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Effect of solids retention time in membrane bioreactors on reverse osmosis membrane fouling

Elizabeth Field

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Elizabeth Field

Civil Engineering

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Approved by the Thesis Committee:

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Chairperson

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Effect of Solids Retention Time In Membrane Bioreactors On Reverse Osmosis Membrane Fouling

By

Elizabeth Field

B.S. Environmental Toxicology University of California-Davis, 2004

THESIS

Submitted in Partial Fulfillment of the Requirements for the Degree of

Master of Science
Civil Engineering

The University of New Mexico
Albuquerque, New Mexico

December, 2009
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On Reverse Osmosis Membrane Fouling

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Abstract

As sources of clean fresh water diminish, there is an increasing interest in water reuse. Membrane bioreactors (MBRs) are commonly used in wastewater treatment plants, though reuse of treated wastewater for potable purposes requires more advanced treatment. Reverse osmosis (RO), a water treatment process commonly used for drinking water, provides near complete removal for all inorganic and organic constituents from water. While much research has been conducted on both treatment processes, little research has been done on how the combined processes work together in treating wastewater to high enough standards for potable water reuse.

The MBR-RO treatment process was studied using a bench scale system to treat primary wastewater effluent from a large municipal wastewater treatment plant. The solids retention time (SRT) in the MBRs was adjusted to 2, 10, and 20 days for the three experiments conducted and various parameters were measured. These parameters included inorganics and organics in the water and on the RO membranes, as well as the specific flux across the RO membranes.

While the concentrations of organic and inorganic constituents in the MBR permeate were similar at the different SRTs, they accumulated to different concentrations on the RO membranes, and different rates of RO specific flux decline were observed. The higher the MBR SRT, the faster the decline in RO membrane specific flux. The effectiveness of RO membrane cleaning could not
be correlated to MBR SRT. Confocal microscopy revealed higher ratios of live cells to dead cells and carbohydrates to dead cells at the highest SRT, but no trend was observed.
# Table of Contents

List of Figures ......................................................................................................................... x
List of Tables ............................................................................................................................ xiv
List of Acronyms ....................................................................................................................... xv

Chapter 1: Introduction ........................................................................................................... 1
  Objectives ................................................................................................................................. 2

Chapter 2: Background .......................................................................................................... 4
  Membrane Bioreactor Performance and Effects of Solids Retention Time ...................... 4
  Reverse Osmosis Fouling and Use in Wastewater Treatment ............................ 7
    Inorganic Fouling ................................................................................................................... 8
    Particulate Fouling ................................................................................................................ 9
    Organic Fouling .................................................................................................................... 9
    Biological Fouling ................................................................................................................ 10
  Cleaning of RO Membranes ................................................................................................. 11
  Confocal Laser Scanning Microscopy ............................................................................. 12
  Summary of Previous Work and Relevance to this Project .................................................. 13

Chapter 3: Experimental Methods .................................................................................. 15
  General Experimental Design ............................................................................................. 15
  MBR Configuration .............................................................................................................. 15
  RO Configuration .................................................................................................................. 18
  MBR Operation ..................................................................................................................... 21
  RO Operation ........................................................................................................................ 21
  MBR Cleaning Procedure ..................................................................................................... 22
  RO Membrane Cleaning Procedure .................................................................................. 22
  Sampling ................................................................................................................................. 23
  Organics Analysis .................................................................................................................. 25
  RO Membrane Autopsy ......................................................................................................... 26

Chapter 4: Results ............................................................................................................... 31
  MBR Performance ................................................................................................................... 31
  Comparison between Bench scale and RDO and SWRP Performance .................... 50
  RO Performance .................................................................................................................... 54
<table>
<thead>
<tr>
<th>Chapter</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rejection of Inorganics by the RO System</td>
<td>54</td>
</tr>
<tr>
<td>RO Physical Parameters</td>
<td>62</td>
</tr>
<tr>
<td>Organics Constituents in Aqueous Samples</td>
<td>63</td>
</tr>
<tr>
<td>RO System Recovery</td>
<td>70</td>
</tr>
<tr>
<td>Membrane Autopsy</td>
<td>72</td>
</tr>
<tr>
<td>General Development of RO Foulant Layer</td>
<td>72</td>
</tr>
<tr>
<td>Organic and Inorganic Content on Unused RO Membranes</td>
<td>73</td>
</tr>
<tr>
<td>Inorganic Content on Fouled RO Membranes</td>
<td>74</td>
</tr>
<tr>
<td>Organic Content on Fouled RO Membranes</td>
<td>77</td>
</tr>
<tr>
<td>Confocal Microscopy Results</td>
<td>80</td>
</tr>
<tr>
<td>Specific Flux</td>
<td>95</td>
</tr>
<tr>
<td>Conclusions and Future Work</td>
<td>98</td>
</tr>
<tr>
<td>Conclusions</td>
<td>98</td>
</tr>
<tr>
<td>Future Work</td>
<td>100</td>
</tr>
<tr>
<td>Appendix</td>
<td>105</td>
</tr>
</tbody>
</table>
List of Figures

Figure 1: Schematic of Bench Scale MBR-RO system ........................................ 17
Figure 2: Image of an MBR Prior to Cleaning ................................................. 17
Figure 3: Schematic of Bottom RO Cell Plates .............................................. 19
Figure 4: An Image of the Bench Scale RO Unit ............................................. 20
Figure 5: An Image of the MBR-RO System Setup at the SWRP ................... 20
Figure 6: Diagram of How RO membranes were Cut for Autopsy (Not to scale) 28
Figure 7: DO Concentrations in MBRs during SRT Experiments ................... 32
Figure 8: Measured MBR HRT for SRT Experiments .................................... 33
Figure 9: Measured MBR SRT during SRT Experiments ............................... 34
Figure 10: Measured Water Temperature in the MBRs ................................. 36
Figure 11: Water Temperature in the MBRs during SRT Experiments .......... 36
Figure 12: MLSS and MLVSS Concentrations reaching Equilibrium in MBR prior to 2 day SRT Experiment ........................................................................ 38
Figure 13: MLSS and MLVSS Concentrations reaching Equilibrium in MBR before 10 day SRT Experiment ................................................................. 38
Figure 14: MLSS and MLVSS Concentrations reaching Equilibrium in MBR before 20 day SRT Experiment ................................................................. 39
Figure 15: Percent of MLSS that is Volatile in the MBRs prior to SRT Experiments ........................................................................................................ 39
Figure 16: MBR Permeate pH Values during SRT Experiments ....................... 40
Figure 17: MBR Permeate EC Values during SRT Experiments ...................... 41
Figure 18: MBR Permeate Turbidity during SRT Experiments ....................... 42
Figure 19: Average MBR Yields in Terms of g VSS Produced per g DOC Removed with 1 Standard Deviation ................................................................. 47
Figure 20: MBR #1 Transmembrane Pressure, the tick marks on top denote a MBR cleaning procedures ................................................................. 48
Figure 21: MBR #2 Transmembrane Pressure, the tick marks on top denote a MBR cleaning procedures ................................................................. 49
Figure 22: MBR Normalized Specific Flux for each SRT Experiment ............. 49
Figure 23: Comparison of Effluent inorganics between the bench scale MBR, RDO plant, and the SWRP ................................................................. 51
Figure 24: pH Values for RO Feed and Permeate Samples Taking during SRT Experiments ................................................................. 55
Figure 25: EC Values for RO Feed and Permeate Samples taken during SRT Experiments ................................................................. 55
Figure 26: Concentration of Cations and Silica in the RO Feed for SRT Experiments with Standard Deviation Bars ................................................................. 58
Figure 27: Concentration of Cations and Silica in the RO Permeate for SRT Experiments with Standard Deviation Bars ................................................................. 59
Figure 51: RO Membrane Foulant Layer Thickness with Standard Deviations as a Progression over Time for SRT Experiments ........................................................................ 80
Figure 52: Rotated View of a Syto 9 and Propidium Iodide Stained RO Foulant Layer ................................................................................................................... 82
Figure 53: Cross Section of a Syto 9 and Propidium Iodide Stained RO Foulant Layer ................................................................................................................... 82
Figure 54: CLSM Image of 10 Day SRT Fouled RO Membrane after 4 Days stained with Syto 9 (green) and Propidium Iodide (red). Microorganisms are simple and uniformly distributed .......................................................... 82
Figure 55: CLSM image of 10 Day SRT Fouled RO Membrane after 7 Days Stained with Syto 9 (green) and Propidium Iodide (red). Microorganisms are more complex, often forming groups ................................................................. 83
Figure 56: CLSM image of 10 Day SRT Fouled RO Membrane after 14 Days Stained with Syto 9 (green) and Propidium Iodide (red). .................................................... 83
Figure 57: CLSM image of 10 Day SRT Fouled RO Membrane after 4 Days Stained with ConA (green) and Propidium Iodide (red). ConA stained material appears more dispersed .......................................................... 84
Figure 58: CLSM image of 10 Day SRT Fouled RO Membrane after 14 Days Stained with ConA (green) and Propidium Iodide (red). ConA stained material seems to be more aggregated .......................................................... 84
Figure 59: CLSM image of 10 Day SRT Fouled RO Membrane after 4 Days Stained with FITC (green) and Propidium Iodide (red) .......................................................... 85
Figure 60: CLSM image of 10 Day SRT Fouled RO Membrane after 14 Days stained with FITC (green) and Propidium Iodide (red) .......................................................... 85
Figure 61: Ratio of Syto9 to Propidium Iodide on the Foulant layer for 10 Day SRT after 14 Days over the Depth of the Foulant Layer Starting from the Top of the Foulant Layer to the Foulant Layer-RO Membrane Interface ........................ 87
Figure 62: Ratio of Syto9 to Propidium Iodide on the Foulant layer for 10 Day SRT after 14 Days over the Percent Depth of the Foulant Layer starting from the Top of the Foulant Layer to the Foulant Layer-RO Membrane Interface ........................ 88
Figure 63: Ratio of the Relative Stained Areas of Syto 9 to Propidium Iodide for the 10 Day SRT Experiment ..................................................................................... 88
Figure 64: Ratio of the Relative Stained Areas of ConA to Propidium Iodide for the 10 Day SRT Experiment ..................................................................................... 89
Figure 65: Ratio of the Relative Stained Areas of FITC to Propidium Iodide for the 10 Day SRT Experiment ..................................................................................... 89
Figure 66: Ratio of the Relative Stained Areas of Syto 9 to Propidium Iodide for the 2 Day SRT Experiment ..................................................................................... 91
Figure 67: Ratio of the Relative Stained Areas of ConA to Propidium Iodide for the 2 Day SRT Experiment ..................................................................................... 91
Figure 68: Ratio of the Relative Stained Areas of FITC to Propidium Iodide for the 2 Day SRT Experiment ................................................................. 92
Figure 69: Ratio of the Relative Stained Areas of Syto 9 to Propidium Iodide after 14 Days for the SRT Experiments .......................................................... 93
Figure 70: Ratio of the Relative Stained Areas of ConA to Propidium Iodide after 14 Days for the SRT Experiments .......................................................... 94
Figure 71: Ratio of the Relative Stained Areas of FITC to Propidium Iodide after 14 Days for the SRT Experiments .......................................................... 94
Figure 72: Specific Flux over Time for SRT Experiments ............................... 97
Figure 73: Labview RO Permeate Flow Rate is Related to Measured Flow Rate for 2 Day SRT Experiment ................................................................. 105
Figure 74: Labview RO Permeate Flow Rate is Related to Measured Flow Rate for 10 Day SRT Experiment ................................................................. 105
Figure 75: Labview RO Permeate Flow Rate is Related to Measured Flow Rate for 20 Day SRT Experiment ................................................................. 106
List of Tables

