with a total carbon calibration curve (10 points) of 0-20 mg L$^{-1}$ and an inorganic carbon calibration curve (5 points) of 0-10 mg L$^{-1}$ using standards from the instrument supplier. SRFA concentrations were measured by a UV-Vis spectrometer (Agilent 8453) at a characteristic wavelength of 254 nm where the extinction coefficient was determined to be 0.0218 ± 0.0005 L (mgC cm)$^{-1}$. The pH values were obtained using a pH meter (Thermo Scientific Orion Star A210) that was calibrated with standard buffer solutions (Orion Application Solutions).
CROSS-FLOW FILTRATION EXPERIMENTS

Cross-flow ultrafiltration experiments were carried out with a previously described system [82] using a commercial PES membrane (Koch HFK-131). Briefly, the active membrane area was 8.5 × 4.0 (34) cm², the height of the feed flow channel was 2 mm, and the experiments were completed under a constant permeate flow rate (7.0 ± 1.5 mL min⁻¹) unless the pressure increased significantly (>2 bar) due to severe fouling. To maintain a constant permeate flux, the feed pressure was adjusted accordingly over the range of 0.7-2.0 bar and the feed flow rate was kept constant at 15 ± 1.5 L h⁻¹. Prior to aqueous organic filtration, all membranes were challenged with DI water for at least 15 min until a steady-state flux was achieved (Figure A.). The permeability data in the presence of organic matter was normalized to the DI water steady-state permeability. The duration of most of the UF experiments was 1 h except for the long-term tests (Section 3.4), which lasted 3-9 h i.e., until the permeability was significantly (≥50%) reduced. A new PES membrane was utilized for each experiment. Permeability was measured after the initiation of an organic filtration experiment (2 min) and then recorded again after 15, 30, 45, and 60 min. For TOC analysis, the feed samples were taken at the start and the end of the each experiment, and the permeate samples were only taken at the end of an experiment. All cross-flow filtration experiments were completed in at least duplicate. For clarity, Figure 2.3a-c and Figure 2.7a-b are presented without error bars.

ELECTRO-KINETIC FILTRATION CONDITIONS

After the initial control experiments, the conductive CNT-PVDF membranes were added into the cross-flow system as a capacitive non-Faradaic electrode to evaluate the fouling trend in the presence and absence of an applied potential. Three different electrode-membrane configurations i.e., the order of the porous anode, porous cathode, and PES UF membrane in regard
to permeate flow, were initially evaluated to help elucidate the electrokinetic anti-fouling mechanism (capacitive (C)harge, electric (F)ield, (C)harge+(F)ield). In all configurations, the dense surfaces of the CNT-PVDF and the UF membrane were facing the feed side. The porous CNT-PVDF cathode and a porous anode were connected by a Ti current collector to an external DC power supply (Agilent E3646A). When the porous electrode is in direct contact with the PES membrane (cathode in C, F, and C+F, anode in C), the Ti lead is compressed into the electrode by the top and/or bottom of the filtration cell. When spacers are placed between the electrode and the PES membrane (anode in F and C+F), the connection of the Ti and the electrode is by unpressurized contact. The spacers in configuration F and C+F were two meshes (GE Desal feed spacers); a plastic mesh with square pores of ~ 4 mm² was first placed on top of the UF membrane (F) or the PVDF-CNT film (C+F) and then a nonwoven cloth (dpore < 0.5 mm) was placed on top of the plastic mesh to support the anodic film. For some experiments, a carbon cloth (Carbon Cloth Plain CCP-30, Fuel Cell Earth, Wakefield, MA) (CCP) was used as the an alternative anode material to the CNT-PVDF. The current was continuously monitored by a multimeter (Amprobe 15XP-A). The cathode potential was determined by placing a 1 M Ag/AgCl reference electrode on the feed side of the membrane and measuring the potential difference between the cathode and the reference with a multimeter. The capacitive electrodes were either rinsed thoroughly with 0.1 mM NaOH for 15 min and then DI for at least 30 min or replaced with new materials for each experiment. A new PES UF membrane was used for all experiments. Flux, pressure, and test duration were the same as control experiments (B).
2.3.4 MATERIAL CHARACTERIZATION

IMAGING

Scanning electron microscopy (SEM) of the commercial PES and the produced CNT-PVDF membranes was conducted in Harvard’s Center for Nanoscale Systems on a Zeiss FESEM Supra55VP. The dry samples were prepared by first immersing in isopropyl alcohol for 24 h and then drying in air for 24 h. For the top and bottom surface images of the PES membrane, a ~ 4-nm layer of a conductive metal alloy (Pt or Pd-Pt) was coated onto the film using a sputter coater (Cressington, 208HR). For cross-sectional images, the membrane samples were prepared by first immersing in liquid nitrogen for 60 s and then snapping for a neat break. The cross-sectional samples were not coated before imaging. The acceleration voltage for surface and cross-section imaging was 1.5 kV and 5 kV, respectively. The surface pore size and thickness were analyzed using ImageJ software (NIH) [84] on at least two randomly selected images with at least 10 measurements of each image.

The carbon cloth was analyzed for pore size and surface morphology with an optical microscope (Keyence VHX 2000). The pore size was quantified by projecting light from the bottom of the sample and measuring the transmitted areas. The images are displayed in Figure A.2.

SPECIFIC SURFACE AREA MEASUREMENT

A specific surface area analyzer (Beckman Coulter SA 3100) was used to measure the surface area of the CNT-PVDF, CCP, and PES membrane materials. The samples were degassed by vacuum at 70 °C for 2 h before the adsorption and desorption of nitrogen gas. The 5-point-BET surface area data were used for specific surface area analysis.
CONTACT ANGLE MEASUREMENT

DI water contact angle measurements were completed with a goniometer (Rame-Hart 190CA) following the sessile drop technique. Samples were dried following the same method as the SEM sample preparation. At least 2 random membrane samples were analyzed and at least 12 measurements were done on each sample.

BULK POROSITY MEASUREMENT

The bulk porosity of the CNT-PVDF, PES, and CCP samples were determined by measuring the wet and dry weight of the membrane samples [82, 85]. The wet weight was measured after removing the surficial water with two polyester/cellulose wipers (VWR International), and then dry weight was measured after drying the samples following the SEM preparation method. The porosity was determined by the following equation:

\[ \varepsilon = \frac{m_1 - m_2}{\rho_w V_m} \] (2.1)

where \( m_1 \) and \( m_2 \) (g) are the wet and dry weights, \( \rho_w \) (g mL\(^{-1}\)) is the density of water, \( V_m \) (m\(^3\)) is the membrane volume, and \( \varepsilon \) (%) is the bulk porosity. The volume \( V_m \) was calculated with sample area and the SEM thickness.

ELECTRODE CONDUCTIVITY MEASUREMENT

The cross-sectional conductivity of the CNT-PVDF and the CCP porous materials was calculated by the following equation:

\[ \sigma = \frac{l}{RA} \] (2.2)

where \( \sigma \) (S m\(^{-1}\)) is the conductivity, \( l \) (m) denotes the thickness of the material, \( R \) (\(\Omega\)) is the resistance, and \( A \) (m\(^2\)) is the geometric area of the sample. The resistance was measured by reported
procedures [81]. Briefly, the samples were placed on a flat stainless steel plate and then a 10 kg stainless steel weight with a $5 \times 5$ cm cross-section was placed on top of the samples. The resistance between the stainless steel plate and weight was measured with a multimeter. The limit of detection for the resistance measurement is 1 $\Omega$.

**ELECTROCHEMICAL CHARACTERIZATION**

The electro-capacitive properties of the synthesized CNT-PVDF membrane were evaluated in the cross-flow cell using the C+F configuration in the absence of fluid flow. The solution to be analyzed was filtered through the system for at least 15 min prior to capacitance measurements. Cyclic voltammetry (CV) was completed with an electrochemical workstation (604D, CHI Inc. Austin, TX). The CV scan rate was 100 mV s$^{-1}$ over a potential range of -1.2 V to 1.2 V. The CV scan was started at 0 V in the negative direction and was completed at 0 V after at least 8 cycles. The CNT-PVDF membrane was utilized as the working electrode, a CNT-PVDF or carbon cloth was utilized as the counter electrode, and 1 M Ag/AgCl was used as the standard reference electrode.

### 2.4 RESULTS AND DISCUSSION

#### 2.4.1 CROSS-FLOW SYSTEM CHARACTERISTICS

**MATERIAL CHARACTERIZATION**

Two porous electrode materials were utilized in this study; the synthesized CNT-PVDF membrane and a commercial CCP, both of which have high cross-flow pure water permeability (> 6,000 LMH bar$^{-1}$). The structure of CNT-PVDF membrane by SEM is shown in Figure 2.1a-d. The bright white lines in the SEM images correspond to the CNTs that form the porous frame and
are coated by the grayish PVDF polymer. The CNT frame pores \(\sim 100\) nm and the CNT-PVDF membrane has a mesoporous top layer (Figure 2.1a; \(d_{\text{pore}} \sim 28\) nm) and a macroporous bottom layer (Figure 2.1c; \(d_{\text{pore}} \sim 27\) µm). The CNT-PVDF cross-section has a typical phase inversion asymmetric structure (Figure 2.1d). The CNT-PVDF thickness is \(\sim 90\) µm by SEM, in contrast to \(\sim 48\) µm by digital micrometer value due to compression, in agreement with the highly porous CNT-PVDF structure i.e., 81% bulk porosity (Table 2.1). The CCP is a woven fabric spun from graphitized carbon yarns (27 × 27 yarns inch\(^{-1}\), Fuel Cell Earth). The pore size of the CCP is \(\sim 0.2\) mm as determined by optical microscopy (Figure A.2). The PES membrane has a reported (Koch) nominal molecular weight cut-off (MWCO) of 10,000 Da; however, experiments completed in this study indicated 90% PEG removal when the molecular weight was \(\geq 50,000\) Da (Figure A.3). No visually discernible pores are observed in the aerial PES membrane SEM image (Figure 2.1e) at a magnification of 100 K. From the cross-sectional SEM image (Figure 2.1f), the PES membrane has a dense sponge-like top layer supported by a porous fabric support and is 158 µm thick—similar to the micrometer measurement of 169 µm. The pure water cross-flow permeability of the PES membrane is \(174 \pm 20\) LMH bar\(^{-1}\). In summary, the large pore size and permeability of the porous electrodes as compared to the PES membrane indicates the porous electrodes will have a negligible effect on the overall permeability of the system, but may influence the near PES surface hydrodynamic conditions and electro-kinetic mass transport of organic matter under an applied potential.
Figure 2.1: SEM images of the CNT-PVDF and PES membranes. Top surface of the CNT-PVDF membrane at different magnifications a) ×1k, b) ×100k; c) bottom surface of the CNT-PVDF, ×1k; d) cross-section of the CNT-PVDF, ×1k; e) top surface of the PES UF membrane.
Table 2.1. Properties of the CNT-PVDF, CCP, PES membranes, and mesh spacers.

<table>
<thead>
<tr>
<th>Materials</th>
<th>Thickness* (mm)</th>
<th>Top pore size (nm)</th>
<th>Bulk porosity (%)</th>
<th>Conductivity* (S m⁻¹)</th>
<th>Contact angle (°)</th>
<th>Specific surface area (m² g⁻¹)</th>
<th>Total surface area (m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrode</td>
<td>CNT-PVDF</td>
<td>0.048</td>
<td>10⁻⁵</td>
<td>3.20×10⁻⁴</td>
<td>66.2</td>
<td>16.3</td>
<td>0.781</td>
</tr>
<tr>
<td></td>
<td>CCP</td>
<td>0.203</td>
<td>0.2</td>
<td>&gt;&gt;8×10⁻²</td>
<td>136</td>
<td>5.54</td>
<td>2.59</td>
</tr>
<tr>
<td>Filter</td>
<td>PES</td>
<td>0.169</td>
<td>--</td>
<td>66.1</td>
<td>--</td>
<td>60.9</td>
<td>15.9</td>
</tr>
<tr>
<td>spacer</td>
<td>Cloth</td>
<td>0.236</td>
<td>0.3</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Mesh</td>
<td>0.600</td>
<td>2.0</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

* Thickness data in this table were the measurement results with a digital micrometer (Fowler Tools and Instruments; 0.00005” to 1.0”)

**ORGANIC FOULANT SOLUTIONS**

The reported properties of the organic matter foulants and their measured and estimated aqueous properties are listed in Table 2.2. The measured pH of the 10 ppm organic matter DI water solutions prepared ranged from 4.67 to 6.27. Using the reported organic matter $pK_a$ values and measured pH values, the degree of ionization can be estimated by the following equation:

$$\alpha = \frac{[A^-]}{[HA]+[A^-]} = \frac{10^{pH-pK_a}}{1+10^{pH-pK_a}} \quad (2.3)$$

For alginate, a solution pH of 6.27 and reported $pK_a$ of 3.21 result in a degree of ionization of 0.999 and an estimated charge density of 13.7 meq g⁻¹. The 10 ppm PAA solutions have an average pH of 5.24; however, the reported degree of ionization 0.27 and $pK_a$ of ~ 6.36 do not agree with Eq 3 due to the intramolecular repulsion between charged PAA monomers that limits the charge density resulting in a higher apparent $pK_a$ [86]. The negative charge density of the aqueous PAA is determined to be 7.5 meq g⁻¹. The 10 ppm PEG solutions have a pH of ~ 5.16 and no ionizable groups near this pH and thus are assumed to be of neutral charge. The SRFA is a complex mixture of molecular organic species with a wide molecular weight distribution. Approximately
70% (by weight) of the SRFA carbon is in the molecular weight range of 3,000 Da to 100,000 Da with a median molecular weight ~ 10,000 Da [87]. The 10 ppm SRFA solution pH is 4.66, corresponding to a charge density of 7.1 meq gC\(^{-1}\) according to the titration data of Ritchie and Perdue [88]. Therefore the charge density of the organic matter ranks in the order of PEG < SRFA < PAA < ALG. No further pH adjustment was made prior to filtration experiments. The addition of NaCl did not change the pH values significantly. For solutions with 0.067 mM CaCl\(_2\) and 0.8 mM NaCl, the 10 ppm SRFA solution has the pH of 4.66, similar to the DI solution, and the 10 ppm ALG solution has a pH of 5.56, lower than 6.27 in DI.

Table 2.2: Properties of the organic foulants and their DI water solutions.

<table>
<thead>
<tr>
<th>Organics</th>
<th>Chemical formula</th>
<th>pK(_a)</th>
<th>M(_n) (Da)</th>
<th>10 ppm pH</th>
<th>Ionization degree</th>
<th>Charge density (meq gC(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALG</td>
<td>(C(_6)H(_7)O(_6)Na(_n))</td>
<td>3.21 [89]</td>
<td>--</td>
<td>6.27</td>
<td>0.999</td>
<td>13.9</td>
</tr>
<tr>
<td>PAA</td>
<td>(C(_3)H(_3)O(_2)Na(_n))</td>
<td>~6.36 [86]</td>
<td>8,000</td>
<td>5.24</td>
<td>~ 0.27 [86]</td>
<td>7.50</td>
</tr>
<tr>
<td>PEG</td>
<td>(C(_2)H(_4)O(_n))</td>
<td>--</td>
<td>10,000</td>
<td>5.16</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>SRFA</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>4.67</td>
<td>--</td>
<td>~ 7.1 [88]</td>
</tr>
</tbody>
</table>

2.4.2 EVALUATION OF ELECTRODE AND MEMBRANE CONFIGURATIONS

ELECTRO-KINETIC MEMBRANE CONFIGURATION

As previously mentioned, three modified electrokinetic filtration configurations as compared to the control B (B stands for basic) were evaluated to determine the optimal configuration for UF organic matter fouling reduction. Detailed schematics and photos of the three modified configurations are can be found in Figure A.4. In all three configurations, the synthesized CNT-PVDF membrane was used as both the anode and cathode. The three configurations differ in the order of the electrodes and the PES membrane with regard to permeate flow. When the anode
Figure 2.2: Representative schemes of the basic cross-flow and electro-kinetic systems. B) Basic cross-flow cell; C) negative surface Charge only; F) electric Field; C+F) negative surface Charge and electric Field

and cathode were on the same side of the membrane, spacers were added to prevent short circuiting.
To examine the role of negative surface charge on fouling reduction, the C (C is short for negative surface Charge) configuration was designed such that the cathode was placed on top and next to the PES membrane in an attempt to repel the negatively-charged organic matter and the anode was placed on the bottom side of the membrane. It is of note that an electric field will exist between the electrodes and may affect the trajectories of the charged foulant by electrophoresis [90]. Thus, when the anode is on the permeate side like in C, the electric field may drive organic matter through the membrane. In order to examine the effect of the electric field alone, the configuration F (F is short for electric Field) was examined where the cathode was placed on the permeate side and the anode on the feed side above a spacer. Finally, the configuration C+F was designed such that the cathode was placed as in C to repel negatively-charged organic matter and the anode was also on the feed side as in F to facilitate organic matter electrophoresis away from the PES membrane. Spacers were introduced on top of the PES membrane in F and the CNT-PVDF cathode in C+F as a support for the anode membrane and to eliminate short-circuiting.

**MEMBRANE ORGANIC FOULING EXPERIMENTS**

The time-dependent DI water normalized cross-flow permeability of 10 ppm aqueous SRFA, PEG, and PAA solutions over a period of 1 h; in configurations B (blue), C (red), F (magenta), and C+F (black), in the absence (closed) and presence (open) of a 2 V applied potential are presented in Figure 2.3a-c. The UV$_{254}$ (SRFA) and TOC (PEG and PAA) rejections are shown in Figure 2.3d. The permeability data have also been organized into four sub-plots by configuration as displayed in Figure A.5.

For all three organic matter solutions permeability in the unmodified B configuration, there was significant and rapid (2 min) initial fouling and the 1 hour UF permeability is ~ 0.7 of the DI water. The rapid permeability loss after the initial 2 min required to make the first measurement
can be attributed to concentration polarization [91] and pore blockage [92]. After the initial rapid permeability decline, the gradual accumulation of organic matter over the next hour, either on the membrane surface or in the pores, results in slow and continuous permeability decline i.e, the permeability of the SRFA decreased from 0.86 to 0.66, the PEG from 0.83 to 0.72, and the PAA from 0.79 to 0.69 after 1 h of filtration. The PES membrane is able to reject 56% of SRFA, 24%
of PEG, and 77% PAA (Figure 2.3d). Even though PAA has a smaller molecular weight than PEG, upon hydration PAA has a larger hydrodynamic radius [86] resulting in higher rejection. For SRFA, the measured TOC rejection (unplotted) was 56% as compared to the measured UV$_{254}$ rejection (Figure 2.3d) of 70%, indicating the more aromatic SRFA is rejected to a greater extent than the more aliphatic SRFA. According to Haiber et al. [87], ~50% of the SRFA is <50,000 Da and ~40% is <10,000 Da, which is consistent with the TOC rejection data since the PES membrane MWCO is in the range of 10,000-50,000 Da.

Table 2.3: Fitting results for the configuration- and time-dependent permeability.

<table>
<thead>
<tr>
<th>Organics</th>
<th>B</th>
<th>C</th>
<th>F</th>
<th>C+F</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 V</td>
<td>2 V</td>
<td>0 V</td>
<td>2 V</td>
</tr>
<tr>
<td>SRFA</td>
<td>L</td>
<td>0.147</td>
<td>0.119</td>
<td>0.085</td>
</tr>
<tr>
<td></td>
<td>$k$ (h$^{-1}$)</td>
<td>0.251</td>
<td>0.242</td>
<td>0.248</td>
</tr>
<tr>
<td></td>
<td>$R^2$</td>
<td>0.986</td>
<td>0.884</td>
<td>0.950</td>
</tr>
<tr>
<td>PEG</td>
<td>L</td>
<td>0.187</td>
<td>0.150</td>
<td><strong>0.100</strong></td>
</tr>
<tr>
<td></td>
<td>$k$ (h$^{-1}$)</td>
<td>0.137</td>
<td>0.093</td>
<td>0.115</td>
</tr>
<tr>
<td></td>
<td>$R^2$</td>
<td>0.846</td>
<td>0.800</td>
<td>0.660</td>
</tr>
<tr>
<td>PAA</td>
<td>L</td>
<td>0.196</td>
<td>0.310</td>
<td>0.200</td>
</tr>
<tr>
<td></td>
<td>$k$ (h$^{-1}$)</td>
<td>0.160</td>
<td>1.003</td>
<td>0.330</td>
</tr>
<tr>
<td></td>
<td>$R^2$</td>
<td>0.975</td>
<td>0.902</td>
<td>0.958</td>
</tr>
</tbody>
</table>

* The lowest two values for $L$ and $k$ are in bold, and if one of the two values is significantly lower than the other, it is placed in italics.

To quantitatively evaluate the electrode-membrane configurations, the normalized time-dependent permeability data are fit to modified empirical fouling kinetic models. There are a number of quantitative membrane fouling kinetic models [92-94], and an exponential model was used here (Eq 2.4) [93] to describe the permeability change with time.

$$\log J_t = \log J_0 - kt$$  \hspace{1cm} (2.4)

where $J_t$ is the permeability at time $t$ (h) normalized to the clean membrane permeability prior to organic solution filtration, $J_0$ (= 1) stands for normalized DI permeability, and $k$ (h$^{-1}$) is the fouling
rate constant. However, the exponential model in Eq 2.4 cannot describe the initial rapid permeability decline observed in the majority of our experiments; thus another constant $L$ (dimensionless) indicating the initial (2 min) permeability loss is included, yielding the modified exponential model (Eq 2.5) below:

$$\log J_t = \log (1 - L)J_0 - kt$$

The solid lines in Figure 2.3a-c represent the experimental data fits to Eq 2.5, where the lighter colors correspond to open symbols (no applied cell voltage) and normal colors correspond to the closed symbols (2 V applied cell voltage). The fitting results for $L$, $k$, and $R^2$ are listed in Table 2.3. More than 70% of the $R^2$ values are $\geq 0.8$. The lower $R^2$ values are observed for the cases where the time-dependent fouling is minimal or decreasing and by visual examination the fitting results in all cases are decent.

HYDRODYNAMIC EFFECTS ON FOULING: MESH SPACERS AND THE CNT-PVDF MEMBRANE

Configuration C has minimal positive and sometimes negative effects on permeability and rejection in the absence of applied potential as compared to the control B. For SRFA, C has the second highest $L$ (0.119) and $k$ (0.242 h$^{-1}$), lower than or close to those of B. For PEG, C has the second highest $L$ (0.150), but lowest $k$ (0.093 h$^{-1}$). However, for the four configurations without external voltage, the $L$ and $k$ values are quite similar and thus the PEG data are not indicative of the hydrodynamic effects discussed in this section. For PAA, configuration C has the highest $L$ (0.310) and $k$ (1.219 h$^{-1}$), both greater than B of 0.196 and 0.235 h$^{-1}$, respectively. Configuration C also has the lowest rejection of PEG (11%) and PAA (66%) and second lowest next to C+F for SRFA (59%). The high fouling and low rejection indicate that when the CNT-PVDF membrane is directly placed on the PES membrane it may negatively affect mass transport via internal
concentration polarization i.e., the fluid velocity inside the CNT-PVDF is lower than in the feed channel, thus the back transport of the organic matter will be slower. An internal concentration polarization mechanism is supported by the fact that the colloidal PAA with slower back diffusion has a more negative effect than the molecular SRFA. Alternatively, the significant loss of membrane performance of configuration C in the absence of voltage with PAA may also be due to the increased hydrophobicity of the CNT-PVDF membrane, which will increase the tendency of PAA to deposit and foul the membrane. For example, the contact angle of a clean CNT-PVDF membrane (66°) is higher than that of the PES membrane (61°) (Table 2.1), and after 1 h filtration of PAA in configuration C, both the PES and the CNT-PVDF membrane contact angle decreased by ~ 5-20° (data not listed), indicating PAA attachment to both surfaces. However, the absolute contact angle decrease was typically higher for PES than the CNT-PVDF, in agreement with the higher PES organic rejection, indicating internal concentration polarization is likely the predominant factor for the reduced performance of C configuration in the absence of applied potential.

The optimal configuration in regard to fouling reduction in the absence of applied potential was F, where the mesh spacer directly contacts the surface of the PES membrane e.g, the magenta open points and light lines (Figure 2.3a-c). For SRFA without potential, the $L$ of F is 0, indicating no initial permeability loss, and the $k$ is 0.125 h$^{-1}$, much lower than 0.251 and 0.242 h$^{-1}$ of both B and C configurations. For PAA without potential, the values of $L$ and $k$, 0.059 and 0.050 h$^{-1}$ in F are the lowest for all configurations. The rejection of SRFA, PEG, and PAA in configuration F is 85%, 47%, and 95%, respectively, highest among the configurations without applied potential. The increased permeability and rejection of F relative to the other configurations in the absence of
applied potential can be attributed to the spacer mesh on top of the membrane that creates near surface turbulence reducing fouling [95].

The time-dependent fouling and rejection performance of C+F in the absence of applied voltage is intermediate to C and F. The C+F time-dependent permeability trends are close to those of F for SRFA (turbulent mixing) and C for PAA (internal concentration polarization). The rejection of SRFA (51%), PEG (14%), and PAA (77%) in C+F are also intermediate to C and F. In summary, the fouling and rejection results indicate that having the mesh spacer on the PES membrane (F) reduces fouling by producing near-surface turbulence, having the CNT-PVDF membrane on the PES membrane (C) increases fouling due to internal concentration polarization, and having the mesh spacer on the PVDF-CNT on the PES membrane (C+F) has a result that is organic matter property dependent.

ELECTRO-KINETIC EFFECTS ON FOULING: SURFACE CHARGE VS. ELECTRIC FIELD

The application of an applied total cell voltage of 2 V generally increases the normalized time-dependent permeability and organic matter rejection. The measured current in these experiments was \(<0.5 \text{ mA},\) corresponding to a low current density of \(<0.15 \text{ A m}^{-2}\) \((0.015 \text{ mA cm}^{-2})\) indicating Faradaic electron transfer reactions are negligible as expected from the insulating PVDF electrode coating. Thus, electron transfer reactions will be neglected and only electro-kinetic effects will be considered in regard to the anti-fouling mechanism.

Configuration C was designed to examine the ability of surface charge alone towards UF fouling mitigation. Comparing the open and closed red symbols in Figures 3a-c, the normalized time-dependent permeability was significantly higher when a potential was applied for PAA and no significant change in permeability was visually observed for SRFA and PEG. The minimal surface charge effects on organic fouling suggest that negative hydrodynamic effects dominate. In
the C configuration at 2 V applied potential, the fouling constant $L$ for SRFA (0.085), PEG (0.100), and PAA (0.200) were 0.035, 0.050, and 0.110 lower, respectively, than those with no cell voltage. The $k$ is similar for SRFA and PEG with and without applied voltage, yet decreases greatly from 1.003 to 0.330 h$^{-1}$ for PAA when voltage is applied. The significant fouling reduction with applied voltage in the PAA experiments is due to the high PAA charge density (7.5 meq gC$^{-1}$, Table 2.2) among the three foulants. Even though SRFA has a similar charge density (7.1 meq gC$^{-1}$), due to its wide molecular weight distribution [87] the smaller molecules could be electrophoretically driven into and through the PES membrane e.g., under an applied potential a decrease in SRFA rejection from 59 to 39% was observed. The 1 h rejection data for PEG and PAA in configuration C increased from 11% to 17% and from 66% to 75% with applied voltage.

Configuration F was designed to examine the ability of electric field alone towards UF fouling mitigation. Visual examination of the normalized time-dependent permeability data of F at 0 V and 2 V (Figure 2.3) indicates a minimal reduction in fouling with applied potential and that hydrodynamic effects of the spacer are dominant. The $L$ values for all organic matters are within 10% of each other at 0 and 2 V. The fouling rate constant $k$ decreased in all cases under applied potential: SRFA (0.125 to 0.079 h$^{-1}$), PEG (0.101 to 0.019 h$^{-1}$), and PAA (0.050 to 0.004 h$^{-1}$). However, there was no obvious visual improvement in permeability since the fouling constants all have low absolute values. The rejection of SFRA and PAA in configuration F was similar in the absence and presence of applied voltage, whereas the PEG rejection improved from 47 to 65%; however, the mechanism of enhanced PEG rejection is unknown.

Configuration C+F was designed to examine the combined ability of capacitive charge and electric field towards UF fouling mitigation. At 2 V cell potential, the C+F configuration had the best normalized time-dependent permeability for negatively-charged SRFA and PAA and was
among the best for the neutral PEG. For SRFA and PAA, the initial (2 min) fouling constant $L$ is quite small (<0.03) and the fouling rate constant $k$ is negative indicating an increase in permeability with time (Figure 2.3a and c). The negative fouling trend for SRFA and PAA may be caused by electro-osmosis along surfaces of particles deposited on the cathode film similar to observed during electro-filtration experiments [90]. The organic matter rejection ratio in the C+F configuration increased with applied potential as well, from 51% to 73% for SRFA, 14% to 18% for PEG, and 77% to 97% for PAA, but the overall rejections are still slightly lower than F in the presence or absence of cell voltage.

It is of note that among the three foulants, PEG always had the lowest and PAA generally had the highest response to applied potential in terms of both time-dependent permeability and organic matter rejection. PEG is minimally charged and PAA has the highest charge density among the three organic matters indicating that electro-kinetic processes are the predominant fouling reduction mechanism when potential is applied. Even though SRFA has a high charge density, its heterogeneous molecular charge and size distribution may reduce the efficacy of electro-kinetic processes on fouling reduction.

In summary, C+F is the optimal configuration due to moderate hydrodynamic effects and notable electrokinetic enhancements to the time-dependent permeability and rejection of negatively-charged organic matter by UF membranes. The performance enhancements of the C+F configuration are best evaluated with PAA due to its large response to an applied potential. Therefore, the investigation of the electro-kinetic anti-fouling mechanism in the following section will be carried out with the C+F configuration using PAA as a representative foulant.
2.4.3 CAPACITIVE ANTI-FOULING MECHANISM STUDY

In order to quantitatively understand the fouling reduction mechanism upon application of potential to the porous CNT-PVDF cathode, a fundamental study was conducted by examining the PAA 1 hour permeability reduction as a function of anode material, ionic strength, and applied potential. The experiments were supported by calculation of the capacitive surface charge by cyclic voltammetry and physical modeling of the system using DLVO theory.

ELECTRICAL DOUBLE-LAYER CAPACITANCE OF THE CNT-PVDF ELECTRODE

Cyclic voltammetry is an electrochemical technique that can be used to characterize the Faradic reactivity and non-Faradic capacitance of electrodes. Cyclic voltammetry of 10 ppm PAA in 0.1-20 mM NaCl solutions over the working potential range of -1.2 V to 1.2 V is displayed in Figure 4a. No redox peaks are observed and the current density is quite low with a maximum of 0.67 A m⁻² (0.067 mA cm⁻²) indicating the CNT-PVDF membrane is non-Faradaic [81]. The current vs. voltage curve maintained a relatively full and rounded shape and is representative of an electrochemical double-layer capacitor. Both the anodic and cathodic current increased with increasing NaCl concentration.

A simplified physical model is adopted to calculate the capacitance of the cathodic film from the CV curves. Assuming charge transport is rapid in the electrode and the solution as compared to the interfacial charge transfer, the electrode response can be modeled as a capacitor and resistor in parallel. The capacitance of the double-layer can thus be determined accordingly with the following equation [96].

\[ C = \frac{l_a - l_c}{2 \times s} \] (2.6)
where $I_a$ and $I_c$ (A) are the anodic and cathodic current at the open circuit potential and $s$ (V s$^{-1}$) is the potential sweep rate. Other studies have simplified the electrode double-layer model into a single capacitor [97, 98] and determined the capacitance using the following equation [98]:

$$C = \frac{q_a - q_c}{\Delta V}$$  \hspace{1cm} (2.7)

where $q_a$ and $q_c$ (C) represent the anodic and cathodic charge and $\Delta V$ (V) denotes the potential sweep range.

Figure 2.4: Electrical capacitance of the CNT-PVDF as a function of ionic strength. [PAA] = 10 ppm and [NaCl] = 0.1-20 mM. C+F configuration: a) cyclic voltammetry of the conductive CNT-PVDF with a scan rate of 100 mV s$^{-1}$ and b) capacitance of the CNT-PVDF electrode calculated from Eq 2.6.

