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MECHANISMS OF MS2 BACTERIOPHAGE REMOVAL BY ULTRAFILTRATION
MEMBRANE WITH DIFFERENT FOULANTS

BY

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THESIS

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ABSTRACT

An ultrafiltration unit with a polyvinylidene fluoride (PVDF) membrane of 40 nm nominal pore size was used to study bacteriophage MS2 removal under different membrane conditions. The membrane conditions were subject to fouling of soluble microbial products (SMP), which was extracted from membrane bio-reactor (MBR) feed water, and cleaning methods including backwashing and chemical cleaning. The membrane conditions tested were pristine membrane, membrane fouled by SMP, backwashed membrane, and chemically cleaned membrane. The order of MS2 removal by these membranes was: fouled membrane > backwashed membrane > chemically cleaned membrane $\approx$ pristine membrane. A linear correlation between membrane relative permeability and MS2 removal was found. MS2 mass balance analysis for the ultrafiltration unit showed a higher percentage of MS2 in the concentrate for the fouled membrane compared to that for the pristine membrane. Quartz crystal microbalance (QCM) results showed faster kinetics of MS2 adhesion to the pristine membrane compared to the SMP-fouled membrane. In agreement with QCM results, an attractive force between MS2 and the pristine membrane was detected using an atomic force microscope (AFM), while a repulsive force was detected for the interaction between MS2 and the fouled membrane. The presence of SMP on the membrane surface led to higher rejection of MS2 due to both pore blocking and repulsion between MS2 and the SMP layer. Chemical cleaning removed most of the SMP foulant and as a result led to a lower MS2 removal.

To Xinlei and Baba, Mama

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TABLE OF CONTENTS

CHAPTER 1: INTRODUCTION	1
CHAPTER 2: MATERIALS AND METHODS.....	4
2.1 Chemicals and Reagents.....	4
2.2 MS2 Bacteriophage Preparation.....	4
2.3 SMP Extraction.....	5
2.4 Ultrafiltration Unit Setup.....	5
2.5 MS2 Removal Experiment.....	7
2.6 Adhesion Kinetics Measured with Quartz Crystal Microbalance (QCM)...	8
2.7 Membrane Topography and Interaction Force Studied with AFM	9
2.8 Electrophoretic Mobility and Hydrodynamic Diameter of SMP and MS2..	12
CHAPTER 3: RESULTS.....	13
3.1 MS2 and SMP Characterization.....	13
3.2 MS2 Removal by Ultrafiltration Membrane.....	17
3.3 Adhesion Kinetics.....	20
3.4 AFM Imaging and Interaction Force Measurement.....	22
CHAPTER 4: DISCUSSION.....	27
CHAPTER 5: CONCLUSIONS.....	29
REFERENCES	31

CHAPTER 1

INTRODUCTION

In 2006, an estimated 2.6 billion gallons of wastewater was reused per day in America, and this number is rising.[1] WaterReuse Foundation conducted a survey in 2004, in which nearly all respondents showed their concern about microbiological safety for water reuse as indirect potable source.[1] Pathogens including human enteric virus have been detected in raw wastewater at concentrations as high as 10^9 virus/L.[2] To protect public health, the state of California requires 5 log removal of viruses for recycled water.[3] However, conventional wastewater treatment, which relies on activated sludge process, is not able to achieve this stringent requirement.[4] Membrane bioreactor (MBR) has the ability to meet the increasingly stringent regulations for recycled water, including virus removal.[5] Significant removal of human enteric viruses by MBRs has been reported for full scale MBR wastewater treatment plants.[2, 6, 7] A MBR combines low-pressure microfiltration (MF) or ultrafiltration (UF) with the activated sludge process.[5] Virus removal in MBRs was attributed to both membrane filtration and adsorption to activated sludge.[8, 9] Due to the virus' nanometer size, ultrafiltration membrane alone had low virus removal.[8] Decreasing membrane pore size certainly improved virus removal[10] but the corresponding increase in trans-membrane pressure means higher energy consumption. Furthermore, membrane imperfection also resulted in viruses breaking through the membrane.[11] In addition to size exclusion through membrane, viruses can also be removed together with the MBR biomass after they adsorb to the biomass.[8, 12] Thus, transmission of virus through recycled water could be reduced significantly by proper treatment.[2, 13]

Foulant is unavoidable in MBRs and has been shown to play an important role for virus removal.[8, 14-17] Based on the observation that virus removal by fouled membranes with different nominal pore sizes was similar,[14] the formation of a foulant layer on the membrane surface was the dominating factor controlling virus removal.[17] MBR foulants were classified into three categories: removable, irremovable, and irreversible according to membrane cleaning methods.[18] Removable foulant, or cake layer, can be removed by backwash, while irremovable foulant, or gel layer, can only be removed by chemical cleaning. Irreversible foulant cannot be removed and is permanently associated with the membrane.[16, 18] The irremovable foulant was found to contribute more significantly to virus removal compared with removable foulant.[16] However, studies on interactions between viruses and the membrane surface or different foulant layers are rare. Knowledge on virus interactions with membrane and the foulant layer will allow precise prediction of virus removal for different membrane conditions and thus improve the performance of wastewater treatment.

Soluble microbial product (SMP) produced by biological activities in the MBRs[19] has been identified as an important foulant.[20, 21] Both pore blocking and cake layer formation by SMP were observed at the initial stage of MBR operation, while cake layer formation was found to be dominant in the long term operation.[22] Virus interaction with the foulant layer rather than with the pristine membrane surface is expected to control virus removal by MBR. The objectives of this study are to determine interactions between viruses and pristine or fouled membrane surfaces, and to relate these interactions to the observed virus removal by an ultrafiltration unit. Bacteriophage MS2 is used as a conservative virus surrogate in this study because of its smaller size

compared to human enteric virus.[23, 24] MS2 removal were studied under different membrane conditions, because membrane surface characteristics changed due to SMP fouling, backwashing, and chemical cleaning. The adhesion kinetics of MS2 onto membrane and foulant surfaces and MS2 interaction forces with these surfaces were studied by quartz crystal microbalance (QCM), and atomic force microscope (AFM), respectively. The QCM and AFM findings were used to explain the MS2 mass balance and removal by the ultrafiltration unit.

CHAPTER 2

MATERIALS AND METHODS

2.1 Chemicals and Reagents

All solutions in this study were prepared with deionized (DI) water with resistivity higher than 17.5 M Ω ·cm at 25 °C. The NaHCO₃ and NaCl were from Fisher Scientific (Pittsburgh, PA). The CaCl₂ was from Sigma-Aldrich (St. Louis, MO). The polyethylene glycol (PEG) 6,000 for MS2 purification is molecular biology grade from Calbiochem (Darmstadt, Germany). The dialysis membrane for SMP extraction was from Thermo Scientific (Rockford, IL). The 0.01% NaOCl solution for chemical cleaning is diluted from Clorox (Oakland, California) splash-less concentrated regular bleach by DI water and the ethylenediaminetetraacetic acid (EDTA) was from Fisher Scientific (Pittsburgh, PA). 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) was from Fisher Scientific (Pittsburgh, PA). Poly-L-lysine (PLL) was from MP Biomedicals (Solon, OH). Membrane filtration, QCM for MS2 adhesion, and AFM force profiles were conducted at pH = 8.0 buffered by NaHCO₃.