Table 1: Effectiveness of Different Technologies in Removing COD, DOC, UV254 absorbance and NH4-N. RO treatment consistently outperforms all other treatment technologies. (Adapted from Cao et al. [9]) .............................................8
Table 2: Design Information for the Bench Scale MBRs .................................................. 18
Table 3: Design Information for the Bench Scale RO unit ....................................... 19
Table 4: Measured Parameters, Methods and Instrumentation .................................. 24
Table 5: Frequency and Location of Sampling .......................................................... 24
Table 6: MLSS and MLVSS Concentrations during SRT Experiments ................. 40
Table 7: MBR Feed Water Quality Data ................................................................... 43
Table 8: MBR Permeate Water Quality Data .......................................................... 44
Table 9: Removal of Organic Constituents from the MBR ...................................... 44
Table 10: Comparison of Organic Constituents from the bench scale MBR to effluent from the RDO plant and SWRP ............................................................. 51
Table 11: Comparison of Percent DOC as Protein and Carbohydrates between Bench Scale MBR Effluent and Effluent from the RDO Plant and SWRP ............. 53
Table 12: Percent Removal of Protein and Carbohydrates from RO Membrane Cleaning Procedure .......................................................... 79
Table 13: Percent Flux Recovery ............................................................................. 98
### List of Acronyms

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
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<tr>
<td>CLSM</td>
<td>Confocal Laser Scanning Microscopy</td>
</tr>
<tr>
<td>ConA</td>
<td>Concanavalin Alexa Fluor 488 conjugate</td>
</tr>
<tr>
<td>DI</td>
<td>Deionized</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>DO</td>
<td>Dissolved Oxygen</td>
</tr>
<tr>
<td>DOC</td>
<td>Dissolved Organic Carbon</td>
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<tr>
<td>EC</td>
<td>Electrical Conductivity</td>
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<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic</td>
</tr>
<tr>
<td>EPA</td>
<td>Environmental Protection Agency</td>
</tr>
<tr>
<td>EPS</td>
<td>Extracellular Polymeric Substance</td>
</tr>
<tr>
<td>FISH</td>
<td>Fluorescent in Situ Hybridization</td>
</tr>
<tr>
<td>FITC</td>
<td>Fluorescein isothiocyanate</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier Transform Infrared Spectroscopy</td>
</tr>
<tr>
<td>HRT</td>
<td>Hydraulic Retention Time</td>
</tr>
<tr>
<td>kDa</td>
<td>kilo Dalton</td>
</tr>
<tr>
<td>lps</td>
<td>liters per second</td>
</tr>
<tr>
<td>MBR</td>
<td>Membrane Bioreactor</td>
</tr>
<tr>
<td>MLSS</td>
<td>Mixed Liquor Suspended Solids</td>
</tr>
<tr>
<td>MLVSS</td>
<td>Mixed Liquor Volatile Suspended Solids</td>
</tr>
<tr>
<td>NIH</td>
<td>National Institute of Health</td>
</tr>
<tr>
<td>NOM</td>
<td>Natural Organic Matter</td>
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<tr>
<td>NTU</td>
<td>Nephelometric Turbidity Unit</td>
</tr>
<tr>
<td>PCR-DGGE</td>
<td>Polymerase Chain Reaction Denaturing Gradient Gel Electrophoresis</td>
</tr>
<tr>
<td>PLFA</td>
<td>Phospholipid Fatty Acid Analysis</td>
</tr>
<tr>
<td>PVC</td>
<td>Polyvinyl chloride</td>
</tr>
<tr>
<td>RAS</td>
<td>Return Activated Sludge</td>
</tr>
<tr>
<td>RDO</td>
<td>Rio Del Oro</td>
</tr>
<tr>
<td>RO</td>
<td>Reverse Osmosis</td>
</tr>
<tr>
<td>SDS</td>
<td>Sodium Dodecyl Sulfate</td>
</tr>
<tr>
<td>SMP</td>
<td>Soluble Microbial Products</td>
</tr>
<tr>
<td>SRT</td>
<td>Solid Retention Time</td>
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<tr>
<td>SUVA</td>
<td>Specific Ultraviolet Absorbance</td>
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<tr>
<td>SWRP</td>
<td>Southside Water Reclamation Plant</td>
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<td>WHO</td>
<td>World Health Organization</td>
</tr>
</tbody>
</table>
Chapter 1: Introduction

As populations continue to grow, water resources are increasingly strained. Many areas around the world are turning to water reuse to augment their potable water supply. Modern biological wastewater treatment technologies are capable of removing suspended solids, organic constituents and nutrients such as nitrogen and phosphorous. However, they do not completely remove many constituents of concern such as pathogenic bacteria and viruses, and trace constituents such as pharmaceuticals and personal care products (PPCPs) and endocrine disrupting compounds (EDCs). Other technologies must be added to the wastewater treatment train to remove these harmful constituents.

Reverse Osmosis (RO) treatment has traditionally been used for seawater and industrial desalination processes. RO is a membrane based treatment technique that uses high pressure to separate dissolved constituents by preferential diffusion through a semipermeable membrane. A high quality permeate stream is generated along with a concentrated waste stream. RO processes provide excellent removal of both inorganic and organic constituents including PPCPs and EDCs.

Because of its ability to achieve a high degree of removal of nearly all dissolved constituents from water, RO has recently been considered for post-treatment of MBR permeate in treating wastewater to drinking water standards for water reclamation applications. Many water utilities are considering using MBR-RO processes as a means of treating their wastewater to drinking water standards, especially with regard to removing emerging contaminants such as PPCPs. However, there is limited data on the performance of combined MBR-RO processes for reuse applications, particularly with regard to membrane fouling.