The capacitance was calculated from the CV data using both Eq 2.6 and 2.7 and the results from Eq 2.7 were consistently around 70% of those from Eq 2.6. The simulation of the CV curve from the capacitor and resistor in parallel model, Eq 2.6, yields a spindle shape while that from the single capacitor model, Eq 2.7, yields a rectangular shape, and the former shape is closer to the experimental curve in Figure 2.4a. Hence, Eq 2.6 was utilized for this study. The specific capacitance (F gC$^{-1}$) of the CNT-PVDF cathode is then calculated by dividing $C$ by the CNT mass.
i.e., the electro-active fraction of the CNT-PVDF membrane (Figure 2.4b). The calculated specific capacitance of the PVDF-CNT cathode increases with NaCl concentration, ranging from 0.656 F gC\(^{-1}\) at 0.1 mM NaCl to 2.4 F gC\(^{-1}\) at 20 mM NaCl, slightly lower than the reported range of 4-135 F g\(^{-1}\) [99] for CNT electrodes due to the insulating PVDF coating.

**CATHODE POTENTIAL DISTRIBUTION**

According to the model, the surface charge of the cathode can be calculated by its capacitance and potential. Relations between electrode capacitance, overall cell capacitance, surface charge, and potentials are summarized in Eq 2.8-10.

\[
Q = C_{cell} \times V_{cell} = C_{cat} \times V_{cat} = C_{ano} \times V_{ano} \quad (2.8)
\]

\[
V_{cell} = V_{cat} + V_{ano} \quad (2.9)
\]

\[
\frac{1}{C_{cell}} = \frac{1}{C_{cat}} + \frac{1}{C_{ano}} \quad (2.10)
\]

where \(Q\) (C) is the capacitor charge accumulation; \(C_{cell}\), \(C_{cat}\), and \(C_{ano}\) (F) are the capacitance of the overall cell, cathode, and anode, respectively, and \(V_{cell}\), \(V_{cat}\), and \(V_{ano}\) (V) are the potentials of the cell, cathode, and anode, respectively. Ideally, when the same CNT-PVDF membrane is used on both the anode and cathode, the cell voltage should be equally distributed on the cathode and anode (Eq 2.7); yet in our system, the cathode potential was only -0.3 V vs. 1 M Ag/AgCl at a 2 V cell voltage in a 10 mM NaCl solution. This is due to a configuration limitation since the anode connection to the Ti current collector is an unpressurized contact, in contrast to the cathode connection that is a pressurized contact. Also, the CNT-PVDF membrane has a relatively low conductivity of \(3.2 \times 10^{-4}\) S m\(^{-1}\). As a result, the measured \(C_{ano}\) is less than \(C_{cat}\), resulting in a \(V_{cat}\) lower than ideal expectations. Based on this observation, an improvement to the C+F configuration was made by replacing the CNT-PVDF anode with a CCP anode. As shown in Table 2.1, the CCP
has a conductivity > 0.08 S m\(^{-1}\) (2 orders of magnitude > PVDF-CNT) and the reported conductivity of similar carbon clothes were 10\(^2\)-10\(^5\) S m\(^{-1}\) [100]. The high CCP conductivity ensures a low potential drop within the capacitive anode. Additionally, the total surface area of the CCP is 2.59 m\(^2\) (Table 2.1), 3.3 times the CNT-PVDF surface area of 0.781 m\(^2\), and will also increase cathodic double-layer capacitance i.e., increased \(C_{ano}\) and \(V_{cat}\). These effects are confirmed experimentally as the cathode potential at 2 V total cell voltage is increased to \(V_{cat} = -1.2\) V with a CCP anode as compared to -0.3 V with a PVDF-CNT anode.

**IONIC STRENGTH EFFECTS ON FOULING REDUCTION**

![Figure 2.5](image)

Figure 2.5: Anti-fouling performance as a function of ionic strength and cathode potential. CNT-PVDF cathode and CNT-PVDF or CCP anode: a) the 1 h normalized permeability vs. aqueous NaCl concentration; [PAA] = 10 ppm, b) the 1 h normalized permeability vs. cathode potential; [PAA] = 10 ppm and [NaCl] = 1 mM. Solid line is the linear correlation of the 1 hr normalized permeability vs cathode potential.

Natural surface waters will contain not only organic contaminants, but also dissolved salts. The typical conductivity of natural surface water is 150-500 \(\mu\)S cm\(^{-1}\) according to US EPA 2012 [101], corresponding to a NaCl concentration range of 1-4 mM. In previous studies, NaCl concentrations of 0-20 mM have been utilized to examine organic matter UF fouling [53, 102-
105]. Here, 10 ppm PAA and 0-20 mM NaCl aqueous solutions were used to examine the electro-
kinetic fouling reduction at 2 V cell voltage for the two anode materials. The cathode potential was 
in the range of -0.19 to -0.27 V for the CNT-PVDF anode and -1.16 to -1.32 V for the CCP anode.
The currents observed in these experiments increase with NaCl concentration and all currents are 
lower than 1.5 mA or a maximum current density of 0.44 A m$^{-2}$ (0.044 mA cm$^{-2}$). Only 1 h 
permeability data were displayed in Figure 2.5 for clarity.

After 1 h of 10 ppm PAA in DI water filtration, the normalized permeability was 0.96 and 
1.08 for the CCP anode and the CNT-PVDF anode, respectively, indicating that the CNT-PVDF 
is effective for nearly complete fouling reduction in DI-PAA solutions for both anode materials. 
The addition of NaCl decreases the 1 h permeability for both anodes. It is of note that the electrode 
capacitance increases with NaCl concentration (Figure 2.4a and b) with resulting increases in 
surface charge density. However, the data indicates the compression of electrical double-layer 
prevails over the increased capacitance. The CNT-PVDF anode system begins fouling at 0.1 mM 
NaCl, at which the 1 h permeability decreases to 0.70, while the CCP anode system does not show 
a similar decrease in 1 h permeability until near 10 mM NaCl. At higher ionic strengths of 10 and 
20 mM NaCl, the 1 h UF permeability dropped to 0.54-0.64 for both anodes similar to the control 
results of 0.60-0.71 in configuration B (data not plotted), again indicating the reduction of the 
Debye layer at high ionic strength can negate capacitive fouling resistance. In summary, increased 
ionic strength lowers the electro-kinetic anti-fouling performance and the CCP anode system has 
 improved salt tolerance as compared to the CNT-PVDF anode.

**CATHODE POTENTIAL EFFECTS ON FOULING REDUCTION**

As discussed in the previous section, utilization of a CCP anode results in a more negative 
CNT-PVDF cathode potential that in turn yields improved anti-fouling performance with 10 ppm
PAA and 0.1-2.0 mM NaCl feed solutions. In order to determine the correlation between the cathode potential and the CNT-PVDF anti-fouling performance, a set of experiments were conducted with 10 ppm PAA, 1 mM NaCl feed solutions, and a CCP anode over a range of cathode potentials (Figure 2.5b). For all experiments, the current remained less than 1.5 mA (current density < 0.44 A m\(^{-2}\) or 0.044 mA cm\(^{-2}\)). The total cell voltage was not increased to values greater than 2.5 V to avoid Ti current collector oxidation and Faradaic electrochemistry. The cathode potential (vs. 1 M Ag/AgCl) became more negative (0.12 V to -1.23 V) with increasing total cell voltage (0 to 2.5 V). The relation between the cathode and cell potential is displayed in Figure A.6. At the highest cathode potential of -1.23 V, minimal fouling is observed with a normalized 1 h permeability of 0.92. The normalized 1 h permeability is inversely and linearly (\(R^2 = 0.99\)) correlated to the cathode potential (\(J_c/J_0 = -0.28V_{cat} + 0.59\)). When the cathode potential with the CCP anode is similar to that of the CNT-PVDF anode at 2 V total cell voltage (overlaid red & black points in Figure 2.5b), the 1 h normalized permeability has a similar value of 0.65. Hence, the electro-kinetic fouling mitigation is closely related to the cathode potential and the effect of anode material on fouling reduction is attributed to the corresponding shift in cathode potential.

**THEORETICAL ANALYSIS: DLVO INTERACTION ENERGY AND COLLISION EFFICIENCY**

The observed electrokinetic fouling reduction is a function of feed solution ionic strength and cathode potential and both will affect the electrical double-layer and the capacitive surface charge. Alternative fouling reduction mechanisms such as organic matter adsorption to the anode and electrophoresis can be excluded. For example, recovery of the feed organic matter content is calculated by dividing the feed TOC at the end of 1 h test by that at the beginning to receive 103 ± 8%. The >100% result is caused by permeate sampling to monitor flow rate and measure TOC
and confirms that organic matter uptake by the anode or any other part of the cross-flow system is negligible. Electrophoresis of the organic matter is also expected to be negligible because the electric field is weak as a result of the low current density; (Ohm’s Law; Eq 2.11).

\[ J = \sigma E \]  

(2.11)

where \( J \) (A m\(^{-2}\)) is the current density, \( \sigma \) (S m\(^{-1}\)) is the solution conductivity, and \( E \) (V m\(^{-1}\)) is the electric field strength. In a 1 mM NaCl solution at 2 V, the electric field strength was \( \sim 20 \) V m\(^{-1}\); orders of magnitude smaller than typical values (kV m\(^{-1}\)) utilized during electro-filtration [90] and in agreement with the limited 1 h fouling reduction in configuration F under applied potential.

Elucidation of the electrokinetic anti-fouling mechanism is assisted by quantitative analysis of the effect of ionic strength and cathode potential on the normalized 1 h permeability. Under the aqueous solution conditions here, both the organic matter and the CNT-PVDF cathode will be negatively charged and therefore it is expected that electrostatic repulsion will play an important role in organic matter fouling reduction. DLVO is a well-accepted theory that describes the interactions between charged particles and surfaces in aqueous media and the total DLVO interaction energy for organic colloids can be calculated by the sum of the van der Waals and the electrostatic double layer forces. It is assumed that the PAA colloids are spherical particles, the CNT-PVDF membrane is a flat plate particle collector with a total area of the measured BET surface area, and that there are no direct interactions between the PAA and UF membrane. Thus, two equations are selected to quantify the van der Waals, Eq 2.12, and electrostatic, Eq 2.13, interactions between the PAA and CNT-PVDF [106]:

\[ V_E = \frac{128\pi a n_0 c kT}{k^2} \gamma_1 \gamma_2 \exp(-\kappa h) \]  

(2.12)

\[ V_A = -\frac{A}{6} \left[ \frac{a}{h} + \frac{a}{h+2a} + \ln\left(\frac{h}{h+2a}\right) \right] \]  

(2.13)
where $V_E$ and $V_A$ (J) are the potential energies for the electrostatic and van der Waals interactions, respectively, $a$ (m) is the particle radius, $h$ (m) is the particle to surface separation distance, $n_o$ (# m$^{-3}$) is the bulk number density of ions (# m$^{-3}$), $\gamma_1$ and $\gamma_2$ denote reduced surface potential ($\text{tanh}(ze\varphi/kT)$) for the particle and the plate (dimensionless), $\kappa$ (m$^{-1}$) represents the reciprocal Debye length, $k$ (m$^2$ kg s$^{-2}$ K$^{-1}$) is the Boltzmann constant, $A$ (J) denotes the Hamaker constant, and $T$ (K) is the solution temperature. It is of note that measuring the surface potentials of the nano-sized PAA particles (d ~10 nm) or cathodic films with external potential is technically difficult. Hence, simplified approximations detailed in the supplementary information (prior to Table A.1) were adopted to estimate the Debye length and surface potential values. The Hamaker constant is assumed to be $10^{-19}$ J as this is within the range of natural and engineered aqueous systems [106].

The DLVO interaction energy profile of PAA with the charged CNT-PVDF cathode (-1.2 V) over a range of ionic strengths (NaCl) is plotted in Figure 2.6a. The primary energy barrier height is in qualitative agreement with experimental data (Figure 2.5a). At low ionic strength e.g., 0.1 mM NaCl, there is high energy barrier that would prevent deposition of PAA. As the salt concentration increases, the barrier height decreases. The theoretical threshold for the disappearance of a deposition energy barrier is between 2 and 10 mM NaCl, in agreement with experimental results indicating no fouling reduction when [NaCl] $\geq$ 10 mM when the total interaction energy curve approaches the van der Waals energy curve.
Complementary to the DLVO approach, Elimelech has developed a semi-quantitative dimensionless constant ($N_{col}$, Eq 14) to predict the collisional efficiency of colloid particles on charged surfaces [107]:

$$N_{col} = \frac{\kappa A}{\epsilon_0 \epsilon f \psi_p \psi_c}$$  \hspace{1cm} (2.14)

The collisional efficiency, $\alpha$, is related to $N_{col}$ by the following equation:

$$\alpha = B (N_{col})^n$$  \hspace{1cm} (2.15)

where $B$ and $n$ are empirical constants. For the ionic strength and cathode potential dependent fouling reduction experiments, $N_{col}$ was calculated and correlated to the normalized 1 h permeability data, Figure 2.6b. There is an inverse correlation between the 1 h normalized permeability data with the calculated $N_{col}$ values. Therefore, the agreement of the calculated DLVO interaction energies and collisional efficiencies with the experimental data results in our conclusion that the electrokinetic fouling reduction mechanism is due to capacitive generation of negative surface charge on the PVDF-CNT cathode.
2.4.4 PRACTICAL IMPLICATIONS

To demonstrate the potential of capacitive fouling reduction for drinking water applications, extended UF experiments were carried out with synthetic solutions mimicking natural surface waters utilizing the optimal anti-fouling system: the C+F configuration with a CCP anode and a CNT-PVDF cathode. SRFA and ALG were chosen as representative molecular and colloidal organic matter. A moderate ionic strength of 1 mM NaCl was used for comparison to DI solutions. Calcium, another common cation in natural surface water, was also used. Unlike the monovalent sodium ion, the divalent calcium ion can act as a bridge between organic matter and a membrane surface [58, 108, 109], in particular if carboxylate groups are present, resulting in increased fouling [69]. In order to evaluate the effect of capacitive surface charge on organic matter fouling in the presence of calcium, 0.067 mM CaCl$_2$ and 0.8 mM NaCl were added to the 10 ppm SRFA and ALG solutions to yield the same ionic strength as a 1 mM NaCl solution. The normalized time-dependent cross-flow permeabilities (Figure 2.7a and b) were fitted to Eq 2.5 for quantitative comparison with the control (B) experiments and the results are listed in Table 2.4.

Capacitive fouling reduction at 2 V total cell voltage as compared to no applied potential was observed under all solution conditions (Figure 2.7). The fitted values for $L$ and $k$, which represent the initial permeability loss and the time-dependent fouling rate constant, are consistently smaller in the C+F configuration as compared to the control system. For SRFA in configuration B, the initial permeability loss $L$ is 0.150-0.235 for all solutions and the fouling rate $k$ is $0.189$ h$^{-1}$ for unsalted solution and much smaller, $0.022-0.030$ h$^{-1}$, for the salted solutions. Increased SRFA fouling with ionic strength has been reported because of double layer compression [91]. In contrast, the reduced fouling at increased ionic strength observed here is likely due to SRFA compaction at increased ionic strength resulting in lower rejection (69% no salt, 58% 1 mM NaCl,
Extended time-dependent permeabilities for the C+F and control configurations over a range of solution conditions. a) [SRFA] = 10 ppm, b) [ALG] =10 ppm with no salt, [NaCl] = 1 mM, and [CaCl$_2$] = 0.067 mM + [NaCl] = 0.8 mM, in configuration C+F with CCP anode and CNT-PVDF cathode at a cell voltage of 2V or in configuration B, c) Organic matter removal percentage under different solution conditions in the cross-flow filtration, and d) photos of the membrane after 3 h test in [SRFA] = 10 ppm and [NaCl] = 1 mM with and without 2 V cell voltage.

The fouling was capacitively reduced to similar levels for all the SRFA solutions when a 2 V cell voltage was applied. The capacitive $L$ values are all $<0.026$; 6 to 15-fold less than the control (B) values indicating the C+F configuration effectively eliminates initial rapid organic matter accumulation near and/or on the membrane.
surface. The fouling rate constant, $k$, decreases significantly as well from 0.189 to 0.021 h$^{-1}$ for the unsalted, from 0.022 to 0.015 h$^{-1}$ for the NaCl, and from 0.030 to 0.016 h$^{-1}$ for the CaCl$_2$ solutions.

The capacitive SRFA fouling reduction can also be visually observed in the images of the control and C+F membrane surfaces (Figure 2.7d) after 3 h of SRFA cross-flow ultrafiltration.

Table 2.4: Fitting results for the extended time-dependent permeability experiments.

<table>
<thead>
<tr>
<th>Organics</th>
<th>No salt</th>
<th>[NaCl] = 1 mM</th>
<th>[CaCl$_2$] = 0.067 mM</th>
<th>[NaCl] = 0.8 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>C+F-2 V</td>
<td>B</td>
<td>C+F-2 V</td>
</tr>
<tr>
<td>SRFA</td>
<td>$L$</td>
<td>0.150</td>
<td>0.024</td>
<td>0.165</td>
</tr>
<tr>
<td></td>
<td>$k(h^{-1})$</td>
<td>0.189</td>
<td>0.021</td>
<td>0.022</td>
</tr>
<tr>
<td></td>
<td>$R^2$</td>
<td>0.951</td>
<td>0.988</td>
<td>0.731</td>
</tr>
<tr>
<td>ALG</td>
<td>$L$</td>
<td>0.206</td>
<td>-0.027</td>
<td>0.370</td>
</tr>
<tr>
<td></td>
<td>$k(h^{-1})$</td>
<td>0.118</td>
<td>0.084</td>
<td>0.294</td>
</tr>
<tr>
<td></td>
<td>$R^2$</td>
<td>0.671</td>
<td>0.976</td>
<td>0.877</td>
</tr>
</tbody>
</table>

* The lowest two values for $L$ and $k$ are in bold, and if one of the two values is significantly lower than the other, it is placed in italics.

Capacitive fouling reduction is also observed during ALG cross-flow filtration (Figure 2.7b). Similar to PAA, ALG displayed a greater response to capacitive fouling reduction due to its higher negative charge density of 13.9 meq gC$^{-1}$ (Table 2.2). For the ALG control experiments in configuration B, the addition of salt resulted in more severe fouling. The initial fouling $L$ increased from 0.206 for the DI to 0.370 for NaCl and 0.295 for CaCl$_2$ and the fouling rate constant, $k$, increased from 0.118 h$^{-1}$ for the DI to 0.294 h$^{-1}$ for NaCl and 0.532 h$^{-1}$ for CaCl$_2$, respectively. For the ALG capacitive anti-fouling experiments, the initial fouling $L$ is close to zero at -0.027 for the DI and 0.020 for NaCl and increases to 0.131 for the CaCl$_2$ and in all cases is significantly lower than the control experiments. The ALG capacitive fouling rate constant, $k$, values (0.082-0.194 h$^{-1}$) are also decreased (29-85%) as compared to the control. The results again confirm that the 2 V
capacitive CNT-PVDF negative surface charges are able to reduce organic matter deposition onto and fouling of UF membranes and also indicate that the electrically-induced surface charge is not very active towards calcium ion organic-membrane bridging phenomenon that could result in increased fouling.

The extended fouling experiments indicate that significantly less backwashing will be required with the capacitive anti-fouling system as compared to the control. For example, if it is assumed that backwash is needed when $J_t = 0.5J_0$, then backwash would be required once every 4 h for the capacitive CNT-PVDF system with a 10 ppm ALG & 1 mM NaCl solution and every 6 h for the capacitive CNT-PVDF system with 10 ppm ALG & 0.067 mM CaCl$_2$ + 0.8 mM NaCl as compared to every 0.5 h for the control PES membrane under the same solution conditions.

The capital and O&M costs of the capacitive anti-fouling system are compared to the control system by analysis of the time-dependent permeability data. According to Schafer et al., 4.2-4.6 US$/1000 m^3$ is the operating cost for surface water ultrafiltration and the membrane cost is 11.2 US$/1000 m^3$ assuming a 5-year membrane lifetime [53]. Operating the capacitive electrodes at 2 V, the energy required for the UF of SRFA and ALG solutions is reduced by 84% and 54% in 1 mM NaCl, and 75% and 37% in the 0.067 mM CaCl$_2$ and 0.8 mM NaCl solution according to the integration of the energy cost over the 3 h experiments, saving 0.7-2.9 US$/1000 m^3$. In addition, backwash frequency can be significantly reduced (<1/4 to 1/8 of the control) resulting in more net water production and less chemical consumption. This will further cut the energy costs by approximately 0.4 US$/1000 m^3$ with the assumption that the backwash consumes ~10% of the purified water production. In comparison, the increased energy consumption by the capacitive CNT-PVDF charging is negligible (~ 0.1 US$/1000 m^3$). Thus, the total O&M energy savings is 1.0-3.2 US$/1000 m^3$. The increased material cost of the CNT-PVDF is also considered. The CNT-
PVDF membrane has a CNT load of 0.7 g m\(^{-2}\), corresponding to an increased cost of \(~0.1\) US$/m\(^2\) considering the commercial MWNT price of 100 US$/kg [46]. The commercial carbon cloth cost is \(~5.0\) US$/m\(^2\), resulting in a total material cost increase of 5.1 US$/m\(^2\) on an industrial scale, comparable with a recent study where 2.5 US$/m\(^2\) cost increase was expected from the addition of a thin CNT deposition layer on top of a tight NF membrane to reduce biofouling [80]. This is equivalent to \(~15\text{-}30\%\) of the total UF membrane cost of 15-30 US$/m\(^2\) based on current industrial prices [53]. In summary, it will take 1.5-5 years to pay back the additional material cost from O&M energy savings assuming a membrane flux of 120 LMH and an energy cost reduction of 1.0-3.2 US$/1000 m\(^3\).

The capacitive electrodes examined have potential to be incorporated into practical modules, such as the spiral wound and the plate and frame modules, for which the membrane-spacer-membrane stack is well established. Further research will be needed for scale-up and optimization. In particular, design to ensure homogenous potential distribution [110] such as optimization of current collector geometry and CNT-PVDF conductivity will be necessary for larger area membranes. The CCP counter electrode used here is expensive and alternatives should be explored. The capacitive electrodes can also be fabricated as an active layer [80] on the UF membrane to reduce CNT material cost and negative hydrodynamic effects. Finally, the energy budget of the capacitive anti-fouling system requires more systematic research, for instance, the hydrodynamics of a commercial UF module are typically better than the laboratory module used in this study [95] and a natural feed solution will contain a range of impurities that could influence the performance of this system.
2.5 CONCLUSIONS

A cathodic CNT-PVDF membrane on a PES UF membrane is able to capacitively-reduce negatively-charged organic matter fouling. The greatest electrokinetic fouling reduction was observed when the permeate first flows through the porous anode, then the porous cathode, and finally the UF membrane. The electrokinetic fouling reduction is increased as the negative charge on the organic matter increases. A systematic study as a function of ionic strength and cathode potential using PAA as a model foulant indicated the fouling reduction is due to negative capacitive charging of the cathodic CNT-PVDF membrane and confirmed by calculations of the theoretical DLVO electrostatic energy barrier and collision efficiency between the organic colloids and the cathode surface. Extended ultrafiltration experiments with Suwanee River fulvic acid and alginate solutions demonstrated that capacitive system has increased operational energy-efficiency as compared to the control and that the induced surface charge does not undergo ion bridging with calcium. The operational energy costs of the capacitive system are 37-84% lower than the control system depending on solution conditions and accounting for the increased capital (material) cost the payback time is 1.5-5 years.
CHAPTER 3  
SEMIQUANTITATIVE PERFORMANCE AND MECHANISM EVALUATION OF  
CARBON NANOMATERIALS AS CATHODE COATINGS FOR MICROBIAL  
FOULING REDUCTION  

3.1 ABSTRACT  

In this study, we examined bacterial attachment and survival on a titanium (Ti) cathode coated with various carbon nanomaterials (CNM): pristine carbon nanotubes (CNT), oxidized carbon nanotubes (O-CNT), oxidized-annealed carbon nanotubes (OA-CNT), carbon black (CB), and reduced graphene oxide (rGO). The carbon nanomaterials were dispersed in an isopropyl alcohol-Nafion solution and then dip-coated onto a Ti substrate. *Pseudomonas fluorescens* was selected as the representative bacterium for environmental biofouling. Experiments in the absence of electric potential indicate that increased nanoscale surface roughness and decreased hydrophobicity of the CNM coating decreased bacterial adhesion. The loss of bacterial viability on the non-charged CNM coatings ranged from 22% for the CB to 67% for the OA-CNT and was dependent on the CNM dimensions and surface chemistry. For electrochemical experiments, the total density and percent inactivation of the adhered bacteria was semi-quantitatively analyzed as a function of electrode potential, current density, and hydrogen peroxide generation. Electrode potential and hydrogen peroxide generation were the dominant factors in regard to short-term (3 h) bacterial attachment and bacterial inactivation, respectively. Extended electrochemical experiments (12 h) indicated that in all cases the total deposited bacterial density increased linearly with time and that the rate of bacterial attachment was decreased 8-10 fold when an electric
potential was applied. In summary, this study provides a fundamental rationale for selection of CNM as a cathode coating to reduce microbial fouling in the absence and presence of an electric potential.

3.2 INTRODUCTION

Biofilm formation is ubiquitous in aquatic environments and undesirable for industrial systems such as heat exchangers and ship hulls [111] as well as engineered environmental systems such as membrane filters [9] and water distribution pipelines. A critical initial stage of biofouling involves microorganism adhesion and formation of the primary slime layer that allows for continued biofilm development [112, 113]. Thus reducing microbial fouling may offer opportunity for biofouling control.

Continuous research efforts have been devoted to microbial fouling control from antimicrobial surface development [114] to bacterial biofilm ecology disturbance by quorum sensing [115, 116]. In regard to the antimicrobial surface, the interfacial energy between a surface and water has been identified as a key factor and hydrophilic surfaces generally hinder protein adsorption and in turn reduce microbial attachment [117]. A highly charged cationic surface has also been demonstrated to kill bacteria and reduce fouling [118]. However, as microbes have a complex and species-dependent surface chemistry, a permanently modified surface will not reduce biofouling for all microbes and decreased antifouling performance is often observed in complex environments. One solution may be active biofouling reduction that can be tuned in situ to reduce biofouling for a range of species. Active chemical fouling reduction typically relies on the slow release of biocides from a surface [114], yet this method arouses many environmental concerns and is restricted to a limited lifetime after the majority of the biocide is released. In contrast, photo- or electro-chemical methods are among the most effective, easily automated, and environmentally-
friendly ways to reduce microbial fouling [20, 21] since the amount and time-dependent release of chemicals can be controlled via a feedback loop. Photo- and electro-chemical fouling reduction studies can be broadly divided into two categories—fundamental mechanism and novel material investigations.

In regard to the electrochemical mechanism of biofouling reduction, the influence of electric current or potential on bacterial adhesion [119], detachment [20, 120-122] and inactivation [19, 37, 122] has been widely examined. The most commonly accepted theories are that the cathode repels bacteria via electrostatic interactions and the anode inactivates bacteria through direct and indirect oxidation [26, 113]. However, some of the fundamental phenomena are still not clearly understood. For instance, Kerr et al. [119] observed reduced cathodic bacterial attachment; however, the magnitude of reduction was independent of cathode potential in disagreement with the classical electrostatic repulsion model i.e., Derjaguin-Landau-Verwey-Overbeek (DLVO) theory. Limited attention has been paid to the possibility of cathodic surface disinfection e.g., the reduction of O$_2$ to reactive oxygen species (ROS). Also, most of the electrochemical biofouling reduction literature provides only qualitative relations between fouling reduction performance and electrochemical parameters. Therefore, one primary objective here is to provide (semi-)quantitative relationships between bacterial attachment/inactivation and electrochemical parameters.

Recent electrochemical biofouling research has also focused on the synthesis and application of novel electrode materials. Electrochemistry occurs at the electrode-electrolyte interface and thus for non-conductive surfaces, a conductive coating is required. Carbon-based materials like carbon black (CB) and graphite doped in a polymer matrix [19, 26] have been previously used as electrode coating materials since they are stable and inexpensive. More
recently, the investigation of engineered carbon nanomaterials such as carbon nanotubes (CNT) have shown promise for electrochemical biofouling reduction due to superior antifouling properties i.e., increased cytotoxicity and conductivity [123, 124]. The nanosize also allows for dispersion in a range of matrices and a reduced material demand [125]. For instance, de Lannoy et al. (2013) dispersed CNT into a polyamide thin film (~50 nm) to produce electrically-conductive polymer-nanocomposite nanofiltration membranes that significantly reduced biofilm formation [126]. Nevertheless, there is more to exploit in this area since CNT properties can be tuned via chemical modification to increase cytotoxicity [127-129] and conductivity [130]. It is also of interest to compare CNT with conventional CNM such as CB and emerging CNM such as graphene or reduced graphene oxide (rGO) i.e., CB, CNT, and rGO have different number and magnitude of dimensions on the nanoscale and in turn the coating morphology is variable. Thus, another primary objective of this research is to compare the electrochemical biofouling reduction of engineered carbon nanomaterial coatings with different structures and surface chemistries.

Here, *Pseudomonas fluorescens* (*P. fluorescens*, ATCC® 700830™) is utilized as a representative bacterium for bacteria attachment and potential biofilm development [131-133] and three CNT, CB, and rGO are utilized as the cathode coating on a Ti substrate for electrochemical biofouling reduction. All experiments were carried out in an artificial aqueous environment of 0.9% (155 mM) NaCl saline solution containing a 1/8 dilution of tryptic soy broth (TSB), which is a relative nutritious aqueous condition compared to natural environment, but close to some extreme circumstances like food processing wastewater where severe biofouling may take place. A detailed comparison of these aqueous conditions is listed in Table B.1. First, bacterial adhesion and inactivation on the CNM coatings in the absence of applied potential was evaluated as a control for electrochemical fouling reduction. Next, a negative voltage (-0.4 to -2.0 V) vs. counter
electrode (carbon cloth anode) was applied to the CNM coated Ti cathode and the attached bacterial surface density and percent inactivation were semi-quantitatively correlated to electrode potential, current density, and hydrogen peroxide generation. Finally, extended electrochemical anti-biofouling experiments were completed over 12 h to evaluate the kinetics of electrochemical microbial fouling reduction.

3.3 MATERIALS AND METHODS

3.3.1 MATERIALS

Carbon nanotubes (C-grade; multi-walled; powder and bulk paper) were purchased from NanoTechLabs, Inc. (Yadkinville, NC). The oxidized CNT (O-CNT) were prepared by sparging O$_3$ (5-10 mg L$^{-1}$) into an NaOH solution (pH 10) and filtering the O$_3$-NaOH solution through CNT buckypaper in a commercial filtration casing (Whatman) while applying an anodic current of 3.2 mA cm$^{-2}$ (40 mA total; effective filtration area of 12.5 cm$^2$; total voltage varied from 4 to 8 V) for 60 min using a direct current (DC) power supply (Agilent E3646A). The O-CNT were annealed at 600 °C (Thermolyne, 21100) under an inert (Ar) atmosphere for 30 min to produce the OA-CNT [128]. CB (Vulcan XC72-R) was kindly donated by Cabot (Boston, MA) and used as received. Reduced graphene oxide (rGO) was used as a representative 2D carbon nanomaterial and synthesized using a modified Hummer’s method and subsequently photoreduced following previously reported methods [134]. Nafion® 117 solution (~5% in a mixture of lower aliphatic alcohols and water) and formaldehyde (35% in water) was purchased from Sigma Aldrich (St. Louis, MO). *P. fluorescens* (ATCC® 700830™, Manassas, VA) was utilized in the microbial attachment and inactivation experiments. Bacto™ tryptic soy broth (TSB; Becton Dickinson), NaCl (reagent grade), isopropyl alcohol (IPA), and ethanol (laboratory grade) were purchased
from VWR International (West Chester, PA). The fluorescence staining reagents of 4',6-
diamidino-2-phenyindole dilactate (DAPI) and propidium iodide (PI) were acquired from Life
Technologies (Grand Island, NY). Deionized (DI) water (>18 MΩ) was produced by a Nanopure
Infinity Ultrapure Water System (Barnstead/Thermolyne) and was used to prepare all solutions
and rinse containers.