2.2 MS2 Bacteriophage Preparation

The bacteriophage MS2 strain used for this study was ATCC 15597-B1. A MS2 stock was prepared following the protocol provided in Gutierrez et al.[25] *Escherichia coli* (ATCC 15597) was grown in tryptic soy broth liquid media and then infected by MS2. The MS2 suspension was first centrifuged at 5,000 rpm for 15 minutes and then filtered by 0.22 μ m hydrophilic membrane (Millipore Co., US) to remove cell debris. The broth was washed away by 1mM NaHCO₃ solution in a Millipore ultrafiltration unit (Whatman Nucleopore, USA) with a 100-kDa membrane (Koch Membranes, USA). The

MS2 was further purified by precipitation with 10% (w/v) polyethylene glycol (PEG) 6,000 and 0.5 M NaCl.[26] The mixture was centrifuged at 10,000 rpm for 60 min. The supernatant was discarded and the MS2 pellet was resuspended and stored in pre-sterile 1mM NaHCO₃ solution. The purity of this MS2 stock was examined using SDS-PAGE. The concentration of MS2 stock (2×10^{13} PFU/mL) was determined by the standard Plaque Forming Unit (PFU) assay.[25, 27]

2.3 SMP Extraction

Soluble microbial product (SMP) was extracted from the activated sludge of a membrane bio-reactor (MBR) in Traverse City Regional Wastewater Treatment Plant. Activated sludge was collected in October 2011 and stored at 4 °C before treatment. Solids in the sample were removed by centrifugation at 5,000 rpm for 15 min. The supernatant was filtered with a 0.22 µm hydrophilic membrane (Millipore Co., US) and the filtrate was dialyzed against deionized (DI) water using a 3,500 Da dialysis membrane until the conductivity of SMP was below 15 µS/cm. The concentration of the extracted SMP was determined by measuring total organic carbon (TOC = 22.5mg/L).

The concentration of cations in the activated sludge sample and extracted SMP were measured by ICP-MS (Perkin Elmer, USA). The pH of the activated sludge was measured to be 8.0. All MS2 and SMP solutions in this study were buffered to pH = 8.0 by NaHCO₃. Other solutions at pH = 8.0 were also buffered with NaHCO₃.

2.4 Ultrafiltration Unit Setup

A single-membrane unit nearly identical to the one described by Sweity et al.,[28] was pressure driven by a Masterflex peristaltic pump (77202-60) to filter MS2 solution

through a hollow fiber in dead-end flow operation (Figure 1). The PVDF membrane cut from a ZW-10 unit (Zenon, Canada) was enclosed in a polyvinyl chloride pipe having a total volume of 75 mL. The nominal pore size of the membrane was 40 nm reported by the manufacturer. The effluent flow rate and the trans-membrane pressure were measured by an electronic balance and a pressure gauge connected to a computer and recorded in 30-second intervals.

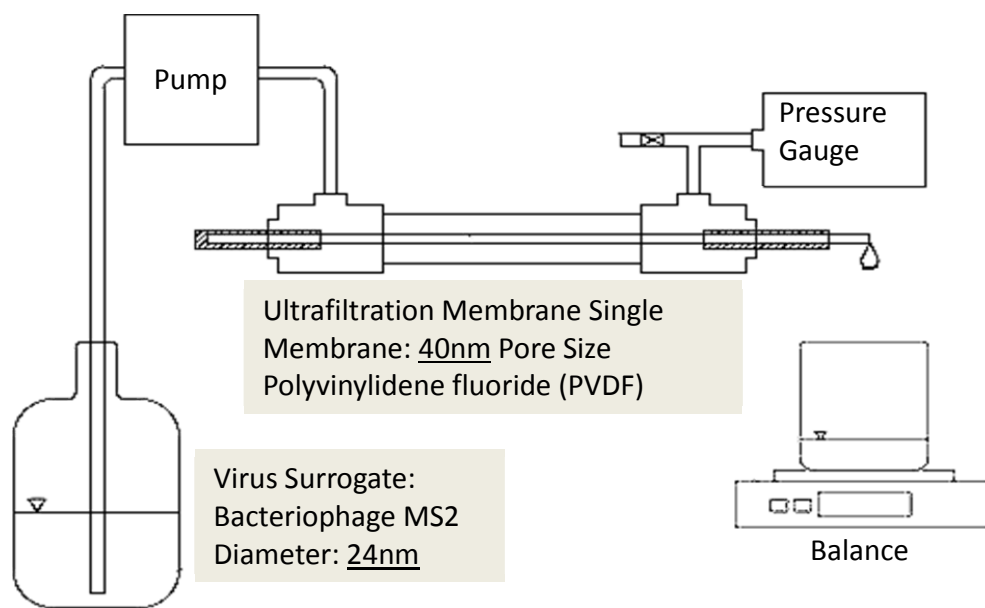


Figure 1. Schematic diagram of single membrane filtration unit. Flow rate was set to 1.90 mL/min and the pressure was in the range of 0.4-0.8 bar.

The MS2 removal was tested in the four membrane conditions: pristine, fouled, backwashed and chemically cleaned membrane as explained below. The pristine membrane was tested for MS2 removal after cleaning the membrane sequentially with 0.01% NaOCl for 30 min, deionized (DI) water for 1 hr, 5 mM ethylenediaminetetraacetic acid (EDTA) for 30 min, and finally DI water for 1 hr. This cleaning method will be referred to as chemical cleaning. The pristine membrane was

fouled by pumping 96 mL of SMP solution (22.5 mg C/L) in solution containing 3mM CaCl_2 at pH = 8.0 through the membrane in a closed loop for 9-12 hr. This membrane will be referred to as fouled membrane. The fouled membrane was backwashed with 1 mM NaHCO_3 solution pumped in the reverse flow direction. The permeability of this membrane was tested periodically until the flux stabilized. This membrane will be referred to as backwashed membrane. The backwashed membrane was chemically cleaned as described above and will then be referred to as chemically cleaned membrane.

To evaluate the molecular weight cutoff (MWCO) of the membrane in different conditions, aqueous solutions of dextran at different molecular weights (150, 450 – 650, 2000k)[29] were pumped through the membranes. Concentrations of dextran in the influent and the effluent were measured with a total organic carbon analyzer (TOC-VWP, Shimadzu, Japan) to determine dextran rejection by the membrane. The dextran rejection was further validated by detecting the increase in TOC of the concentrate.