There are four types of RO fouling: inorganic, particulate, biological, and organic. Inorganic and particulate RO fouling can be controlled by using antiscalents and by following simple RO cleaning procedures. Biological and organic RO fouling were more difficult to control and can lead to irreversible
fouling and thus reduced RO membrane lifespan. The RO feed water quality in an MBR-RO process depends on how the MBR process is operated. RO membrane fouling and the rate of RO specific flux decline are therefore dependent on how the MBR is operated. Solids retention time (SRT) is the average amount of time that the mixed liquor is retained in the membrane bioreactor. Because SRT affects the MBR microbial communities and permeate quality, MBR SRT is expected to impact RO membrane fouling. SRT is expected to affect biological and organic fouling on the RO membranes. Therefore, it is desirable to optimize MBR operation to minimize biological and organic fouling on the RO membranes.

Objectives
The main objectives of this study were to:

- Determine whether membrane bioreactor (MBR) solids retention time (SRT) affects:
  - Dissolved organic carbon (DOC), protein, or carbohydrate concentrations in the MBR permeate
  - The high molecular weight fractions of DOC, protein and carbohydrates in the RO feed
  - Reverse osmosis (RO) fouling rates
  - Inorganic or organic concentrations in the foulant layer on the RO membranes
  - Effectiveness of RO membrane cleaning (in terms of removal of inorganic and organic constituents and specific flux recovery)
- Visualize and compare the spatial and temporal development of the RO foulant layer for the SRT conditions tested

This study complements a previously conducted pilot study, which examined the performance of RO membranes in treating effluent from an MBR, lagoon, and conventional wastewater treatment process.

A bench scale study was conducted in order to study the interactions between MBR operation and RO performance. Two MBRs were operated at
different SRTs, and the permeate from the MBR was fed to an RO unit. Water quality parameters were measured in the MBR feed, MBR permeate, RO feed, and RO permeate. The fractional removal of each constituent was determined for the MBR and RO treatment processes. RO membranes were extracted from the RO unit over the course of the run during each experiment and autopsied for organic and inorganic constituents on the RO foulant layer. Staining of the RO foulant layers using Syto 9, ConA, FITC and propidium iodide combined with confocal laser scanning microscopy (CLSM) was done to enable characterization of the spatial and temporal development of the foulant layer. Finally, trends in the MBR permeate quality and RO membrane fouling due to changes in SRT were compared with the performance of the RO unit, defined as the rate of specific flux decline.

A central feature of this research was the examination of the foulant layer on the RO membrane using CLSM. In confocal microscopy, a laser controls the focal plane, allowing the microscope to create images at various depths in the foulant layer. The result is a 3-dimensional view of the distribution of proteins, carbohydrates, and live and dead microbial cells. CLSM can be used to measure the thickness of the foulant layer on the RO membrane and, qualitatively (visual identification), the presence of proteins, carbohydrates, live cells, and dead cells. CLSM examinations were done three times- after 3 to 4 days, after 6 to 7 days, and finally after 14 days of operation. The evolution of the fouling layer was then determined.
Chapter 2: Background

Membrane bioreactors (MBRs) have recently been combined with reverse osmosis (RO) to generate high quality water in water reuse applications. There is limited knowledge of how these two technologies interact with each other, particularly with regard to how MBR operation affects MBR permeate quality and RO membrane fouling. This section presents a discussion of MBR performance and the affects of solids retention time (SRT) on RO membrane fouling and the use of RO in wastewater treatment, RO membrane cleaning, and confocal laser scanning microscopy (CLSM) combined with fluorescent staining and its use as a diagnostic tool in RO membrane fouling.

Membrane Bioreactor Performance and Effects of Solids Retention Time

MBRs are a variation of the suspended culture biological wastewater treatment process which uses membrane filtration instead of gravity sedimentation to achieve solids removal. MBRs are more compact than the traditional activated sludge and clarifier design and they allow operators to have complete control over hydraulic retention time (HRT) and solid retention time (SRT) and result in lower permeate turbidity. The HRT controls the substrate loading to the MBR, and the SRT can be used to control the MLSS concentration in the MBR. MLSS concentration increases with increasing SRT. MBR permeate contains undegraded or partially degraded constituents in the wastewater and soluble products of microbial degradation and cell growth. SRT is an important operating parameter in MBRs that can greatly impact fouling of the microfilters in the MBR, the microbial community in the MBR, and the MBR permeate water quality.

MBRs typically foul more quickly when operated at low SRTs. Low SRTs result in higher food to microorganism (F/M) ratio, and bound EPS when MLSS concentrations are below 5,000 mg/L. These factors contribute to an increase in transmembrane pressure required to maintain MBR permeate flow and a decline in MBR specific flux [1]. Bound or suspended EPS is the portion of EPS bound
to flocs in the MBR and can be filtered out of the mixed liquor, and dissolved EPS is the portion of EPS dissolved in the aqueous phase which cannot be filtered. EPS consists primarily of carbohydrates and protein. Polysaccharides are a greater constituent in EPS than protein [2]. Bound EPS has been implicated in MBR fouling. However, dissolved EPS was not found to contribute significantly to MBR fouling [3]. Evidence that bound EPS increases MBR fouling is that filtration index, the ratio of sludge permeate flux to clear water flux, decreased by 80 percent when the bound EPS concentration increased from 15 to 90 mg/L [2]. The increase of bound EPS at low SRTs could be a result of increased EPS production, reduced EPS degradation, or decreased EPS solubility [1]. The mechanism by which bound EPS increases MBR fouling may be by changing MBR floc structure. Some research has shown that EPS increases the size and density of MBR flocs [3]. However, Schmid et al. were not able to relate the activated sludge floc size or structure to the fractions of carbohydrates, humic substances, proteins or DNA [4].

SRT affects microbial communities in MBRs. Changes in microbial communities would be expected to impact MBR permeate water quality. A lot of research has been conducted to characterize MBR microbial communities. A number of different techniques can be used to classify bacterial communities. These techniques include phospholipid fatty acid analysis (PLFA), polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) on extracted 16s rRNA, fluorescent in situ hybridization (FISH), and staining with microscopy. Biomass viability and production rate are higher at low SRTs. A PLFA analysis was conducted by Cicek et al. on the mixed liquor from MBRs operated at different SRTs. They found that microbial communities changed with SRT. Ratios of eukaryotic organisms, gram positive bacteria, and yeast cells also changed with SRT. They also evaluated whether the ability of microorganisms to use different carbon sources was affected by SRT. BIOLOG analysis results showed that the ability of the microorganisms present in the MBR mixed liquor to utilize different carbon substrates was not affected by SRT [5].
Other factors besides SRT can affect MBR microbial communities. Oxygen concentrations were found to affect the microbial community. Biofilm thickness was nearly three times larger in MBRs with high DO concentration (6 mg/L) than with low DO concentration (<0.1 mg/L) [6]. The microbial community attached on the membranes was the same as those in the mixed liquor. The oxygen demands of MBRs operating at higher SRTs were not substantially higher than oxygen demands of MBRs operating at lower SRTs [1].

Changes in SRT affect the MBR permeate quality as well. MBR permeate DOC concentration increased with increasing SRT values. Dissolved EPS released by cells or floc are included in the DOC concentration [3]. Excellent turbidity removals of greater than 99 percent were observed as well as 97.3 and 95.9 percent removals of COD and UV\textsubscript{254} absorbance, respectively [7]. Increasing SRT was shown to decrease MBR permeate protein and carbohydrate concentrations. An MBR operating at a 23 day SRT had 40 times greater protein concentration and 5 times greater carbohydrate concentrations as an MBR operating at 40 days.

SRT can also impact the concentrations and forms of inorganic nitrogen by changing nitrification rates. Nitrification is the biological oxidation of ammonia to nitrite and then to nitrate. Nitrification was hindered when MBRs were operated at SRTs lower than 5 days [5]. However, complete nitrification has been seen at SRTs as low as 5 days. Nitrification rates in MBRs are affected by factors other than SRT, including pH, alkalinity, DO concentration, substrate concentration, temperature, and the carbon to nitrogen ratio. The carbon to nitrogen ratio in the MBR feed water has been shown to have a more profound positive effect on nitrification rates than SRT [8]. To remove nitrogen from the MBRs, the denitrification process is used in which heterotrophic bacteria use organic carbon as their electron donor and nitrate as the electron acceptor to reduce nitrate to nitrogen gas. This requires a selector operated at anoxic conditions.
Reverse Osmosis Fouling and Use in Wastewater Treatment

Use of RO in wastewater is of increasing interest because of its ability to produce high quality water with very low concentrations of nearly all inorganic and organic constituents. These constituents include bacteria, viruses, NOM, EPS, inorganics and radionuclides. Cao et al. examined the amount and removal of COD, DOC, UV$_{254}$ absorbance and NH$_4$-N by different technologies. This data is presented in Table 1. RO out performs all other technologies at removing these compounds, with DOC and UV$_{254}$ absorbance removal of 88.2 and 99 percent, respectively. Cao et al. conducted toxicity tests on fish using the water samples in Table 1. RO treated wastewater reduced fish mortality from 90 percent when in the untreated secondary wastewater to less than 10 percent post RO treatment. RO treated wastewater had one of the lowest values for genotoxicity, similar to ozone treated wastewater at doses at or greater than 8.5 mg/L. Hatch rates for fish were greatest for the RO treated wastewater, and no developmental issues were seen in fish in RO treated wastewater [9]. Although RO is very effective at removing constituents from water, some NOM can pass through RO membranes. FTIR analysis has shown that RO permeate consists mostly of hydrophilic constituents, whereas the RO foulant layer consists primarily of hydrophobic compounds. This holds true regardless of the pretreatment used for the RO unit [10].