3.3.2 COATING THE TI ELECTRODE WITH CARBON NANOMATERIALS

The CNT, O-CNT, and OA-CNT were first dispersed in IPA with Nafion by probe
ultrasonication and then dip-coated onto a Ti electrode [135, 136]. Briefly, 20 mg of CNT and 400
µL of the 5% Nafion solution were added to 20 mL of IPA and ultrasonicated (Branson, Sonifier
S450D) at 20 W (1000 W L⁻¹) for 15 min. The solution was covered by a piece of aluminum foil
to avoid excessive solvent evaporation. Similar procedures were followed for CB and rGO
dispersions, except that the concentration of the carbon material was increased from 1 to 5 mg mL⁻¹
(with the same carbon/Nafion ratio) to ensure full surface coverage. The CNM dispersions were
then allowed to cool to room temperature. The Ti coupons (L:W:H; 4 × 2 × 0.15 cm) used as the
electrode substrate were first polished with sandpaper (3M 413Q; grit rating 220), placed in an
ultrasonication bath (Branson 2100) for 5 min to remove attached particles, rinsed with copious
amounts of tap water, DI water, and ethanol in series, and finally dried with compressed air. The
clean Ti coupons were coated by immersion in freshly prepared CNM solutions for 5 s and then
hung vertically in an oven at 60 °C for 2 min. The coated electrodes were kept in sterile containers
until bacterial experiments or material characterization was completed.
3.3.3 BACTERIAL ATTACHMENT EXPERIMENTS

*P. fluorescens* was cultured in TSB at 30 °C and harvested at the mid-exponential phase (18 h). After centrifugation and resuspension twice in 0.9% NaCl saline solution, the bacterial stock solution was diluted to OD$_{600} = 0.15$ in saline solution (~$10^8$ mL$^{-1}$ according to fluorescence microscopy enumeration). Finally, 1/8 the volume of TSB was added to the bacterial saline solution to provide essential nutrients, and the resulting solution contained 2.1 g L$^{-1}$ pancreatic casein digest, 0.4 g L$^{-1}$ papaic soybean digest, 0.3 g L$^{-1}$ dextrose, 0.3 g L$^{-1}$ K$_2$HPO$_4$, and 9.6 g L$^{-1}$ NaCl.

Figure 3.1: Schematic of the experimental setup to examine bacterial deposition and inactivation. Typically, the Ti with CNM coating was used as the cathode and the CCP was used as the anode. The bacteria solution was placed in 30 °C water bath and agitated with a magnetic bar at 90 rpm.

A scheme of the bacterial attachment experimental setup is displayed in Figure 3.1 and a photo of the system is shown in Figure B.1. Approximately 25 mL of the bacterial suspension was added to a 30 mL glass beaker and maintained at 30°C using a water bath. The suspension was agitated by a magnetic stir bar at 90 rpm. The electrochemical experiments were carried out using the classical two-electrode system. The CNM coated electrode was used as the working electrode and a piece of carbon cloth (CCP; L:W:H; 4 × 2 × 0.02 cm; Carbon Cloth Plain CCP-30; Fuel Cell Earth; Wakefield, MA) was used as the working electrode and a piece of carbon cloth
(CCP;L:W:H, 4 × 2 × 0.02 cm; Carbon Cloth Plain CCP-30; Fuel Cell Earth; Wakefield, MA) was used as the counter electrode. The two electrodes were pre-wetted by a 10/90 (v/v) EtOH/DI water solution to eliminate air bubbles, rinsed with DI water, and then fixed onto a glass slide with rubber bands prior to insertion into the bacterial suspension. The distance between the electrodes was 1.2 cm and the total applied voltage (-0.4 to -2.0 V) was controlled by a DC power supply (Agilent E3646A). The sign given to the total voltage (-/+) indicates the polarity of the CNM coated electrode and in the majority of the experiments the CNM had a negative polarity. The cathode potential (vs. 1 M Ag/AgCl standard reference electrode) was -0.3 to -1.2 V over the total voltage range of -0.4 to -2.0 V. The current of the system was monitored by a digital multimeter (Agilent 34401A). In general, the experiments to evaluate the effect of CNM coating material and electrochemical parameters on *P. fluorescens* attachment and inactivation lasted 3 h. To examine extended bacterial attachment and inactivation kinetics, the electrode surface was examined at 0.5, 3, 6, and 12 h. All bacterial attachment experiments were completed in at least duplicate and two non-charged samples were analyzed for each batch of bacterial solution as a control.

After incubation of the electrodes in a bacterial solution in the absence or presence of electric potential, the cathode was removed from solution and first rinsed to remove the non-attached bacteria by immersion in ~5 mL of saline solution with light agitation for 30 s. Subsequently, the cathode was quickly and lightly wiped with a paper pad to remove excess solution. Finally, the cathode was placed in a beaker containing 10 mL of saline solution and vortexed at ~1500 rpm (VWR 58816-121) for 2 min. Disposable sterile or autoclaved containers were used to handle all solutions. Preliminary experiments (Table B.2) indicated that 2 min of vortexing was able recover >95% of the bacteria from the coupon while retaining the percentage of the inactivated bacteria similar to direct fluorescence observation. A fraction of the vortexed
solution was then vacuum filtered onto a 0.2 µm gray polycarbonate membrane (Sterlitech; Kent, WA) and stained with PI (cells with compromised membranes; excitation/emission at 535/617 nm) for 10 min and DAPI (total cells; excitation/emission at 358/461 nm) for 5 min, respectively. The filtered solution volume was controlled such that there were 100-300 cells in the field of view (278 × 200 µm²). The stained samples were quantified using an epifluorescent microscope (Olympus BX60) with a 40X objective lens. At least 5 images of random locations on each filter were recorded by a digital camera (Diagnostic Instrument Spot RT color) for cell enumeration. The density of the bacteria on the original cathode was calculated using Eq 3.1:

\[ d_{coating} = \frac{N}{A_{photo} V_{vortex} A_{vortex}} \frac{A_{filter} V_{vortex}}{V_{filter}} \]  

(3.1)

where \( d_{coating} \) (bac cm\(^{-2}\)) is the density of the bacteria on the CNM electrode, \( N \) (dimensionless) denotes the bacterial counts from epifluorescent images, \( A_{filter}, A_{photo}, \) and \( A_{vortex} \) (cm\(^2\)) are the area of the polycarbonate membrane, the area of the microscope image, and the area of the coated sample for vortexing, respectively, and \( V_{vortex} \) and \( V_{filter} \) (mL) denote the volume of bacteria solution vortexed and the volume filtered onto the polycarbonate membrane for cell counting, respectively.

### 3.3.4 QUANTIFICATION OF CATHODIC H\(_2\)O\(_2\) ELECTRO-GENERATION

The potential contribution of electrochemical ROS to bacterial viability loss was examined by measuring cathodic H\(_2\)O\(_2\) production. During cathodic electrolysis, H\(_2\)O\(_2\) can be generated by reducing dissolved oxygen [137, 138] and H\(_2\)O\(_2\) is known to be detrimental to bacteria [139, 140]. To promote H\(_2\)O\(_2\) generation and avoid loss via reaction with bacteria or broth, the electrodes were immersed in 0.9% NaCl saline solution that was sparged with pure oxygen in the absence of bacteria and broth. Similar voltages to the bacterial attachment/inactivation experiments were
applied for 5 min. The solution was also more vigorously (120 rpm) stirred to ensure the dispersion of the reactive oxygen species to prevent further reduction to hydroxyl radical or water. After 5 min of electrolysis, 1 mL of the solution was removed for H$_2$O$_2$ analysis following the ammonium molybdate-iodide method [141].

3.3.5 CNM SURFACE COATING CHARACTERIZATION

The CNM surface coatings were characterized by scanning electron microscopy (SEM), atomic force microscopy (AFM), and X-ray photoelectron spectroscopy (XPS). The CNM surface coating contact angle and conductivity was also measured.

SEM was completed on a Zeiss FESEM UltraPlus. Surface images were taken to compare the nanostructure of the different coatings and cross-sectional images were utilized to determine the coating thickness. The surface morphology was also characterized by AFM (Asylum-1 MFP-3D AFM System) to determine surface roughness (estimated from a sample area of 1.5 × 1.5 μm$^2$). SEM was also used to examine the bacteria morphology on the CNT coating without or with -2 V total voltage after 3 and 12 h of contact. The samples were prepared by rinsing for 30 s in saline solution to remove the non-attached cells, placed in a sealed container with formaldehyde for vapor fixation (12 h), followed by dehydration in 40-100% ethanol/DI solutions, and finally completely dehydrated with a critical point drier (Tousimis 931 GL 2.5).

XPS was carried out on a Thermo Scientific K-Alpha XPS. A survey scan was used to determine elemental ratios, and individual element scans of C and O were used to determine individual functional group ratios. Casa XPS software was utilized to quantify the XPS data using Gaussian components after a Shirley back-ground subtraction. The XPS peaks are deconvoluted into four categories; 284.4 - 285.3 eV for the sp$^2$ and sp$^3$-hybridized carbon atoms (C=C, C-C, or C-H) and 286.4-286.6 eV, 287.3-287.4 eV, and 288.8-289.3 eV for carbon atoms bound to oxygen
atoms via one (C-O), two (C=O), and three bonds (O-C=O) [142-144]. DI water contact angle measurements of the clean CNM coating and CNT coatings after bacterial experiments (dried) were completed using a goniometer (Rame-Hart 190CA) via the sessile drop technique. At least 2 random samples were analyzed and at least 12 measurements were completed on each sample.

For the conductivity measurement, the CNM was dip-coated onto a clean glass slide (VWR® Superfrost® Plus Micro Slide) following the procedure used for the polished Ti substrates. The sheet resistance of the coatings was determined with a conductivity analyzer (Keithley 2635A System SourceMeter®) using the four-point probe method. The bulk resistivity was calculated by multiplying the measured sheet resistance by the film thickness (as characterized by SEM cross-sectional images).

3.4 RESULTS

3.4.1 MATERIAL CHARACTERIZATION

The physical-chemical properties of the CNM coating materials and the coatings are listed in Table 3.1. SEM images of the CNT, O-CNT, CB, and rGO coatings are displayed in Figure 3.2. A photo of a polished Ti coupon and coupons with different CNM coatings is shown in Figure B.2.

The CNT surface chemistry is altered by the ozone-electrochemical oxidation and annealing treatments. The O/C ratio of the pure CNM increased from 0.00 for the CNT to 0.09 for the O-CNT, while that for the corresponding coating with Nafion increased from 0.18 to 0.25. The water contact angle of the CNT vs. O-CNT coating slightly decreased from 135.8 ± 2.1° to 129.6 ± 3.0°. Annealing the O-CNT to OA-CNT at 600 °C for 30 min under inert conditions reduced the O/C ratio without Nafion to 0.05, slightly increased of the O/C ratio on the coating with Nafion to
0.27 (likely due to improved interactions of OA-CNT with the Nafion), and increased the contact angle of the coating to 134.5 ± 3.7°. The CNT functional groups are also different according to the Table 3.1: Physical-chemical properties of the carbon nanomaterials and their coatings.

<table>
<thead>
<tr>
<th>Carbon Nanomaterials (w/o Nafion)</th>
<th>O/C</th>
<th>Curve fitting result of C(1s) spectra&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Main dimensions</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNT</td>
<td>0.004</td>
<td>1.000</td>
<td>N/D&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>O-CNT</td>
<td>0.092</td>
<td>0.882</td>
<td>N/D</td>
</tr>
<tr>
<td>OA-CNT</td>
<td>0.054</td>
<td>0.956</td>
<td>0.044</td>
</tr>
<tr>
<td>CB</td>
<td>0.015</td>
<td>1.000</td>
<td>N/D</td>
</tr>
<tr>
<td>rGO</td>
<td>0.404</td>
<td>0.531</td>
<td>N/D</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Coating (with Nafion)</th>
<th>O/C</th>
<th>Contact Angle (°)</th>
<th>Roughness Ra (nm)</th>
<th>Thickness (nm)</th>
<th>Electric Resistivity (Ω cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNT</td>
<td>0.180</td>
<td>135.8 ± 2.1</td>
<td>2.86</td>
<td>150 ± 5</td>
<td>0.029</td>
</tr>
<tr>
<td>O-CNT</td>
<td>0.254</td>
<td>129.6 ± 3.0</td>
<td>4.58</td>
<td>155 ± 5</td>
<td>0.042</td>
</tr>
<tr>
<td>OA-CNT</td>
<td>0.265</td>
<td>134.5 ± 3.7</td>
<td>3.41</td>
<td>120 ± 5</td>
<td>0.056</td>
</tr>
<tr>
<td>CB</td>
<td>0.119</td>
<td>124.5 ± 2.2</td>
<td>4.82</td>
<td>400 ± 5</td>
<td>0.950</td>
</tr>
<tr>
<td>rGO</td>
<td>0.270</td>
<td>96.9 ± 3.2</td>
<td>1.58</td>
<td>&lt;5&lt;sup&gt;e&lt;/sup&gt;</td>
<td>N/A</td>
</tr>
</tbody>
</table>

<sup>a</sup>: There are some discrepancy between the O/C analysis and the deconvolution of the C(1s) spectra, and the difference was within 30% for the O-CNT, OA-CNT, and rGO. The XPS files for the rGO sample can be find in Figure S3 for reference of the two methods used.

<sup>b</sup>: None-detected (<0.001) from the curve fitting result of C(1s) spectra.

<sup>c</sup>: According to supplier specification.

<sup>d</sup>: There is a proportion of 0.055 of the carbon atoms fitted at the peak of 290.9 eV (C-F bond), which are probably the contamination on the sample surface from the fluorinated tubing in the oxidizing system and not listed in this table.

<sup>e</sup>: Below the detect limit (~5 nm) by SEM observation of the cross section.
deconvolution of the XPS C(1s) spectra. The pristine CNT contains minimal oxygen groups, and the O-CNT has a proportion of 0.12 of carbon atoms highly oxidized in the form of O-C=O (higher than the O/C ratio of 0.09 due to discrepancy of different analysis methods). After annealing, 0.044 of the carbon atoms on OA-CNT are bound to oxygen groups via one bond (C-O).

Figure 3.2: SEM images of the 1) CNT, 2) O-CNT, 3) CB, and 4) rGO coatings on Ti substrate.

The morphology of the CNT coatings is influenced by oxidative and thermal treatments. The as-received CNT are 15-20 nm in diameter and 100 µm in length [145]. The O-CNT have a shorter length as compared to the CNT as some tube lengths around 1 µm can be observed by SEM (Figure 3.2). The CNT coating has a nanoscale surface roughness of 2.86 nm and the O-CNT coating of 4.58 nm based on the AFM estimation from an area of 1.5 × 1.5 µm² (roughly the projection area of a single bacterium). The greater nanoscale roughness of the O-CNT is likely related to their shorter length, increased number of tips, and the addition of functional groups. No dimensional changes to the individual tubes was observed for the OA-CNT as compared to the O-CNT, yet the nanoscale surface roughness of the corresponding coating decreased to 3.41 nm, likely due to removal of functional groups on the CNT tips or walls [146]. The CNT, O-CNT, and OA-CNT coatings have a similar thickness of 150 ± 5, 155 ± 5, and 120 ± 5 nm, respectively.
The other CNM i.e., CB and rGO are quite different from the CNT in both surface chemistry and coating morphology. CB is a hydrophobic material with an O/C ratio of 0.02. The contact angle of the CB coating was 124.5 ± 2.2°. The rGO had the highest oxygen content (O/C ratio = 0.40 with 0.07 of the C in the form of O-C=O and 0.40 in the form of C=O) with a contact angle of 96.9 ± 3.2°, significantly lower than the other CNM coatings examined.

In regard to the CB/rGO coating morphology, the nanoscale surface roughness of the CB coating is 4.82 nm, highest among all the CNM utilized in this study since CB consists of spherical nanoparticles with an average diameter of 60 nm. The CB coating is also much thicker (400 ± 5 nm) than the other CNM, which was necessary to achieve full surface coverage. The rGO are 1 µm wide and 1.5 nm thick flakes and formed an extremely thin (<5 nm) and smooth (nanoscale surface roughness = 1.58 nm) coating on the Ti substrate.

The electrical resistivity of the CNT coating is the lowest at 0.029 Ω cm and CNT oxidation and subsequent annealing increased the resistivity to 0.042 and 0.056 Ω cm, respectively. The CB has a bulk resistivity of 0.950 Ω cm, around 30-fold greater than the pristine CNT. The rGO resistivity was nearly 10 orders of magnitude greater than the CNT due to its highly oxidized structure. However, an accurate resistivity measurement for the rGO cannot be made due to the lack of an accurate rGO thickness measurement i.e., the coating was too thin to be accurately determined from the SEM cross-sectional image.

### 3.4.2 BACTERIAL ATTACHMENT IN THE ABSENCE OF APPLIED POTENTIAL

*P. fluorescens* attachment and inactivation after 3 h incubation in the absence of applied potential was initially assessed as a control for the electrochemical experiments and the results are presented in Figure 3.3a.
Figure 3.3: Live/dead bacterial density on the CNM coatings a) in the absence and b) presence of -1 V and -2 V total voltage. Analysis is completed after 3 h incubation at 30°C in 0.9% NaCl saline solution in 1/8 TSB. Shaded sections represent the inactivated bacterial density and the white sections represent the live bacterial density. The inactivated percent is displayed on top of each column.

*P. fluorescens* has the highest affinity for the CNT coating with a total bacterial attachment of \((1.36 \pm 0.20) \times 10^6 \text{ cm}^{-2}\) that decreased to \((0.90 \pm 0.10) \times 10^6 \text{ cm}^{-2}\) and \((0.72 \pm 0.06) \times 10^6 \text{ cm}^{-2}\) for the OA-CNT and O-CNT coatings, respectively. The total bacterial density on the CB and rGO coatings was \((0.65 \pm 0.03) \times 10^6 \text{ cm}^{-2}\) and \((0.63 \pm 0.07) \times 10^6 \text{ cm}^{-2}\), respectively. The percent inactivation of the deposited bacteria is illustrated in Figure 3a on top of each column as the shaded section and the range from low to high was CB (22%) < CNT (44%) < rGO (53%) < O-CNT (61%) < OA-CNT (67%). The bacteria deposition on a polished Ti substrate was \((0.95 \pm 0.20) \times 10^6 \text{ cm}^{-2}\) (lower than CNT and greater than other CNM) and the dead ratio was 23% (slightly greater than CB and lower than other CNM) as a comparison (data not plotted). Welch’s t-test of the total bacterial deposition data showed that the CNT had a significantly \((P < 0.05)\) higher bacterial density than the others and that of the OA-CNT was higher than the CB and rGO (Table B.3).
Statistical analysis was carried out following a multi-linear regression model to study the dependence of bacteria adhesion density on the surface contact angle (as an indicator of surface hydrophobicity) and nanoscale roughness. The results are summarized in Table 3.2. The correlation coefficient for contact angle (º) and surface roughness (nm) was 0.02 and -0.20, with the P-values of 0.08 and 0.12 (i.e., there is a 0.08 chance for the null hypothesis to be true that there is no dependence of bacterial attachment density with contact angle, whereas that for surface roughness is 0.12), respectively. The intercept of the correlation was -1.13 and the corresponding P-value was 0.24. Even though the P-values for the two variables were greater than 0.05, which is generally used as the standard for a significant correlation, they were not far off. The overall $R^2$ value was 0.71 and thus it is quite probable that there is a linear correlation between the total bacteria density in the absence of applied potential and the CNM coating hydrophobicity and nanoscale roughness.

<table>
<thead>
<tr>
<th>Variable 1</th>
<th>Variable 2</th>
<th>Intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contact angle (º)</td>
<td>Roughness (nm)</td>
<td>Bacteria density ($10^6$ cm$^{-2}$)</td>
</tr>
<tr>
<td>Coefficient</td>
<td>0.02</td>
<td>-0.20</td>
</tr>
<tr>
<td>P-value</td>
<td>0.08</td>
<td>0.12</td>
</tr>
<tr>
<td>$R^2$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 3.4.3 BACTERIA ATTACHMENT IN THE PRESENCE OF APPLIED POTENTIAL

After examining the attachment of *P. fluorescens* to the control coatings in the absence of electric potential, bacterial attachment experiments were completed as a function of total applied voltage using the CNM coated Ti substrates as the cathode. The bacterial density and inactivation at -1.0 and -2.0 V total voltage after 3 h incubation are shown in Figure 3.3b. In general, the
bacterial density decreased (by 25-60\% for -1.0 V and 67-90\% for -2.0 V) and the percent inactivation increased (by 8-32\% for -1.0 V and 1-23\% for -2.0 V) in the presence of applied voltage as compared to the non-charged controls. At -1.0 V, the range of \textit{P. fluorescens} average surface density was CB (0.27 × 10^6 cm^-2) < rGO (0.48 × 10^6 cm^-2) < OA-CNT (0.51 × 10^6 cm^-2) < O-CNT (0.53 × 10^6 cm^-2) < CNT (0.70 × 10^6 cm^-2), and the corresponding percent inactivated was 43, 60, 75, 74, and 76\%, respectively. At -2.0 V, the coatings had a further decreased total bacterial deposition ranging from 0.16 × 10^6 cm^-2 for CB to 0.26 × 10^6 cm^-2 for CNT and OA-CNT. The percent inactivation at -2.0 V was close to or less than -1.0 V following the order: CB (38\%) < rGO (61\%) < O-CNT (62\%) < CNT (67\%) < OA-CNT (76\%).

Figure 3.4: Total bacterial surface density normalized to the non-charged control density. Analysis is completed: a) for all carbon nanomaterial coatings with total applied voltage from -2.0 to 0 V (non-charged) and b) for the CNT coating only with total voltage of -2.0 to +2.0 V, after 3 h incubation at 30\°C in 0.9\% NaCl saline solution with 1/8 TSB.

The bacterial deposition density on the various CNM coatings as a function of total voltage from -2 to 0 V after 3 h incubation was normalized to the non-charged controls as displayed in Figure 3.4a. The P-value of the one-way Analysis of Variance (ANOVA) of the normalized densities at different voltages for each CNM is <0.05 (even <0.01), indicating the voltage-
dependent normalized bacterial density variation is significant. The combined data had a linear correlation \( d = 0.37V + 0.98; R^2 = 0.80; \) where \( d \) is the normalized bacterial surface density to the control surface and \( V \) is the total applied voltage in V) between the normalized bacterial density and the applied voltage. To illustrate the effect of electric potential polarity on bacterial adhesion, positive total voltages of +1.0 and +2.0 V were also applied between the CNT coating and the CCP counter electrode (i.e., CNT coated electrode was used as anode), and the relative bacteria density is displayed in Figure 3.4b. The linear correlation of the bacterial surface density with total applied voltage extended to positive surface charges up to +1 V and upon application of increased positive voltages, a decrease in total bacterial density was observed (1.36 of the control at +2.0 V as compared 1.61 at +1.0 V).

The inactivation of \( P. fluorescens \) on the CNM coatings over total voltage range of -2 to 0 V is displayed in Figure 3.5 (black). To elucidate the underlying mechanism for cathodic bacterial viability loss, the current density (blue) and \( \text{H}_2\text{O}_2 \) generation (red) were also displayed in Figure 3.5. The zero point of the direct current (blue) and \( \text{H}_2\text{O}_2 \) generation (red) was set at the same height as the control (uncharged) CNM coating percent inactivation for comparison and the total range was kept consistent.

Unlike the apparent linear decrease in total bacterial adhesion, the percent bacterial inactivation had a non-linear trend. In regard to the CNT and CB coatings, the percent inactivation change with voltage was significant with a P-value of the ANOVA test being <0.05. The percentage slightly decreased from the control in the absence of potential to -0.4 V and increased back to or higher than the control level at -0.7 V, but the deviation was <5%. For both the greatest viability loss was observed at -1.0 V; 76% for the CNT (control: 44%) and 43% for the CB (control: 22%). At more negative potentials, the percent inactivation first decreased at -1.5 V (54%
Figure 3.5: Loss of bacterial viability, direct current density, and H$_2$O$_2$ generation for CNM coatings as a function of total applied voltage. Analysis is completed with total voltage of -2.0 to 0 V (non-charged) after 3 h incubation at 30 ºC in 0.9% NaCl saline solution with 1/8 TSB.

For the CNT and 33% for the CB) and then increased at -2.0 V (67% for the CNT and 38% for the CB). For the O-CNT, OA-CNT, and rGO coatings, the percent inactivation of the controls was relatively high (61%, 67%, and 53%, respectively) and the variation with total voltage was relatively limited (the P-value of the ANOVA test was >0.05). In particular for rGO, the percent
inactivation for all the samples varied by only 13%. The greatest percent inactivation was at -1.5 V for the O-CNT, OA-CNT, and rGO at 78%, 81%, and 66%, respectively.

In general, the current density increased with increasing voltage. For the CNT, O-CNT, and OA-CNT coatings, the current density was as low as <0.01 mA cm$^2$ at total voltages of -0.4 to -1.0 V and increased to a maximum of ~0.15 mA cm$^2$ upon increasing voltage from -1.0 to -2.0 V. The current increase for CB and rGO was less than the three CNT with the highest current densities being observed at -2.0 V of 0.10 and 0.07 mA cm$^2$, respectively.

Despite the varied surface chemistry and morphology of the CNM coatings, all displayed a very similar trend towards H$_2$O$_2$ generation, which peaked at -1.5 V, corresponding to a cathode potential of around -0.9 V vs. 1 M Ag/AgCl reference electrode (+0.23 V vs. SHE; Figure B.4). This is more negative than the theoretical dissolved O$_2$ reduction potential at neutral pH of ~0.10 V vs. 1 M Ag/AgCl (O$_2$ + 2H$^+$ + 2e$^-$ → H$_2$O$_2$(aq), $E^0 = 0.69$ V; Nernst equation) [40] and previously reported values of -0.5 to -0.8 V vs. 1 M Ag/AgCl [39], likely due to a higher overpotential from the Nafion coating hindering electron-transfer. The maximum H$_2$O$_2$ concentration was ~0.01 mM after 5 min electrolysis using saturated oxygen in the absence of bacteria and broth, thus under the bacterial incubation conditions, the cumulative H$_2$O$_2$ generation will be less than 0.07 mM in 3 h. If also considering possible cathodic H$_2$O$_2$ reduction pathways to the hydroxyl radical and water as well as H$_2$O$_2$ loss via reaction with the broth components, the final H$_2$O$_2$ concentration will be lower than minimal concentrations observed to be toxic to bacteria (≥0.15 mM) [147]. In agreement with this expectation, <5% of the suspended bacteria were inactivated under an applied potential of -2 V.

A multi-linear regression model was again applied in an attempt to quantitatively examine the dependence of bacterial inactivation (as compared to uncharged controls) to electric current
Table 3.3: Multi-linear regression result of the bacterial dead percentage increase from controls with current density and H$_2$O$_2$ generation.

<table>
<thead>
<tr>
<th>Variable 1</th>
<th>Variable 2</th>
<th>Intercept</th>
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<th></th>
<th></th>
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<tr>
<td>Current density (mA cm$^{-2}$)</td>
<td>H$_2$O$_2$ concentration (mM)</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Coefficient</td>
<td>1254.3</td>
<td>5.43</td>
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<tr>
<td>P-value</td>
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<td>0.11</td>
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<tr>
<td>R$^2$</td>
<td>0.12</td>
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</table>

Figure 3.6: Bacterial growth on the CNT surface as a function of total voltage and incubation time. a) Total bacterial deposition on the CNT surface with a total voltage of 0, -1.0, and -2.0 V after 0.5, 3, 6, and 12 h incubation; b) SEM images of bacterial morphology on CNT cathode at 1) 3 h, 0 V, 2) 3 h, -2.0 V, 3) 12 h, 0 V, and 4) 12 h, -2.0 V at 30 °C in 0.9% NaCl saline solution with 1/8 TSB.
and H$_2$O$_2$ generation. The results are summarized in Table 3.3. The overall correlation was not significant with the R$^2$ = 0.12 and the P-values of both variable inputs are >0.05. However, the correlation of the percent bacterial inactivation with H$_2$O$_2$ generation was notably greater than with the current density with a 0.11 and 0.88 chance for the null hypothesis to be true, respectively.

The effect of electrochemistry on extended bacterial attachment was also evaluated. The total *P. fluorescens* cell density on the CNT cathode coatings with 0, -1, and -2 V total voltage after 0.5, 3, 6, and 12 h incubation is displayed in Figure 3.6a. In addition, *Escherichia coli* (*E. Coli, ATCC® 700830™*) has been used in the up-to-12 h attachment experiments to confirm the trend observed for *P. fluorescens*, as shown in Figure B.5. SEM analysis was completed on the non-charged control and -1 and -2 V CNT coatings after 3 and 12 h of incubation (Figure 3.6b and Figure B.6).

Despite the inherent CNT cytotoxicity, the bacterial density on the control surface in the absence of electric potential was significantly greater at (4.4 ± 1.8) × 10$^6$ cm$^{-2}$ after 12 h than in the presence of an electric potential of -1 and -2 V at (1.0 ± 0.1) × 10$^6$ cm$^{-2}$ and (0.8 ± 0.1) × 10$^6$ cm$^{-2}$, respectively. Thus, the application of electric potential reduced the total bacterial density to 0.18 of the control at -1 V and 0.14 at -2 V after 12 h, which was less than after 3 h when the relative total cell density compared to the control was 0.45 and 0.16 at -1 and -2 V, respectively. In all cases, the total bacterial surface density increased linearly with time. In the absence of electric potential, the bacterial density followed the equation; $D_{0V} = 0.32t + 0.61$, where $D$ is the bacterial density in 10$^6$ cm$^{-2}$ (distinguished from $d$ representing normalized bacterial density compared to the control), $t$ is the incubation time in h, and the slope indicates the rate of increase in bacterial attachment density. The bacterial attachment kinetics in the presence of electric potential at -1 V was $D_{-1V} = 0.04t + 0.70$ and at -2 V was $D_{-2V} = 0.03t + 0.43$. Thus, the rate of
bacterial deposition on the CNT coating upon application of -1 and -2 V was 8-10 fold less than the uncharged control and in turn it would be expected that biofilm formation and subsequent biofouling kinetics would also be significantly reduced.

Interestingly, the cell density on both the -1 and -2 V cathode surface decreased from 0.5 h to 3 h incubation indicating the initial adhesion of the bacteria is reversible [120]. The underlying reason for the decrease is still not clear, but may be due to the time necessary for the electric double layer to develop i.e., the capacitive current stabilized after 15 min, delaying organic matter/bacteria attachment/detachment. Welch’s t-test showed that application of -1 and -2 V to the electrode resulted in a significantly lower total bacterial deposition than the controls at 3 h and 12 h incubation (Table B.4).

From the SEM analysis in Figure 3.6b after 3 h of incubation, the intact bacteria cells look similar and no obvious cell appendages can be observed in either the 0 V (Figure 3.6b-1) and or the -2 V (Figure 3.6b-2) experiments. However, after 12 h, the cells on the 0 V control surface (Figure 3.6b-3) have developed some appendage structure [148] adhering the cells to the surface as well as each other, whereas the cells on the -2 V (Figure 3.6b-4) cathode still have a smooth surface.

3.5 DISCUSSION

3.5.1 BACTERIAL ATTACHMENT IN THE ABSENCE OF ELECTRIC POTENTIAL: I) TOTAL DENSITY

The difference in bacterial attachment in the absence of electric potential on the CNM coatings is a function of their hydrophobicity and surface roughness. Hydrophobic interactions have been demonstrated to drive cell adhesion to surfaces [149, 150] since bacteria have
hydrophobic surface components e.g., lipid bilayer membranes, despite commonly having an overall negative surface charge [16]. Surface roughness on the µm or larger scale typically results in increased microbial adhesion due to an increased surface area [150] and enhanced bacteria-substrate interactions [151]. However, recent studies have indicated that nanoscale surface roughness may reduce bacterial attachment [152, 153]. The results of the multi-linear regression model on the dependence of bacterial attachment density on coating contact angle and nanoscale roughness agree with the previous studies.

3.5.2 BACTERIA ATTACHMENT IN THE ABSENCE OF ELECTRIC POTENTIAL: II) PERCENT INACTIVATION

Upon contact with the surface CNM coating, a fraction of the bacteria will be inactivated due to membrane degradation and oxidative stress [154-156].