2.5 MS2 Removal Experiment

Each time before an MS2 removal test was performed for a membrane, the flux through the membrane was stabilized by pumping 3 mM CaCl_2 solution at pH=8.0 through the membrane. MS2 solution was made by seeding 3 mM CaCl_2 solution at pH=8.0 with MS2 to a final concentration of 10^9 PFU/mL. Four permeate samples were taken at one-hour intervals during filtration. The concentrate in the unit was collected into a pre-sterilized bottle after filtration had ceased. After filtration experiment, 1 mM NaHCO_3 solution was pumped into the membrane unit in the reverse direction to remove MS2, which were reversibly adhered to the membrane surface. The volume of the

permeate (V_p), the concentrate (V_c) and the backwash solution (V_b) was converted from mass measured by a balance. Volume of the influent (V_i) was the sum of V_p and V_c . MS2 concentration in the influent (C_i), the permeate (C_p), the concentrate (C_c) and the backwash solution (C_b) was measured by the plaque forming unit assay. The equation of MS2 mass balance in the ultrafiltration unit is as below:

$$V_i C_i = V_p C_p + V_c C_c + V_b C_b + MS2_{irreversibly\ adhered} \quad (1)$$

The MS2 removal under each membrane condition was determined in log value by the equation:

$$MS2\ removal\ (logs) = -\log \frac{MS2_{permeate}}{MS2_{influent}} \quad (2)$$

The flux through the ultrafiltration membrane at each condition was calculated and normalized over the flux through the pristine membrane ($J_{pristine}$) by the equation:

$$J_{normalized} = \frac{J_x}{J_{pristine}} \quad (3)$$

The MS2 removal tests were conducted in triplicate for each membrane condition.

2.6 Adhesion Kinetics Measured with Quartz Crystal Microbalance (QCM)

A QCM system (QCM-D300, Gothenburg, Sweden) was used to measure MS2 adhesion to pristine and SMP fouled PVDF surface. The pristine PVDF sensor with a fundamental resonant frequency of 5 MHz (QSX999) was obtained from Q-Sense (Sweden). Following a cleaning protocol of the sensor developed previously,[28] the sensor was soaked in 5 mM EDTA solution for 0.5 h, rinsed thoroughly with DI water and dried with ultra-high purity N_2 . The temperature of the QCM chamber was set at 25

°C. Flow in the chamber was controlled by a syringe pump (KD Scientific Inc., Holliston, MA) at 0.1 mL/min to maintain a laminar flow with a Reynolds number of 1.0 and a Peclet number of 1.7×10^{-8} . [30]

To measure MS2 adhesion on PVDF surface, a baseline was acquired with DI water until the frequency shift change was less than 1 Hz in 30 min. Following the baseline, the sensor was equilibrated with 3 mM CaCl₂ solution at pH = 8.0 for 20 min. After this equilibration, the MS2 solution at a concentration of 2×10^{11} PFU/mL in 3 mM CaCl₂ at pH = 8.0 was pumped into the QCM chamber. To measure MS2 adhesion on the SMP fouled PVDF sensor, a baseline was obtained with 3 mM CaCl₂ solution at pH = 8.0. The SMP solution containing 22.5 mg C/L and 3 mM CaCl₂ at pH = 8.0 was pumped into the chamber for 20 min to allow for SMP adhesion to the PVDF surface. Excess SMP was washed away by 3 mM CaCl₂ solution at pH = 8.0 before the same MS2 solution as used above was injected into the chamber. The MS2 adhesion kinetic on either pristine or SMP fouled PVDF surface was calculated based on the change of frequency shift at the 3rd tone (f_3) over time at the initial slope of adhesion. [31]

2.7 Membrane Topography and Interaction Force Studied with AFM

The topography of the pristine, fouled, backwashed and chemically cleaned membrane surface was determined by AFM imaging in the tapping mode at room temperature in air with a MFP-3D AFM instrument (Asylum Research, USA). The probe used for topography imaging was Tap300Al-G (BudgetSensor, Bulgaria). For each membrane condition, three images were randomly taken and the size of each image was 5

$\mu\text{m} \times 5 \mu\text{m}$. The roughness of each image was calculated as the standard deviation of measured height for the whole image area.

The interactions between MS2 and the studied membranes were determined by force profiles taken in AFM contact mode. A silica sphere with $1 \mu\text{m}$ in diameter glued to a tipless cantilever with a spring constant of 0.06 nN/nm was obtained from Novascan Technologies (USA). This is referred to as an AFM probe. MS2 was coated onto this AFM probe by a layer-by-layer method. A positively charged poly-L-lysine (PLL) layer was coated on the probe by immersing the probe into 0.1 g PLL/L solution in HEPES buffer for 90 min. The PLL-coated probe was then rinsed with DI water and immersed in the MS2 stock solution at 10^{11} PFU/mL overnight. The MS2 coated probe was rinsed with DI water right before using for force measurement. The actual spring constant of the cantilever was calibrated before each experiment using the thermal tuning method in AR-MFP-3D software (Asylum Research, USA). Based on the calibrated spring constant, the deflection of the cantilever measured was converted to force.[32]

All AFM force measurements between MS2 and different membranes were conducted in 3 mM CaCl_2 solution at $\text{pH} = 8.0$. For each membrane surface, the force measurement was conducted at multiple randomly selected locations, and more than 10 replicates were taken at each location. Force profiles were recorded when the AFM probe was approaching the membrane surfaces. The interaction was determined to be repulsive if the approaching force profile remained positive. The decay lengths of repulsive force profiles were determined by fitting approaching force profiles following the equation $F = B \times \exp(h\kappa)$ in which F is the force and h is the distance between the probe and the surface. B is a pre-exponential constant and κ^{-1} is the repulsion force decay length. By

fitting the linear part of $\ln(F)$ as a function of h , κ was calculated as the slope.[33, 34]

The repulsive force decay length was compared with the Debye length calculated according to the Debye-Hückel theory. The Debye length in NaCl solution was calculated as a function of ionic strength: $\kappa^{-1}(nm) = \frac{0.304}{\sqrt{I(M)}}$, in which I is the ionic strength in mol/L.[35] The interaction was determined to be attractive if negative force was detected in the approaching force profiles. The maximum attraction force was determined from each attractive force profile. The numbers of force profiles analyzed were 118, 132, 92 and 107 for pristine, chemically cleaned, backwashed and SMP fouled membranes, respectively.

To ensure the reliability of force measurements between MS2 and different membranes, two control experiments were conducted. The first control experiment tested interactions between the bare silica sphere and 3 types of surfaces: the highest quality grade V1 mica disc (Ted Pella, USA) as a reference surface, the pristine PVDF surface, and the SMP fouled PVDF surface. We used a HFM-180 PVDF ultrafiltration membrane (Koch Membrane, USA) as a pristine PVDF surface. The SMP fouled PVDF surface was prepared by pipetting 100 μ L SMP solution, which did not contain $CaCl_2$ or $NaHCO_3$, on the pristine PVDF membrane surface. This surface was kept at 4 °C overnight for the foulant layer formation. The force curves were taken in NaCl solutions at pH = 8.0 for four ionic strengths: 3 mM, 10 mM, 30 mM and 100 mM. For the pristine and SMP fouled PVDF surfaces, the force curves were taken at 3 different locations randomly selected on the surface in each ionic strength. For the mica surface, the force curves were taken at 1 location per ionic strength. At least 5 replicates were taken at each location. The second control experiment for MS2-coated probe and mica surface was conducted in

NaCl solutions at pH 8.0 for three ionic strengths: 3 mM, 10 mM and 30 mM. The force curves were taken at three different locations on the mica surface at each ionic strength. At least 5 replicates were taken at each location. The decay length of repulsive approaching force profiles obtained in these control experiments was determined and compared with the decay length obtained for MS2 and fouled surface.