Problems in RO applications include cost, fouling, and concentrate management. RO membrane fouling is problematic because it decreases membrane flux and salt rejection, increases the required feed pressure to the RO units and energy requirements, and leads to membrane degradation [11]. The degree of RO membrane fouling is determined by the thickness, composition, and structure of the foulant layer. Understanding how the RO foulant layer develops in terms of these factors is important.

RO membrane fouling has been classified into four categories: inorganic (or scaling), particulate, organic and biological. Adjusting SRT in MBRs is not expected to significantly affect the inorganic and particulate RO fouling, so the focus of this discussion will be on organic and biological fouling. RO membrane
cleaning procedures are necessary to prolong the operational life of the RO membranes. The cleaning procedure used is dependent on the type of RO membrane fouling observed, which is dependent on the RO feed water quality. Membrane autopsies are conducted to determine the type of fouling and to adjust the cleaning procedure to optimize its effectiveness.

Table 1: Effectiveness of Different Technologies in Removing COD, DOC, UV254 absorbance and NH4-N. RO treatment consistently outperforms all other treatment technologies. (Adapted from Cao et al. [9])

<table>
<thead>
<tr>
<th>Treatment Type</th>
<th>DOC</th>
<th>UV254 absorbance</th>
<th>NH4-N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration (mg/L)</td>
<td>% Removal</td>
<td>Concentration (mg/L)</td>
</tr>
<tr>
<td>Secondary Effluent</td>
<td>7.9</td>
<td>15.2</td>
<td>1.9</td>
</tr>
<tr>
<td>Coagulation and Sand Filtration</td>
<td>6.4</td>
<td>13.7</td>
<td>9.9</td>
</tr>
<tr>
<td>UV (40 mJ/cm²)</td>
<td>7.7</td>
<td>13.0</td>
<td>14.0</td>
</tr>
<tr>
<td>UV (84 mJ/cm²)</td>
<td>7.6</td>
<td>12.8</td>
<td>15.4</td>
</tr>
<tr>
<td>Chlorination (5 mg/L)</td>
<td>7.6</td>
<td>13.3</td>
<td>12.5</td>
</tr>
<tr>
<td>Chlorination (10 mg/L)</td>
<td>7.1</td>
<td>11.9</td>
<td>21.8</td>
</tr>
<tr>
<td>Ozonation (2 mg/L)</td>
<td>6.4</td>
<td>11.7</td>
<td>22.9</td>
</tr>
<tr>
<td>Ozonation (3.8 mg/L)</td>
<td>6.1</td>
<td>10.0</td>
<td>34.3</td>
</tr>
<tr>
<td>Ozonation (6.5 mg/L)</td>
<td>6.0</td>
<td>9.0</td>
<td>60.2</td>
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<tr>
<td>Ozonation (11.1 mg/L)</td>
<td>5.7</td>
<td>4.7</td>
<td>68.8</td>
</tr>
<tr>
<td>Ozonation (15 mg/L)</td>
<td>5.5</td>
<td>4.9</td>
<td>67.5</td>
</tr>
<tr>
<td>Ultrafiltration (50kDa)</td>
<td>6.2</td>
<td>11.7</td>
<td>23.1</td>
</tr>
<tr>
<td>RO</td>
<td>0.9</td>
<td>0.2</td>
<td>99.0</td>
</tr>
</tbody>
</table>

Inorganic Fouling

Inorganic fouling, also known as scaling, occurs when the concentrations of an inorganic constituents in the RO feed water increases to the point that precipitation occurs on the RO membrane. Fouling due to the presence of iron in the RO feed water is nearly as common as biofouling. Silica and aluminum are also common RO membrane foulants, though less common than iron. The trivalent forms of aluminum and iron are commonly used as coagulants in water treatment, and also help precipitate silica. Calcium carbonate and calcium sulfate are typically not common contributors to RO membrane fouling. Calcium fluoride and barium sulfate, while frequently at concentrations sufficient to cause scaling issues, rarely contribute to RO membrane fouling. Fouling due to calcium phosphate is rare and only observed in locations with high calcium phosphate in the groundwater, but could be a bigger problem in wastewater applications due to higher phosphate concentrations in wastewater than groundwater. Scaling problems can typically be avoided by use of antiscalants [12]. Antiscalants work
by hindering crystal formation and growth at supersaturated conditions [13]. No antiscalants were added upstream of the RO unit in this project.

**Particulate Fouling**

Particulate fouling, also known as colloidal fouling, is caused by inorganic or organic materials which can plug the RO membrane and lead to cake formation. Particulate fouling can be more problematic in RO applications than in membrane filtration because of the unfeasibility of RO membrane backwashing, but it can be easily prevented by proper pretreatment. Prefiltration using coagulation with filtration or microfiltration can reduce or prevent colloidal fouling [13]. Thus, using MBR permeate would be expected to prevent RO particulate fouling.

**Organic Fouling**

Organic fouling precedes biological fouling on RO membranes [14]. Subramani et al. found that the organics in the RO foulant layer have a high electron donor capacity, which leads to an acid-base repulsion between the RO membrane and the cells. However, organic compounds in the RO foulant layer increased surface roughness subsequently facilitating microbial attachment. EPS is particularly problematic in organic fouling. Some EPS constituents attach more efficiently than others. For example, even when protein in the RO feed water was three fold more concentrated than the carbohydrates, the carbohydrate concentration on the RO membrane was three times higher than the protein concentration in the EPS foulant layer on the RO membrane [15].

Calcium increases organic fouling on RO membranes. The presence of calcium at 0.5 mM concentration increased the adsorption of polysaccharides and DNA by 2 and 3 times, respectively. The protein concentrations did not change when in the presence of calcium [15]. Calcium binds to carboxylic acid groups and forms an intermolecular bridge, crosslinking organic matter in the
foulant layer on the RO membrane [16]. This increases the hydraulic resistance of the organic fouling layers [14].

Water samples can be divided into different molecular weight fractions by vapor pressure osmometry, field flow fractionation, analytical ultrafiltration or size exclusion chromatography [17]. Organic compounds greater than 10kDa have been implicated as a contributing factor to organic fouling.

**Biological Fouling**

Biological fouling, also known as biofouling, is the result of microbial growth on the membrane surface. Biofilms are the stable result of a four stage process that consists of microbial attachment, maturation of the biofilm, maintenance of the biofilm, and detachment and microbial dispersal [18]. In order for a biofilm to form, an initial layer of organic molecules must first adhere to the RO membrane surface. After this organic layer is established, bacteria adhere to the membrane surface, reproduce, and produce EPS. EPS, a type of organic foulant, has been implicated in causing significant irreversible RO membrane fouling. Colonization of the RO membrane by microorganisms can occur in less than a day, with a loose biofilm containing microorganisms embedded in EPS within 3 days, with a completely developed, dense biofilm apparent by 7 days [19]. Active bacterial cells appear to concentrate at the RO membrane surface, which may be due to the concentration polarization effect leading to higher nutrient concentrations at the RO membrane surface [20]. Most RO biofilms contain similar bacteria, including the slime forming Pseudomonas genus [12]. Unlike the findings of Kim et al. for MBRs, Ivnitzsky et al. found that the dominant bacteria in the RO feed water were not always found in the RO membrane biofilm [19]. A study was conducted on an RO unit where the RO concentrate was recycled back to the RO feed tank. Cells attached to the RO membrane were shown to have a faster growth rate than the cells in the RO feed tank [20]. Biofouling on RO membranes is particularly problematic at temperatures greater than 25 degrees C [11]. Out of the 150 RO membranes autopsied in a study conducted by Darton et al., more than 50 percent had significant biofouling. Pseudomonas aeruginosa is a species of bacteria that has
been used to study the development of biofilms on RO membranes. Using these bacteria, the two mechanisms causing RO membrane fouling by biological organisms were elucidated. First, the bacterial cells on the RO membrane prevent salt back diffusion resulting in increased osmotic pressure. This leads to a decline in salt rejection and permeate flux. Second, the EPS that bacteria produce lead to RO permeate flux decline due to hydraulic resistance to permeate flow [15].