For coatings with similar surface morphology i.e., the CNT, O-CNT, and OA-CNT, the percent inactivation is a result of the material surface chemistry. Pasquini et al. [128] observed that oxidative acid treatment and subsequent annealing increased the cytotoxicity of the multi-walled CNT due to increased CNT reactivity, which was closely related to number of surface defect sites such as oxygen-containing groups that change in speciation post-annealing. Similarly, Akhavan and Ghaderi [157] examined the bacterial toxicity of graphene oxide and reduced graphene oxide and assigned the higher toxicity of the reduced graphene oxide to sharpened nanowall edges that had stronger interactions with the cell membrane and/or better charge transfer resulting in increased cell membrane damage. Thus, the increase of percent of inactivation from CNT to O-CNT (44 to 61%) is due to the introduction of functional surface defects e.g., surface oxy-groups such as carboxylates or quinones, on the walls and tips of the tubes and in turn an increase in reactivity [128]. Annealing can either remove the functional groups to reveal undecorated defects
and sharper tips [146] or alter the speciation of the functional surface groups resulting in higher/lower reactivity [158]. Since the OA-CNT here only contain C-O type functional groups according to XPS characterization, which is usually assigned to alcohols or ethers that have not been reported to be highly reactive [143], it is likely that the former reason is responsible for the observed increase in percent inactivation of OA-CNT (67%).

For materials with similar surface chemistry, Kang et al. [156] observed that CNT size (diameter) is a key factor governing their antibacterial effects and that CNT of smaller diameter (single-walled) had more irreversible cell morphology damage than that of larger diameter (multi-walled). It has also been observed that the size of the graphene oxide aggregates is related to bacterial toxicity and that smaller aggregates had a greater antibacterial activity [155]. In this study, the CB and pristine CNT contain minimal surface oxygen functional groups; however, the CB particles are roughly spherical and have an average diameter of 60 nm that is greater than the CNT diameter of 15 nm, thus the CB have less potential for physical membrane perturbation resulting in less bacterial inactivation (22%) than the CNT (44%). In comparison, Yang et al. [159] compared the toxicity of CB (d = 12 nm) and CNT (d = 8 nm) dispersions to primary mouse embryo fibroblasts and observed the CNT had a slightly higher cytotoxicity. Therefore, the diameter of the CB vs. the CNT is likely the governing factor towards cytotoxicity.

In regard to the rGO coating, an increased bacterial toxicity might be expected due to high amount of introduced defect sites and the significantly smaller material dimension (thickness of 1.5 nm) of the graphene flakes. However, the overlaid structure of the rGO coating (smooth surface with a roughness of 1.58 nm) may not allow for sufficient contact between the rGO nano-edges and the bacterial cells reducing anti-microbial activity. The percent inactivation of rGO coating (53%) is greater than the CB and CNT, but less than the O-CNT and OA-CNT.
3.5.3 BACTERIA ATTACHMENT IN THE PRESENCE OF APPLIED POTENTIAL: I) NORMALIZED DENSITY

The relative attached bacterial density at 3 h deceased monotonically with increasing applied negative working electrode potential. As briefly mentioned above, most bacteria are negatively charged at neutral pH in an aqueous environment [15, 16], thus the magnitude of the electrostatic interaction between the bacterial cell membrane and the cathode surface should decrease with electrode potential according to classical DLVO theory, which has widely been used to predict colloid and bacterial deposition [15]. A more negative electrode potential will result in a more negative surface charge and thus a stronger electrostatic repulsion between the electrode surface and bacterial cells. According to a DLVO energy calculation following a previous established method [50] (Figure B.7), an energy barrier exists between the bacteria (-10.8 mV; measured with Malvern zen3600, UK) and the cathode surface at all total applied voltages (-0.4 to -2.0 V).

Alternative mechanisms have also been proposed for bacterial desorption from an electrode surface. For instance, Poortinga et al. (2001) applied a potential of -0.9 V (-800 µA) to +1.9 V (800 µA) vs. Ag/AgCl reference electrode to an indium tin oxide electrode of 21 cm² and found that the bacteria desorption probability increased with applied current. Apart from the electrostatic interaction between the electrode surface and the bacteria, it was demonstrated that the charged surface also caused an electroosmotic effect, which resulted in bacterial movement in parallel with the electrode surface [121]. In contrast, Kang et al. (2011) monitored bacteria translational motion on an anode surface using a particle tracking method, but found that the averaged mean square displacement of the bacterial cells was independent of the applied current density in the experimental range of 7.5-30 µA cm⁻² [160]. The current density in this study was intermediate to
these two studies ranging from -158 to +101 µA cm\(^{-2}\) for total voltages of -2.0 to +2.0 V, thus an electroosmotic mechanism may also contribute to the bacterial fouling reduction, especially for the observed decrease in \(P.\) fluorescens adhesion at the CNT coating at +2 V total voltage as compared to +1 V (Figure 3.4b). Yet the quantitative contribution of the electroosmotic force to the bacterial adhesion is still not clear and requires further study.

### 3.5.4 BACTERIA ATTACHMENT IN THE PRESENCE OF APPLIED POTENTIAL: II) PERCENT INACTIVATION

Numerous studies have observed bacterial disinfection when an anodic current is applied [19, 37]. For instance, when CNT were used as an anode, two possible bacterial inactivation pathways were identified: direct inactivation at the CNT surface and indirect inactivation by electrochemical production of Cl\(^-\) or HO\(^-\) [38]. However, cathodic bacterial inactivation, in particular for CNM, has not been well studied.

Indirect disinfection of the attached bacteria at the cathode via ROS formation is one possible mechanism [41]. Hydrogen peroxide is a common cathodic electrolysis product from a CNM electrode via reduction of dissolved oxygen and its presence is generally accepted as evidence for production of more reactive species like \(\cdot\)OH [41]. According to the multi-linear regression model of the dependence of bacterial inactivation on electric current and \(H_2O_2\) generation, the inactivation of \(P.\) fluorescens is significantly more likely related to the \(H_2O_2\) generation than the total coulombs delivered to the electrode-solution interface, supporting an indirect inactivation mechanism by ROS rather than direct inactivation by electron transfer from the cathode to the bacteria. However, it should be noted that the \(H_2O_2\) generation during the 3 h experiments within the ambient environment is estimated to be lower than the toxic level if all the ROS are dispersed in the bulk solution and in agreement, no significant percent inactivation (<5%)
of the suspended bacteria was observed. One reason for this phenomenon is that the electrochemical reactions in a batch reactor are typically a mass-transfer-limited process [161] and thereby the H$_2$O$_2$ or other ROS are generated rapidly at the electrode-solution interface and will likely react before diffusing into the bulk solution resulting in a relatively high interfacial ROS concentration.

The relatively weak correlation (P-value = 0.11) between the percent of deposited bacteria that were inactivated and the H$_2$O$_2$ generation may be related to alternative reaction mechanisms, for instance, the scavenging of ROS by broth ingredients. A notable decrease of the contact angle of the CNT coating (135.8º) after 3 h incubation in the bacterial solution with no (27.3º) or -2 V (30.2º) total voltage was observed here, implying a layer of organic matter was accumulated on the CNT surface, which may compete with the bacteria for reaction with cathodic ROS.

3.5.5 BACTERIA ATTACHMENT IN THE PRESENCE OF APPLIED POTENTIAL: III) DEPOSITION KINETICS

The enhanced microbial fouling inhibition i.e., significantly (8-10 fold) reduced bacterial deposition kinetics, on the CNM electrodes in the presence of an electric potential is related to the bacterial attachment process that is generally described by two primary steps [149]. The first step involves bacterial transport close to a surface allowing for initial adhesion via van der Waals, electrostatic, and hydrophobic interactions. The next step is the irreversible attachment of the cells to the surface by production of appendages such as exo-polysaccharides and pili or fimbriae. Thus, it is likely that the local cathode surface environment at -2 V not only causes loss of bacterial viability to some cells, but also inhibits the development of or degrades cell appendages and in turn reduces irreversible adhesion. Studies on this phenomenon are still limited, yet the recent research by Gall et al. (2013) on bacterial adhesion to electrode surface using a quartz crystal
microbalance with dissipation analysis may help elucidate the observations here. By monitoring the frequency shift from a reference resonance frequency as well as the dissipation of the piezoelectric crystal oscillation magnitude, information was collected on the rigidity/softness of the deposited bacteria. Subsequently, a model was proposed that on a negatively charged electrode surface, a positively charged electric double layer is developed, which causes bacterial cell surface appendages to aggregate and in turn the cell appears more rigid [162]. Following this model, it can be speculated that when exposed to a negative cathode potential the bacterial appendages may not extend and thus bacteria attachment is further reduced. The electrochemical fouling reduction over extended time periods elucidates some of discrepancies between the voltage-dependence of bacterial deposition and classical DLVO theory, for instance, the magnitude of reduction was independent of cathode potential in long term experiments lasting for a week [119].

It is also possible that cell appendages on the control CNT surface after 12 incubation (Figure 3.6b-3 and Figure B.6-1 and 2) were from cell wall extension (remains after cell division), which indicated that some cells on the control may keep dividing and reproducing while those on the cathode surface were inhibited, thus corresponded to the slower kinetics on CNM electrodes in the presence of a negative potential.

3.5.6 IMPLICATION AND APPLICATION OF CATHODE POTENTIAL REDUCED BACTERIAL ADHESION AND VIABILITY

From the experimental results here, the use of CNM cathode coatings to electrochemically reduce bacterial attachment and viability seems quite promising. In regard to the CNM selection, a comparison is discussed below in regard to bacterial attachment on the non-charged control surface, electrochemical inhibition, and cost analysis.
First, the bacterial attachment on the control coating in the absence of applied potential is a key factor determining the fouling potential, which functions as the baseline for electrochemical biofouling reduction. The conventional CB serves as a good coating material in this study due to its surface morphology. The pristine CNT has higher cytotoxicity towards the deposited bacteria, but the relatively smooth and hydrophobic surface lead to greater bacterial adhesion. After oxidation and annealing, the CNT properties can be tailored to reduce bacterial adhesion and increase surface toxicity. The rGO coating has low bacterial adhesion as a result of the lesser hydrophobic surface and relatively high cytotoxicity. Improved design of the rGO surface orientation, for instance to allow for more exposure of graphene edges to the bacteria [157] may increase the surface cytotoxicity. Thus, CNM surface properties should carefully considered when designing an anti-biofouling surface.

Second, the electrochemical bacterial attachment reduction is much less dependent on the intrinsic properties of different CNM and the application of a negative electric potential both reduces bacterial adhesion and increases bacterial inactivation. The electric conductivity of the coating material may be important when this technique is used to promote the anti-biofouling of non-conductive material surface since an even potential distribution across the lateral surface is required. Thus, the various CNT would yield a more effective coating, whereas the application of rGO to a non-conductive surface is likely not viable for electrochemical bacterial fouling reduction unless the conductivity can be greatly enhanced e.g., via thermal/chemical reduction. It will also be of interest to develop new materials with high and/or selective electrochemical properties e.g., a greater number of reactive oxy-defect sites while still maintaining conductivity, that allow for increased inactivation of the attached bacteria.
Finally, the commercial and material cost should be taken into consideration selecting surface coating material. On one hand, CB still has a great advantage over the other materials in terms of commercial price and availability i.e., the bulk industrial CB market price is $<0.1 \text{ US}$ kg$^{-1}$. The industrial price for CNT has been brought down to as low as $100 \text{ US} \text{ dollar kg}^{-1}$ [163] according to recent literature report, which will favor its growing application, while the commercial market for graphene production and application is still under development. On the other hand, the material cost for a uniform functional nano-thin coating greatly encourage the development of novel CNM coatings, especially graphene-like materials since significantly less material is necessary to produce an electroactive coating i.e., the rGO coating was $<5 \text{ nm}$ thick while the CNT coatings were 100-150 nm thick, and the CB coating was 400 nm thick.

3.6 CONCLUSIONS

*P. fluorescens* adhesion and inactivation on a Ti cathode coated with CNT, O-CNT, OA-CNT, CB, and rGO was systematically and semi-quantitatively analyzed. For the CNM coatings in the absence of applied potential, the total bacterial attachment is correlated to the surface coating hydrophobicity and nanoscale roughness, while the loss of bacterial viability is related to the CNM nanoscale dimensions and surface chemistry. For electrochemical biofouling reduction, the bacterial surface density decreased linearly with increasing applied negative potential for all CNM coated cathodes indicating electrostatic repulsion as the dominant mechanism. The electrochemical inactivation of the deposited bacteria was best correlated with $\text{H}_2\text{O}_2$ generation and the inactivation was only effective towards adhered as compared to suspended bacteria likely due to a high near surface concentration of cathodic ROS. Extended (12 h) experiments indicated that application of a negative potential (-1 and -2 V) to the CNT coated electrode reduced the bacterial deposition kinetics by 8-10 fold as well as the production of appendages by the adhered
cells likely reducing irreversible attachment. In conclusion, the use of CNM as cathode coating material is a promising method to actively reduce microbial fouling and the study here provides semi-quantitative insights into the material properties and electrochemical parameters most relevant to biofouling reduction, which can become fundamental support for future research and applications.
CHAPTER 4

CNT COMPOSITE CATHODE MEMBRANE BIOFOULING REDUCTION DURING FORWARD OSMOSIS OF SYNTHETIC AND ACTUAL WASTEWATER

4.1 ABSTRACT

A thin carbon nanotube (CNT) composite layer was cast-coated on commercial forward osmosis (FO) membrane surface. The composites were made from CNT and Nafion (CNT) or CNT, carbon black (CB), and Nafion (CNT+CB), with or without mechanical pressing. Fouling experiments using synthetic wastewater containing P. fluorescens of $10^7$-$10^9$ bacteria mL$^{-1}$ (PF) as feed were carried out in the absence and presence of flushing to evaluate and compare the fouling rate reduction and reversibility over 84 h. The pressed p(CNT+CB) had the optimal antifouling performance of 44% fouling rate reduction and 76-195% fouling resistance recovery ($FRR$) increase as compared to the control, and the material cost was $\frac{1}{3}$ lower than the CNT electrodes. Both PF and actual wastewater (WW) were used as feed to challenge the p(CNT+CB) membranes for fouling mitigation during filtration with short-interval (2 min every 40 min) flushing. The fouling mechanism for PF and WW was different due to solution matrix difference; however, the p(CNT+CB) cathode at 2 V could reduce the initial flux loss by 63% for WW fouling as well as the fouling rate by $\sim$50% for both the PF and WW fouling in comparison to the control. Application of electrical voltage was necessary to reduce fouling, confirming the electro-active fouling mitigation mechanism.
4.2 INTRODUCTION

Forward osmosis (FO), or osmosis, describes the transport of water from a solution of low osmotic pressure to a solution of high osmotic pressure through a semi-permeable membrane. This phenomenon has a long history, but the rapid growth of FO in engineered processes initiated in the 1990s with the development of highly selective and permeable FO membranes [8]. Since, FO has been evaluated for various applications such as water purification and energy production [164].

In the field of water treatment, FO can be divided into two categories, open-loop and closed-loop, depending on whether the draw solution or solute requires recycle. For instance, in open-loop FO, naturally abundant seawater or brackish water is used as draw solution, the waste can be directly discharged, and the energy demand is very low in comparison to the pressure-driven membrane processes of similar selectivity, such as nanofiltration (NF) and reverse osmosis (RO) [165]. In closed-loop FO, the energy cost is notably increased due to draw solution regeneration via heating [166], membrane distillation (MD) [167], NF [168], and RO [169]; however, the overall process has advantages of low feed quality requirement and high product water purity. The most pronounced advantage of FO in both open- and closed-loops is the low fouling propensity [170-172] and high fouling reversibility [173, 174] due to the lack of hydraulic pressure. Therefore, FO has been investigated as a promising alternative technique for wastewater treatment, where severe biofouling occurs.

FO has been used for the treatment of synthetic [172], municipal [175] wastewater (WW), and anaerobic digester centrate [169]. The hybridization of FO and membrane bioreactor (MBR) is also of research interest [176-179]. Biofouling i.e., the accumulation of bacteria and the subsequent formation of biofilm on membrane surfaces, results in decreased permeability and cleaning efficacy during FO [171, 172, 176, 180]. The fouling behavior depends on the draw and feed
solution properties, membrane characteristics, and hydraulic conditions, thus the fouling control can be carried out using all these aspects.

To date, most studies have focused on biofouling behavior and mechanism of FO as well as comparison to pressure-driven membranes. There have also been some studies on FO fouling reduction. For example, the use of an intermittent flushing by elevated water flow [176] or air scouring [178] on the feed side increases boundary layer turbulence resulting in reduced fouling. A periodic osmotic backwash has also been observed to recover flux [176, 181]. However, these methods may not be optimal since the former is energy-intensive and may reduce membrane longevity [180] and the latter requires additional operation and maintenance. There are novel antifouling techniques, which have been recently developed for other membrane processes that might be useful to reduce FO fouling, such as electro-active fouling mitigation.

CNT is a carbon nanomaterial with outstanding properties such as stability, intrinsic biotoxicity, and conductivity. CNT can be fabricated into a free-standing membrane or a thin porous layer on a substrate with a pore size of 10s-100s nm and has been investigated for membrane applications. For example, a CNT layer was found to reduce organic fouling on pressure-driven membranes due to adsorption of the foulant before they reach the membrane surface [76, 182]. Additionally, inherent CNT biotoxicity can inactivate bacteria [154, 183, 184] before contacting the membrane surface. CNT membranes have also been used in combination with an electrical potential e.g., an anodic CNT filter was effective for bacteria and virus removal and inactivation [38], a CNT-polymer composite cathode on ultrafiltration membranes was effective for organic fouling reduction [185], and a AC-CNT electrode under the selective layer of RO membranes was effective for biofouling reduction [80]. CNT as well as other carbon nanomaterial (CNM) coating has also been observed to be effective for reduction of microbial
attachment on surfaces via electrostatic repulsion and electrochemical inactivation [186], which has the advantages of simple fabrication and automation and thus may have potential application in FO.

Here, a thin CNT composite layer was cast-coated on a commercial FO membrane. CNT and CNT+CB Nafion composites (unpressed or pressed) were then characterized and compared in various FO operation modes using synthetic WW as feed. The efficacy of p(CNT+CB) fouling control during FO of synthetic and actual WW was examined and the fouling reduction mechanism for each WW was discussed.

4.3 MATERIALS AND METHODS

4.3.1 MATERIALS

Carbon nanotubes (CNT; C-grade, multiwall, powder, >95% purity) were purchased from NanoTechLabs, Inc. (Yadkinville, NC). Carbon black (CB; Vulcan XC 72-R) was kindly provided by Cabot (Boston, MA). Nafion 117 solution (~5% in a mixture of lower aliphatic alcohols and water) and formaldehyde (ACS reagent, 37 wt.% in water) were purchased from Sigma-Aldrich (St. Louis, MO). NaCl (reagent grade), ethanol (EtOH), isopropyl alcohol (IPA) (laboratory grade), BD Bacto™ tryptic soy broth (TSB), and tryptic soy agar (TSA) were purchased from VWR International (West Chester, PA). Deionized (DI) water (>18 MΩ) was produced by a Nanopure Infinity ultrapure water system (Barnstead/Thermolyne) and was used to prepare solutions and to rinse containers.

The synthetic WW was prepared by adding bacteria to diluted culture broth. *P. fluorescens* ATCC 700830 (ATCC, Manassas, VA) was the model bacterium for biofouling evaluation due to its high biofilm formation and fouling potential [131-133]. The bacteria were cultivated in full
strength TSB broth for 18 h, harvested, resuspended twice, and calibrated to an optical density at 600 nm (OD$_{600}$) of 0.15 in DI water, which has a concentration of ~$10^8$ CFU mL$^{-1}$. Then 300 mL bacterial stock solution and 50 mL sterilized full-strength TSB were added to 2650 mL DI, yielding an initial concentration of $10^7$ mL$^{-1}$ bacteria and 500 mg L$^{-1}$ TSB. The actual WW was collected from the primary sedimentary effluent in Deer Island WW Treatment Plant (Boston, MA) in August 2015. The synthetic and actual WW will be denoted as PF and WW solution for short in the text, respectively.

The FO membrane was purchased from Hydration Technology Inc. (HTI; Albany, Oregon). The thin-film composite (TFC) membrane was developed for FO applications with an ultrathin polyester screen mesh support to reduce internal concentration polarization [187]. The membranes were thoroughly rinsed and bath ultrasonicated in DI and IPA for 15 min separately prior to usage.

### 4.3.2 FABRICATION OF CNT COMPOSITE LAYER ON MEMBRANE SURFACE

Four types of CNT composite coating were prepared for characterization and FO experiments, namely CNT, CNT+CB, pCNT, and p(CNT+CB), where p indicates they are mechanically pressed.

For the CNT samples, a CNT-Nafion solution was prepared by probe ultrasonicating 60 mg CNT, 300 mg 5% Nafion solution, and 30 g IPA for 15 min at a power density of ~1,000 W L$^{-1}$. Since the density of IPA was 0.80 g mL$^{-1}$, the resulting solution has 1.6 mg mL$^{-1}$ CNT and 0.4 mg mL$^{-1}$ Nafion. For the CNT+CB samples, a similar procedure was followed except that 60 mg CNT was replaced with 40 mg CNT + 20 mg CB, thus the final cast solution contained 1.1 mg mL$^{-1}$ CNT, 0.5 mg mL$^{-1}$ CB, and 0.4 mg mL$^{-1}$ Nafion. After the sonicated solution cooled to room temperature, a clean FO coupon with an area of $5.7 \times 11.4$ cm$^2$ was mounted on a horizontal
platform and the excess water was gently removed with a Kim wipe. Then 1 mL CNT or CNT+CB solution was added, which naturally spread over the membrane surface to form a relatively uniform layer. After 10 min, another 1 mL solution was cast, which together with the first layer generated a very uniform coating. The second layer required ~15 min to dry. The CNT and CNT+CB samples were then characterized dry, stored in DI water, or further treated to make the pressed (p) coatings.

To prepare the pCNT and p(CNT+CB) coatings, an unpressed sample was first sandwiched between 2 pieces of parchment paper, facing the shiny side. The 3 layers were then sandwiched with 2 pieces of silicon rubber (7 × 11.4 cm², 1.6 mm thick; McMaster Carr, Robbinsville, NJ). Finally, the 5 layers were mechanical pressed (Carver 4386) at 6 MPa (60 bar) for 15 min at room temperature.

4.3.3 FO SYSTEM SETUP

FO experiments were performed in a bench-scale crossflow FO system (Figure 4.1). The primary components included a feed and draw solution reservoir (4 L with a removable lid), a peristaltic pump (Masterflex L/S Variable-Speed) with 4 heads, a digital scale (Sartorius Quintix 5101-1S) connected to a PC for continuous weight monitoring, a commercial FO filtration module (CF042A-FO), and an air/water flushing automatic switch. For the electro-active experiments, a DC power supply (Agilent E3646A) was used to provide 2 V total voltage.
Figure 4.1: Scheme of the FO system

A flat-sheet membrane coupon with an effective filtration area of $8.5 \times 4.0 \text{ cm}^2$ was used for all experiments. The height of the feed and draw channel was 2 mm and the experiments were completed at a constant flow rate of 73 mL min$^{-1}$ (1.5 cm s$^{-1}$ in the flow compartment, Re = 60) for both the feed and draw solution unless otherwise specified. The membrane was placed with dense side facing the feed. A diamond-patterned spacer (2.0 mm pore size; 0.6 mm thickness) was placed on top of the membrane surface in the feed compartment. To compare the control and electro-active experiments under the same conditions, a carbon cloth (0.2 mm pore size; 0.2 mm thickness) and a separator (0.2 mm pore size; 0.3 mm thickness) were placed on top of the spacer in all experiments. For the electro-active experiments, the cathode (CNT composite) and anode (carbon cloth) in the filtration module were connected to the DC power supply using Ti leads. The feed reservoir was magnetically stirrer at 120 rpm during fouling experiments, and it was covered with $\sim 10 \text{ cm}^2$ ventilation window to prevent excess water evaporation as well as provide air
exchange. The draw reservoir was placed on the digital balance and weight was recorded every 20 s. Since both inlet and outlet water flows were open to the atmosphere, the differential pressure inside the system was < 0.04 bar.

At the start of each experiment, the FO membrane was wetted by spraying ~2 mL 50% EtOH/DI on the membrane surface. It was then equilibrated with the draw solution (1 M NaCl) and foulant-free feed (DI) water to achieve a stable water flux. During the fouling experiments, the draw solution volume will increase as a result of water uptake from the feed, thereby the 1 M NaCl solution was replaced every 24 h. As a result of the bacterial activity in the PF feed, the TOC will decrease over time. To maintain a relatively stable nutrient level, 25 mL of sterilized TSB solution was added to the feed and the volume was adjusted to 3 L every 24 h. TOC and conductivity of the feed and draw solution were measured at the start and end of every 24 h cycle. Turbidity and pH were measured at the start and end of the 84 h experiments. Fouling experiments were completed at least in duplicate.

Since the osmotic driving force for water flux constantly decreased as a result of draw solution dilution and feed solution concentration, the membrane fouling needs to be distinguished from the change in osmotic driving force in regard to the overall flux decline. A baseline experiment was completed to quantify the flux decline in the absence of fouling [173]. A simple correction of the raw filtration data with the solution conductivity change (Eq 4.1 and 4.2) can provide a good prediction of the flux from the change in osmotic driving force (Figure C.1; <12% difference), thus all the normalized data utilized this correlation:

\[
J_t = j_t' \times \frac{k_{D0} - k_{F0}}{V_{DF} - V_{FF}}
\]

\[
J_t = j_t / j_0
\]
where \( j_i \) and \( j'_i \) (LMH) are the corrected and raw flux data at \( t \) (h), \( k \) (\( \mu \text{S cm}^{-1} \)) and \( V \) (mL) are the conductivity and volume of the feed and draw (subscript of F and D) at time 0 or \( t \) (h) (subscript of 0 and \( t \)). The capitalized \( J_i \) is the normalized flux at \( t \) (h).

Flushing was completed with increased feed solution velocity (858 mL min\(^{-1}\); 17.6 cm s\(^{-1}\), \( \text{Re} = 701 \)) plus air flow (1 L min\(^{-1}\), 2.3 m s\(^{-1}\) at the diffuser outlets at the entrance of the feed compartment, \( \text{Re} = 156 \); 9 diffuser outlets in total) for 2 min after fouling.

### 4.3.4 MODE OF FOULING EXPERIMENTS

The fouling experiments were conducted in three different modes based on the flushing frequency, namely no-flushing, long-interval-flushing (6× 2 min every 40 min at the end of each 24 h cycle), and short-interval-flushing (2 min every 40 min throughout the whole experiment).

The no-flushing experiments were carried out using PF solution as feed under relatively constant conditions. The CNT and CNT+CB cathode at 2 V were compared with the control to evaluate the influence of CNT composition on the fouling rate.

The long-interval flushing experiments were also completed using PF solution as feed. The CNT+CB and p(CNT+CB) cathode at 2 V were compared to the control to evaluate the effects of mechanical pressing on the fouling reversibility after 24, 48, and 72 h fouling unless otherwise stated.

The short-interval flushing were completed to compare the p(CNT+CB) electrode fouling reduction using both the PF and WW solutions as feed with periodic flushing frequency similar to typical wastewater treatment facility operation. The electrodes at 2 V were compared to uncharged electrodes (0 V) and uncoated control membranes for mechanism analysis.
4.3.5 SOLUTION CHARACTERIZATION

The feed and draw samples were evaluated for total organic carbon (TOC), inorganic carbon (IC), conductivity, pH, and turbidity using Shimadzu TOC-Vw, Oakton CON 11, Orion pH meter, and Hach 2100Q portable turbidimeter, respectively. The bacterial density was examined by a CFU method on TSA at 30°C. The feed solution was imaged under optical microscope by spreading ~50 µL solution on a glass slide sealed with a cover slip.

Zeta potential of the *P. fluorescens* in the synthetic WW was measured by a Malvern Nano ZS Particle Sizer.

4.3.6 MEMBRANE CHARACTERIZATION

Membrane cross-sections and surfaces were imaged by a Zeiss FESEM UltraPlus in Harvard’s Center for Nanoscale Systems (CNS) using a 1 nm Pt/Pd (80/20) sputter coating. The fouled membranes were fixed with formaldehyde vapor and dehydrated in EtOH/DI solutions containing 40-100% EtOH prior to SEM imaging. AFM analysis was completed on an Asylum-1 MFP-3D AFM system in Harvard’s CNS. The electrical resistance of the CNT composite layers was measured by Kiethly multimeter using a 2-point probe measurement. Contact angle was measured using a goniometer (Rame-Hart 190CA) by the sessile drop method. All the image analysis including surface porosity, pore size, contact angle, and cross-section thickness was done with Image J (National Institutes of Health). All characterization measurements were completed at least in duplicate.
4.4 RESULTS AND DISCUSSION

4.4.1 CHARACTERISTICS OF THE WASTEWATER AND MEMBRANES

PROPERTIES OF WASTEWATER SOLUTIONS

The chemical, biological, and physical properties of the PF and WW are summarized in Table 4.1. According to the TSB manufacturer specification, the PF solution contains 283 mg L\(^{-1}\) pancreatic casein digest, 50 mg L\(^{-1}\) papaic soybean digest, 42 mg L\(^{-1}\) dextrose, 42 mg L\(^{-1}\) K\(_2\)HPO\(_4\), and 83 mg L\(^{-1}\) NaCl, and the measured TOC and IC are 183.1 ± 19.8 and 3.4 ± 0.8 mg L\(^{-1}\), similar to synthetic WW in previous studies [178, 188]. At the end of each 24 h, the TOC decreased by ~30-50% due to bacterial consumption, therefore 25 mL sterile TSB broth (full strength) was added to maintain the organic level. The average final TOC was 146.0 ± 22.2 mg L\(^{-1}\). The IC at the end of experiments increased to 40.5 ± 7.6 mg L\(^{-1}\), likely due to bacterial activity as well. The pH remained relatively stable in the range of 6.9-7.4. PF solution conductivity increased from 345 ± 45 to 3190 ± 653 µS cm\(^{-1}\) after 84 h, of which the contribution of IC should be <7% assuming all the IC was in the form of NaHCO\(_3\). Thus, the conductivity increase was primarily due to reverse salt diffusion. Significant bacterial growth (>2 orders of magnitude) was observed during the experiments; the CFU increased from \(1.3 \times 10^7\) to \(3.4 \times 10^9\) CFU mL\(^{-1}\) and the turbidity increased from 0.9 to 187.7 NTU. Note that there was typically <800 mL feed volume change at the end of each 24 h cycle, thus <30% concentrating effect on the feed properties should be taken into consideration.

The WW solution has different chemical components as compared to the PF; TOC (82.5 ± 25.4 mg L\(^{-1}\)), IC (49.6 ± 2.8 mg L\(^{-1}\)), and conductivity (2924 µS cm\(^{-1}\)) was ~0.5, 15, and 8 times that of the PF solution. There was a slight increase of pH from 6.7 to 8.3 during the experiments,
but the underlying reason was not clear. The TOC \((87.3 \pm 16.2 \text{ mg L}^{-1})\) and IC \((59.0 \pm 5.2 \text{ mg L}^{-1})\) of the WW remained relatively stable after 84 h indicating limited bacterial activity. The measured CFU confirmed a low bacterial concentration in the WW \((3.6 \times 10^5 \text{ to } 3.1 \times 10^6 \text{ CFU mL}^{-1})\). Similar to the PF experiments, the conductivity increased by \(~3900 \mu \text{S cm}^{-1}\) at the end of experiments. It is of note that there are particulates in the WW with an initial turbidity of \(11.7 \pm 1.0 \text{ NTU}\) (as evidenced by the optical microscopy images in 4.4.3). The turbidity increased to an average of \(22.9 \pm 6.7\) by the end, likely due to big particle breaking down during circulation. The bacterial effects on turbidity should be limited at such low concentrations.

Table 4.1: Properties of the synthetic and actual wastewater

<table>
<thead>
<tr>
<th>Properties</th>
<th>PF solution</th>
<th>WW solution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Start of experiment</td>
<td>End of experiment</td>
</tr>
<tr>
<td>Chemical</td>
<td>TOC (mg L(^{-1}))</td>
<td>183.1 ± 19.8</td>
</tr>
<tr>
<td></td>
<td>IC (mg L(^{-1}))</td>
<td>3.4 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>Conductivity (µS cm(^{-1}))</td>
<td>345 ± 45</td>
</tr>
<tr>
<td></td>
<td>pH</td>
<td>6.9 ± 0.1</td>
</tr>
<tr>
<td>Biological</td>
<td>Bacteria density (CFU mL(^{-1}))</td>
<td>((1.3 \pm 0.2) \times 10^7)</td>
</tr>
<tr>
<td>Physical</td>
<td>Turbidity (NTU)</td>
<td>0.9 ± 0.1</td>
</tr>
</tbody>
</table>

**PROPERTIES OF THE CNT COMPOSITE COATING**

The membranes and CNT composite coatings were also characterized as displayed in Figure 4.2 and Table 4.2.