2.8 Electrophoretic Mobility and Hydrodynamic Diameter of SMP and MS2

The electrophoretic mobility and hydrodynamic diameter of SMP and MS2 were measured with a Zetasizer ZS90 (Malvern, UK) at 25 °C. All samples are adjusted to pH = 8.0. Electrophoretic mobility of SMP and MS2 were measured in 3 mM CaCl₂ solution and also NaCl solutions from 1 to 100 mM. The zeta potentials were converted from electrophoretic mobility according to the Smoluchowski equation.[36] Hydrodynamic diameters of SMP and MS2 were measured in the same electrolyte solutions as those for electrophoretic mobility. A 4 mW HeNe laser with a wavelength at 633 nm and a photodiode detector located 90° to the laser beam were applied to measure the hydrodynamic diameter. Hydrodynamic diameter measurements were conducted immediately after samples prepared in the electrolyte solutions and also one day afterwards to ensure no change in hydrodynamic diameter over time. For both electrophoretic mobility and hydrodynamic diameter measurements, three replicates of each condition were measured to ensure reliability. The concentration of MS2 used in these measurements was 2×10^{11} PFU/mL.

CHAPTER 3 RESULTS

3.1 MS2 and SMP Characterization

After purification, protein components of the MS2 stock were tested with SDS-PAGE. The image of SDS-PAGE gel is shown below in Figure 2. Only the maturation protein and the coat protein were observed, which is consistent with past research.[37, 38] The concentration of MS2 stock determined by the standard Plaque Forming Unit (PFU) assay was 2×10^{13} PFU/mL.

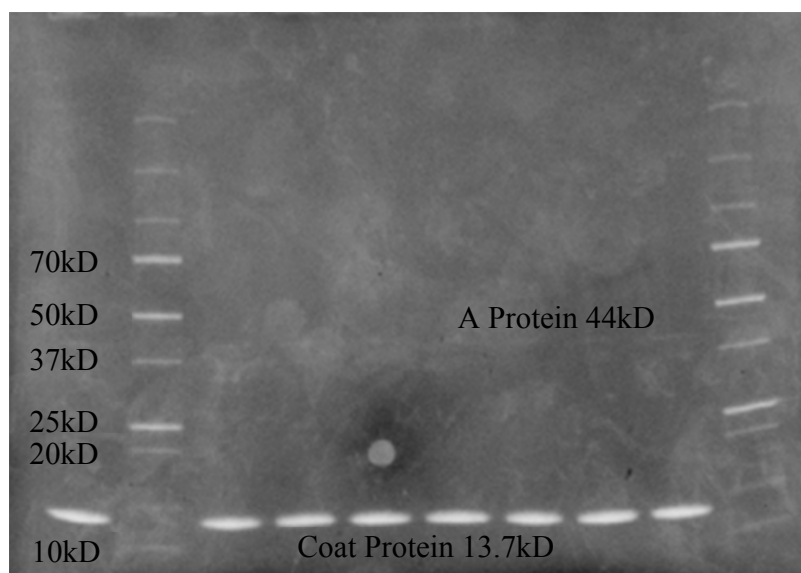


Figure 2. SDS-PAGE image of MS2 proteins stained by Coomassie Brilliant blue showing only A protein and coat protein of MS2.

Cations of activated sludge and SMP solutions after dialysis were measured with ICP-MS (Table 1). Calcium (1.895 ± 0.005 mM) and magnesium (1.500 ± 0.004 mM) were found to be dominant cations in the activated sludge sample, while concentrations of other cations were low enough to be neglected. In the extracted SMP solution, both calcium and magnesium were effectively removed through dialysis. In the following

experiments, 3mM Ca^{2+} was set to be the electrolyte condition to simulate the activated sludge.

Table 1. Cation concentrations determined by ICP-MS

Metal	Unit	Activated Sludge		SMP solution	
		1	2	1	2
Ca	ppm	75.6	76	2.228	2.226
Mg	ppm	36.1	35.9	0.904	0.912
Co	ppb	1.1	1.1	0.289	0.289
Cr	ppb	13.5	13.2	0.895	1.07
Cu	ppb	3.6	3.1	2.03	2.24
Fe	ppb	783	881	607	545
Mn	ppb	73.7	74.3	8.7	8.71
Ni	ppb	10	10.2	3.44	3.49
Ti	ppb	56.4	5.2	2.08	2.07
Zn	ppb	41	57.3	5.14	5.54

The electrophoretic mobility and the zeta potential of SMP and MS2 as a function of ionic strength are shown in Figure 3. The electrophoretic mobility of MS2 in NaCl from 3 mM to 100 mM was negative and became less negative as ionic strength increased. Specifically, the electrophoretic mobility of MS2 was $-2.28 \pm 0.04 \mu\text{mcm/Vs}$ in 3mM NaCl and increased to $-0.84 \pm 0.01 \mu\text{mcm/Vs}$ in 100 mM NaCl at pH = 8.0. This result is consistent with past research.[39] The electrophoretic mobility of SMP in NaCl solution up to 30 mM was negative. In 100 mM NaCl, electrophoretic mobility of SMP

was close to zero, i.e. $-0.0009 \pm 0.009 \mu\text{mcm/Vs}$. As ionic strength increased, SMP electrophoretic mobility in NaCl solution at pH = 8.0 also became less negative. In both cases of MS2 and SMP, the negative charge on MS2 and SMP were screened by counter ions Na^+ as ionic strength increased. The electrophoretic mobility of SMP and MS2 were also measured in 3 mM CaCl_2 solution at pH = 8.0, which was the electrolyte condition used in the ultrafiltration experiment. In this solution, the electrophoretic mobility values of MS2 and SMP were less negative compared to those in 10 mM NaCl solution at the same pH ($-0.81 \pm 0.02 \mu\text{mcm/Vs}$ vs. $-1.96 \pm 0.05 \mu\text{mcm/Vs}$ for MS2 and $-0.86 \pm 0.02 \mu\text{mcm/Vs}$ vs. $-1.39 \pm 0.05 \mu\text{mcm/Vs}$ for SMP). The enhanced negative charge screening by divalent cations Ca^{2+} has been attributed to Ca^{2+} complexation with carboxylate functional groups of MS2 and natural organic matters.[39, 40]

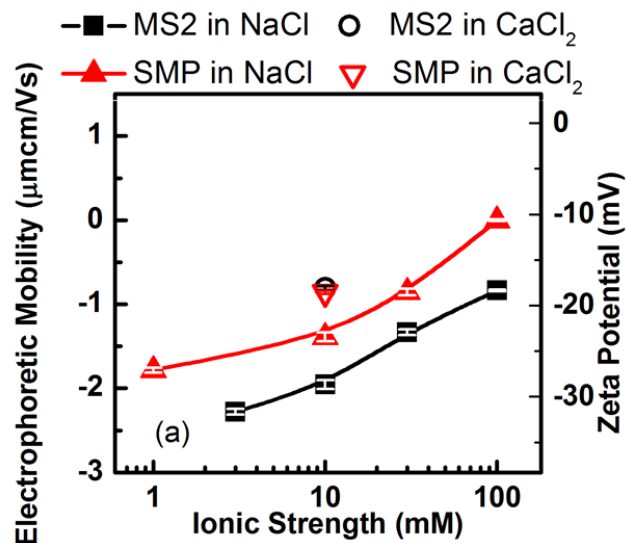


Figure 3. Electrophoretic mobility and zeta potential of MS2 and SMP in NaCl and CaCl_2 solutions. All electrophoretic mobility measurements were conducted at 25 °C at pH = 8.0 buffered by NaHCO_3 . Concentration of SMP was 22.5 mg TOC/L and concentration of MS2 was 2×10^{11} PFU/mL.