The adhesion of microorganisms to RO membranes has been shown to be affected by the ionic strength of the RO feed water [21]. The pH does not seem to affect the attachment of microorganisms on RO membranes. Based on research conducted on RO membranes, a linear relationship was not able to be established between the hydrophobicity of the RO membranes and the degree of microbial attachment on the RO membranes. Efforts have been taken to control biofilm growth on RO membranes by using various disinfectants including chlorine and ozone. Dechlorination is necessary prior to RO treatment due to the sensitivity of RO membranes to chlorine. Chlorination with subsequent dechlorination was shown to be ineffective at preventing biofilm formation. While microorganisms were inactivated by the chlorine, EPS attachment to the RO membranes was not affected and bacterial detachment did not decrease [22]. Ozonation may increase RO fouling by lysing algal cells and releasing EPS, leading to an increase EPS attachment to the RO membrane with a subsequent increase in algal and bacterial cell attachment [23].

**Cleaning of RO Membranes**

RO membrane cleaning is crucial in maintaining adequate specific flux across the RO membrane. Effective cleaning procedures for RO membranes depend on the type of RO fouling. RO membranes are costly, therefore developing effective cleaning procedures to extend their lifespan and minimize waste is important. Cleaning with NaOH alone has been shown to condition organic foulants at the RO membrane surface [12]. SDS, an anionic surfactant, and EDTA, a metal chelating agent, have been shown to be effective in the
removal of organic fouled RO membranes. Cleaning procedures using these chemicals for RO membrane cleaning can be optimized by adjusting concentration, pH and temperature of the solution as well as the cross-flow velocity in the RO unit and the duration of the cleaning. The pH is a critical parameter for EDTA solutions, as the pKa values for the carboxylic groups on EDTA are 1.99, 2.67, 6.16 and 10.26. In order for EDTA to be effective at cleaning, all of the carboxylic groups must be deprotonated, and thus the pH of the solution must be greater than 10.26. Because the pKa of the sulfuric acid group in SDS is 2.12, pH does not play a significant role in the effectiveness of SDS cleaning solutions [16, 24]. A 0.5 mM EDTA solution at a pH of 11 was able to remove nearly 45% of the foulant layer on RO membranes tested [16]. However, SDS concentration is critical for it to be an effective cleaning agent. SDS diffuses into the RO foulant layer and forms micelles around organic matter, which solubilize and break up the foulant layer. For this to occur, the SDS concentration must be above 8.36 mM in DI water, which is the critical micelle concentration for SDS. SDS solutions at concentrations of 10 mM or greater have been shown to be effective in cleaning RO membranes.

Confocal Laser Scanning Microscopy

Confocal laser scanning microscopy (CLSM) is an optical microscopy technique with the ability to focus on multiple independent planes through a specimen, generating high-resolution images at each depth within the sample. When coupled with fluorescent-staining techniques, CLSM allows researchers to visualize three-dimensional images of complex structures such as microorganisms and biofilms. Although CLSM investigations of fouled RO membranes have not been reported in published literature, it has been used to study foulant layers on MF and UF membranes. Chen, et al. used CLSM to examine the foulant layer on a 0.45 µm mixed cellulose ester membrane filtering waste activated sludge from a food processing plant, using ConA, FITC, and calcoflour white as stains. ConA stained carbohydrates-like material, FITC stained protein, and calcoflour-white stains cellulose and chitin in the cell walls of
fungus. They found large spatial variations in the foulant layer. The ConA-stained α-polysaccharides tended to be located near the membrane surface, while the FITC-stained protein and calcoflour-white-stained β-polysaccharides were distributed more heterogeneously [25]. The MBR foulant layer was found to be between 6.8 and 17.8 mm thick [26]. Nanofiltration membranes using tertiary treated wastewater were stained with propidium iodide, staining dead cells, and ConA and analyzed using confocal microscopy. The foulant layer thickness was 20 to 30 µm after 7 days of operation [19]. CLSM data was used in another study to show that biofilm development was strongly dependant on the presence of EPS [27].

**Summary of Previous Work and Relevance to this Project**
While inorganic fouling of the RO membranes may occur, it is not expected to change with SRT. Thus, any differences in specific flux decline between the SRT experiments should not be attributable to inorganic fouling. MBR permeate is not expected to have a significant amount of particles due to high turbidity removal by the MBR. Therefore particulate fouling on the RO membranes should not occur. Even though some studies have seen increases in DOC concentration with increasing SRT, concentrations of protein and carbohydrates were shown to be inversely proportional to SRT. Because protein and carbohydrates are constituents of EPS, which has been implicated in causing RO fouling, more organic fouling and faster specific flux declines are expected at lower SRT conditions. Because organic fouling is a precursor to biological fouling, more biological fouling is expected at lower SRT values. Different constituents in the MBR permeate as a result of the MBR process operating at different SRTs may lead to differences in organic fouling rates and characteristics, and might also cause different biofouling characteristics. Higher organic and biological fouling on the RO membranes at lower SRT conditions may result in thicker or a more dense RO foulant layers, making the RO cleaning procedure less effective. CLSM with fluorescent staining should show
differences in types and amounts of biological fouling over the depth of the RO foulant layer as well as differences in the RO foulant layer thickness over time.
Chapter 3: Experimental Methods

General Experimental Design

A bench scale MBR-RO system was operated at the Southside Water Reclamation Plant (SWRP) in Albuquerque, NM. Two MBRs were constructed and operated at an HRT of approximately 8.5 hrs. Experiments were conducted at SRTs of 2, 10 and 20 days. Experiments were run for 2 weeks. MBRs were operated at the desired SRT for a minimum of 3 SRTs or until a steady state MLSS concentration was achieved. The RO unit was run with deionized (DI) water until a steady state specific flux was achieved before experiments were started.

MBR Configuration

Two bench scale MBRs were constructed to treat wastewater that had been subjected to primary sedimentation. A schematic of the MBR and RO systems is shown in Figure 1. The 114 liter MBR tanks were designed to hold sufficient volume for sample collection, to maintain system stability and to provide adequate flux through the microfiltration unit. The microfiltration units in the MBRs were Puron hollow fiber submerged membranes with outside-in flow donated by Koch. Air was supplied continuously to microfiltration units to provide air scouring for the membranes, and to diffusers designed to provide sufficient air to the MBR to maintain aerobic conditions. A Pondmaster Model AP-100 air pump providing about 4300 L/min was used for air scouring to the membranes and was connected to the microfiltration unit. A Pondmaster Model AP-40 air pump with an air flow of 1350 L/min was used to provide air to the aeration device. This pump was attached to a garden hose that was connected to a manifold constructed of ¾ inch PVC pipe and fine air diffusers. Mixing in the MBRs was provided by the aeration. The air flow from the pumps was split equally to the MBRs. The volume in each MBR was kept constant by a float valve that controlled the flow of primary wastewater effluent into the MBR. A second float valve was installed that was designed to open if the first valve failed.
Wasting lines for each MBR were installed in each tank slightly higher than the top of the microfilter and the flow rate was controlled by a variable speed peristaltic pump that pumped the MBR waste into a waste collection tank. The waste collection tank was open to the atmosphere to prevent pressure buildup and discharged to a sump that pumped waste to the head of the wastewater treatment plant. A level switch was installed above the microfiltration unit to prevent the microfilters from drying out due to low water levels. When the water level dropped to the level switch, the MBR permeate and wasting pumps automatically turned off. A backwash system was not included in the design of the MBRs due to expense and complications in design. Instead, the MBRs were operated with a relaxation time included in the operating cycle. A picture of one of the MBRs before cleaning is shown in Figure 2. A pressure gage and a pressure transducer were installed before the MBR permeate pump and a pulse dampener and then a rotameter were installed after the microfiltration pump. Downstream of the MBR rotameter was a 3 way ball valve which allowed the MBR permeate to be diverted for sample collection and flow measurement. After the 3-way valve was a ¾ inch PVC tee that connected to a 2 foot piece of clear PVC stand pipe which served as a wasting line before the RO tank. During the experiments, some of the MBR permeate was pumped from the bottom of the stand pipe into the RO feed tank. At the end of the pipe was another ¾ inch PVC tee that was connected to tubing that went to the waste collection tank. Downstream of the PVC wasting line was a rotameter with a valve to control flow to the RO feed tank. After the rotameter was another 3-way valve that allowed the flow to be diverted for verification of flow rate using a graduated cylinder and stopwatch. The MBR permeate then flowed to the RO feed tank. Design information for the MBRs is presented in Table 2.
Figure 1: Schematic of Bench Scale MBR-RO system