The pristine FO membrane has a rough surface with 100s nm mushroom-like structures as typically observed on TFC polyamide surfaces [189] and the roughness was reported to be 36-49 nm [190, 191]. The CNT and CNT+CB coatings are much smoother with a surface roughness of 2.7 and 3.5 nm, respectively. The difference between the CNT and CNT+CB coatings is that 1/3
(wt) of the CNT was replaced with CB since the CB could achieve electro-active bacterial fouling reduction at a much lower material cost (<0.1%) [186] while the CNT+CB maintains a similar resistance [192]. Both the CNT (25% porosity, 49 nm pore size) and CNT+CB (29% porosity, 50 nm pore size) coatings are porous in comparison to the dense TFC surface. The CNT and CB are bounded together by Nafion 117 [193-195] with a mass ratio for CNT or CNT+CB to Nafion of 80:20. The C-F bonds of Nafion 117 tend to face outwards when interacting with CB and the threshold for full surface coverage of CB by Nafion was achieved at 70-80% CB. The contact angle of CNT (116 ± 4°) and CNT+CB (106 ± 2°) is lower than our previous samples with a CNM ratio of 50% (136° for CNT and 125° for CB) since the C-F bonds are more hydrophobic than the CNM surfaces [195]. In comparison, the pristine FO membrane is very hydrophilic with a contact angle of 56 ± 3°.

The CNT (740 nm) and CNT+CB (520 nm) coatings are very thin in comparison to the pristine membranes (114-117 μm). As a result of the thin and porous structure of CNT composite coatings, the membrane permeability with CNT (12.4 ± 0.8 LMH) and CNT+CB (12.7 ± 1.0 LMH) was not statistically different from the pristine membranes (12.6 ± 1.2 LMH) when using a 1 M NaCl draw solution. The thickness of the CNT+CB was ~2/3 of the CNT, indicating that the CB was embedded inside the CNT network and had no effect on the thickness. The average electrical resistance of the CNT and CNT+CB on the lesser dimension (4 cm) is 0.3 and 0.4 kΩ when dry and increases to 2.2 and 2.5 kΩ when wet, likely due to swelling of the Nafion when in contact with water [196].
Figure 4.2: SEM images of the pristine, CNT, and CNT+CB coating. Surface view, ×50k magnification.

Table 4.2: Properties of the CNT-CB and CNT electrodes coated membrane and the control membranes.

<table>
<thead>
<tr>
<th>Properties</th>
<th>Unpressed</th>
<th>Pressed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PVDF</td>
<td>CNT</td>
</tr>
<tr>
<td><strong>Morphology</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roughnessa (nm)</td>
<td>36-49b</td>
<td>2.7</td>
</tr>
<tr>
<td>Surface porosity (%)</td>
<td>dense</td>
<td>25 ± 1</td>
</tr>
<tr>
<td>Surface pore diameter (nm)</td>
<td>N/A</td>
<td>42 ± 7</td>
</tr>
<tr>
<td>Contact angle (°)</td>
<td>56 ± 3</td>
<td>116 ± 4</td>
</tr>
<tr>
<td><strong>Permeability</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coating thickness (nm)</td>
<td>N/A</td>
<td>740 ± 30</td>
</tr>
<tr>
<td>Membrane thickness (µm)</td>
<td>118 ± 2</td>
<td>119 ± 3</td>
</tr>
<tr>
<td>Membrane fluxc (LMH)</td>
<td>12.6 ± 1.2</td>
<td>12.4 ± 0.8</td>
</tr>
<tr>
<td>Dry (kΩ)</td>
<td>N/A</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>Wet (kΩ)</td>
<td>N/A</td>
<td>2.2 ± 0.4</td>
</tr>
</tbody>
</table>

a: The Ra within an area of 1.5 × 1.5 µm².
b: The reported Ra within an area of 3 × 3 µm²[190, 191].
c: The membrane flux was measured with DI feed and 1 M NaCl draw solution.

Mechanical pressing of the CNT and CNT+CB coatings was conducted to enhance adhesion on the FO membranes. The pCNT and p(CNT+CB) morphology is similar to the
unpressed samples; the roughness (2.6 and 3.2 nm), surface porosity (22 and 25%), average pore size (40 and 47 nm), and contact angle (106 and 96°) are all slightly (~10%) decreased. The thickness of the pCNT (660 nm), p(CNT+CB) (430 nm), and pressed membrane (78-81 µm) are 11, 17, and 32% less than the unpressed samples, respectively. The permeability of the pCNT (11.7 ± 0.5 LMH) and p(CNT+CB) (12.0 ± 0.9 LMH) is only decreased by ~6%, indicating that the permeability primarily depends on the dense selective layer that is not significantly affected by the mechanical pressing. The coating electrical resistance remains identical when dry and decreases by ~50% when wet. The coating interaction with the TFC membrane was improved by pressing as the pressed coating became more resistant to vigorous flushing (Figure C.3), yet all the coatings remained integrity under all conditions in this study.

4.4.2 CNT COMPOSITE COATING OPTIMIZATION USING PF FEED SOLUTION

The PF solution was first used as feed to evaluate the antifouling performance of the CNT composite electrodes and to optimize the composite electrode fabrication.

COMPARISON OF CNT AND CNT+CB—FLUX DECLINE IN THE ABSENCE OF FLUSHING

Normalized flux decline during the 84 h fouling experiments in the absence of flushing is displayed in Figure 4.3. Significant fouling was observed on the control and the normalized flux decreased from 1 at the beginning to 0.11 ± 0.02 after 84 h. Both the CNT and CNT+CB cathodes at 2 V had slightly less fouling as compared to the control with final normalized fluxes of 0.33 ± 0.02 and 0.35 ± 0.01, respectively. For quantitative comparison, an empirical exponential flux decline was used to model the fouling trend [93, 185] \( J_t = \exp(-kt) \); solid lines). The fouling rate constant \( k (\text{h}^{-1}) \) decreased by 41 and 44% for the CNT (0.016 h^{-1}) and CNT+CB (0.015 h^{-1}) cathode
at 2 V as compared to the control (0.027 h⁻¹), respectively. Even though the exponential fitting depicted the overall trends (R² = 0.96, 0.89, and 0.81), it is of note that the fouling data decayed quite slow for the control and remained quite stable for the CNT and CNT+CB 2 V in the last 24 h. The equilibrium fouling status was likely due to stationary bacterial growth phase after 48 h.

Figure 4.3: The normalized flux of the control, CNT at 2 V, and CNT+CB at 2 V in the fouling experiments using PF solution as feed with no flushing. All experiments were completed using 1 M NaCl draw solution at 1.5 cm s⁻¹ feed and draw flow velocity.

The observed fouling is severe as compared to previous FO fouling studies. In all studies in Table 4.3, the dense layer was in contact with the feed solution, which is more resistant towards fouling due to the lack of internal support layer pore clogging [176, 197]. Inorganic particle fouling (~42-50%) is typically observed within a relatively short operation time (10-15 h) [174, 198], and organic fouling (7-50%) occurs in <18 h dependent upon foulant type as well as presence of coagulating multivalent cations [174, 199]. FO microbial fouling is dependent upon the WW properties and increases with operation time with fouling (4-38%) being observed after ~24 h [172, 175, 190] and significant flux decline (56-68%) occurring after 800 h despite continuous aeration resulting in more near membrane surface turbulence [178].
Table 4.3: Fouling comparison in FO with the dense layer facing the feed.

<table>
<thead>
<tr>
<th>Feed</th>
<th>Main foulants</th>
<th>Inorganics</th>
<th>Draw</th>
<th>Flow rate (cm s⁻¹)</th>
<th>t (h)</th>
<th>Flux decline (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inorganic</td>
<td>400 mg L⁻¹ silica particles of 300 nm</td>
<td>50 mM NaCl</td>
<td>3.0 M NaCl</td>
<td>8.5</td>
<td>15</td>
<td>~42</td>
<td>Lee et al., 2010 [174]</td>
</tr>
<tr>
<td></td>
<td>1 g L⁻¹ silica particles of 139 nm and 24 nm</td>
<td>50 mM NaCl</td>
<td>5 M NaCl</td>
<td>8.5</td>
<td>10</td>
<td>~50</td>
<td>Boo et al., 2012 [198]</td>
</tr>
<tr>
<td>Organic</td>
<td>200 mg L⁻¹ alginate, humic acid, or BSA protein</td>
<td>50 mM total ionic strength (IS) with 1 mM Ca²⁺</td>
<td>5.0 M NaCl</td>
<td>8.5</td>
<td>12-18</td>
<td>~50, 43, or 17</td>
<td>Lee et al., 2010 [174]</td>
</tr>
<tr>
<td></td>
<td>250 mg L⁻¹ alginate of 12-80 kDa</td>
<td>~17 mM IS with 0.5 mM Ca²⁺</td>
<td>2-4 M NaCl</td>
<td>8.5</td>
<td>~18</td>
<td>7-16</td>
<td>Castrillón et al., 2014 [199]</td>
</tr>
<tr>
<td></td>
<td>200, 200, 50, and 50 mg L⁻¹ glucose, sodium acetate, meat extract, and peptone</td>
<td>Tap water, ~3 mM IS with 0.1 mM Fe³⁺, 0.7 mM Ca²⁺, etc.</td>
<td>0.5 M NaCl</td>
<td>120</td>
<td>128</td>
<td>25</td>
<td>Zhang et al., 2012 [176]</td>
</tr>
<tr>
<td></td>
<td>200, 200, 50, and 50 mg L⁻¹ glucose, sodium acetate, meat extract, and peptone</td>
<td>Tap water</td>
<td>0.5-1 M NaCl</td>
<td>NA</td>
<td>~800⁺</td>
<td>56-68</td>
<td>Zhang et al., 2014 [178]</td>
</tr>
<tr>
<td></td>
<td>Sludge</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>92, 79, 17, 122, 116, and 52 mg L⁻¹ urea, sodium acetate, peptone, starch, milk powder, and yeast extract</td>
<td>~10 mM IS with Mg²⁺, Fe²⁺, etc.</td>
<td>0.7 M NaCl</td>
<td>8.6-14.1</td>
<td>20</td>
<td>~5-30</td>
<td>Valladares Linares et al., [175]</td>
</tr>
<tr>
<td></td>
<td>2 × 10⁷ mL⁻¹ bacteria from municipal WW</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sterile wastewater</td>
<td>16 mM IS</td>
<td>1.3 M NaCl</td>
<td>8.5</td>
<td>~24</td>
<td>~10</td>
<td>Kwan et al., 2015 [172]</td>
</tr>
<tr>
<td></td>
<td>2 × 10⁶ mL⁻¹ P. aeruginosa</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 g L⁻¹ sodium acetate</td>
<td>Brackish surface water</td>
<td>2 M NaCl</td>
<td>9.9</td>
<td>24</td>
<td>22-38</td>
<td>Hegab et al., 2015 [190]</td>
</tr>
<tr>
<td></td>
<td>Ambient bacteria</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>283 mg L⁻¹ pancreatic casein digest, 50 mg L⁻¹ papain soybean digest, and 42 mg L⁻¹ dextrose</td>
<td>0.2 mM K₂HPO₄ and 1.4 mM NaCl</td>
<td>1 M NaCl</td>
<td>1.5</td>
<td>84</td>
<td>67-89</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>10⁷-10⁹ mL⁻¹ P. fluorescens</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

a. Experiments were conducted with continuous aeration.
The severe fouling (67-89%) after 24 h in this study should be attributed to the high bacteria concentration (10^7-10^9 mL^-1) and low linear flow velocity in the feed compartment (1.5 cm s^-1). The high microbial concentration here is illustrative of extreme fouling conditions such as in food processing wastewater [200] and WW sludge [201]. The flow velocity was significantly lower than other studies (8.5-120 cm s^-1), however, preliminary experiments at different flow velocities indicate that the intrinsic membrane flux at 1.5 cm s^-1 (12.6 LMH) is only ~20 and 30% lower than at 8.5 (15.2 LMH) and 17.6 (18.0 LMH) cm s^-1, respectively. Additionally, a typical empirical circulation vs. treatment flow rate ratio in industrial water treatment facilities is 40:1 and it is ~100:1 in this study at 73 mL min^-1 (1.5 cm s^-1), thus the hydraulic conditions at such a low flow velocity seem sufficient for FO operation. A ~50 µm biofilm developed on the membrane surface after ~20 h operation using 10^6 mL^-1 initial bacterial concentration, yet the biofilm had a loose structure with vertical channels that did not significantly affect the flux (~10%) [172]. The biofilm could be thicker here after a longer operation time at a higher bacterial concentration, resulting in severe concentration polarization, which is generally considered to be the cause of flux decline in FO [172, 174, 180].

Electrostatic repulsion is one possible mechanism for fouling reduction on the cathodic (2 V) CNT or CNT+CB coated membranes. *P. fluorescens* in broth has a zeta potential of -15.7 mV. The FO membrane surface has a zeta potential of ~0 to -7 mV in 50 mM NaCl within the pH range of 2 to 10 [198]; in comparison, the zeta potential on both the CNT and CNT+CB surfaces at 2 V total potential is more negative (<-50 mV) [185]. Cathodic bacterial inactivation may also occur via electrochemical H_2O_2 generation and other reactive oxygen species [41, 186]. At 2 V total potential, the cathode potential vs. 1 M Ag/AgCl was -0.5 V (Figure C.4), which is optimal for H_2O_2 generation on CNT [39]. Therefore, it is possible that bacterial fouling on the cathodic CNT
or CNT+CB coated membranes is reduced by both electrostatic and electrochemical effects. Since the CNT and CNT+CB had similar morphology and electrical resistance, their antifouling was quite similar.

In summary, cathodic CNT and CNT+CB coating at 2 V reduced membrane fouling under extreme conditions when no flushing applied. Both electrodes showed similar fouling reduction, and the CNT+CB reduced material cost by 1/3, thus only the CNT+CB was evaluated in the following experiments.

**COMPARISON OF PRESSED AND UNPRESSED ELECTRODES—FLUX RECOVERY BY LONG-INTERVAL-FLUSHING**

Significant flux decline necessitates periodic cleaning of the membrane surface. Increased flow rate and air-induced turbulence are both effective methods to recover permeability and the combined liquid-air flushing was used here, as it displayed a notably better performance than either method alone in preliminary experiments.

Normalized time-dependent flux change with long-interval (daily) flushing using PF feed solution is displayed in Figure 4.4a. The fouling-induced resistance recovery (FRR) [10, 202] was calculated using the equations below and is shown in Figure 4.4b.

\[
FRR\% = \frac{R_f - R_c}{R_f - R_0} \times 100\% 
\]

where \( R_f \), \( R_c \), and \( R_0 \) refer to the resistance of the fouled, cleaned, and the original membranes, respectively. Since the normalized flux \( J_t \) does not include the effects of feed and draw solution concentration change during filtration i.e., it is normalized to depict only the fouling trend, the resistance is inversely proportional to \( J_t \). Thus the following equation can be derived:

\[
FRR\% = \frac{J_c - J_f}{J_0 - J_f J_c} = \frac{J_c - J_f}{1 - J_f J_c} \times 100\% 
\]
where $J_c$ and $J_f$ denote the normalized flux of clean and fouled membrane and $J_0$ by definition is 1.

Figure 4.4: The a) normalized flux and b) FRR of the control, CNT+CB at 2 V, and p(CNT+CB) at 2 V in the fouling experiments using PF solution as feed with daily flushing. All experiments were completed using 1 M NaCl draw solution at 1.5 cm s$^{-1}$ feed and draw flow velocity.

From the normalized flux change with time (Figure 4.4a), the cathodic CNT+CB had a higher flux than the control in the first 24 h consistent with the results in Table 4.3 and no greater flux recovery by flushing could be observed after flushing at 24, 48, and 72 h, resulting in a similar
or lower flux (0.35-0.41) than the control (0.36-0.54) over the last 12 h. The control FRR has an overall increasing trend (23 to 46%) with time while that of the cathodic CNT+CB is decreasing (54 to 14%) (Figure 4.4b). The two FRR trends coincided at 36 h as confirmed by a set of complimentary experiments with flushing at 36 h. In comparison, the p(CNT+CB) FRR (68-81%) was 76-195% higher than the control and the normalized flux was >0.53 after the 84 h fouling experiment.

Bacterial morphology on both the fouled and flushed control and CNT+CB surfaces was characterized visually and microscopically and flushing only removes a fraction of attached biofilm instead of an entire layer even though it is difficult to quantify. The FRR change is related to the electrical properties of the CNT composite electrodes. Since the unpressed CNT+CB electrode has a less dense structure, it was more vulnerable to swelling (and possibly fouling as well) with an average resistance increase of 250% (2.5 to 6.4 kΩ) than the p(CNT+CB) with an increase of 60% (1.2 to 1.9 kΩ). Accordingly, the effects of electrostatic repulsion as well as electrochemical inactivation decreased with time for the CNT+CB, while those for the p(CNT+CB) remained relatively constant. Thereby, p(CNT+CB) is selected as the optimal electrode for further antifouling experiments.

4.4.3 ANTIFOULING EVALUATION AND ANALYSIS USING PF AND WW FEED SOLUTION

COMPARISON OF PF AND WW—FLUX CHANGE IN THE PRESENCE OF FREQUENT FLUSHING

Normalized time-dependent flux of the control and p(CNT+CB) at 2 V using PF and WW feed solution with short-interval (every 40 min) flushing is displayed in Figure 4.5a and b,
respectively. For mechanistic analysis, experiments with p(CNT+CB) at 0 V (no charge) were also carried out under the same conditions. The daily average of absolute water flux (x-axis) and reverse salt diffusion flux (y-axis) is displayed in Figure 4.5c. All the membranes had a high TOC rejection (>99%) with no obvious dependence of membrane type or operation method.

The control flux decline using a PF feed solution after 84 h (Figure 4.5a) was much slower in the presence of frequent flushing than that in the absence of flushing (Figure 4.3), with the final normalized flux increased from 0.11± 0.02 to 0.70 ± 0.03. The cathodic p(CNT+CB) at 2 V reduced the fouling by ~50% with a final normalized flux of 0.87 ± 0.02. In comparison, the p(CNT+CB) at 0 V had more fouling than the control and the final normalized flux was 0.50 ± 0.04. The exponential fitting was also applied here for comparison with the no-flushing experiments and the overall fouling rate constant ranks in the order of p(CNT+CB)-0 V (0.008 h$^{-1}$) > control (0.005 h$^{-1}$) > p(CNT+CB)-2 V (0.002 h$^{-1}$). Note that since the fouling rate constant is very low for both the PF and WW feed experiments in the presence of short-interval flushing, the exponential fitting is close to a linear fitting within the experimental time.

The control had a quite different fouling (Figure 4.5b) using WW as compared to the PF feed with a significant drop of flux within in the first 1 h and a slow time-dependent fouling trend afterwards. To illustrate the initial fouling observed in these experiments, the exponential model was modified to include a constant for initial normalized flux loss $L$ (dimensionless) [185] ($J_t = (1-L) \exp(-kt)$). $L$ is the greatest for the control (0.32) and decreases by 63% for p(CNT+CB) at 2 V (0.12) and 0 V (0.11). The fouling rate constant order is the same as the PF feed solution with slightly decreased values: p(CNT+CB)-0 V (0.008 h$^{-1}$) > control (0.004 h$^{-1}$) > p(CNT+CB)-2 V (0.001 h$^{-1}$). The final normalized flux of the cathodic p(CNT+CB) at 2 V with WW feed was the highest (0.83 ± 0.07) due to both minimal initial flux loss and slower overall fouling rate, the
p(CNT+CB) at 0 V (0.49 ± 0.10) is similar to the control (0.51 ± 0.01) as a result of a smaller initial flux loss and greater fouling rate.

Reverse salt diffusion from the draw to feed is an important FO performance measure and is displayed with the daily normalized water flux in Figure 4.5c. In PF feed experiments (open symbols), the control (7.4-11.1 LMH), p(CNT+CB)-2 V (7.4-13.5 LMH), and p(CNT+CB)-0 V (5.4-10.7 LMH) had varying distributions in terms of absolute water flux (x-axis) with relative values in agreement with the normalized flux data. The reverse salt diffusion flux was similar (except for 1 data point for the control) for the control (0.11-0.18 mol m\(^{-2}\) h\(^{-1}\)) and p(CNT+CB)-2 V (0.12-0.20 mol m\(^{-2}\) h\(^{-1}\)). It ranged widely (0.07-0.31 mol m\(^{-2}\) h\(^{-1}\)) and increased with flux decrease over time for the p(CNT+CB)-0 V. In WW feed experiments (closed symbols), only the data for the control and p(CNT+CB) at 2 V were available, which had distinct distributions on both axes—the control had low water flux (4.9-7.2 LMH) and reverse salt flux (0.01-0.13 mol m\(^{-2}\) h\(^{-1}\)), whereas those for the p(CNT+CB)-2 V were both high (6.7-10.2 LMH and 0.09-0.30 mol m\(^{-2}\) h\(^{-1}\)). The averaged reverse salt diffusion of the p(CNT+CB) (0.18 mol m\(^{-2}\) h\(^{-1}\)) using WW feed was slightly greater than the PF experiments (0.13-0.16 mol m\(^{-2}\) h\(^{-1}\)), and that for the control (0.08 mol m\(^{-2}\) h\(^{-1}\)) was notably lower.
Figure 4.5: The water and reverse salt flux of the control, p(CNT+CB) at 0 V, and at 2 V in the fouling experiments using PF and WW solution as feed with flushing every 40 min. a) The normalized flux using PF feed, b) the normalized flux using WW feed, and c) the daily average reverse salt flux vs. water flux. All experiments were completed using 1 M NaCl draw solution at 1.5 cm s$^{-1}$ feed and draw flow velocity. The data of the reverse salt flux of CNT+CB at 0 V in WW filtration (magenta lower triangle, filled) were not available due to conductivity meter malfunction.

**COMPARISON OF PF AND WW—FOULING MITIGATION MECHANISM**

The different fouling and antifouling phenomena are related to the differences between the PF and WW feed solutions. Optical microscopic images of the PF and WW solution are displayed
in Figure 4.6a and b, and the fouled p(CNT+CB) surface at 0 V by PF and WW are displayed in Figure 4.6c and d. All images were acquired after the completion of 84 h experiments.

The PF contained only organics and bacteria (Figure 4.6a) as foulants, and their concentration was ~2 and 30-1000 times higher than the WW, respectively. Organic fouling can occur over short time period (<18 h), yet its contribution to flux loss should be limited due to the frequent flushing and lack of coagulating cations. Bacterial accumulation is primary reason for flux decline due to the larger number of bacteria present on all the membrane surfaces after 84 h experiments (Figure 4.6c). The p(CNT+CB) is more prone to fouling than the control, likely due to its greater hydrophobicity and less roughness on nm scale [186]. This is also in agreement with the long-interval flushing experiments, where the FRR of the unpressed CNT+CB decreased to be lower than the control as the electrical resistance increased over time. The fouling reduction with p(CNT+CB) can thereby be concluded to be related to the electrochemical and electrokinetic effects on the attached bacteria or biofilm.

The WW had a more complicated solution matrix than the PF, including various organics, inorganic ions, particulates of sub-micron sizes, and bacteria (Figure 4.6b). Even though the underlying mechanism is complicated, it is likely that the WW fouling immediately occurred due to inorganic particle accumulation rather than organic and bacterial fouling. The former could be severe enough for the control such that the pristine membrane surface was blocked and both water flux and reverse salt flux decreased significantly (~30%) at the start of experiments. The network of the p(CNT+CB) seemed to reduce the accumulation of particles on the membrane and less initial flux loss was observed for the p(CNT+CB) at both 2 V and 0 V. After the initial fouling, gradual bacterial fouling occurred and the fouling rates were consistent with the PF experiments. However, the WW bacterial concentration was low and the solution chemistry was not clear, thus there might
be other factors contributing to the fouling constant change as well, like electrochemically mineral scaling reduction [203]. As shown in the SEM photo (Figure 4.6d), a none-uniform fouling layer compositied of various foulants was found on the p(CNT+CB) 0 V after WW fouling.

Figure 4.6: Microscopic images of the PF and WW solution and the fouled membrane surfaces. The optical microscopic images (×40 objective lens) of the PF and WW solutions after 84 h experiment at 0 V are shown in a) and b), prepared by dropping ~50 µL solution onto a glass slide, adding the cover slip, and then removing the excess solution. The SEM images (×10k) of the p(CNT+CB) electrode surfaces after 84 h experiment at 0 V using PF and WW as feed solution are shown in c) and d). All experiments were completed using 1 M NaCl draw solution at 1.5 cm s⁻¹ feed and draw flow velocity.

4.5 CONCLUSIONS

1) Severe biofouling occurs in FO systems with the membrane dense layer facing feed solution at low flow velocity (1.5 cm s⁻¹). Normalized flux decreased by 67-89%, 47-65%, and 13-
50% after 84 h in the operation modes with no, long-interval, and short-interval flushing, respectively, using a feed solution of PF containing 146-183 mg mL\(^{-1}\) TOC and 10\(^7\)-10\(^9\) mL\(^{-1}\) \textit{P. fluorescens}. The normalized flux decline was 17-51% in presence of short-interval flushing using a feed solution of WW, which contains 83-87 mg mL\(^{-1}\) TOC, 10\(^5\)-10\(^6\) mL\(^{-1}\) bacteria, and particles of a turbidity of 12-23.

2) CNT composite electrodes were fabricated to evaluate the electro-active method for biofouling reduction on FO membrane surface. The pressed CNT+CB cathode at 2 V showed the best performance in terms of fouling rate reduction (44%) and FRR increase (76-195%) in comparison with the control.

3) The biofouling and antifouling mechanism was different for the PF and WW FO experiments. Bacterial accumulation should be the primary cause of fouling using PF feed due to its high bacterial concentration, and the fouling mitigation with p(CNT+CB) cathode at 2 V is due to the electro-active effects on bacterial attachment and biofilm formation while the p(CNT+CB) at 0 V had a greater propensity to fouling than the control. Initial flux loss occurred for the WW FO, probably due to particulate deposition on the control membrane surface, which was mitigated with the p(CNT+CB) in the absence and presence of electrical potential. The gradual fouling rate of WW can also be reduced with the electro-active effects by ~50%, similar to the PF experiments.
CHAPTER 5

INSULATED INTERLACED SURFACE ELECTRODES FOR BACTERIAL
INACTIVATION AND DETACHMENT

5.1 ABSTRACT

Effective and environmentally-friendly antibiofouling surfaces have long been of research interest. In this study, we proposed, fabricated, and evaluated a surface coated with insulated interlaced electrodes for bacterial fouling reduction. The electrodes were printed Ag filaments with 100 µm width and 400 µm spacing over an area of 2 × 2 cm². The insulating coating material on top of the Ag electrodes was PDMS or TPU with a thickness of 10 to 40 µm. To evaluate the antibiofouling potential, the inactivation of bacteria after 2 min contact with the surface and the detachment of bacteria after 15 and 40 h growth were examined. The inactivation was found related to the insulating material, coating thickness, and applied voltage (magnitude and AC/DC). The highest inactivation (84.5%) was achieved with 50 V AC 10 kHz on the 10 µm TPU. The detachment of bacteria after 15 and 40 h incubation was completed with simultaneous rinsing and AC treatment on 20 µm TPU. Higher AC voltages and longer rinsing times resulted in cleaner surfaces and the detachment efficiency decreased after extended bacterial growth time. Theoretical analysis indicated that the 10 V electric field strength above the electrode surface is non-uniform (~6,000-17,000 V m⁻¹ for the 20 µm TPU) and dielectrophoresis should play a key role in bacterial detachment. The trends observed in this study indicate that this technique may have merits for future antifouling surface development.
5.2 INTRODUCTION

Microbial attachment onto surfaces and subsequent biofilm formation are critical issues for many environmental and engineering applications. Biofouling is when a biofilm results in unacceptable losses to process performance or product quality/quantity [1, 2]. Thus, the development of antibiofouling surfaces or techniques is needed.

Bacterial attachment, viability, and detachment from the surface are important factors mediating biofouling. Attachment is generally recognized as a physicochemical process where the interfacial chemistry plays a key role. For example, to reduce attachment surfaces have been designed to be super hydrophilic to sterically hinder proteins and microbes [117] or to electrostatically repel bacteria [186]. After the bacterial attachment, the cells gradually grow into a biofilm with a protective structure resistant to external stimuli. A surface that can inactivate bacteria will also reduce biofilm formation, for instance, silver has been used to inactivate bacteria since 1000 BC [204] and active photo- or electro- chemical methods have been used reduce surface bacterial viability [21]. In regard to bacterial detachment, methods involve electric repulsion [121] and mechanical stress such as electrically-generated bubble elevation [20] or near-surface turbulence [205].

Electro-active methods are an important category of antibiofouling techniques implemented with electrodes at the surface-solution interface that can prevent bacterial attachment and/or promote inactivation and detachment. In a conventional electro-active system (AC or DC), the surface is used as an anode [26, 113] and/or a cathode [186] with a separate counter electrode to inactive or repel bacteria. Electric field can also cause bacterial cell damage and pulsed electric field (PEF) have found sterilization applications in the food processing industry for decades [206, 207]. However, one working electrode with an inactive counter electrode is not an ideal design,
especially when both the electrodes as well as the electric field could be beneficial for antibiofouling. A single surface integrating both electrodes, such as interlaced electrodes, is therefore proposed for electro-active antifouling.

Interlaced electrodes have been used in dielectrophoretic (DEP) studies where cells or cell components are separated and/or concentrated [208], primarily in microelectromechanical systems (MEMS) [32, 33]. However, this technique has limitations such as the high voltage degradation of the uncoated microelectrodes, the electric field decay reducing the concentrating efficacy [209, 210], and the high cost of photo-lithography limiting scale-up. Little attention has been paid to interlaced electrodes for biofouling reduction. A recent study used insulated interlaced metal wire electrodes beneath a microfiltration membrane to reduce clay particle accumulation via AC DEP [34]. However, alignment of the individual metal wires is time consuming and material intensive. The electric field should also decrease penetrating the membrane. To address all the issues above, a direct ink write (DIW) printing method [51] was adopted to rapidly and inexpensively produce interlaced electrodes (µm scale) over a relative large area (cm scale) and a thin insulating coating was casted on top of the electrode pattern to prevent electrode degradation with limited electric field decay. The fabricated surface may find potential application on many surfaces such as ship hull, medical tubings, and water distribution pipelines.

Here, we for the first time demonstrate the antimicrobial fouling potential of insulated interlaced surface electrodes. First, a conductive interlaced array of Ag electrodes (100 µm width, 400 µm spacing, and 2 × 2 cm² area) was printed onto a glass slide substrate and coated with insulating polymer using a mold of controlled depth. Second, bacterial inactivation under DC or AC potential was examined. Then, bacterial detachment under AC was evaluated after 15 and 40 h growth and a theoretical study of the electro-inactivation and detachment phenomena using
COMSOL modeling was completed. Finally, potential scale-up and lamination of the electrodes are discussed.