MS2 hydrodynamic diameters in 3 mM NaCl, 10 mM NaCl, 30 mM NaCl, 100 mM NaCl and 3 mM CaCl_2 were 39 ± 1 nm, 35 ± 1 nm, 35 ± 1 nm, 34 ± 1 nm and 33

nm $\pm$ 1nm (N = 3), respectively. These results are consistent with previous measurement for MS2.[39] Assuming that SMP aggregates were spherical, the hydrodynamic diameter of the SMP aggregates varied from 192 $\pm$ 7 nm in 30 mM NaCl to 211 $\pm$ 10nm in 1 mM NaCl. Hydrodynamic diameters of MS2 and SMP in all the conditions tested were stable within 1 day.

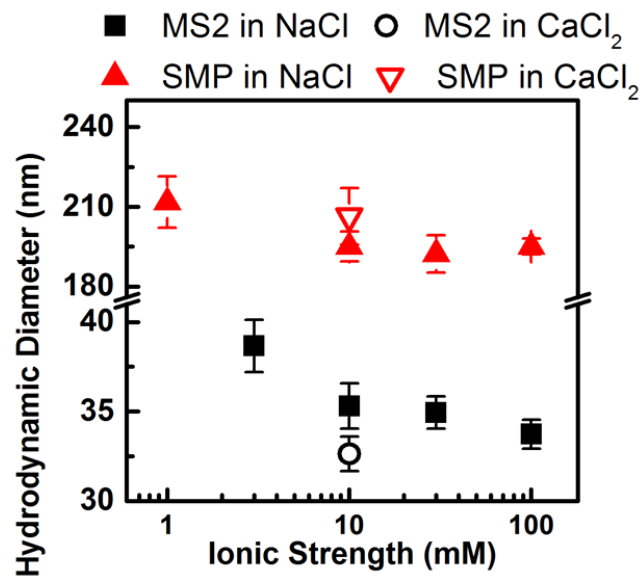


Figure 4. Hydrodynamic diameter of SMP and MS2 in NaCl and CaCl₂ solutions. All hydrodynamic diameter measurements were conducted at 25 °C at pH = 8.0 buffered by NaHCO₃. Concentration of SMP was 22.5 mg TOC/L and concentration of MS2 was 2 $\times$ 10¹¹ PFU/mL.

3.2 MS2 Removal by Ultrafiltration Membrane

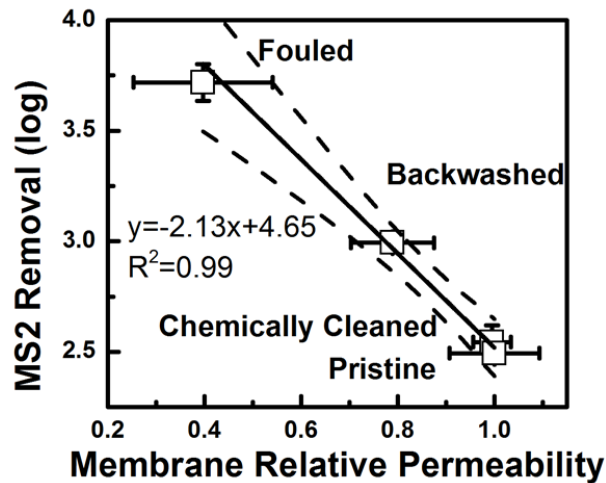


Figure 5. MS2 removal in logs as a function of relative permeability for pristine, fouled, backwashed and chemically cleaned membrane. Linear fitting equation was shown.

A linear correlation was found between the MS2 log removal and relative permeability with R^2 of 0.99, as shown in Figure 5. The pristine membrane had a 2.49 ± 0.03 log removal of MS2. SMP foulant caused an increase in MS2 removal to 3.72 ± 0.08 log and a decrease in the relative permeability to 0.40 ± 0.14 . Backwashing led to MS2 removal of 2.99 ± 0.03 log at the relative permeability of 0.79 ± 0.09 . The chemically cleaned membrane performed similarly to the pristine membrane in terms of both MS2 removal and relative permeability. A previous report showed that a lower frequency of chemical cleaning contributed to higher virus removal in MBRs.[8] Similar performance of chemically cleaned and pristine membranes suggested that irreversible foulants in SMP, which cannot be removed by either backwashing or chemical cleaning, showed no influence to both MS2 removal and relative permeability. The difference in MS2 log removal between the backwashed compared with the fouled membrane and between the chemically cleaned compared with the backwashed can be attributed to the roles of

removable and irremovable foulants. The contribution of removable foulants to MS2 removal was 0.73 log compared to 0.55 log by irremovable foulant. Similar findings were reported for MBR activated sludge,[41] indicating that either SMP is the main contributor to virus removal or SMP fouling can be used as a good indicator for all factors controlling virus removal in MBRs.

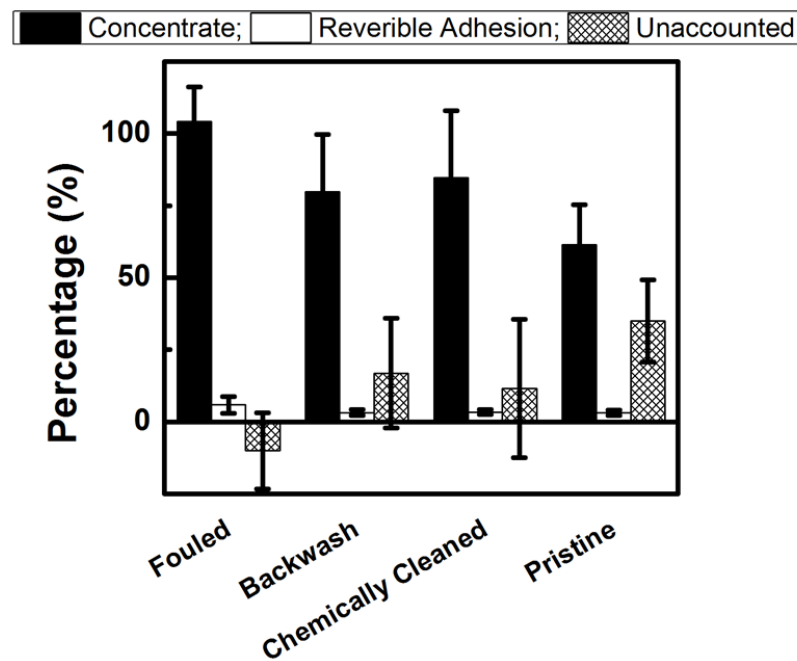


Figure 6. Mass balance of MS2 in the ultrafiltration unit for four membrane conditions.