Figure 2: Image of an MBR Prior to Cleaning
Table 2: Design Information for the Bench Scale MBRs

<table>
<thead>
<tr>
<th>Design Variable</th>
<th>SRT (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>HRT (Hours)</td>
<td>8.5</td>
</tr>
<tr>
<td>Volume (L)</td>
<td>88</td>
</tr>
<tr>
<td>MF surface area (m(^2))</td>
<td>1.5</td>
</tr>
<tr>
<td>Flowrates (mL/min)</td>
<td></td>
</tr>
<tr>
<td>Influent</td>
<td>192</td>
</tr>
<tr>
<td>Effluent</td>
<td>158</td>
</tr>
<tr>
<td>Wasting</td>
<td>33.9</td>
</tr>
<tr>
<td>Flux (LMH)</td>
<td>6.31</td>
</tr>
</tbody>
</table>

**RO Configuration**

A custom flat sheet RO unit was designed by Dr. Kerry Howe and constructed by the UNM Physics department. A schematic of the bottom RO cell plates is shown in Figure 3 and the RO unit is shown in Figure 4. The RO unit consisted of 6 cell plates and a flat sheet RO membrane was placed between each set of two cell plates. This design allowed up to five membranes to be tested simultaneously and taken out for autopsy at different times over the course of a test. Membranes were taken out after 3 to 4 days, after 7 to 8 days, and after 14 days. Osmonics AG RO membranes, proprietary polyamide thin film membranes, were cut to the appropriate size to fit between each set of two cell plates. The cell plates were held within a support frame that consisted of 2.54 cm thick metal plates on the top and bottom and 8 1.27 cm stainless steel screws spanning the unit. RO feed water was pumped into the bottom membrane cell. The concentrate from each RO membrane was used to feed the next RO membrane in the series. Concentrate from the last RO membrane was returned to the RO Feed tank. Permeate from each RO membrane was combined and wasted. The RO feed pressure was controlled by a metering valve on the concentrate line downstream of the RO unit. Design information for the RO unit is provided in Table 3. The entire MBR-RO system setup at the SWRP is shown in Figure 5.
<table>
<thead>
<tr>
<th>Design Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RO membrane length (m)</td>
<td>0.200</td>
</tr>
<tr>
<td>RO membrane width (m)</td>
<td>0.080</td>
</tr>
<tr>
<td>Channel depth (m)</td>
<td>0.000508</td>
</tr>
<tr>
<td>Effective membrane area (m$^2$)</td>
<td>0.016</td>
</tr>
<tr>
<td>Feed channel cross sectional area (m$^2$)</td>
<td>4.06E-05</td>
</tr>
<tr>
<td>Feed flow velocity (m/s)</td>
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</tr>
<tr>
<td>Permeate flux (LMH)</td>
<td>20</td>
</tr>
<tr>
<td>Flow (L/min)</td>
<td></td>
</tr>
<tr>
<td>Feed flow</td>
<td>0.366</td>
</tr>
<tr>
<td>Permeate flow (per RO membrane sheet)</td>
<td>0.02667</td>
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<tr>
<td>Concentrate flow</td>
<td>0.360</td>
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<tr>
<td>Recovery (per RO membrane sheet)</td>
<td>0.0146</td>
</tr>
<tr>
<td>Units in series</td>
<td>5</td>
</tr>
<tr>
<td>Total feed flow (L/min)</td>
<td>0.37</td>
</tr>
<tr>
<td>Total permeate flow (L/hr)</td>
<td>1.60</td>
</tr>
</tbody>
</table>

Table 3: Design Information for the Bench Scale RO unit

Figure 3: Schematic of Bottom RO Cell Plates
Figure 4: An Image of the Bench Scale RO Unit

Figure 5: An Image of the MBR-RO System Setup at the SWRP
**MBR Operation**

The MBRs were operated on a 10-minute cycle consisting of 9 minutes of filtration and one minute of relaxation. The cycle was controlled by a ChronTrol XT table top timer. During the one minute membrane relaxation time, both MBR wasting and permeate pumps were turned off. In addition to membrane relaxation, manual backwashes were performed using tap water to prevent MBR flux decline due to cake layer formation. Backwashes were found to be largely ineffective in preventing MBR flux decline and were time consuming. By the beginning of July, backwashes were eliminated from the MBR operation and replaced by cleaning the MBR with a low concentration sodium hypochlorite solution. The MBR cleaning procedure is described in detail later in the MBR Cleaning Procedure section. MBR waste and permeate flow rates were adjusted to maintain desired HRT and SRT. MBRs were operated for a minimum of 3 SRTs at the SRT that would be used for each experiment to allow the MBRs to reach steady state MLSS concentrations. The MBRs were operated at a constant volume with continuous aeration and wasting.

**RO Operation**

The RO unit was operated using DI water continuously for several days before the experiment. No disinfectants or scale inhibitors were added to assess a worst case scenario in terms of RO membrane fouling. The RO permeate flow rate was controlled by changing the RO feed pressure by adjusting the concentrate flow control valve. MBR permeate flow rate to the RO tank was controlled by a variable speed peristaltic pump and measured using a rotameter. The volume in the RO feed tank was kept constant by an overflow at the top of the RO feed tank. By controlling these flow rates, the MBR permeate was concentrated in the RO feed tank to provide a recovery similar to that seen by the final RO element in a full scale RO system operating at 75% recovery. The RO membranes were sequentially removed from the membrane support apparatus.
over the course of each experiment to characterize the extent and nature of fouling through the autopsy procedure.

**MBR Cleaning Procedure**

The MBRs were cleaned when the transmembrane pressure became high (approximately 0.3 bar) and before the MBR permeate flow rate became too low to maintain the desired HRT of 8.5 hrs. The mixed liquor in the MBR and the diffuser assembly were transferred to another tank and covered. The mixed liquor was aerated continuously during MBR cleaning. The MBR was rinsed several times with treated wastewater and drained. The MBR was then filled with treated wastewater. Sodium hypochlorite was added at concentrations of between 1000 to 2000 mg/L to the MBR. The MBR was allowed to soak in the sodium hypochlorite solution for at least 4 hours. Then, the MBR was drained and refilled with tap water. A small amount of sodium thiosulfate was added to ensure that no sodium hypochlorite remained in the MBR. The MBR was drained and the mixed liquor and the aeration apparatus were transferred back into the MBR. Air for membrane scouring was supplied continuously during the MBR cleaning procedure.

**RO Membrane Cleaning Procedure**

At the end of each experiment, the remaining RO membrane in the six-plate membrane support apparatus was cleaned to determine the affect of SRT on RO membrane cleaning. The RO cleaning solution used was a 1% EDTA (34.2 mM) solution made with tap water and adjusted to a pH of between 10 and 11 with NaOH. The cleaning solution was prepared the day before cleaning. First, the RO pump was turned off and the RO feed tank was drained, rinsed, and filled with 10 L of this cleaning solution. Next, the RO pump was turned back on and the cleaning solution was allowed to circulate in the system for one hour at a pressure of roughly 100 psi. The cleaning solution was then drained and the RO feed tank was rinsed out with treated wastewater. Once thoroughly rinsed, the
RO feed tank was refilled with DI water and for the first minute of operation, fluid in the concentrate and permeate lines was wasted to ensure that the cleaning solution was removed from the system. DI water was then allowed to circulate through the system for a few hours. Data was recorded for calculation of specific flux and the RO pump was turned off. RO membranes were extracted from the RO unit before and after the cleaning procedure and brought back to the university to conduct the membrane autopsy.