5.3 MATERIALS AND METHODS

5.3.1 MATERIALS

The Ag electrodes were made with Ag flakes purchased from Inframat Advanced Materials (Manchester, CT). The insulating coating was thermoplastic polyurethane (TPU; Elastollan®) purchased from BASF (Florham Park, NJ) and polydimethylsiloxane (PDMS; Sylgard® 184) from Dow Corning (Midland, MI). N-n-dimethylormamide (DMF) was acquired from Sigma (St. Louis, MO). *Escherichia coli* (*E. coli*) ATCC® 27325™ and *Pseudomonas fluorescens* (*P. fluorescens*) ATCC® 700830™ (Manassas, VA) were utilized in the microbial inactivation and detachment experiments, respectively. BD Bacto™ tryptic soy broth (TSB) and tryptic soy agar (TSA), NaCl (reagent grade), ethanol (EtOH) (laboratory grade), and crystal violet staining solution (BD DIFCOTM and BBLTM) were purchased from VWR International (West Chester, PA). The fluorescence staining reagents 4’, 6-diamidino-2-phenyindole dilactate (DAPI) and propidium iodide (PI) were acquired from Life Technologies (Grand Island, NY). Deionized (DI) water (>18 MΩ) was produced by a Nanopure Infinity ultrapure water system (Barnstead/Thermolyne) and was used to prepare solutions and to rinse containers.
5.3.2 SURFACE FABRICATION

Figure 5.1: Scheme of the experimental setup. a) The fabricated surface with interlaced electrodes on glass slide (actual photo, top; schematic plot not to scale, bottom); b) the inactivation test setup; c) the experimental procedure (top) and the setup (actual photo, bottom left; schematic plot, bottom right) of bacterial detachment experiment, consisting of (1) inlets and outlets, (2) a polycarbonate cover, (3) a rubber channel, (4) a fabricated surface for testing, (5) a rubber cushion, and (6) a polycarbonate bottom, all mechanically sealed with screws.
The Ag electrodes were printed onto plain glass slides using a custom-built 3D printer (ABG 10000, Aerotech Inc., Pittsburgh, PA, USA). The filament width was \( \sim 100 \) \( \mu \text{m} \) and the spacing between the electrode filaments was \( 400 \) \( \mu \text{m} \). The whole array is \( 2 \times 2 \) cm\(^2\) with two antenna extending 0.5 to 1 cm for connection to an external circuit (Figure 5.1a). The glass slides with Ag electrodes were dried in an oven overnight at \( 80 \, ^\circ \text{C} \). The electric resistance of the dried electrodes was determined by a digital multimeter (34401A, Agilent).

The PDMS solution was prepared by mixing the precursor solution and curing agent (10:1) and was used within 30 min after mixing. The TPU solution was prepared by dissolving TPU pellets in DMF and kept in a sealed container prior to usage. A \( \sim 2.5 \times 2.5 \) cm\(^2\) mold was made around the printed Ag electrodes array using 1 to 4 layers of Scotch\textsuperscript{\textregistered} tape (3M, St. Paul, MN) to control depth. The thickness of a single layer of tape was \( 51 \pm 1 \) \( \mu \text{m} \) by digital micrometer (Fowler Tools and Instruments; 1.27 to 25,400 \( \mu \text{m} \)). Then, the polymeric casting solution was poured into the mold and spread with a clean razor blade. After a smooth layer of polymer solution was coated on the Ag electrodes, the tape was removed and the slides were transferred to a hot plate to cure overnight at \( 80 ^\circ \text{C} \).

### 5.3.3 BACTERIAL SOLUTION PREPARATION

*E. coli* and *P. fluorescens* strains were grown on TSA plates and the colonies were transferred from plates to a TSB solution for cultivation. The *E. coli* was incubated at \( 37 ^\circ \text{C} \) and harvested after 4 h and the *P. fluorescens* was incubated at \( 30 ^\circ \text{C} \) and harvested after 18 h (exponential growth phase) at 120 rpm rotation. After centrifugation (Heraeus\textsuperscript{TM} Pico\textsuperscript{TM} microcentrifuge) at 10,000 rpm for 2 min and resuspension (Mini Vortexer, VWR) twice in 155 mM (0.9 % wt) NaCl saline solution, the bacterial stock solution was diluted to an optical density...
of 0.15 at 600 nm (OD$_{600}$ = 0.15) in NaCl saline solution (10$^8$ bacteria mL$^{-1}$ according to fluorescence microscopy enumeration).

The zeta potential of the *E. coli* and *P. fluorescens* in 155 mM NaCl was measured by a Malvern Nano ZS Particle Sizer. The conductivity of the 155 mM NaCl solution was determined by Oakton® CON 11 handheld conductivity/TDS meter.

## 5.3.4 BACTERIA INACTIVATION EXPERIMENTS

Bacterial inactivation experiments were completed using *E. coli* as the model bacterium. First, a solution of 10$^6$ bacteria mL$^{-1}$ in 155 mM NaCl was diluted from the stock, 2 mL of the prepared solution was vacuum filtered onto a gray polycarbonate membrane (0.2 µm pore size, Sterlitech; Kent, WA) with a round filtration area of 2.2 cm$^2$ (d = 1.7 cm), and the membrane was inverted and placed on the insulated interlaced surface electrodes (Figure 5.1b). Next, DC or AC voltages were applied to the electrodes and 0.5 mL 155 mM NaCl saline was spread onto the back of the membrane to keep moist. DC was supplied by an Agilent N5750A (150V/5A, 750W) and AC was generated by a customized inverter (DC to a square-wave AC) with the V$_{rms}$ equivalent to the DC and the frequency (10 kHz) set by an arbitrary waveform generator (Agilent 33120A). After 2 min, the gray membrane was gently removed for PI/DAPI fluorescence staining and microscopy. No significant change in bacteria density was noted from transporting the gray membrane according to preliminary tests (D.1 ). Both DAPI and PI bind to DNA and fluoresce upon excitation. PI will only stain inactivated cells with perturbed membranes, while DAPI will stain both live and inactivated cells. The *E. coli* solution was used within 1 h of preparation and a sample exposed to the surface in the absence of voltage was used as a control. All experiments were completed at least in duplicate. The bacterial inactivation by DC and AC compared to the uncharged controls was calculated following Eq 5.1:
\[
Inactivation \% = \left( \frac{N_{PI} - N'_{PI}}{N_{DAPI} - N'_{DAPI}} \right) \times 100\%
\] (5.1)

where \( N_{PI} \) and \( N_{DAPI} \) are the average numbers of PI and DAPI stained bacteria for the treated samples, and \( N'_{PI} \) and \( N'_{DAPI} \) are that for the control samples.

### 5.3.5 BACTERIAL DETACHMENT EXPERIMENTS

Bacterial detachment experiments were completed using \( P. \) fluorescens. An image of test cell is shown in Figure 5.1c. The flow cell consisted of (1) plastic inlets and outlets, (2) a 1 cm thick polycarbonate plate top cover, (3) a 1.5 mm thick rubber with hollowed channel in the middle, (4) the test surface, (5) another rubber layer as a cushion, and (6) a 1 cm thick polycarbonate plate bottom. The layers were mechanically sealed by four screws. The dimensions of the flow channel were 40 mm (length), 15 mm (width), and 1.5 mm (depth). There were two inlets and two outlets with a diameter of 3 mm and separation of 7 mm that were connected to an influent multichannel peristaltic pump (Masterflex® console drive) and an effluent waste container open to the atmosphere, respectively.

Prior to fouling and detachment experiments, the flow channel and the tubing were thoroughly cleaned with detergent solution, DI water, 70% EtOH, DI water, and then pre-conditioned with 155 mM NaCl for 30 min. The seeding of bacteria was completed by flowing \( 5 \times 10^7 \) mL\(^{-1} \) \( P. \) fluorescens solution (prepared by adding 50 mL TSB to 50 mL \( 10^8 \) mL\(^{-1} \) suspension) at 0.49 mL min\(^{-1} \) (3.6 \times 10^{-4} \) m s\(^{-1} \), \( Re = 1.10 \)) for 2 h. Then the surface was briefly rinsed with 155 mM NaCl saline at 4.9 mL min\(^{-1} \) for 2 min to remove any unattached bacteria. Next, half-strength TSB solution was flowed through the system at 0.49 mL min\(^{-1} \) at room temperature to promote surface bacterial growth. After 15 or 40 h cultivation, the surface was treated with AC power while rinsing with 155 mM NaCl saline for 2 to 20 min at an increased flow rate of 4.9 mL min\(^{-1} \). Finally,
the sample surface was removed and stained with DAPI (or crystal violet in some preliminary experiments. The bacterial detachment was not affected by the flow direction relative to electrode alignment and the majority of the data presented were collected with the electrode filaments in parallel with the flow direction. The bacterial areal coverage and brightness intensity (0-255 for black-white; as an indicator of bacterial density) were quantified using Image J (National Institutes of Health, details in D.2 ). Each experiment was completed in at least duplicate. The AC treatment efficiency was calculated according to Eq 5.2.

$$\varepsilon \% = \left(1 - \frac{c_t}{c_t'}\right) \times 100\%$$

(5.2)

where $c_t$ and $c_t'$ (%) are the bacteria coverage on an AC treated sample and an uncharged control after the same rinsing time $t$, respectively.

5.3.6 SURFACE CHARACTERIZATION

The DI water contact angle of the PDMS and TPU coating was measured using a goniometer (ramé-hart model 190 CA) via the sessile drop technique. At least 2 random samples were analyzed and at least 12 measurements were performed on each sample.

The negative surface charge of the PDMS and TPU was quantified following the toluidine blue procedure of Tiraferri and Elimelech [211] (details in D.3 ).

Scanning electron microscopy was carried out on a Zeiss UltraPlus field emission SEM (FESEM). The samples were coated with 2 nm of Pt/Pd (80/20) using an EMS 300T D Dual Head Sputter Coater.

Atomic force microscopy (AFM) was completed using the Cypher S AFM from Asylum Research (Oxford Instrument).
5.4 RESULTS AND DISCUSSION

5.4.1 CHARACTERIZATION

The dried Ag filaments have a thickness of 5 ± 2 µm (digital micrometer). The electric resistance over 2 cm was determined to be 5.8 ± 0.2 Ω, corresponding to a bulk resistivity of 1.2 × 10⁻⁷ Ω m. As the electrode surface was insulated from the electrolyte solution, current was only observed under AC voltage. The I_{rms} ranged from 0.05 mA to 28 mA with 5 to 50 V AC voltage (V_{rms}), yielding < 0.4% potential drop along the Ag filaments.

The insulating coating thickness [34] and relative permittivity (dielectric constant) will affect the electric field strength. The PDMS and TPU coating thickness was dependent on mold depth, polymer type, and concentration. Using a 51 µm thick mold, the PDMS had a dry coating thickness of 38-41 µm (PDMS-40) while the TPU had a thickness of 8-10 µm (TPU-10). To examine the effect of thickness on bacterial inactivation by electric field, the TPU was also coated using molds of 2× and 4× the initial mold thickness, resulting in dry coating thicknesses of 17-20 (TPU-20) and 36-40 µm (TPU-40), respectively. The relative permittivity of TPU and PDMS was from manufacturer specification of 4.0-8.0 and ~ 2.7, respectively (Table 5.1).

As mentioned previously, surface properties of the insulating coating should have an influence on bacterial attachment in the absence of electric field as a result of interfacial interactions. The contact angle, negative surface charge, and surface roughness were thus measured and summarized in Table 5.1. The TPU had a lower contact angle and a higher negative surface charge density than the PDMS. Both polymeric coatings had a clear and smooth surface by visual examination. The surfaces are also uniform by SEM (50,000×, Figure D.1) with TPU having 10s nm ridges, likely due to solvent evaporation while drying. The AFM roughness of the PDMS and TPU is 0.38 and 0.81 nm.
The zeta potential of *P. fluorescens* in 155 mM NaCl was -10.8 mV and of *E. coli* was -14.5 mV. The conductivity of the 155 mM NaCl solution was determined to be ~15,300 µS cm\(^{-1}\) and the addition of bacteria did not affect conductivity.

Table 5.1: Characterization of the polymeric insulating coating.

<table>
<thead>
<tr>
<th>Materials</th>
<th>Dry thickness (µm)</th>
<th>Relative permittivity (10 kHz)</th>
<th>Contact angle (°)</th>
<th>Negative surface charge (nm(^{2}))</th>
<th>Surface roughness (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDMS</td>
<td>38-41</td>
<td>2.68-2.72(^{a})</td>
<td>92.3 ± 1.5</td>
<td>0.38 ± 0.10</td>
<td>0.38</td>
</tr>
<tr>
<td>TPU</td>
<td>8-10, 17-20, 36-40</td>
<td>4.0-8.0(^{b})</td>
<td>71.9 ± 3.8</td>
<td>1.42 ± 0.11</td>
<td>0.81</td>
</tr>
</tbody>
</table>

\(^{a}\) Manufacturer specification.

\(^{b}\) Commonly reported range.

5.4.2 BACTERIA INACTIVATION WITH DC AND AC

Bacterial inactivation was examined by applying DC or AC for 2 min (Figure 5.2a-DC, b-AC). *E. coli* was selected as the model bacteria since it has a short incubation time and is ubiquitous in the environment [212, 213]. Preliminary experiments with *P. fluorescens* showed similar inactivation trends to the *E. coli* (Figure D.2).

The optimal electric-field inactivation over control was 67.8 ± 4.9 and 84.5 ± 3.1% on TPU-10 at 50 V DC and AC, respectively. For all surfaces, the inactivation percentage increased with voltage for both DC and AC and the AC inactivation was greater than DC at the same voltage. To quantitatively compare, a linear regression (fixed intercept = 0, \(R^2 \geq 0.9\)) was performed for inactivation percentages <50%, and at least three data points were included in each regression (Table 5.2). Once the inactivation was >50%, the curves started to plateau. No obvious spatial difference in activation was observed since all the interlaced array has a greater dimension (l = 2 cm) than the bacterial deposition area (d = 1.7 cm).
Figure 5.2: *E. Coli* inactivation for PDMS and TPU coating of different thicknesses by (a) DC and (b) AC of 5, 10, 20, and 50 V (Vrms for AC) for 2 min. All the data were calculated by extracting the dead ratio on the uncharged controls (Eq 5.1), and thereby the average value was 0 for all samples at 0 V. Dashed lines are the linear regression of the inactivation percent (<50%) with voltage.

Table 5.2: Summary of the linear regression results of inactivation (<50%) with DC/AC voltages on PDMS and TPU surfaces with different thicknesses.

<table>
<thead>
<tr>
<th></th>
<th>TPU-10</th>
<th>TPU-20</th>
<th>TPU-40</th>
<th>PDMS-40</th>
</tr>
</thead>
<tbody>
<tr>
<td>DC</td>
<td>Slope (V⁻¹)</td>
<td>3.72</td>
<td>2.11</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td>R²</td>
<td>0.99</td>
<td>0.91</td>
<td>1.00</td>
</tr>
<tr>
<td>AC</td>
<td>Slope (V⁻¹)</td>
<td>4.90</td>
<td>5.37</td>
<td>2.22</td>
</tr>
<tr>
<td></td>
<td>R²</td>
<td>0.90</td>
<td>0.96</td>
<td>0.95</td>
</tr>
</tbody>
</table>

The DC inactivation vs. voltage slope decreases from 3.72 to 0.89 V⁻¹ (~4-fold) as the TPU coating thickness increases from 10 to 40 µm. For the DC-TPU and DC-PDMS with the same
thickness of 40 µm, the slope of the TPU-40 (0.89 V⁻¹) is nearly 2 times of the PDMS (0.50 V⁻¹), similar to their difference in relative permittivity. The AC slope decreases from 4.90 to 2.22 V⁻¹ (~2-fold) as the TPU thickness increases from 10 to 40 µm. Even though the TPU-20 has a greater slope than the TPU-10, the inactivation for TPU-10 is higher than TPU-20 at voltages of 20 and 50 V. The AC-PDMS-40 has a slope of 0.94 V⁻¹, which is also approximately half of the AC-TPU-40, again similar to the difference in relative permittivity. All the AC slopes are 30-150% higher than those of DC under similar conditions.

Few quantitative studies have investigated the inactivation dependence on voltage or electric field for the insulated electrodes. A previous study using PEF to sterilize milk [206] observed that the log inactivation of *E. coli* appeared linear within the range of log 2.0 to 8.0 at a nominal electric field strength of 1,500,000 to 3,000,000 V m⁻¹. In this study, the max log inactivation was ~1 at a nominal electric field strength of 167,000 V m⁻¹, and the log inactivation also followed a linear trend when <0.25 (44%). The nonlinear inactivation vs voltage beyond 50% might be due to dead bacteria on the control surface (*N'PI/N'DAPI* = 12.8 ± 2.6% for TPU and 16.3 ± 5.4% for PDMS), thus subtracting it from *NPI/NDAPI* of even 100% would yield a maximum inactivation of ~80-90%. The nominal electric field strength is one magnitude lower than that in most PEF studies where a similar inactivation percent was observed (90%, log inactivation = 1), suggesting a different inactivation mechanism.

PEF bacterial inactivation studies typically utilize nominal electric field strengths > 1,500,000 V m⁻¹ for 10s to 100s µs to achieve a notable log inactivation (>0.5-1) of suspended bacteria in solution [206, 207]. The PEF underlying mechanism has not been conclusively elucidated although electroporation i.e., the electric-field induced formation of membrane pores appears important. Strong electric fields (>2,500,000 V m⁻¹) result in an irreversible effect and
ultimately cell death [207, 214, 215]. However, in those cases, the bacteria were suspended between the electrodes, whereas in this study, the bacteria were filtered on a membrane facing the insulated interlaced surface electrodes. Experiments were completed to evaluate whether suspended bacteria could be inactivated, 10 µL (25 µm) of $10^8$ bacteria mL$^{-1}$ solution was placed on the interlaced electrodes, and a very low inactivation percent vs. the control ($<6\%$) could be observed at 50 V AC. Thus, the phenomena in this work is specific for attached bacteria on or between the electrodes where the electric field is the strongest. Apart from electroporation, attached bacteria may also experience shear stress caused by the electrophoretic force or other electrokinetic phenomena as discussed in following sections due to more constrained surroundings. Resistive heating and corresponding surface morphology change might contribute to the inactivation as well since some TPU-10 surfaces deformed by visual examination at 50 V AC, but the temperature change on the surface over 2 min inactivation was not detectable by a digital thermocouple ($\pm 0.1^\circ C$).

5.4.3 BACTERIAL DETACHMENT WITH AC

*P. fluorescens* was used as the model bacteria for the detachment experiments as they have a greater propensity to attach than *E. coli* [186] to develop into a biofilm [131-133]. The optimal surface, TPU-20 was evaluated for bacterial fouling and AC detachment (the comparison of TPU-20 and PDMS-40 can be found in Figure D.3). TPU-10 was not selected as the testing surface since the thin surface coating occasionally corroded at coating defects or deformed at high voltages and the AC-TPU-20 inactivation results were similar to AC-TPU-10. AC was applied due to the better performance than DC in the inactivation and some preliminary detachment experiments.

Bacterial areal coverage is an important parameter in regard to biofilm formation [25, 133, 178, 216]. The bacterial coverage and AC treatment efficiency as a function of applied voltage and
rinsing time are displayed in Figure 5.3a and b, respectively. Representative optical microscopy images of the TPU-20 surfaces with DAPI stained bacteria are displayed in Figure 5.3c.

After 15 h of incubation at room temperature, the average bacterial coverage (left y axis; bars) was 11.3 ± 5.6% for TPU-20 after 2 min rinsing in the absence of voltage (control). After treatment with 5, 10, 20, and 50 V AC, the TPU-20 had a decreased bacterial coverage from 2.9 ± 2.4 to 0.7 ± 0.1% and the corresponding AC treatment efficiency increased from 74.7 ± 23.4 to 93.9 ± 1.1%. The 10 V AC of moderate performance was selected to evaluate the detachment dependence on rinsing time as well as bacterial growth time. The bacterial coverage decreased from 11.3 ± 5.6 to 7.3 ± 1.2% after 2 to 20 min rinsing in the absence of voltage after 15 h bacteria growth. The AC treatment efficiency slightly increased from 89.5 ± 1.1 to 96.5 ± 1.0% with the increased rinsing time. After 40 h incubation, the bacterial coverage (18.5 ± 4.6 to 13.3 ± 2.8% after 2 to 20 min rinsing) increased by ~50-100% and the AC treatment efficiency (73.6 ± 10.5 to 85.1 ± 7.0 after 2 to 20 min rinsing) decreased by ~10-15% from that after 15 h incubation, respectively.

Images of the TPU-20 samples after 15 (left) and 40 h (right) incubation and 2 min rinsing in the absence (top) and presence (bottom) 10 V AC are presented in Figure 5.3c. Bacterial coverage and average DAPI fluorescence intensity are reported below the images. The fluorescence intensity was used as an indicator of bacterial density on the surface. For the 15 h incubation experiment post-rinsing, the bacterial coverage was 10.1% for the control and decreased to 2.1% at 10 V AC. The average bacterial fluorescence intensity (0-255, black-white) was quite similar for the control (38) and the 10 AC (37) sample, although it seemed that most individual bacterial cells were removed by the AC treatment rinsing and only some aggregates remained. For the 40 h incubation experiment post-rinsing, a more developed biofilm structure and
a higher bacterial coverage (20.0%) was observed on the control with an average intensity of 46
than the 15 h experiment. At 10 V AC, the bacterial coverage decreased to 5.9% and the average
intensity increased to 58 with again larger aggregates remaining on the surface compared to the 15
h experiment post-rinsing. Therefore, the attached bacteria became more resistant to rinsing and
AC treatment upon increasing incubation time from 15 to 40 h.

Comparison to previous literature report on electro-active bacterial detachment is not
straightforward since this is the first work evaluating the antimicrobial fouling potential of
insulated interlaced surface electrodes. For example, Du et al. [34] applied 200 V AC of 200 kHz
to metal wires with a 30 µm insulating coating and ~1.5 mm spacing and increased the normalized
permeability of membrane above the electrodes from 41 to 69% after 400 min filtration. However,
the electrodes were placed below the membrane, the foulant was clay particles, and only
permeability performance was examined instead of the foulant attachment density. Despite the
experimental differences, the treatment efficiency of 49.5 to 93.7% in this study is in agreement if
not significantly better than their work under comparable conditions.
Figure 5.3: *P. fluorescens* detachment on the control and AC treated TPU-20 surfaces. a) The voltage dependence (0-50 V) of bacterial coverage and treatment efficiency at 0 V (control) and 10 V AC after 15 bacterial growth; b) the rinsing time dependence (2-20 min) of bacterial coverage and treatment efficiency by 2 min rinsing after 15 h or 40 h bacterial growth; c) the optical microscopy images of the control (top) and 10 V AC (bottom) treated of TPU-20 after 15 (left) and 40 h (right) bacterial growth and 2 min rinsing, noted with the bacteria coverage and average bacterial fluorescence intensity (0-255, black-white).
Control, 15 h incubation, 2 min rinsing
Bacteria coverage: 10.1%; avg intensity: 38

Control, 40 h incubation, 2 min rinsing
Bacteria coverage: 20.0%; avg intensity: 46

10 V AC, 15 h incubation, 2 min rinsing
Bacteria coverage: 2.1%; avg intensity: 37

10 V AC, 40 h incubation, 2 min rinsing
Bacteria coverage: 5.9%; avg intensity: 58

Figure 5.3 (Continued).
5.4.4 THEORETICAL MODELING AND ANALYSIS

To further investigate the bacterial inactivation and detachment results, a simplified model using COMSOL electrostatics simulation was developed. The relative permittivity of PDMS and TPU was assumed to be in the center of the reported range as 2.7 and 6.0, respectively. According to the assumption of no electric current, the model is only valid for the initial charging of the system. Once an electric potential is applied, both the insulating polymer layer and the electrolyte solution will be polarized and an electric double layer (EDL) will develop at the polymer-solution interface. Considering the EDL as a capacitor and the solution as a resistor, the high-pass filter effect exists such that the effective voltage within solution increases with AC frequency and decreases with polymer thickness [34]. Theory and experiments have shown an AC frequency of $\geq \sim 10$ kHz is able to prevent measurable EDL screening of the electric field [217], explaining the better performance of AC vs. DC since DC exerts only temporary forces whereas AC exerts continuous forces due to rapidly switching polarities.

The electric field (magnitude in gray contour and direction in yellow streamline) for TPU-20 at 10 V total voltage over the interlaced electrodes is shown in Figure 5.4a. A none-uniform electric field is found between the electrodes decreasing with the distance to the Ag filament edges. Since the solution has a different relative permittivity (80) from the TPU polymer (6.0), the electric field displays a distinct transition at the polymer-solution interface. The magnitude of the electric field strength was 6,000-17,000 ($\pm 500$) V m$^{-1}$ at the TPU-20 surface. Similarly, the electric field strength at other coating surfaces was simulated to be 2,000-4,000, 3,500-8,000, and 10,000-
Figure 5.4: COMSOL modeling of the electric field strength. a) The magnitude (grey contour) and direction (yellow streamline) of electric field over the cross-section of a pair of electrodes near the middle of the electrode array with 10 V using electrostatic simulation on TPU-20 (note that the aspect ratio of the dimensions was not 1:1); b) the bacterial inactivation and detachment by 10 V DC or AC vs. simulated electric field strength.
29,000 V m\(^{-1}\) for PDMS-40, TPU-40, and TPU-10, respectively, indicating that a thinner coating with a higher relative permittivity resulted in a greater electric field strength.

The average electric field strength was plotted with the inactivation and detachment experimental results in Figure 4b. It was found that the DC inactivation percent was almost linearly correlated to the electric field strength (slope = 0.0021 m V\(^{-1}\) and \(R^2 = 0.94\)). The AC inactivation percent also followed a linear correlation to the electric field strength (slope = 0.0044 m V\(^{-1}\) and \(R^2 = 0.99\)) except for the TPU-10 data, probably due to the surface malfunction such as electrode corrosion as mentioned previously. The linear dependence of inactivation on electric field strength agreed with the results in Figure 5.2 and Table 5.2, where different voltages were applied to the same polymer coating. The AC detachment efficiency was only examined on TPU-20 (Figure 3) and PDMS-40 (SI), which indicated that a higher electric field strength was better for detachment after 15 or 40 h bacterial growth, although the correlation did not look linear with an intercept = 0.

There are a number of physical mechanisms governing bacterial mobility in an aqueous electric field.

First, bacteria generally have a negative surface charge and will transport in electric field due to electrophoretic mobility. The governing equation is:

\[
v = \mu E
\]

where \(v\) (m s\(^{-1}\)) is the electrophoretic velocity, \(\mu\) (m\(^2\) V\(^{-1}\) s\(^{-1}\)) is the electrical mobility, and \(E\) (V m\(^{-1}\)) is the magnitude of the applied electric field. According to the zeta potential measurement, the bacterium used in this study are negatively charged and should exhibit negative electrophoretic mobility. The typically reported electrophoretic mobility of \(E.\ coli\) and \(P.\ fluorescens\) are in the range of around -0.5 to \(-2.5 \times 10^{-8}\) m\(^2\) V\(^{-1}\) s\(^{-1}\) in low ionic strength solutions and are usually 1-2
fold less when the ionic strength is increased to the 155 mM [218]. The bacteria should have an electrophoretic mobility on the scale of $\mu$m s$^{-1}$ in the solution between the electrodes from the above assumptions. However, 10 kHz AC would not generate notable bacterial movement i.e., bacteria electromigrate $\sim$0.001 $\mu$m before the polarities are switched. DC bacterial electromigration was not observed, likely due to the high-pass filter effect of the EDL. However, notable electromigration of positively-charged crystal violet dyes was observed for TPU-20 (5 min, 10 V DC, Figure S3), confirming EDL development on the interlaced electrodes.

Another potential mechanism is dielectrophoresis i.e., the mobility of polarizable microparticles by non-uniform electric fields. Cell components, cells, and cell aggregates having dimensions of the order of <1-100 $\mu$m are all considered microparticles [27-29]. The magnitude of the induced charge is usually small, equivalent to around 0.1% of a microbe’s net surface charge and can be generated within a $\mu$s [32]. The well-known expression for the DEP force on a dielectric sphere in a dielectric medium is [30, 31]:

$$F = 2\pi R^3 \varepsilon_0 \varepsilon_m Re[K] \nabla E^2$$

(5.4)

where $R$ (m) is the radius of the sphere, $\varepsilon_m$ is the relative permittivity of the electrolyte medium, $\varepsilon_0$ is the permittivity of free space, $E$ is the local electric field, and $Re[K]$ is the Clausius-Mossoti function determining the effective polarizability of the particle. When $Re[K]$ is positive, the polarized particles move towards the more intense electric field and the opposite otherwise. As the electric field is represented by $\nabla E^2$, the polarity of the electrode does not affect the DEP direction, thus the particle bacteria will always transport in a single direction within an AC field. The Clausius-Mossoti function is:

$$Re[K] = Re\left(\frac{\delta_p - \delta_m}{\delta_p + 2\delta_m}\right)$$

(5.5)
where $\delta_p^*$ and $\delta_m^*$ are the complex AC conductivity of the particle and suspending medium, respectively:

$$\delta^* = \delta - \frac{j\sigma}{\omega}$$

(5.6)

where $\delta$ (S m$^{-1}$) is the conductivity of the particle or the medium, $\omega$ (rad s$^{-1}$) is the angular frequency of the applied electric field, and $j$ is the imaginary unit. [30, 31, 48]

Markx et al. (1994) determined the effective conductivity of a number of bacteria strains within the frequency range of 10-100 kHz [31] and found many gram-negative bacteria including *E. coli* had an effective conductivity $<1,000$ µS cm$^{-1}$, lower than the effective solution conductivity of 15,300 µS cm$^{-1}$ in this study, suggesting a possible negative $Re[K]$. In another study using DI water as a very resistive suspension medium, a numeric simulation of a live cell yields a negative $Re[K]$ of -0.5 at 10 kHz [32], and it is generally recognized that negative $Re[K]$ tends to occur with higher solution conductivity [219]. Since the electric field weakens with distance from the electrode (Figure 5.4), the bacteria are expected to transport away from the electrode surface.

In terms of DEP magnitude, the typical electric field strength required for bacterial confinement in a microfluidic system is $\sim$30,000-60,000 V m$^{-1}$ [210]. The electric field above the TPU-20 is close to the lower boundary of this range. In accordance with the spatial magnitude distribution of electric field, both horizontal and vertical movement (out of focus plane) of bacteria above the TPU-20 surface at 20 V AC ($<1$ µm s$^{-1}$) was observed (Figure D.5) by an optical microscope. Cell rotation may also occur as *P. fluorescens* is not a sphere but a rod-like shape [27], which may further increase the probability of bacteria detachment from the surface.

Finally, electroosmotic flow on the surface between the Ag electrodes [24, 220] may contribute to the bacterial mobility since TPU carries slightly negative charge (-1.42 nm$^{-2}$) while the PDMS surface is closer to neutral (-0.38 nm$^{-2}$) according to Table 5.1. Electroosmotic flow
was suggested to occur on inorganic membrane surface at 500 to 4,500 V m\(^{-1}\) increasing microfiltration flux and will increase with electric field strength \([35]\), thereby with >6,000 V m\(^{-1}\) on the TPU-20 samples at 10 V AC, electroosmosis might also be a factor affecting bacterial detachment.

### 5.4.5 OUTLOOK AND IMPLICATION

The bacterial inactivation and detachment phenomena on insulated interlaced surface electrodes have shown potential for antibiofouling applications. However, the practical application of this technology is largely dependent on the scale-up and versatility potential.

In this work, the electrodes examined had an effective area of 2 \(\times\) 2 cm\(^2\), and the pattern should be able to extend in area by a few fold given the potential drop over the Ag filaments of <0.4% at 50 V AC. Assembly of multiple parallel electrodes arrays is another option for scale-up in practical applications.

![Figure 5.5: Delamination and relamination of the insulated interlaced electrode surface in 2 steps of a) detaching the surface from glass after rinsing with EtOH and b) attaching the surface to the interior surface of a polypropylene centrifuge tube.](image)

There is need for versatility in terms of insulated electrode array transfer. For example, medical tubing and water distribution pipelines would require printing the electrodes inside a
cylinder, which would be difficult. One potential method is delamination from the glass substrate and relamination to an interior substrate. For example, the TPU-20 with Ag electrode pattern can be easily detached from the glass substrate with EtOH and then re-applied to the interior surface of a centrifuge tube (Figure 5.5a and b). Blow-drying at ~65°C for 5 min seals the TPU layer on the internal polypropylene centrifuge tube surface. Thus, it is possible to extend the application of the insulated interlaced electrodes to surfaces of different properties and curvatures that may not be viable for direct printing.

Apart from the scale-up and lamination possibilities, this technique can be improved in a few aspects including insulating polymer permittivity, thickness, sealing, and surface properties. Optimization of the electrode pattern can be exploited in the future as well.