The mass balance of MS2 in the ultrafiltration unit is shown in Figure 6. Since the percentage of MS2 in the effluent was below 0.35% in every membrane condition, it is not shown in the figure. For all four membrane conditions tested, the percentages of reversible adhered MS2 were stable between 3% and 6%. The percentages of MS2 in the concentrates were $62 \pm 14\%$ and $104 \pm 12\%$ for pristine and fouled membranes, respectively. The results of an unpaired t-test showed that these percentages were significantly different (power $1 - \beta > 85\%$, significant level $\alpha = 0.05$). Thus, the mass

balance showed that the percentage of irreversibly adhered MS2 for the pristine membrane was higher than that for the fouled membrane. The percentages of MS2 irreversibly adhered to backwashed and chemically cleaned membranes were in between those for the pristine and fouled membranes. MS2 adhesion onto the pristine and fouled membrane surfaces was further studied by QCM and AFM and described below to complement the mass balance data.

3.3 Adhesion Kinetics

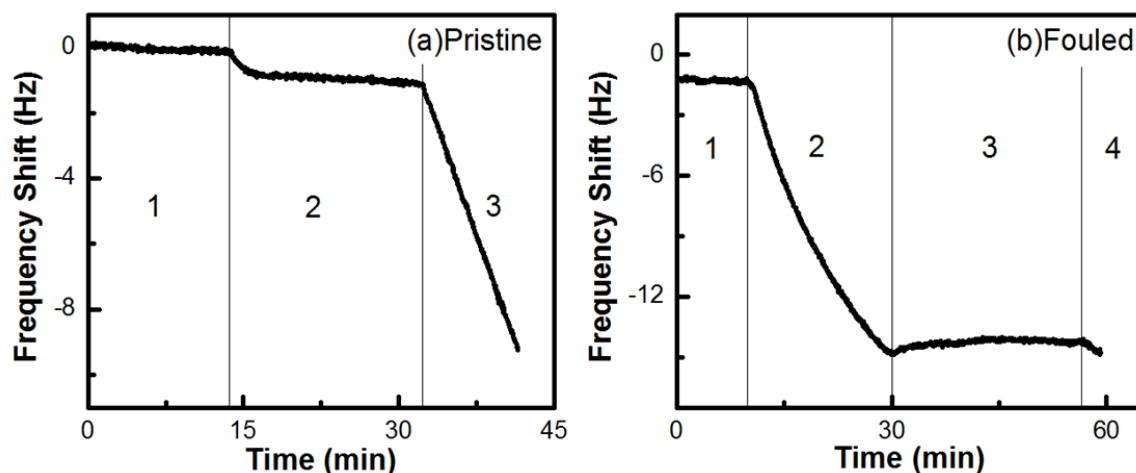


Figure 7. QCM frequency shift over time of MS2 adhesion on (a) pristine PVDF and (b) SMP fouled PVDF in 3 mM CaCl_2 solution. Concentration of MS2 solution was 2×10^{11} PFU/mL. (a) Step 1 was obtaining baseline with DDI water. Step 2 was equilibrating with CaCl_2 solution. MS2 adhesion was in step 3. (b) Step 1 was baseline with CaCl_2 solution. Step 2 was SMP adhesion on PVDF. Extra SMP was washed away in step 3 by CaCl_2 solution. MS2 adhesion on SMP covered PVDF surface was in step 4.

MS2 adhesion kinetics on pristine and fouled PVDF surfaces were determined by the QCM technique. As shown in Figure 7(a), the stable frequency of the pristine PVDF surface was obtained in DI water and subsequently in 3mM CaCl_2 at pH = 8.0. MS2 injection resulted in a frequency decrease indicating MS2 adhesion to the pristine PVDF surface. The initial slope of the frequency shift versus time was 0.66 ± 0.13 Hz/min for three replicates. In Figure 7(b), the stable frequency of the PVDF surface was obtained in 3 mM CaCl_2 electrolyte solution at pH = 8.0. A sharp decrease of frequency shift after the injection of SMP solution at stage 2 indicated SMP adhesion onto the PVDF surface. The subsequent step for washing SMP off the PVDF surface with 3 mM CaCl_2 solution (pH = 8.0) did not change the frequency shift significantly, suggesting the SMP layer stayed on the sensor.

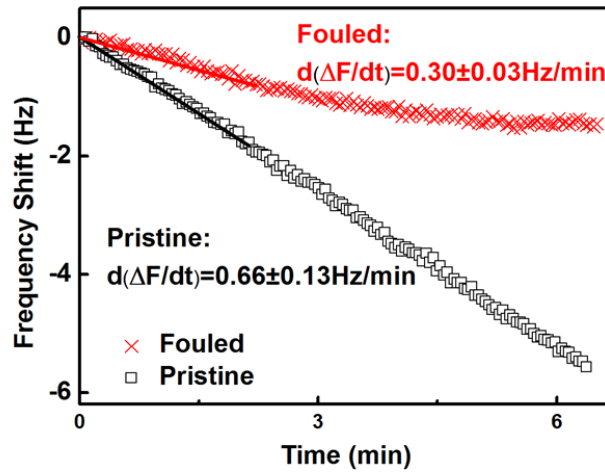


Figure 8. Adhesion kinetics of MS2 onto pristine and SMP fouled PVDF surface.

MS2 adhesion was also observed in the final stage, where the initial slope of the frequency shift versus time was 0.30 ± 0.03 Hz/min as observed for three replicates. MS2 adhesion kinetics in 3 mM CaCl_2 solution at pH = 8.0 on the pristine PVDF was statistically higher than on the SMP fouled PVDF ($1 - \beta = 95\%$, $\alpha = 0.05$). Ca^{2+} cations were expected to complex with carboxylate groups of both SMP and MS2 to cause higher adhesion of MS2 on SMP compared to adhesion on PVDF.[31][42] The observed higher adhesion of MS2 to PVDF than on SMP fouled PVDF may suggest that either carboxylates are not a predominant group in SMP, and/or other interaction mechanisms, such as steric repulsion controls MS2 adhesion on the SMP layer. On the other hand, strong repulsion resulting in low MS2 adhesion on the SMP layer is consistent with high concentration of MS2 found in the concentrate of the filtration experiments.