**Sampling**

Grab samples of water were taken from the MBR feed, MBR tank, RO feed tank, and RO feed and permeate lines. The pH and EC were measured daily. Alkalinity, TOC, UV absorbance, protein, carbohydrate, MLSS and MLVSS were measured 2 to 3 times per week. Inorganics were measured 3 times per experiment. RO flow rate, RO concentrate temperature, and MBR and RO feed pressure were measured continuously and recorded using Labview. Organic and inorganic material on the RO membranes was characterized and quantified once per membrane after each membrane had been extracted from the RO unit. Various parameters were measured using CLSM, which was conducted once per extracted membrane. Grab samples of effluent before chlorination from the RDO plant and the SWRP were measured for all water quality parameters and compared with permeate from the bench scale MBR. The methods and instrumentation used are identified in Table 4. Table 5 shows the frequency of sampling.
### Table 4: Measured Parameters, Methods and Instrumentation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method</th>
<th>Instrument</th>
</tr>
</thead>
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<td>pH</td>
<td>SM 4500-H⁺ B</td>
<td>Oakton pH/CON 10 Series Meter</td>
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<tr>
<td>EC</td>
<td>SM 2510 B</td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>SM 2550 B</td>
<td></td>
</tr>
<tr>
<td>DO</td>
<td>SM 4500-O G</td>
<td>Hach HQ40d DO Meter</td>
</tr>
<tr>
<td>Alkalinity</td>
<td>SM 2320 B</td>
<td>NA</td>
</tr>
<tr>
<td>DOC</td>
<td>SM 5310 C</td>
<td>Tekmar Dohrmann Phoenix 8000</td>
</tr>
<tr>
<td>UV absorbance</td>
<td>SM 5910 B</td>
<td>Varian Cary 50 Conc UV-Visible Spectrophotometer</td>
</tr>
<tr>
<td>Protein</td>
<td>Modified Lowry Protein Assay (Dubois et. al, 1956)</td>
<td>Modified Lowry Protein Assay Kit from Pierce (Product #23240)</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>phenol-sulfuric acid</td>
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</tr>
<tr>
<td>MLSS</td>
<td>SM 2540 G</td>
<td>NA</td>
</tr>
<tr>
<td>MLVSS</td>
<td>SM 2540 G</td>
<td>NA</td>
</tr>
<tr>
<td>Turbidity</td>
<td>SM 2130 B</td>
<td>Hach 2100AN Turbidimeter</td>
</tr>
<tr>
<td>Membrane Inorganics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anions</td>
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### Table 5: Frequency and Location of Sampling

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<td>Alkalinity</td>
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<td>Turbidity</td>
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<td>TOC (filter MBR feed)</td>
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<td>Carbohydrate</td>
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<td>Membrane Organics</td>
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<td>CLSM</td>
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Organics Analysis

Samples were analyzed for dissolved organic carbon (DOC), protein, carbohydrates, and UV\textsubscript{254} absorbance. The RO feed samples were fractionated by filtration through a 10 kDa ultrafiltration membrane prior to analysis, and analyses were done on both the total and <10 kDa fractions. Specific UV absorbance (SUVA) was calculated as the ratio of UV\textsubscript{254} absorbance divided by the DOC, multiplied by 100.

All glassware used for organic analyses was washed with soap and water, rinsed with tap water, soaked in a 10 percent nitric acid bath for at least 1 hour, and rinsed with DI water. The mouths of bottles were sealed with aluminum foil and the glassware was baked at 550 °C for at least 1 hour. All plasticware used for organic analyses was washed with soap and water, rinsed with DI water, and allowed to air dry.

RO permeate samples were not filtered. Samples that passed through the MBR filters (nominal pore size 0.05 µm) should not have significant particulate matter, however, additional filtration was performed to ensure particle removal. For all samples other than RO permeate, (untreated wastewater, samples from full-scale treatment facilities) samples were filtered through 42.5-mm diameter Whatman GF/F glass-fiber filters (binder free, 0.7 µm nominal pore size). The filters were baked at 550 °C for at least 1 hour prior to use. A minimum of 600 mL of DI water was passed through the filter prior to sample filtration. During sample filtration, the first 25 mL of filtrate was discarded and the subsequent filtered sample was collected into plastic bottles that were previously rinsed with DI water and sample water.

Fractionation was performed with 63.5-mm diameter Millipore Amicon YM10 ultrafiltration membranes. Filters were prepared by soaking in a 5 percent NaCl solution for at least 30 minutes, followed by soaking in DI water for a minimum of 1 hour with the water being changed 3 times. The filter was placed in an Amicon 8200 stirred cell and the cell was connected to a feed reservoir containing DI water. DI water was filtered through the membrane at 350 kPa for at least 5 minutes. The remaining DI water was dumped from the feed reservoir.
and stirred cell, and the sample was placed directly in the stirred cell. The sample was filtered at 300 kPa with the stirring at the maximum setting. The first 25 mL of sample was discarded and the next 120 mL was collected directly into three 40-mL TOC vials.

DOC, protein, and carbohydrate concentrations and \( \text{UV}_{254} \) absorbance were analyzed to characterize and quantify organics present in the water samples and foulant layer of autopsied RO membranes. DOC was analyzed according to Standard Method 5310-C (Persulfate-ultraviolet oxidation method) using a Tekmar-Dohrmann Phoenix 8000 TOC Analyzer. Two standards were used to verify that the instrument was working correctly and the standard curve was still good. \( \text{UV}_{254} \) absorbance was analyzed according to Standard Method 5910-B using a Varian Cary 50 UV/vis spectrophotometer and a 1 cm quartz cuvette without pH adjustment. Protein was analyzed using the Modified Lowry Protein Assay Kit by Pierce, a Varian Cary 50 UV/vis spectrophotometer reading at 750 nm and a 1 cm quartz cuvette. In this method, proteins reacted with a cupric-sulfate tetrate solution to form tetradentate copper-protein complexes. Six bovine serum albumin (BSA) standards were used to create a standard curve at a concentration of 0, 2.5, 5, 10, 15, and 20 mg/L. Volumes in the procedure supplied by Pierce were multiplied by four to ensure sufficient volume for analysis. Carbohydrate concentrations were analyzed by the Sulfate-Phenol method developed by Dubois et. al. using dextrose standards, a Varian Cary 50 UV/vis spectrophotometer reading at 490 nm and a 1 cm quartz cuvette. Six dextrose standards were used at a concentration of 0, 10, 20, 30, 40, and 50 mg/L for the calibration curve.

**RO Membrane Autopsy**

The RO membranes taken from the RO unit were put in a clean, plastic container on top of a strip of parafilm. DI water moistened wet paper towels were put below the parafilm to prevent the RO membranes from drying out. The membranes were then transported to the Environmental Laboratory at UNM, where the RO membranes were cut into pieces using a quilting mat and rotary
cutter. Six 4 cm$^2$ pieces were cut for inorganic and protein and carbohydrate analyses. Six smaller pieces, measuring roughly 3 cm$^2$, were cut for staining and CLSM analysis. A diagram of how the RO membranes were cut up for autopsy is shown in Figure 6.

Membranes for inorganic analysis were delivered the Earth and Planetary Sciences analytical chemistry laboratory under the senior research scientist Dr. Abdul-Mehdi Ali for digestion and ICP-MS analysis. Each 4 cm$^2$ membrane piece was cut into nine pieces and placed in a 40 mL teflon tube. The membrane pieces were then digested with aqua regia (3 mL of HNO$_3$ and 1 mL HCl) on a heating block until the volume was reduced to about 1 mL. The digested membrane was mixed using a vortex mixer for 30 seconds. Then, the digested membrane was filtered into a 50 mL volumetric flask using a 11.0 cm Whatman 40 filter paper. The teflon tube was rinsed at least 3 to 4 times with approximately 5 mL volumes of 18 MΩ water, vortexed for 10 seconds, and filtered into the 50 mL volumetric flask. The volumetric flask was filled to volume with 18 MΩ water and transferred into a 10 mL 16x100 borosilicate glass culture tube for ICP-MS analysis. Blanks were analyzed by conducting the same procedure with a piece of unused membrane.
Figure 6: Diagram of How RO membranes were Cut for Autopsy (Not to scale)

RO membrane pieces cut for protein and carbohydrate analysis were further cut in half and placed in 40 mL TOC vials. A 2.5 mL volume of 1% SDS was pipetted on top of each RO membrane piece and sonicated for one hour. A 7.5 mL volume of DI water was then pipetted on top of the RO membrane pieces, bringing the volume to a total of 10 mLs. The TOC vials were vortexed for 5 seconds to mix the membrane extract with the DI water and the membranes. The membrane extract was then transferred to another set of TOC vials. From there, the membrane extract was subjected to protein and carbohydrate analysis. Blanks were analyzed by conducting the same procedure with a piece of unused membrane.

RO membrane pieces were stained for detection of live and dead cells, protein, and carbohydrates with a confocal laser scanning microscope. A piece of RO membrane was cut from both ends of the RO membrane for each stain. RO membrane pieces cut for staining and autopsy were placed on top of a piece of parafilm, which was on top of a circular piece of DI wetted paper towel, which was laid in a glass petri dish. The glass petri dishes were kept in the absence of light during staining to prevent photobleaching of the stained membranes. The
RO membrane pieces were stained with three sets of stains: syto 9 with propidium iodide, concanavalin Alexa Fluor 488 conjugate (ConA) with propidium iodide, and fluorescein isothiocyanate (FITC) with propidium iodide. The stains could not all be used together because Syto 9, ConA, and FITC have overlapping emission bands. Syto 9 stains all cells, while propidium iodide only stains cells with damaged membranes. When these two dyes are used together, the propidium iodide displaces Syto 9, resulting in live bacteria staining with Syto 9 (green) and dead bacteria staining with propidium iodide (red). ConA binds to α-mannopyranosyl and glucopyranosyl residues and was used in this project to identify the carbohydrate fraction of the foulant layer. FITC binds to proteins.