5.5 CONCLUSIONS

Interlaced surface Ag electrodes with insulating polymer coating was fabricated and evaluated for antimicrobial fouling performance in terms of bacterial inactivation and detachment. The bacteria in contact with the surface after 2 min DC/AC could be inactivated following a linear regression with the voltages applied at <50% inactivation and the highest inactivation compared to the control was up to 84.5% on the TPU-10 with 50 V AC 10 kHz. Bacterial detachment was achieved with simultaneous rinsing and AC treatment after 15 and 40 h bacterial growth. The detachment was found to be dependent on the applied voltage, rinsing time, and the bacteria growth time. For TPU-20 surface with 15 h bacteria growth, the optimal microbial coverage could be reduced to 0.7% after 2 min rinsing at 50 V. Both experimental results and theoretical analyses revealed that the insulating polymer coating material as well as thickness have notable influence on the antifouling performance. DEP might be the principle mechanism for the bacterial
detachment. While there are many improvement possibilities, this technique seems promising in terms of the performance as well as scale-up potential and versatility for future applications.
CHAPTER 6
INTERLACED CNT SURFACE ELECTRODE BACTERIAL FOULING REDUCTION
OF MICROPOROUS MEMBRANES

6.1 ABSTRACT

Novel antibacterial membranes with Interlaced Carbon nanotube Surface Electrodes (ICSEs) were prepared by vacuum filtering a well-dispersed CNT-Nafion solution through a laser-cut acrylic stencil onto a polyvinylidene fluoride (PVDF) commercial microfiltration (MF) membrane. The electrochemically active ICSEs was hypothesized as an effective way to inhibit bacteria growth and accumulation on the MF membranes. Dead-end filtration tests were carried out using $10^7$ and $10^8$ bacteria mL$^{-1}$ to study the effects of ICSEs on the bacterial density and morphology as well as to evaluate the bacterial fouling trend and backwash (BW) efficacy of the system. In addition, a simplified COMSOL model was built to describe the electric field above the ICSEs. At 2 V DC and AC, the average bacterial log removal (feed to permeate) of the ICSE-PVDF increased by ~1 log compared to the control PVDF (3.5-4 log). Bacterial surface density on the ICSEs was altered in the presence of electric potential, the cathode had a lower bacterial density than the control (87-90%) and the anode (59-93%) after filtration, and BW could further reduce the density with the cathode again displaying highest performance. The optimal operating conditions reduced the fouling rate by 75% from the control during filtration at 2 V AC and achieved up to 96% fouling resistance recovery ($FRR$) by BW at 8 V AC using 155 mM NaCl solution. Finally, the COMSOL model indicated that electrophoresis and dielectrophoresis both may play an important role in the bacterial fouling reduction and BW efficacy increase.
6.2 INTRODUCTION

Microfiltration (MF) is an important separation technique widely used in water treatment and industrial processing. MF typically has a pore size distribution ranging from 100 nm to 10 µm and removes suspended particles and microbial cells by mechanical sieving [7]. Accumulation of bacteria on the membrane surface is common during MF. At short timescales, high bacterial concentrations as in the case of recovering chemicals of biological origin [221] or in membrane bioreactor systems [222] will result in concentration polarization and cake layer formation, greatly reducing the membrane permeability. At long timescales, even low bacterial concentrations may result in biofilm formation on the membrane surface decreasing flux and backwash (BW) efficacy [223]. Since biofouling increases operation and maintenance (O&M) costs and reduces membrane lifetime, fouling mitigation is of practical importance and research interest.

Bacterial fouling control can be divided into categories based on the mechanism of membrane surface or structure modification, boundary condition control, and exertion of external forces [24]. For instance, modification of microporous polysulfone membrane with a sulfonated polyether-ethersulfone/polyethersulfone block copolymer [224], the addition of silver nanoparticles [225], operating at critical flux [13, 226], sonication [227], and electric field [228, 229] have all been reported to be able to reduce MF microbial fouling. Among these methods, electrically-based method, utilizing electrokinetics and electrochemistry, has the advantages of easy automation and control. Electrophoresis and electroosmosis play an important role in the electrical systems since most bacteria are negative charged in natural aqueous conditions despite their complex surface chemistry [15]. Dielectrophoresis of bacterial cells [31, 230] is an alternative mechanism, although it has seldom been discussed in bacterial fouling reduction. The formation
of micro-bubbles at an electro-active membrane surface has also been demonstrated to remove the cake filtration layer [229].

The electrode material largely determines the success of electro-active method; it needs to be anti-corrosive, inexpensive, and capable of serving as both an electrode as well as a separation membrane [24]. Among all researched materials, the development of a carbon nanotube (CNT) electrochemical filter [38] provides a possible solution to electrode fabrication. The CNT electrode surface has been shown to be able to inactive bacteria in the presence of both oxidative [38] and reductive [186] potentials. The industrial price of CNT has decreased to 100 US$ kg$^{-1}$ [46], allowing for many commercialization applications. The multiwalled CNT network has a pore size around 100 nm and is relatively stable at low electrochemical voltages.

In conventional electrochemical filtration systems, the two electrodes are typically placed in parallel, on both sides [24, 47, 231] or both on the feed side [49, 50] of the membrane surface, complicating the filter design and hindering scale-up. On top of this, typically only one electrode was functional whereas the other was simply a counter electrode. Du et al. developed an interlaced electrode design where 0.6 mm diameter cylindrical metal wires with a 0.03 mm insulating polyurethane coating were placed in an alternating-anode-and-cathode manner beneath the MF membrane to reduce clay particle fouling at 200 V AC (200 kHz) by dielectrophoresis [34]. However, the insulating layer thickness for anti-corrosion necessitates the use of high voltage and frequency and the membrane thickness also limits the dielectrophoretic force. Interlaced CNT surface electrodes (ICSEs) on membrane surface would address in various extents the previously mentioned issues.

Here, ICSEs were fabricated by vacuum filtration of a well-dispersed CNT-Nafion solution through a laser-cut acrylic stencil onto a commercial MF membrane. Next, the effects of the ICSEs
on bacterial density distribution and morphology change were evaluated using $10^7$ mL$^{-1}$ bacterial solution in a dead-end filtration cell. Filtration with $10^8$ mL$^{-1}$ bacterial solution was carried out in the same device to examine the anti-bacterial fouling of the system and to optimize BW conditions. Finally, a COMSOL model of the electric field above the electrodes surface was used to elucidate the underlying anti-biofouling mechanisms.

6.3 MATERIALS AND METHODS

6.3.1 MATERIALS

CNT (C-grade, multiwalled, powder, >95% purity) was purchased from NanoTechLabs, Inc. (Yadkinville, NC). Nafion117 solution (~5% in a mixture of lower aliphatic alcohols and water) and formaldehyde (ACS reagent, 37 wt. % in water) were purchased from Sigma-Aldrich (St. Louis, MO). Pseudomonas fluorescens (P. fluorescens) ATCC 700830 (ATCC, Manassas, VA) was utilized in the bacterial fouling experiment. BD Bacto™ tryptic soy broth (TSB) and tryptic soy agar (TSA), NaCl (reagent grade), isopropyl alcohol (IPA; laboratory grade) and ethanol (EtOH; laboratory grade) were purchased from VWR International (West Chester, PA). The fluorescence staining reagent 4’, 6-diamidino-2-phenyindole dilactate (DAPI) was acquired from Life Technologies (Grand Island, NY). Deionized (DI) water (>18 MΩ) was produced by a Nanopure Infinity ultrapure water system (Barnstead/Thermolyne) and was used to prepare solutions and to rinse containers.

Polyvinylidene fluoride (PVDF) membrane (0.3 µm pore size according to manufacturer specification; GE Osmonics JX) and polycarbonate (PC) membrane (0.2 µm pore size, 47 mm diameter according to manufacturer specification, grey) were purchased from Sterlitech (Kent,
WA). The PVDF membrane was cleaned by ultrasonicating in IPA for 5 min, in DI for 5 min, and kept in IPA prior to use. The PC membrane was used directly without pre-treatment.

6.3.2 FABRICATION OF THE INTERLACED CNT ELECTRODES ON MEMBRANE SURFACE

The ICSEs were fabricated by vacuum filtering CNT-Nafion in IPA solution through an acrylic stencil onto commercial MF membranes. The CNT-Nafion solution was prepared by probe ultrasonicating 60 mg CNT and 300 mg Nafion solution in 30 g IPA at 50% magnitude (~1,000 W L$^{-1}$) for 15 min (Branson Sonifier S450-D). Since the density of the IPA was 0.80 g mL$^{-1}$, the resulting solution has a concentration of 1.6 mg mL$^{-1}$ CNT and 0.4 mg mL$^{-1}$ Nafion in IPA. A piece of aluminum foil was used to cover the container to avoid excessive solvent evaporation. The solution was uniform, well-dispersed, and stable for up to 2 months. The acrylic stencil was prepared by a laser cutter (VersaLaser cutting/engraving system). The thickness of the acrylic plate was 3 mm, and the filament dimensions were $18 \times 1.5$ mm$^2$ with 0.5 mm edge-to-edge spacing. The main pattern without the two antenna leads has an area of $2.1 \times 2.1$ cm$^2$. The protective adhesive brown paper was kept on the acrylic plate during cutting as well as CNT-Nafion solution filtration to help seal the system. The filtration setup for ICSEs fabrication is shown in Figure 1. After a specific volume (1.0 to 2.2 mL) of the CNT-Nafion solution was added to the stencil, ~0.5 bar vacuum was applied and maintained for 5-10 min. The samples were then dried in a sterile petridish at room temperature.
Figure 6.1: Schematic of the system for interlaced CNT electrodes fabrication on MF membrane: 1) top case, 2) stencil, 3) MF membrane, 4) porous sinter, 5) bottom case, 6) MF membrane with interlaced CNT electrodes, 7) container case, 8) pump connection, 9) container, 10) base.

6.3.3 BACTERIA FILTRATION EXPERIMENT

MF membranes undergo compaction during initial filtration [232, 233], here mechanical pre-compaction was adopted by compressing the membrane with or without ICSEs for 45 min (Carver 4386) between two pieces of 1.6 mm silicon rubber at the pressure of 4 MPa (40 bar). Compacted membranes had a relative constant permeability at pressure of 0-1.1 bar and the permeability was close to the membranes compacted by DI filtration (Figure E.1a and b). Note the rubber provided a smooth compaction surface and reduced the actual pressure exerted on the membranes. In addition, parchment paper was placed between the membrane and rubber to protect against rubber stretching of rubbers during the mechanical press.
*P. fluorescens* was cultured in TSB by seeding from a TSA plate at 30°C and harvested at mid-exponential phase (18 h). After centrifugation and resuspension twice in 155 mM NaCl solution, the bacterial stock solution was diluted to an optical density of 0.15 at 600 nm (OD$_{600}$ = 0.15) in saline solution (~10$^8$ mL$^{-1}$ according to fluorescence microscopy). Finally, the solution was either used directly or diluted to 10$^7$ bacteria mL$^{-1}$.

All filtration experiments were completed in dead-end mode using a peristaltic pump at a constant flow rate of 1.2 mL min$^{-1}$ with an effective filtration area of 4.4 cm$^2$ (similar to the CNT pattern area). The membranes were first wetted with ~8 mL of IPA/DI 50/50 and then rinsed with DI water for >10 min (typically 30 min). The inlet pressure was monitored and the outlet was open to the atmosphere. Prior to bacterial filtration, the membrane pure water permeability (PWP, L m$^{-2}$ h$^{-1}$ bar$^{-1}$) of the membranes was determined by the following equation:

$$PWP = \frac{\Delta V}{(\Delta t A_{eff} \Delta P)}$$

(6.1)

where $\Delta V$ (mL) is the permeate volume collected over a time $\Delta t$ (min), $A_{eff}$ (cm$^2$) is the effective filtration area of the membrane and $\Delta P$ (bar) is the transmembrane (= inlet) pressure.

Bacterial filtration tests were carried out with either 10$^7$ or 10$^8$ bacteria mL$^{-1}$ solution in 155 mM NaCl, with or without ICSEs, in the absence or presence of applied voltage. The 10$^7$ mL$^{-1}$ bacteria was used for bacterial density and morphology evaluation. The 10$^8$ bacteria mL$^{-1}$ was used to examine the fouling and BW efficacy. DC was applied by an Agilent N5750A (150V/5A, 750W) power supply and AC was generated by a customized inverter (DC to square-wave AC) with $V_{rms}$ (2 or 8 V) equivalent to DC and frequency (10 kHz) set by an arbitrary waveform generator (Agilent 33120A). The current of the system was monitored by the power supply with 1 mA limit of detection. The $\Delta P$ was recorded at 2, 10, and every 10 min afterwards and aliquots of
the permeate and feed were collected in sterile glass vials at 30 min for bacterial concentration measurement.

As the influent concentration was quite high \((10^7-10^8 \text{ bacteria mL}^{-1})\) in the dead-end filtration experiment, a 10 min BW was carried out every 60 min or when the \(\Delta P\) reached 1.1 \(\pm 0.1\) bar. The BW solution was either DI water or 155 mM NaCl and the flow rate was the same as the filtration rate \((1.2 \text{ mL min}^{-1})\). For filtration in the presence of voltage, the BW was carried out in the presence of voltage as well with \(V_{\text{rms}} = 8 \text{ V}\) (DC or AC). For experiments with \(10^8 \text{ mL}^{-1}\) bacterial solution, DI water was filtered for 10 min after each BW to determine the fouling resistance recovery \((FRR)\) following Equation 2\([10, 202]\) below:

\[
FRR\% = \left[\frac{(\Delta P_f - \Delta P_c)}{(\Delta P_f - \Delta P_0)}\right] \times 100
\]  

(6.2)

where \(\Delta P_f, \Delta P_c,\) and \(\Delta P_0\) (bar) are the transmembrane pressure after fouling, after BW, and before fouling respectively. It is also of note that for \(10^7 \text{ bacteria mL}^{-1}\), the DC polarity of BW was reversed in comparison to the filtration in an attempt to evaluate the BW effects on bacterial density; and for \(10^8 \text{ bacteria mL}^{-1}\), the DC potential was set in one polarity in the first 5 min and in the opposite polarity in the second 5 min to allow for equal cleaning on both electrodes. A diagram displaying the experimental procedures is in Figure E.2.

**6.3.4 MEMBRANE CHARACTERIZATION**

Membranes prior to filtration were evaluated for volumetric pore size distribution, mean pore size, and wet curve flow with a gas-liquid displacement porometer (POROLUX\textsuperscript{TM} 100) \([234]\), for volumetric porosity \((\mathcal{E}_V)\) with a pycnometer and balance \([235]\), and for superficial pore size, porosity, and cross-section thickness measurement with a Zeiss UltraPlus field emission SEM.
Analysis of the SEM images was completed using ImageJ (National Institutes of Health, Bethesda, MD).

6.3.5 **BACTERIA CHARACTERIZATION AND QUANTIFICATION**

The bacterial concentration in the feed solution was pre-determined by OD$_{600}$ as previously mentioned. To evaluate the bacterial rejection, the feed and permeate solution were characterized using the plate-counting method to quantify colony-forming unit (CFU).

After $10^7$ bacteria mL$^{-1}$ filtration, the membranes were examined by fluorescence microscopy to determine surface bacterial density and by SEM for confirmation of bacterial density as well as to analyze morphology. The PVDF membranes after $10^8$ bacteria mL$^{-1}$ fouling and BW were only analyzed by SEM. Fluorescence microscopy was completed by staining the sample with DAPI (excitation/emission 358/461 nm) for 2-5 min and then imaged by an inverted microscope (Olympus BX60). To prepare for SEM, the bacterial samples were fixed with formaldehyde vapor for at least 12 h, dehydrated with 40-to-100% EtOH-DI solutions, dried at room temperature, and then coated with 2 nm of Pt/Pd (80/20) (EMS 300T D Dual Head Sputter Coater).

6.4 **RESULTS AND DISCUSSION**

6.4.1 **MEMBRANE CHARACTERIZATION**

The PVDF and/or CNT electrodes were characterized by SEM for cross-sectional and superficial morphology (Figure 6.2a-e), by two-point probe for electric conductivity (Figure 6.2f), and by gas-liquid displacement for volumetric pore size and mean pore size ($d_V$, 50% of the cumulative number of pores) (Figure 6.2g), and the results are summarized in Table 6.1.
Figure 6.2: The cross-sectional and superficial morphology of the PVDF membrane and CNT electrodes. SEM photos include a) the cross-section of an unpressed PVDF, b) the top surface of an unpressed PVDF, c) the top surface of a pressed PVDF, d) the cross-section of a pressed PVDF with ICSEs on top, e) the top surface of the CNT electrodes. f) The electric resistance of CNT electrodes with different CNT-Nafion solution volumes and g) the cumulative filter flow, differential filter flow, and wet curve flow of the unpressed PVDF, pressed PVDF, and pressed ICSE-PVDF measured by gas-liquid displacement method.
Table 6.1: Morphological characteristics (thickness, $\delta$; superficial and volumetric porosity, $\varepsilon_S$ and $\varepsilon_V$; superficial and volumetric mean pore diameter, $d_S$ and $d_V$) and filtration properties of PVDF membranes with or without ICSEs.

<table>
<thead>
<tr>
<th>Membranes</th>
<th>Morphological characteristics</th>
<th>Filtration measurements</th>
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<td></td>
<td>$\delta$ (um)</td>
<td>$\varepsilon_S$ (%)</td>
</tr>
<tr>
<td>PVDF unpressed</td>
<td>181 ± 6</td>
<td>15.7 ± 1.4</td>
</tr>
<tr>
<td>PVDF pressed</td>
<td>119 ± 3</td>
<td>8.1 ± 0.9</td>
</tr>
<tr>
<td>ICSE pressed</td>
<td>14 ± 1</td>
<td>25.5 ± 4.1</td>
</tr>
<tr>
<td>ICSE-PVDF pressed</td>
<td>123 ± 6</td>
<td>NA</td>
</tr>
</tbody>
</table>

From the SEM images, the unpressed PVDF membrane has a thickness ($\delta$) of about 181 ± 6 $\mu$m, a surface porosity ($\varepsilon_S$) of 15.7 ± 1.4%, and a pore diameter ($d_S$) of 44 ± 4 nm. After mechanical compaction, surface morphology of the pressed PVDF membrane was mostly reserved while the $\delta$, $\varepsilon_S$, and $d_S$ decreased to 119 ± 3 $\mu$m, 8.1 ± 0.9%, and 31 ± 4 nm, respectively. The CNT network after compaction is more porous than the PVDF in regard to surface porosity ($\varepsilon_S = 25.5 ± 4.1$%) and pore size ($d_S = 52 ± 10$ nm).

From the volumetric pore size characterization, the unpressed PVDF has a wide and big peak ($d_v = 722 ± 59$ nm) for the differential filter flow, while the peak for the pressed PVDF is narrower and shifted towards smaller values ($d_v = 412 ± 13$ nm). It was also observed that the pressed ICSE-PVDF has two peaks, one in coincidence with the pressed PVDF and the other has a smaller pore size ($d_v = 175 ± 7$ nm) from the ICSEs. The mean pore size of the pressed ICSE-PVDF ($d_v = 245 ± 14$ nm) was in-between. The volumetric porosity also decreased from 39.0 ± 4.5 to 31.4 ± 7.5% with the addition of ICSEs.

From pre-filtration tests with DI (Table 6.1), the compaction reduced the $PWP$ of the PVDF membranes from 2950 ± 323 to 935 ± 71 L m$^{-2}$ h$^{-1}$ bar$^{-1}$. The pressed PVDF had a stable and
similar permeability to the DI-compacted PVDF with filtration (Figure E.1). The addition of 14 µm ICSEs on top of the PVDF did not change the permeability (902 ± 12 L m⁻² h⁻¹ bar⁻¹) significantly (with P value of 0.31 by Welch’s t test). The small change (~33 L m⁻² h⁻¹ bar⁻¹) between permeability for the PVDF with and without ICSEs was further confirmed by the wet curve flow (Figure 6.1g, on the right), and the volumetric pore size difference resulted in a greater permeability difference only at higher pressures.

The electric conductivity of the ICSEs was also characterized. As shown in Figure 6.2F, the resistance of a CNT electrode filament decreased from 130 ± 18 to 59 ± 6 Ω when the volume of the CNT-Nafion solution was increased from 1.0 to 2.2 mL. The reciprocal of the average resistance was linearly correlated to the amount of CNT deposited. For the filtration experiments, 2.8 mg CNT was deposited on a sample area of 4.4 (2.1×2.1) cm². The CNT electrode filaments were 18 mm in length, 1.5 mm in width, 14 µm in height, and 0.5 mm apart. The bulk resistivity (\(\rho = R A/l\); where \(\rho\) (Ω cm), \(R\) (Ω), \(A\) (cm²), and \(l\) (cm) are bulk resistivity, filament resistance, cross-sectional area, and filament length, respectively) of the CNT network was calculated to be 0.008 Ω cm, lower than the value of 0.029 Ω cm reported in our previous study using CNT-Nafion coating [186] due to the reduced Nafion/CNT ratio (by 3-fold). At the low resistance of 72.9 Ω, the potential drop over a single filament will be negligible (0.07 V given a maximum of 1 mA at 2 V), indicating the CNT electrodes are sufficiently conductive for the electrochemical filtration.

### 6.4.2 BACTERIAL REMOVAL BY THE MEMBRANES

The compacted PVDF membranes should be able to remove bacteria cells by a sieving mechanism \((d_e = 31 ± 4\) nm) and the removal is shown in Figure 6.3. The log removal of the control PVDF was 3.5 ± 0.6 and 4.1 ± 0.9 for \(10^7\) and \(10^8\) bacteria mL⁻¹, respectively. In the absence of electric potential, the addition of ICSEs did not significantly alter the log removal, being slightly
higher at 4.6 ± 0.8 for 10⁷ bacteria mL⁻¹ and lower at 3.6 ± 0.6 for 10⁸ bacteria mL⁻¹. At 2 V DC and AC, the log removal increased by ~1 log from the control PVDF to 4.5 ± 0.5 and 4.7 ± 0.6 for the 10⁷ bacteria mL⁻¹ and 5.1 ± 0.6 and 5.2 ± 0.9 for the 10⁸ bacteria mL⁻¹. However, according to Welch’s t-test (Table E.1) in comparison, none of these results have a P-value < 0.05, thus additional experimental data is required to determine if there is a statistical difference.

Figure 6.3: Bacterial removal by the PVDF membranes with or without ICSEs in the presence or absence of potential. Experiments were completed by filtering 10⁷ or 10⁸ bacteria mL⁻¹ in 155 mM NaCl at the flow rate of 1.2 mL min⁻¹. Feed and permeate samples were taken at 30 min of filtration to determine the CFU.

6.4.3 BACTERIAL DENSITY ON THE MEMBRANE SURFACE

The bacterial density distribution on the membrane surface as displayed in Figure 6.4 and Table 6.2 was affected by the applied electric potential to the ICSEs. The bacterial density on the control PVDF was 202.1 ± 29.1 and 86.6 ± 6.2 × 10⁵ bacteria cm⁻² by SEM. In comparison, there were 62.8 ± 5.6 and 25.9 ± 3.2 × 10⁵ bacteria cm⁻² on the anode and cathode after electrochemical filtration at 2 V, 69 and 87% lower than the control. BW was carried out with DI water at an elevated voltage (8 V) at the opposite polarity. During BW, water splitting (2H₂O → 2H₂ + O₂,
Figure 6.4: Bacterial density and morphology on the PVDF and CNT electrodes after filtration with or without BW. a) Filtration control, b) BW control, c) Filtration anode, d) BW cathode, which was the anode during filtration, e) filtration cathode, and f) BW anode, which was the cathode during filtration. Experiments were completed by filtering $10^7$ bacteria mL$^{-1}$ in 155 mM NaCl solution at the flow rate of 1.2 mL min$^{-1}$ for 60 min and BW at the same flow rate for 10 min with DI water. For the PVDF with ICSEs, 2 V DC and 8 V DC with the reversed polarity was applied during filtration and BW, respectively.
4.92 eV) [236] as well as other electrochemical reactions have occurred and gas bubbles (<1 mm in diameter) were observed on both electrodes during BW, which may remove deposited bacteria as well [43, 44]. After BW, the bacterial density on the cathode \( (2.7 \pm 1.1 \times 10^5 \text{ bacteria cm}^{-2}) \) and anode \( (21.7 \pm 2.1 \times 10^5 \text{ bacteria cm}^{-2}) \) was 97 and 75% lower than the control, respectively.

Table 6.2: Bacterial density on the PVDF and PC membrane surfaces with or without ICSEs using \( 10^7 \) bacteria mL\(^{-1} \) filtration and DI BW.

<table>
<thead>
<tr>
<th>Surfaces on PVDF and PC</th>
<th>Condition</th>
<th>Characterization technique</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Filtration</td>
<td>SEM for PVDF membranes (10(^5)/cm(^2))</td>
</tr>
<tr>
<td>Control PVDF and PC</td>
<td>Filtration</td>
<td>202.1 ± 29.1</td>
</tr>
<tr>
<td></td>
<td>BW</td>
<td>86.6 ± 6.2</td>
</tr>
<tr>
<td>ICSEs on PVDF and PC</td>
<td>Filtration anode, 2 V</td>
<td>62.8 ± 5.6</td>
</tr>
<tr>
<td></td>
<td>BW cathode, 8 V (filtration anode)</td>
<td>2.7 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>Filtration cathode, 2 V</td>
<td>25.9 ± 3.2</td>
</tr>
<tr>
<td></td>
<td>BW anode, 8 V (filtration cathode)</td>
<td>21.7 ± 2.1</td>
</tr>
</tbody>
</table>

By optical microscopy, there were 18.0 ± 2.3 and 3.1 ± 0.5 \( \times 10^5 \) bacteria cm\(^{-2} \) on the control PC before and after BW. In comparison, the bacterial density was increased by 41% on the anode (25.3 ± 4.9 bacteria cm\(^{-2} \)) and significantly reduced by 90% on the cathode (1.8 ± 0.3 \( \times 10^5 \) bacteria cm\(^{-2} \)) of ICSE-PC after filtration, which were further reduced after BW as cathode (0.3 ± 0.1 bacteria cm\(^{-2} \)) and anode (0.4 ± 0.1 \( \times 10^5 \) bacteria cm\(^{-2} \)), being 90 and 87% lower than the control, respectively.

In summary, the bacterial density was control > anode > cathode for the PVDF (SEM) and anode > control > cathode for the PC (optical) membranes after 60 min of filtration. Theoretically, \( 1.64 \times 10^8 \) (1640 \( \times 10^5 \)) bacteria cm\(^{-2} \) should be sieved by the membrane considering the bacterial removal during filtration, which is one and two orders of magnitude higher than observed by SEM.
and microscopy. Therefore, only a small fraction of the sieved bacteria were deposited on the membrane surface and this fraction will be dependent on the surface properties. The PVDF, PC, and CNT have notable difference in surface hydrophobicity (DI water contact angle was 83.5, 65.9, and 118.7°, respectively) and surface roughness (smooth, shiny & very smooth, and rough, respectively), thus the resulting bacteria deposition on them might be different. There was consistently a lower bacterial density on the cathode than the anode before (59-93%) and after BW (25-88%), indicating the electrokinetics affected bacterial deposition more than oxidant production.

6.4.4 BACTERIAL MORPHOLOGY ON THE MEMBRANE SURFACE

The bacteria morphology was also affected by electrochemical filtration and BW (Figure 6.4 insets). The filtered bacteria on the control membrane surface were close to the rod-like structure, with some stretching and broken cells observed. After 60 min filtration at 2 V, the bacterial cells on the anode of ICSE-PVDF appeared flattened and dehydrated. The direct electro-oxidization of E. Coli by CNT at 2 V for 30 s has been reported to alter the cell morphology and cause > 80% inactivation [38], in agreement with the bacteria morphology change observed after 60 min of electrochemistry and inactivation of the deposited cells would be expected. The bacteria on the cathode preserved membrane integrity and morphology after 60 min filtration, also in agreement with our previous findings [186].

After BW, the bacteria remaining on the control membrane surface were still mostly rod-like, whereas after electrochemical BW at 8 V on the CNT, significant degradation of cells on the anode was observed of BW and cells on the cathode also looked rougher than before, but not as degraded as the anode.
6.4.5 FOULING TREND

The $10^7$ bacteria mL$^{-1}$ experiments indicated the bacterial density and morphology change during filtration and BW, but the $\Delta P$ did not change notably after 60 min. To evaluate the antifouling performance of the ICSEs, experiments using $10^8$ bacteria mL$^{-1}$ were completed under similar conditions. The fouling trend is shown in Figure 6.5a. An empirical exponential fitting of the pressure increase was completed to quantitatively compare the fouling trends using Eq 6.3 [50, 93] and the fitting results are summarized in Table 6.3.

\[ \Delta P_t = \Delta P_0 \exp(kt) \]  

(6.3)

where $\Delta P_t$ and $\Delta P_0$ (bar) are the transmembrane pressure at time $t$ (min) and 0, and $k$ (min$^{-1}$) is the fouling rate constant (Table 6.3).

For all membranes prior to fouling, the PWP was determined and the corresponding $\Delta P$ of DI filtration was $0.13 \pm 0.02$ bar. In the first 60 min filtration (Filtration-1), the $\Delta P$ increased to 0.75, 0.66, 0.54, and 0.25 bar with a fouling rate constant $k$ of 0.029, 0.033, 0.019, and 0.012 min$^{-1}$ for the control PVDF and ICSE-PVDF at 0 V, 2 V DC, and 2 V AC, respectively. The fouling rate for ICSE-0 V is slightly greater than the PVDF, but that of the ICSE-DC and ICSE-AC is 34 and 68% lower.

BW with DI water was then conducted for 10 min in an attempt to recover the flux in the absence (PVDF and ICSE-0 V) and presence (ICSE-DC and ICSE-AC) of electric potential at 8 V. After BW-1, the DI filtration $\Delta P$ was 0.64, 0.36, 0.36, and 0.22 bar for the PVDF, ICSE-0 V, ICSE-DC, and ICSE-AC, respectively. An interesting phenomenon is that the $\Delta P_0$ for Filtration-2 was 6-33% lower than the $\Delta P$ of DI filtration right after BW-1, while that for Filtration-1 was in agreement with the PWP prior to membrane fouling. The underlying mechanism is not clear, but the bacterial electric double layer (EDL) is likely to play an important role. The bacterial zeta
Figure 6.5: The fouling trend on different membranes. A) The \( \Delta P \) increase with filtration time on different membranes, including control PVDF and ICSE-PVDF in the absence of voltages (0 V), and ICSE-PVDF in the presence of 2 V DC and 2 V AC 10 kHz using DI BW; the SEM images of the membrane surface after 2 rounds of filtration and BW, including B) control membrane, C) ICSE-PVDF at 2 V DC, D) ICSE-PVDF at 2 V AC using DI BW, and E) ICSE-PVDF at 2 V AC using 155 mM NaCl BW. Experiments were completed using \( 10^8 \) bacteria \( \text{mL}^{-1} \) filtration for 60 min or up to 1.1 bar. A 10-min BW was carried out after each round of filtration in the absence or presence of 8 V DC (for the 2 V DC filtration sample, polarity changed at 5 min) and 8 V AC (for the 2 V AC filtration sample).

Potential will decrease by \(~2/3\) from solution of low (0.1 mM) to high ionic strength (\(~150\) mM) due to EDL compaction [218, 237]. Thus one hypothesis is that the electrostatic repulsion between

153
bacteria decreased during filtration using 155 mM NaCl solution, resulting in a thinner fouling layer and thus a higher permeability than DI.

The $\Delta P$ for most membranes increased to $1.1 \pm 0.1$ bar (maximum allowed) during $10^8$ bacteria mL$^{-1}$ filtration in Filtration-2, while the ICSE-AC had a $\Delta P$ of 0.81 bar after another 60 min. The average operational time for the control PVDF was 67 min, while that of the ICSE-0 V (85 min), ICSE-DC (100 min), and ICSE-AC (projected to be 129 min in the absence of additional BW) increased by 27, 49, and 93%, respectively. The fouling rate constant $k$ for the control PVDF increased by 3-fold from Filtration-1 ($0.029$ min$^{-1}$) to Filtration-2 ($0.118$ min$^{-1}$). A similar but much smaller increase of $k$ (0.5 to 1.5-fold) was observed in experiments using ICSE-PDVF in the absence and presence of potential and the $k$ for ICSE-0 V ($0.049$ min$^{-1}$), DC ($0.046$ min$^{-1}$) and AC ($0.029$ min$^{-1}$) was 58, 61, and 75% lower than the control PVDF. The additional fouling resistance in Filtration-2 might be caused by the compaction of the unremoved bacteria on membrane surface by BW-1, thus the smaller increase of $k$ from Filtration-1 to Filtration-2 in ICSE-PVDF experiments implies a higher removal by BW-1 than the control.