3.4 AFM Imaging and Interaction Force Measurement

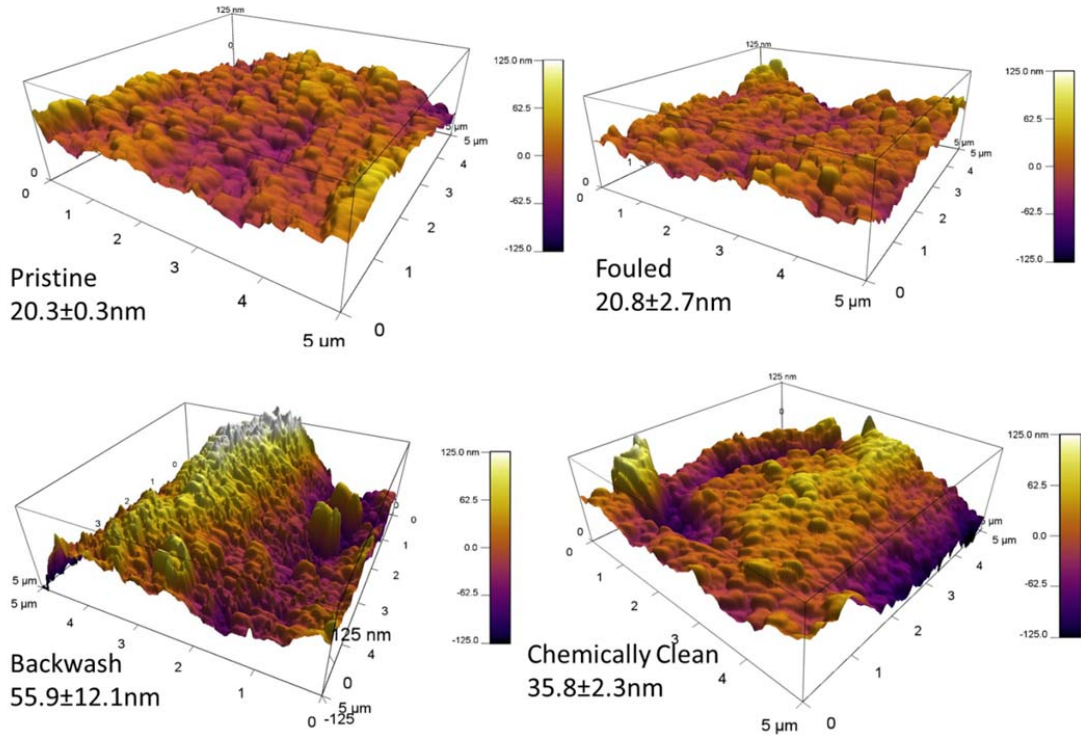


Figure 9 Topography of pristine, SMP fouled, backwashed and chemically cleaned membrane by AFM tapping mode in air. Roughness calculated as standard deviation of height. Average roughness and standard deviation were from 3 replicates on each membrane.

The topography of four membrane conditions was shown in Figure 9. The roughness of pristine, SMP fouled, backwashed and chemically cleaned membranes were 20.3 ± 0.3 nm, 20.8 ± 2.7 nm, 55.9 ± 12.1 nm and 35.8 ± 2.3 nm, respectively. The roughness of the SMP fouled membrane showed no significant difference from that of the pristine membrane, but the roughness of the backwashed and the chemically cleaned membrane was higher. SMP foulants formed a cake layer on the membrane surface, as reported in previous research.[43] The cake layer formation was consistent with the hydrodynamic size of SMP. In 3 mM CaCl_2 , SMP with a diameter of 206 ± 11 nm was

larger than the 40 nm nominal pore size of the PVDF membrane. The roughness of the backwashed membrane became larger because the cake layer was probably crushed due to shear force applied during backwashing. After chemical cleaning, the roughness decreased to 35.8 ± 2.3 nm but was still higher than that of the pristine membrane, suggesting the presence of irreversible foulants on the membrane surface.

Attraction forces were detected between MS2 and the pristine membrane from all AFM force profiles. The force profiles between MS2 and the SMP fouled membrane were, however, all repulsive. Similar to the fouled membrane, interaction between MS2 and the backwashed membrane was also repulsive, consistent with the backwashed membrane topography showing the presence of irremovable foulants. For the chemically cleaned membrane, 62% approaching force curves showed attraction while others showed repulsion. The presence of both attraction and repulsion in the force profiles for chemically cleaned membrane suggested that chemical cleaning may partially remove foulants on the membrane surface. After chemical cleaning, part of the pristine membrane was exposed but the other part was still covered by irreversible foulants. The distribution of maximum attraction forces obtained for both pristine and chemically cleaned membranes is shown in Figure 10. The means of MS2 attraction force on the pristine and the chemically cleaned membrane were not significantly different (two-tailed t test $\alpha = 0.05$, 198 degrees of freedom). Thus, the attraction forces on the chemically cleaned membrane were probably detected for the membrane surface that was completely cleaned of SMP foulant and was recovered to the pristine condition.

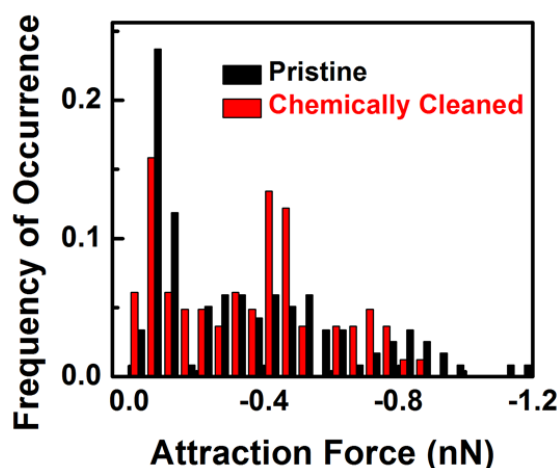


Figure 10. Distribution of maximum attraction force between MS2 and chemically cleaned and pristine membrane in 3 mM CaCl_2 solution.

The fouled, backwashed and some part of the chemically cleaned membranes showed repulsive forces when MS2 was approaching the surface. The distribution of decay lengths determined from the approaching curves is shown in Figure 11. For force profiles between MS2 and backwashed or chemically cleaned membranes, the decay length was below 10 nm. The overlapping distributions of decay length for backwashed and chemically cleaned membranes indicated that foulants on the backwashed and chemically cleaned membrane were similar. The repulsive force decay lengths for the fouled membrane were from 12 to 50 nm and were significantly larger than those determined for the backwashed and chemically cleaned membranes.

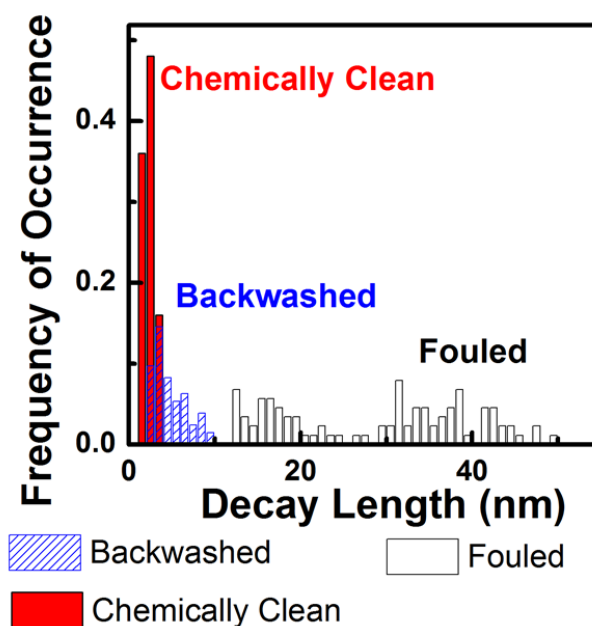


Figure 11. Distribution of decay length of approaching force curves between MS2 and chemically cleaned, backwashed and fouled membrane.