The $FRR$ (Table 6.4) provides further evidence for the removal efficacy. Using DI as the BW solution, the $FRR$ was 18 and 43% for the PVDF and increased to 57 and 65% for the ICSE-0 V in BW-1 and BW-2, respectively. A similar more reversible fouling with CNT layer modification on ultrafiltration membrane was observed with natural organic matters filtration in another study, which was attributed to the surface charge and surface roughness of the CNT [182]. All the ICSE-PVDF had a higher $FRR$ than the PVDF (by 55-216% and 1-51% in BW-1 and BW-2, respectively), ranking in the order of $0 \text{ V} > \text{DC} > \text{AC}$. The reduced $FRR$ for ICSE-DC and ICSE-AC in comparison to the ICSE-0 V is likely due to their reduction of reversible fouling during filtration.
Table 6.3: Exponential fitting results of the control PVDF and ICSE-PVDF using $10^8$ bacteria mL$^{-1}$ filtration and DI BW.

<table>
<thead>
<tr>
<th>Membrane</th>
<th>Filtration-1</th>
<th>Filtration-2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\Delta P_0$ (bar)</td>
<td>$k$ (min$^{-1}$)</td>
</tr>
<tr>
<td>PVDF</td>
<td>0.14</td>
<td>0.029</td>
</tr>
<tr>
<td>ICSE-0 V</td>
<td><strong>0.10</strong></td>
<td>0.033</td>
</tr>
<tr>
<td>ICSE-DC</td>
<td>0.15</td>
<td>0.019</td>
</tr>
<tr>
<td>ICSE-AC</td>
<td>0.12</td>
<td><strong>0.012</strong></td>
</tr>
</tbody>
</table>

*The lowest values in each column are in bold.

Table 6.4: The $\Delta P$ and $FRR$ values for the control PVDF and the ICSE-PVDF using $10^8$ bacteria mL$^{-1}$ filtration and DI or 155 mM NaCl BW.

<table>
<thead>
<tr>
<th>BW solution</th>
<th>Membrane</th>
<th>BW-1</th>
<th>BW-2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\Delta P$ before BW (bar)</td>
<td>$\Delta P$ after BW (bar)</td>
<td>$FRR$ (%)</td>
</tr>
<tr>
<td>DI</td>
<td>PVDF</td>
<td>0.75</td>
<td>0.64</td>
</tr>
<tr>
<td></td>
<td>ICSE-0 V</td>
<td>0.66</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>ICSE-DC</td>
<td>0.54</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>ICSE-AC</td>
<td><strong>0.25</strong></td>
<td><strong>0.22</strong></td>
</tr>
<tr>
<td>155 mM NaCl</td>
<td>PVDF</td>
<td>0.56</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>ICSE-AC</td>
<td><strong>0.26</strong></td>
<td><strong>0.11</strong></td>
</tr>
</tbody>
</table>

*The lowest pressure before and after BW using DI and 155 mM NaCl are in bold and the highest pressure recovery using DI and 155 mM NaCl BW are in bold and italic.

In an attempt to further increase the $FRR$ of ICSE-AC, BW were also completed using 155 mM NaCl solution and an $FRR \geq 96\%$ in both BW-1 and BW-2 were observed. The $FRR$ change is confirmed by the SEM analysis of the membrane surfaces post-BW. As displayed in Figure 6.5b-e, multilayer bacterial deposition was still found on the control PVDF and ICSE-DC post DI BW, while the ICSE-AC had less than a monolayer of bacteria on the ICSEs post DI (mostly covered with bacteria) and 155 mM NaCl (mostly bare CNT surface) BW. The Figure 6.5d-2 and e-2 were at lower magnification on the edge of the CNT electrodes, indicating that there was an increasing bacterial surface density gradient from edge to center and that the bacterial density was almost uniform at $>\sim 100 \mu m$ apart from the edge.
It should be noted that even for the control PVDF, a higher FRR was observed with 155 mM NaCl BW, being 86 and 82% for BW-1 and BW-2, respectively. Therefore, the enhanced FRR is mainly attributed to none-electrochemical factors, for example, the bacterial fouling layer might be compacted and thin in 155 mM NaCl but got loose and thick in DI BW due to the EDL increase as previously mentioned, and the removal of the former bacterial structure was more efficient than the latter.

### 6.4.6 ELECTRIC FIELD MODELING ABOVE THE ICSES

![Figure 6.6: COMSOL simulation of the electric field strength and electric potential distribution above ICSEs.](image)

The total voltage over the two adjacent filaments is set as 2 V. The electric potential is shown in color change from blue (-1 V) to red (+1 V) and the norm of electric field strength is presented with contour from light grey (4500 V m⁻¹) to dark grey (500 V m⁻¹). The green streamlines depict the direction and the electric field strength. No electric double layer and the fluid condition effects are considered.

A simplified COMSOL model describing the electric field above the CNT electrode surface and illustrating the possible forces on the bacterial cells was simulated (Figure 6.6).
One mechanism of the bacterial migration may be electrophoresis. The *P. fluorescens* cells have a zeta potential of -10.8 mV in 155 mM NaCl according to our previous study (in preparation for publication), thus should be mobile in an electric field. The estimated velocity in the vertical direction based on the flow rate and the filter area \( v = \text{flow rate/filter area} \) is \( \sim 45 \, \mu \text{m s}^{-1} \). The electric field strength during filtration (2 V DC) at \( \sim 0.1-0.5 \) and \( 0.8-0.9 \) mm above the surface is 1000 and 500 V m\(^{-1}\), respectively. Supposing the electrostatic mobility of the bacterial cells is \( 1.0 \times 10^{-8} \, \text{m}^2 \, \text{V}^{-1} \, \text{s}^{-1} \) [218], the bacterial horizontal velocity should be \( \sim 10 \) and \( 5 \, \mu \text{m s}^{-1} \), on a similar magnitude to the vertical velocity. Theoretically, at \( < 0.05 \) mm (50 \( \mu \)m) from the edge on the CNT cathode, the electric field of \( > 4500 \, \text{V m}^{-1} \) should be able to prevent bacterial cells from deposition. It is of note that no EDL effect on the CNT electrode surface was considered in this model, which is affected by the ionic strength and flow conditions, therefore the actual electrophoresis may be less than this estimation as no clean ICSE was found after filtration and even BW in the presence of 2 and 8 V DC, respectively.

Another possible mechanism is dielectrophoresis (DEP) as the electric field distribution on the surface is not uniform, becoming less intensive further away from the electrodes, especially at the edges. Negative dielectrophoresis was very likely to take place for the bacterial cells in 155 mM NaCl and even DI solution [32, 238], as a result of which, the bacteria tend to move away from the intense electric field. Besides, the DEP force is independent on the direction of the applied potential, thus is effective with both DC and AC potential [27, 208]. In fact, since 10 kHz AC effectively overcomes the EDL screening [217], the DEP force will be greater in the presence of AC potential and thus better anti-biofouling performance can be anticipated. The bacterial density gradient on the CNT electrode edges (Figure 6.5d-2 and e-2) provides evidence for the DEP forces during BW at 8 V AC potential. The DEP velocity can be significantly affected (2-3 order of
magnitude) by the bacterial properties and solution conductivity [239] and the quantification studies are quite limited. According to the experimental data in this study, DEP on the scale of μm s⁻¹ might be possible near the CNT electrode surface.

6.5 CONCLUSIONS

In summary, ICSEs on commercial PVDF MF membrane were fabricated by vacuum filtering CNT-Nafion solution through a laser-cut stencil. The ICSE-PVDF membrane showed negligible permeability loss at < 1.1 bar and 25-36% higher average removal (~1 log) compared to the control. Potentials of 2 V and 8 V DC were applied to the ICSEs during filtration using 10⁷ bacteria mL⁻¹ and BW using DI, respectively. The deposited bacterial density on the ICSEs was altered in the presence of electric potential, the cathode had a lower bacterial density (3-14 fold) than the control and anode, and BW could further reduce the density with cathode again displaying highest performance. Bacterial morphology was also affected by the applied potential; the deposited cells appeared dehydrated and deformed on the anode at 2 and 8 V and rougher on the cathode only at 8 V, indicating inactivation to different extents. The ICSEs resulted in a lower fouling rate (34-75%) and a longer operation time (49-93%) than the control PVDF using 10⁸ bacteria mL⁻¹ in the presence of 2 V DC and AC and the optimal FRR was 96% by BW at 8 V AC with 155 mM NaCl. Electrophoresis and dielectrophoresis might be the underlying mechanism for the fouling reduction according to electric field modeling and theoretical analysis.
CHAPTER 7

CONCLUSIONS

This dissertation is focused on the electrokinetic and electrochemical methods for microbial and organic fouling mitigation. Fouling experiments were completed on a solid substrate or a porous membrane surface covered by a cathode or interlaced surface electrodes. The electroactive fouling mitigation was studied via both (semi-)quantitative evaluation of the bacterial attachment, inactivation, detachment, and membrane filtration performance in the absence and presence of bacterial or organic fouling under various conditions and theoretical analysis of the underlying mechanism. Detailed conclusions can be found at the end of each chapter and a few key results and possible extensions are highlighted below.

First, electrostatic interaction plays an important role in the microbial and organic foulant attachment on the cathode surface. In a non-Faradaic system, the capacitive charge on the cathode CNT-PVDF membrane reduced negatively-charged organic matter fouling on a PES UF membrane. The antifouling performance increased with negative charge of the foulant and the cathode and decreased with solution ionic strength, in agreement with DLVO theory. The capacitive system reduced O&M energy cost by 37-84% as compared to the control depending on solution conditions (CHAPTER 2). In a Faradaic system, the bacterial surface density of \textit{P. fluorescens} decreased linearly with increasing applied negative potential for all CNM coated cathodes and 67-90% bacterial attachment reduction was found after 3 h fouling at 2 V (CHAPTER 3).

Second, electrochemical inactivation via cathodic reactions on CNT and other CNM surface helps reduce microbial fouling. Up to 81\% of attached \textit{P. fluorescens} was inactivated on
CNM cathode surfaces after 3 h at 0 to -2 V as a result of intrinsic CNM toxicity and electrochemical inactivation and the latter can be correlated with \( \text{H}_2\text{O}_2 \) generation. The inactivation was only effective towards adhered as compared to suspended bacteria likely due to a high near-surface concentration of cathodic ROS. In 12 h fouling experiments, the application of a negative potential (-1 and -2 V) to the CNT coated electrode reduced the bacterial deposition kinetics by 8-10 fold as well as the production of appendages by the adhered cells, which likely reduced irreversible attachment (CHAPTER 3). The microbial FO fouling by synthetic \( P. \text{fluorescens} \) solution and actual wastewater was reduced by \(~50\%\) and the rising reversibility was increased by 76-195\% in comparison to the control using a composite CNT+CB coating on the membrane surface in 84 h filtration experiments at 2 V (CHAPTER 4).

Third, interlaced surface electrodes can be used to reduce microbial fouling via electrokinetic mechanisms such as dielectrophoresis and electrophoresis in the non-uniform electric field above the electrodes and electroosmosis on a charged substrate surface as well as electrochemical mechanisms such as electrochemical inactivation and microbubble generation. In a non-Faradaic system, the insulated interlaced Ag electrodes resulted in up to 84\% \( E. \text{coli} \) inactivation and 94\% \( P. \text{fluorescens} \) detachment with 2 min treatment at 50 V AC (10 kHz) (CHAPTER 5). In a Faradaic system, ICSEs on top of the PVDF MF membranes altered the \( P. \text{fluorescens} \) density and morphology on the cathode and anode at 2 V DC. The microbial fouling rate of the ICSE-PVDF was reduced by up to 75\% as compared to the control at 2 V AC and a high \( FRR \) of up to 96\% could be achieved by BW at 8 V AC using 155 mM NaCl solution (CHAPTER 6).

Last, the electrode material and fabrication are important for the electro-active fouling control and its future application. CNT was utilized for most electrodes fabrication in this
dissertation, showing high versatility and superb properties. By doping CNT in PVDF (5 wt. %), a conductive and non-Faradaic electrode of 90 µm thickness and ~28 nm superficial pore size can be made via the phase inversion method (CHAPTER 2). By mixing CNT with Nafion (1:1 to 4:1 w/w) in IPA, a uniform CNT solution can be prepared, which can be cast on various substrates to form a conductive, electrochemically-active, and porous (~50 nm pore size) layer of 100s nm thickness (CHAPTER 3 and CHAPTER 4) or vacuum filtered through a stencil on a MF membrane to make interlaced electrodes of 10s µm thickness and 1.5 mm width (CHAPTER 6). In addition, the insulated Ag electrodes fabricated by 3 D printing of 5 µm thickness and 100 nm width are highly conductive and can be delaminated and relaminated on other substrates (CHAPTER 5).

Although these electrodes and systems are promising in solving many fouling problems, future work is still needed to promote the development and application, such as the exploration of new electrode materials with low cost and high anti-fouling performance, the scale-up and configuration optimization for specific practical applications, and the performance and longevity evaluation under more realistic conditions.
APPENDIX A

SUPPLEMENTARY INFORMATION FOR CHAPTER 2

A.1 ADDITIONAL MEMBRANE CHARACTERISTICS

Figure A.1: DI permeability change with a new membrane in the cross-flow UF setup. The permeability is relatively constant during the 1 h experiment and 15 min filtration with DI prior to main experiment is enough to achieve a stable permeability.

Figure A.2: Optical microscopy images of CCP. a) Back-transmitted image by projecting light from the bottom of the sample and measuring the transmitted areas (bright white part) for pore size; b) a zoomed-in optical microscopy image of the CCP surface.
Figure A.3: Molecular weight cut off (MWCO) of the PES membrane completed with 10 ppm PEG solutions in the cross-flow UF cell with the basic filtration setup. The 90% TOC rejection is achieved with PES with Mn of 50,000.

A.2 PHOTOS OF THE EXPERIMENTAL SETUP
Figure A.4: Detailed schemes and photos of the three electro-kinetic systems. C) negative surface Charge only; F) electric Field; C+F) negative surface Charge and electric Field.
Figure A.4 (Continued).
Figure A.4 (Continued).
A.3 ADDITIONAL DATA FOR THE E-FILTRATION EXPERIMENTS

Figure A.5: UF membrane fouling and rejection as a function of electrode configuration and specific organic matter. Time-dependent organic membrane permeability normalized to DI permeability for aqueous solutions within different configurations: B) Basic cross-flow cell; C) negative surface Charge only; F) electric Field; C+F) negative surface Charge and electric Field.
Figure A.6: Cathode potential distribution in C+F configuration, CCP anode, CNT-PVDF cathode in 10 ppm PAA and 1 mM NaCl solution. Determined by changing the overall cell voltage stepwise from 0 to 2.5 V with the step of 0.5 V and measuring the cathode potential vs. 1 M Ag/AgCl electrode with an electrochemical workstation (604D, CHI Inc. Austin, TX).

Figure A.7: Rejection of PAA as a function of NaCl concentration in C+F configuration, with CCP or CNT-PVDF anode, CNT-PVDF cathode, at a cell voltage of 2 V.
A.4 ESTIMATION OF THE PES MEMBRANE SURFACE PORE SIZE

The identification of $M_n$ (Dalton) of the contaminants that the membrane is able to remove helps understand the real pore size of membrane in solution. If constant density of the contaminant molecules is assumed, the volume of a molecule will vary linearly with the molecular weight. Depending on the shape of the molecule, the diameter can be expressed by the equation below [56].

$$d = \beta (MW)^n$$  \hspace{1cm} (A.1)

where $d$ (nm) is the hydrodynamic diameter of the molecule and $\beta$ (nm) is the proportionality constant. $n$ is a function of the molecular shape; and it varies from 0.33 for spheres to nearly 1.0 for linear long-chains. The specific equation for PEG is:

$$d = 0.09 (MW)^{0.44}$$  \hspace{1cm} (A.2)

According to the results of MWCO, the UF membrane is able to remove 90% of the PEG with $M_n$ of 50,000 Da, and should have a hydrodynamic diameter of 10.5 nm.

A.5 SURFACE POTENTIALS OF THE PAA AND THE CNT-PVDF CATHODE

Equations to calculate the surface potential of PAA and cathode film:

$$\phi = -\frac{Ne}{\kappa \varepsilon_0} \exp(-h\kappa)$$  \hspace{1cm} (A.3)

$$\frac{1}{\kappa} = \frac{\lambda \varepsilon_0 kT}{\sqrt{n_\infty v^+(v^++v^-)e^2}}$$  \hspace{1cm} (A.4)

where $\kappa$ (m$^{-1}$) is the reciprocal Debye length, $\lambda$ (dimensionless) denotes the dielectric constant (80 for water at room temperature), $\varepsilon_0$ (F·m$^{-1}$) is for the dielectric permittivity of vacuum, $h$ (m) represents the distance from cathode film surface, $n_\infty$ (# m$^{-3}$) is the bulk number density of ions, and $v^+$ and $v^-$ (dimensionless) denote valence of the electrolyte ions. The $Ne$ (C m$^{-2}$) is the surface charge density. For PAA, $Ne$ is calculated by assuming the 1/2 of deprotonated groups are at the
spherical particle surface and the particle diameter is 10.5 nm, same as the pore size of UF membrane estimated by Eq A.2, since almost all TOC can be removed in UF experiments with 10 ppm PAA.

Table A.1: Calculated Debye length and surface potential of PAA and CNT-PVDF (with a cathode potential of 1.2 V) in NaCl solutions of 0.1-20 mM concentration.

<table>
<thead>
<tr>
<th>[NaCl] (mM)</th>
<th>Debye length (nm)</th>
<th>Surface potential (mV)</th>
<th>PAA</th>
<th>CNT-PVDF</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>30.1</td>
<td>-299.4</td>
<td>-104</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>9.67</td>
<td>-94.7</td>
<td>-58.8</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>6.83</td>
<td>-67.0</td>
<td>-53.1</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>3.06</td>
<td>-29.9</td>
<td>-34.1</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>2.16</td>
<td>-21.2</td>
<td>-27.3</td>
<td></td>
</tr>
</tbody>
</table>

The $N_e$ of CNT-PVDF film is determined by assuming all the capacitive charge is evenly distributed on the BET surface area. The calculated values of Debye length and surface potential of PAA and CNT-PVDF are listed above.
## APPENDIX B

### SUPPLEMENTARY INFORMATION FOR CHAPTER 3

## B.1 ADDITIONAL BACKGROUND INFORMATION

Table B.1: Water quality in some representative aqueous environments

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>Conductivity (ms/cm)</th>
<th>Total organic carbon (TOC, mg L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/8 TSB in 0.9% NaClᵃ</td>
<td>7.48</td>
<td>17.55</td>
<td>~900</td>
</tr>
<tr>
<td>Charles River water [240]</td>
<td>7.29</td>
<td>0.55</td>
<td>8.3</td>
</tr>
<tr>
<td>Untreated domestic wastewater [241]</td>
<td>NA</td>
<td>NA</td>
<td>160</td>
</tr>
<tr>
<td>Baker’s yeast wastewater [200]</td>
<td>7.80</td>
<td>19.20</td>
<td>1061</td>
</tr>
<tr>
<td>Wine distillery effluent [242]</td>
<td>NA</td>
<td>NA</td>
<td>8300</td>
</tr>
</tbody>
</table>

ᵃ: Measured in lab with a pH probe (Thermo Scientific Orion™ 9810BN), conductivity meter (Oakton CON 150), and a TOC analyzer (Shimadzu TOC-Vw).
B.2 PHOTOS OF THE EXPERIMENTAL SETUP

Figure B.1: A photo of the bacterial test setup.

B.3 PRELIMINARY EXPERIMENTS

Table B.2: The recovery of the vortexing.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Vortex 0.5 min</th>
<th>Vortex 1 min</th>
<th>Vortex 2 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovery (%)</td>
<td>43%</td>
<td>87%</td>
<td>96%</td>
<td></td>
</tr>
<tr>
<td>Dead ratio (%)</td>
<td>15%</td>
<td>4%</td>
<td>10%</td>
<td>18%</td>
</tr>
</tbody>
</table>

The experiments were carried out by depositing the *P. fluorescens* on a CNT membrane with 2 psi vacuum filtration and the control was done by directly observing the CNT surface. It was probably that the dead cells were vacuum filtered harder onto the CNT membrane and thus less effectively removed by vortexing.
### B.4 ADDITIONAL STATISTICAL DATA

Table B.3: The Welch’s t-test summary of the total bacterial deposition on the control coatings.

<table>
<thead>
<tr>
<th>P-value</th>
<th>CNT</th>
<th>OA-CNT</th>
<th>O-CNT</th>
<th>CB</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OA-CNT</td>
<td>0.005</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O-CNT</td>
<td>0.000</td>
<td>0.112</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CB</td>
<td>0.000</td>
<td>0.049</td>
<td>0.308</td>
<td></td>
</tr>
<tr>
<td>rGO</td>
<td>0.000</td>
<td>0.034</td>
<td>0.119</td>
<td>0.737</td>
</tr>
</tbody>
</table>

a: The average bacterial density on the control coatings ranged from high to low is CNT > OA-CNT > O-CNT > CB > rGO.

b: The P-values lower than 0.05 are in **bold**.

Table B.4: The Welch’s t-test summary of the total bacterial deposition on a CNT coating as a function of voltage and incubation time.

<table>
<thead>
<tr>
<th>P-value to the control</th>
<th>0.5 h</th>
<th>3 h</th>
<th>6 h</th>
<th>12 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>-1 V</td>
<td>0.323</td>
<td>0.017</td>
<td>0.187</td>
<td>0.028</td>
</tr>
<tr>
<td>-2 V</td>
<td>0.132</td>
<td><strong>0.008</strong></td>
<td>0.224</td>
<td><strong>0.010</strong></td>
</tr>
</tbody>
</table>

a: The P-values lower than 0.05 are in **bold**.

b: The 6 h data showed less significance (higher P-value) due to relative greater variations on the control.

### B.5 ADDITIONAL SAMPLE CHARACTERISTICS

Figure B.2: A photo of polished Ti coupons with and without CNM coatings. From left to right is CNT, O-CNT, OA-CNT, CB, rGO, and bare Ti surface, respectively.
Figure B.3: The XPS files for the survey and C(1s) scan of the rGO sample without Nafion.
Cathode potential vs. 1 M Ag/AgCl (V)

Total voltage (V)

0 0.5 1.0 1.5 2.0 2.5

Figure B.4: Cathode potential change of the CNT coating with total voltage. Analysis was completed in 0.9% NaCl saline solution with 1/8 TSB.

B.6 ADDITIONAL SEM AND THEORETICAL RESULTS

Figure B.5: *Escherichia coli* (E. Coli, ATCC® 700830TM) growth on the CNT surface as a function of total voltage and incubation time. Total bacterial deposition on the CNT surface with a total voltage of 0, -1.0, and -2.0 V after 0.5, 3, 6, and 12 h incubation at 30 ºC in 0.9% NaCl saline solution with 1/8 TSB.
Figure B.6: More SEM images of bacterial morphology on CNT cathode after 12 h incubation. a & b) In the absence of voltage, c) at -1.0 V, and d) at -2.0 V at 30 °C in 0.9% NaCl saline solution with 1/8 TSB.

The DLVO energy was calculated according to previously reported method [50]. Briefly, it is assumed that the bacteria cells are spherical particles, the CNT coating cathode is a flat plate particle collector with a total area of the measured BET surface area, and that there are no direct interactions between the bacteria and coated surface. Thus, two equations are selected to quantify the van der Waals, Eq B.1, and electrostatic, Eq B.2, interactions:

\[
V_E = \frac{128\pi a n_o kT}{h^2} \gamma_1 \gamma_2 \exp(-kh)
\]  

(B.1)

\[
V_A = -\frac{A}{6} \left[ \frac{a}{h} + \frac{a}{h+2a} + \ln\left(\frac{h}{h+2a}\right) \right]
\]  

(B.2)
where $V_E$ and $V_A$ (J) are the potential energies for the electrostatic and van der Waals interactions, respectively, $a$ (m) is the particle radius (assumed to be $1 \times 10^{-6}$ for bacteria cells), $h$ (m) is the particle to surface separation distance, $n_\infty$ (# m$^{-3}$) is the bulk number density of ions (# m$^{-3}$), $\gamma_1$ and $\gamma_2$ denote reduced surface potential ($\tanh(ze\phi/kT)$) for the particle and the plate (dimensionless), $\kappa$ (m$^{-1}$) represents the reciprocal Debye length, $k$ (m$^2$ kg s$^{-2}$ K$^{-1}$) is the Boltzmann constant, $A$ (J) denotes the Hamaker constant, and $T$ (K) is the solution temperature and is assumed to be $10^{-20}$ J.

![Graph of DLVO energy](image)

Figure B.7: DLVO energy for the electrode and bacteria interaction at a cathode potential of -0.4 V.

The zeta potential of the $P. fluorescens$ cells is -10.8 mV and is used as the surface potential directly. The potential of the electrode surface is calculated according to the following equations.

$$\varphi = -\frac{Ne}{\kappa \lambda \varepsilon_0} \exp(-h\kappa) \quad (B.3)$$

$$\frac{1}{\kappa} = \sqrt{\frac{\lambda \varepsilon_0 kT}{n_\infty v^+(v^++v^-)e^2}} \quad (B.4)$$

where $\lambda$ (dimensionless) denotes the dielectric constant (80 for water at room temperature), $\varepsilon_0$ (F·m$^{-1}$) is for the dielectric permittivity of vacuum, and $v^+$ and $v^-$ (dimensionless) denote valence
of the electrolyte ions. The $N_e$ (C m$^{-2}$) is the surface charge density measured by cyclic voltammetry method.
C.1 BASELINE EXPERIMENT RESULT

Figure C.1: The baseline experiment and the predicted flux showed relatively good consistency (<12% difference).
C.2 MEMBRANE MORPHOLOGY BEFORE AND AFTER PRESSING

Figure C.2: The SEM of the CNT and (CNT+CB) before and after pressing. a) CNT, b) pCNT, c) CNT+CB, and d) p(CNT+CB).

Figure C.3: The photo of the (CNT+CB)p (left) and (CNT+CB) (right) after 24 h experiment and vigorous flushing with running tap water. The (CNT+CB)p coating remained mostly intact while the (CNT+CB) had obvious deterioration due to flushing, installation, and uninstallation.
C.3 MEMBRANE ELECTROCHEMICAL CHARACTERISTICS

The open circuit potential (OCP) of the cathode (CNT composite) and anode (carbon cloth) vs. the standard reference (1 M Ag/AgCl) was measured by setting the total voltage with a DC power supply and monitoring the OCP-t with an electrochemical workstation (604D, CHI Inc.).

![Graph](image)

Figure C.4: Open circuit potential of the cathode p(CNT+CB) and anode carbon cloth vs. 1 M Ag/AgCl reference electrodes with the total applied voltage of 0-2.5 V in PF and WW solution under static conditions.
APPENDIX D

SUPPLEMENTARY INFORMATION FOR CHAPTER 5

D.1 PRELIMINARY EXPERIMENTS

Bacteria density with \((1.34 \pm 0.32 \times 10^6 \text{ cm}^{-2})\) or without \((1.42 \pm 0.22 \times 10^6 \text{ cm}^{-2})\) contacting the test surface in the inactivation experiments was measured by fluorescence microscopy and showed no significant difference.

D.2 BACTERIAL AREAL COVERAGE ANALYSIS PROTOCOL

The images were processed with Image J to measure the bacteria coverage on the surface and the average intensity of the bacteria on the scale of 0 (black)-255 (white). First, the background interferences from Ag filament (brighter area) was removed by “background subtraction”. Then, the threshold of bacteria vs. background was found by visual examination. The bright areas correspond to the bacterial coverage, and the percentage was calculated. The average intensity of bacteria refers to the mean bacterial brightness.

D.3 NEGATIVE SURFACE CHARGE MEASUREMENT PROTOCOL

Briefly, around 4×4 cm PDMS and TPU samples on glass slides were taped such that 9 cm2 of the top surface of the polymer coatings was exposed. The top surfaces were then submerged in 0.5 mM toluidine blue and 15 mM NaCl solution at pH 6-7 for ~60 s. The dye solution was washed off with copious amounts of 15 mM NaCl and the coating surfaces were placed in a 15 mM NaCl water bath for 4 h to dissolve any loosely bound dye. After washing was completed, the exposed area was cut and peeled off the glass slide surface and placed in a 20-mL glass vial that contained 10-mL of 0.2 M NaCl at pH 2, adjusted with HCl. Then the vial was stirred in a rotating
shaker (NewBrunswick Scientific, E24) for at 50 rpm for 30 min at 35°C to protonate any negatively-charged surface groups and to release the positively-charged dye, which was then quantified by UV-vis spectroscopy ($\lambda_{\text{max}}=634$ nm; $\varepsilon=45,200$ cm$^{-1}$ M$^{-1}$). The negative surface charge density ($n^-$, nm$^{-2}$) was quantified using the equation; $n^-=(A \times V \times N)/(\varepsilon \times SA)$ where $A$ is the absorption at 634 nm, $V$ (L) is the volume of extraction solution, $N= 6.022 \times 10^{23}$ is Avogadro's number, $\varepsilon$ is the extinction coefficient of toluidine blue at 634 nm, and $SA=9 \times 10^{14}$ nm$^2$ is the surface area of the measured coating surface. At least 3 samples of each polymer were evaluated for negative surface charge.

D.4 SEM OF THE POLYMER SURFACE

Figure D.1: SEM images of the a) PDMS and b) TPU surfaces at 50,000 X magnification.
Figure D.2: *P. fluorescens* inactivation on TPU-20 by DC and AC for 2 min. All the data were calculated by extracting the dead ratio on the uncharged controls, and thereby the average value was 0 for all samples at 0 V.
Figure D.3: *P. fluorescens* removal on the control and electrically treated PDMS-40 and TPU-20 surfaces with AC inputs. a) The treatment performance after 2 min rinsing with 155 mM NaCl and voltages of 0 (control), 5, 10, 20, and 50 V; b) and c) the treatment performance with 10 V after 2, 5, 10, and 20 min rinsing on surfaces of 15 h and 40 h bacterial growth, respectively; d) optical microscopy images of the control (top) and 10 V treated (bottom) after 2 min rinsing on the PDMS-40 surface after 15 (left) and 40 h (right) bacterial growth. The images were processed with Image J to measure the bacteria coverage on the surface and the average intensity of the bacteria on the scale of 0 (black) - 255 (white). The background interferences from Ag filament (brighter area) was removed by “background subtraction”.
Figure D.3 (Continued).
Figure D.3 (Continued).
Figure D.4: Migration of positively charged crystal violet dyes on the TPU-20 electrodes with 10 V DC. The surface was a) stained with crystal violet solution for 30 min and then b) treated with 10 V DC for 5 min in contact with DI water. The migration of the positively charged dyes on TPU-20 is a direct evidence for the electrophoresis of charged species on the fabricated surface with DC voltages.

Figure D.5: Trajectory of *E. Coli* cells migration in the solution above TPU-20 in the presence or absence of AC 20 V under optical microscope. The cells were stained with crystal violet for 15 min and then resuspended in DI solutions of ~10^6 mL^-1. The vertical movement corresponds to the cells moving out of focus plane. The red circles indicated the location change of a bacterial aggregate during the 3 min observation.
E.1 PRELIMINARY EXPERIMENTS

Figure E.1: Permeability of the mechanically pressed PVDF membranes in comparison to the unpressed. a) Flux and permeability change with pressure for a pressed PVDF and b) permeability change with time for unpressed and pressed PVDFs. After >100 min DI filtration, the unpressed membranes had a similar stable permeability to the pressed ones.
E.2 FILTRATION EXPERIMENTAL PROTOCOL

Figure E.2: A diagram of the biofouling filtration experiments protocol.
### E.3 ADDITIONAL STATISTICAL DATA

Table E.1: Welch’s t-test summary of the bacterial removal under different conditions.

<table>
<thead>
<tr>
<th>Influent</th>
<th>PVDF</th>
<th>ICSE-0 V</th>
<th>ICSE-DC</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^7$ mL$^{-1}$</td>
<td>ICSE-0 V</td>
<td>0.134</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ICSE-DC</td>
<td>0.070</td>
<td>0.251</td>
</tr>
<tr>
<td></td>
<td>ICSE-AC</td>
<td>0.134</td>
<td>0.89</td>
</tr>
<tr>
<td>$10^8$ mL$^{-1}$</td>
<td>ICSE-0 V</td>
<td>0.655</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ICSE-DC</td>
<td>0.409</td>
<td>0.247</td>
</tr>
<tr>
<td></td>
<td>ICSE-AC</td>
<td>0.423</td>
<td>0.286</td>
</tr>
</tbody>
</table>
REFERENCES