The decay lengths determined for a silica probe approaching mica or PVDF surfaces and for a MS2-coated probe approaching mica surface were consistent with the Debye prediction (Figure 12). However, the decay lengths for the silica probe with SMP foulant were substantially higher in all ionic strength conditions tested (Figure 12). As shown in Figure 11, the decay length detected for MS2 and SMP foulants in 3 mM CaCl_2 at pH = 8.0 was from 12 nm to 50 nm. This range in decay length was also higher than 9.6 nm predicted by the Debye equation for 10 mM ionic strength. The agreement between the repulsive force decay length and theoretically predicted Debye length suggested that electrostatic force is the dominant detectable force between the silica probe with mica or PVDF and even between MS2 and a mica surface. The longer decay length obtained with SMP foulants means the repulsive force is taking place at a longer distance than the Debye length and thus should be a result of a non-electrostatic interaction. We suggest that the polymeric and compressible nature of SMP rejected MS2

at a longer distance than the electrostatic double layer. Repulsive steric force has been detected between five bacteria strains and glass surfaces.[44] Extracellular polymeric substances bound to bacterial cells may be hydrolysed and dissolved to form SMP.[19] It is possible that this fraction of SMP possess the steric force detected in our AFM force profiles.

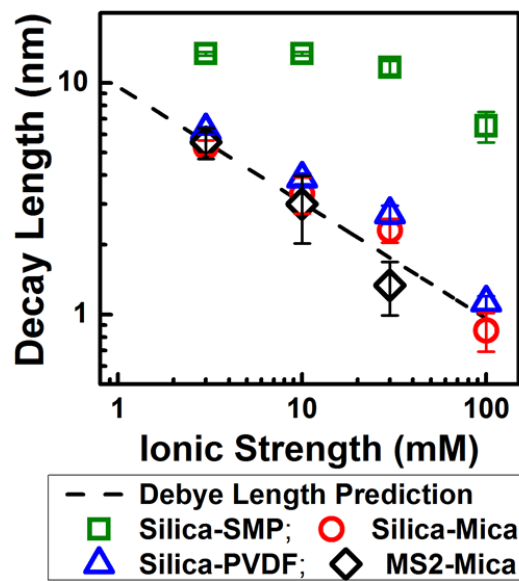


Figure 12. Decay length of approaching force profiles between 1 μ m silica probe and SMP/ mica/ PVDF surface in NaCl solution from 3 to 100mM. Debye length predicted by Debye-Hückel theory is shown in dash line.

CHAPTER 4

DISCUSSION

The AFM force measurement detected only repulsion for the fouled and backwashed membranes, while only attraction was detected for the pristine membrane. Both repulsion and attraction were detected for the chemically cleaned membrane. Thus, when SMP foulants are present on the fouled, backwashed and part of the chemically cleaned membrane, the foulants on the membrane surface acted as a barrier inhibiting MS2 particles approaching the membrane surface and thus increased MS2 removal. The attractive interaction between MS2 and the pristine membrane became repulsion after the membrane was fouled by SMP as measured by AFM. In agreement with the AFM data, the QCM results showed that MS2 adhesion kinetics to the membrane surface became lower when the membrane was fouled. This trend was similar to the decrease in the percentage of irreversibly adhered MS2 observed in the MS2 filtration experiments.

In MS2 filtration experiment, the strong correlation between relative permeability and MS2 log removal may be attributed to membrane pore clogging and porous layer formation above the membrane surface as suggested by previous study.[45] In this study, the nominal pore size of the pristine membrane is 40 nm which is bigger than the size of MS2 particles but MS2 adhered to the pristine membrane surface to achieve removal of 2.49 logs. As evident from the topographies of membrane surfaces by AFM, the foulants entirely or partially covered the surfaces of the chemically cleaned, backwashed, and fouled membranes. Pore blocking of the membranes was examined by dextran rejection. The pristine membrane rejected 5% dextran at 150 kDa MW while the fouled, backwashed, and chemically cleaned rejected 10%, 18%, and 11%, respectively. For 450 – 650 kDa MW dextrans, the pristine membrane rejects 12% while the fouled,

backwashed, and chemically cleaned rejected 24%, 28%, and 26%, respectively.

Compared with the pristine membrane, the pores of other membranes were blocked by foulants as evidenced by higher dextran rejection. In addition, the AFM and QCM data showed that the foulants, which block the pore, also inhibits MS2 adhesion. The combination of pore blocking and repulsion between MS2 and the foulants controlled MS2 removal by the chemically cleaned, backwashed and fouled membranes. This is the first research showing that the presence of SMP foulant on the membrane surface inhibits virus adhesion.

The results presented here show that ultrafiltration membranes fouled by SMP had a higher MS2 removal, and MS2 was found in the concentrates. Chemical cleaning of MBRs should be conducted carefully because of the decrease in MS2 removal achieved by chemically cleaned membrane. The correlation between MS2 removal and relative permeability loss has the potential to predict virus removal with permeability as a rapid indicator in WWTPs.

CHAPTER 5

CONCLUSIONS

SMP fouled ultrafiltration membrane increased MS2 removal by 1.2 logs compared to the pristine membrane. Most MS2 was detected in the concentrate for the SMP fouled membrane while a significant portion of MS2 irreversibly adhered to the pristine membrane as shown in the MS2 mass balance in the ultrafiltration unit. Faster MS2 adhesion onto pristine PVDF surface than onto SMP fouled PVDF surface was evidenced by the QCM measurement. The difference in adhesion kinetics was explained by the AFM force profiles. The interaction between MS2 and the pristine membrane was attraction while it was repulsion between MS2 and the SMP fouled membrane. The decay length of MS2-SMP repulsive force was longer than the Debye prediction in all electrolyte conditions tested, which indicates that steric force is the dominant mechanism in MS2 - SMP interaction.

The influence of membrane cleaning methods to MS2 removal was also studied. The membrane surface topographies studied by AFM showed effective removal of removable and irremovable foulant by backwashing and chemical cleaning. MS2 removal by the backwashed membrane decreased by 0.7 logs compared to the fouled membrane. After chemical cleaning, the MS2 removal decreased to the same as the pristine membrane. Irreversible foulants showed little influence in MS2 removal. A linear relationship between MS2 removal and relative permeability was found in the four membrane conditions studied. The AFM force profiles showed that after backwashing, the decay length of MS2-foulant interaction significantly decreased. Part of the force profiles between MS2 and chemically cleaned membrane showed attraction while others showed repulsion. The distributions of maximum attraction force were not significantly

different for MS2 – pristine membrane interaction and MS2 – chemically cleaned membrane interaction. Irreversible foulants of SMP did not fully cover the membrane surface and the uncovered part of membrane behaved similarly to the pristine membrane versus MS2.

Chemical cleaning of MBR should be conducted carefully because of the decrease in MS2 removal achieved by chemically cleaned membrane. The correlation between MS2 removal and relative permeability loss has the potential to predict virus removal with permeability as a rapid indicator in WWTPs. The interaction of MS2 and membrane surface was identified here as an important factor considering MS2 removal by ultrafiltration membrane.

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