After 1 hour of staining in the absence of light, all RO membrane pieces were rinsed three times with 1 mL volumes of phosphate buffered saline (PBS), and transferred to plastic disposable petri dishes on top of carbon tape and covered in 1X PBS solution. These petri dishes were covered with aluminum foil to prevent photobleaching until the RO membrane pieces were ready to be viewed under the microscope. They were then taken to the Keck Confocal Microscopy Facility for CLSM analysis. Samples were viewed at 50X and 630X magnification. The 630X magnification involved the use of a water immersible 63X lens. The CLSM analysis generated a series of images at 630X magnification at preset thicknesses from the top of the foulant layer to the RO membrane surface (or before the RO membrane surface if the foulant layer itself obstructed the view to the RO membrane surface). Each series of images is called an image stack. Four image stacks from two areas on the RO membrane were taken per stain per membrane. CLSM analysis allowed qualitative observation of the development of the RO foulant layer spatially and temporally.

The Syto 9 and Propidium iodide were purchased in the LIVE/DEAD BacLight Bacterial Viability Kit for microscopy and quantitative assays from Invitrogen. Syto 9 stains bacteria with intact cell walls fluorescent green with an excitation/emission maxima of 480/500 nm. Propidium iodide stains cells with a compromised cell membrane a fluorescent red and has a excitation/emission maxima of 490/635 nm. Equal volumes (3 mL) of SYTO 9 (3.34 mM) and
Propidium iodide (20 mM) dyes were diluted in 1 ml of 1X PBS solution and vortexed for 3 seconds. The Live/Dead stain was then applied to the membranes and allowed to sit in the dark for one hour before rinsing with PBS and analyzed using CLSM.

Carbohydrates were visualized using ConA from Invitrogen. The 5 mg of ConA was dissolved in 5.175 mL of 0.1M bicarbonate solution adjusted to a pH of 8.3. The ConA solution was centrifuged at 10,000 x g for 30 sec, and then aliquoted into 167 µL volumes, transferred to 1 mL microcentrifuge tubes and stored at -20 °C. The final concentration of ConA used on the membranes was 0.97 mg ConA/mL. The microcentrifuge tubes containing ConA were thawed and used as needed. After thawing, 0.5 µL of propidium iodide from the LIVE/DEAD BacLight kit was added to the ConA and vortexed for 3 seconds. The ConA/propidium iodide solution was applied to the membranes and allowed to sit in the dark for one hour prior to rinsing with PBS and imaging by CLSM.

A 0.1 M Sodium bicarbonate solution adjusted to a pH of 8.5 was pipetted onto the RO membrane pieces that were to be stained with FITC and was allowed to sit on the membranes for 15 minutes. A 10 mg vial of FITC was thawed and diluted with 1 mL dimethyl sulfoxide (DMSO) to make a 10 mg/mL solution of FITC. 3 µL of propidium iodide from the LIVE/DEAD BacLight kit was added to the FITC solution and vortexed for 3 seconds. The FITC/propidium iodide solution was then pipetted onto the top of the RO membrane pieces and incubated in the dark for 1 hour. Stains were washed off by rinsing with 1X PBS buffer three times, and examined under the confocal microscope.
Chapter 4: Results

MBR Performance

Construction of the bench scale MBR-RO system was completed at UNM in late November of 2008, and the apparatus was relocated to the Southside Water Reclamation Plant (SWRP) and started up in early December. The MBRs were seeded with return activated sludge (RAS) from the secondary clarifiers. MLSS concentration of the RAS was approximately 5000 mg/L. MBR #1 was seeded with 20 L of RAS and flows were adjusted to achieve an SRT of 2 days. MBR #2 was seeded with 45 L of return RAS and flows were adjusted to achieve an SRT of 20 days. After the 20 day SRT experiment was completed, the SRT in MBR #2 was set for 10 days and allowed to reach steady state for a month.

Initially, the MBRs were supplied air through the Koch microfilter unit for membrane scouring and system aeration. This did not provide sufficient aeration to keep DO concentrations above 2 mg/L, the target DO concentration. Another air pump was installed and attached to coarse air diffusers purchased at a fish supply store. The second air pump increased the DO concentration in the MBRs, but was still insufficient to maintain aerobic conditions. Another aeration design was employed, this time using coiled drip irrigation tubing. This design proved to be better at supplying oxygen to the MBRs, but was still not enough. Finally, an air pump was purchased with higher capacity to replace the second air pump and the drip irrigation pipe was replaced with a PVC header with 12 fine air diffusers for each MBR. This design provided sufficient air to the MBRs, so it was the aeration supply used for all SRT experiments.

The DO concentration in the MBRs was monitored to make sure DO concentration remained above 2 mg/L. When the MBRs were started, the only aeration to the system was provided by the porous stones at the base of the microfiltration unit. This did not supply sufficient aeration to the MBRs, so a series of adaptations were made to aeration system as described previously. The DO concentration was above 3 mg/L at all times for all experiments, as
shown in Figure 7. The DO concentration in the 2 and 10 day SRT experiments were similar, while the DO concentration for the 20 day experiment was slightly lower than for the other experiments. The lower DO concentration may have been due to a higher DO consumption by the higher biomass concentrations in the 20 day SRT MBR. However, minor differences observed in DO concentrations for the different SRT conditions were not expected to affect MBR permeate quality, as the MBRs for all SRT conditions were operated under aerobic conditions.

![Figure 7: DO Concentrations in MBRs during SRT Experiments](image)

The HRT was kept constant at about 8.5 hours. This value is a little higher than typical for MBR plants, but was used over the initially chosen value of 5 hours due to limitations in MBR permeate pumping rate. This dropped the MBR flux from the desired 10 to 15 LMH to between 6 and 8 LMH. The MBR permeate pumps achieved MBR permeate flow rates sufficient to provide a 5 hour HRT when vacuum pressures were low. However, when MBR membrane filters became fouled, the transmembrane pressure became too high to obtain a sufficient MBR permeate flow rate to provide the MBRs with a 5 hour HRT. This was most problematic with the MBR operating at a 2 day SRT, as microfilter
membrane fouling was more problematic at lower SRTs. Observed variations in HRT, shown in Figure 8, were due to changes in MBR permeate flow rate. Average measured HRT values were between 8.45 and 8.58 hours for the three experiments.

![Figure 8: Measured MBR HRT for SRT Experiments](image)

MBR SRTs were chosen to determine how MBR SRT affects RO fouling. 2, 10, and 20 day SRTs were chosen to represent a low, medium, and high SRT values. MBRs frequently operate at SRTs greater than 20 days, however, the amount of time necessary for an MBR to achieve steady state operation is related to the SRT. The rule of thumb for starting MBRs is that steady state can be achieved after 3 SRTs. Operating the MBRs at a SRT greater than 20 days was not feasible given the timeline of the project. Operating the MBRs at very low SRTs was problematic. A 2 day SRT was initially chosen as the lowest experimental SRT because it is near the maximum specific growth rate of mixed cultures in the activated sludge process. When the MBR was run at a 2 day SRT, MLSS concentrations did not seem to reach steady state, and appeared to be slowly decreasing over time. Concern that the MBR was washing out led to a
decision to decrease the MBR wasting rate, which increased the SRT for the 2 day SRT experiment. The SRT was adjusted closer to 2.5 days to avoid washout, and MLSS concentrations were better stabilized thereafter. Measured average SRTs for the three experiments were 2.4, 10.5, and 21.7 days. All measured SRT values for each SRT value during the experiments are shown in Figure 9. Larger deviations were seen in the longer SRTs because they had smaller wasting flow rates. The smaller the wasting flow rate, the larger the percent change in SRT for the same change in flow rate.

The 2 day SRT experiment was run between July 30th and August 13th, 2009 and was the second experiment. The 10 day SRT experiment was run between September 3rd and September 18th, 2009, and was the last experiment. The 20 day SRT experiment was conducted between June 16th and July 1st, 2009, and was the first experiment. The 20 day SRT experiment was done first because it took the longest to achieve steady state. If the MBR operation had been upset, the MBR with the 20 day SRT would have taken the longest to reseed with RAS and reach steady state MLSS concentrations.
Seasonal changes affect MBR operation and permeate quality. Seasonal changes include changes in water quality and temperature. Temperature can affect the MBR microbial growth rates, yield and therefore removal efficiencies in the MBR. Temperature will also affect the viscosity of water and therefore the membrane flux. The water temperature for both MBRs is shown in Figure 10. MBR temperature during the experiments is shown in Figure 11. Average MBR temperatures for the 2, 10, and 20 day SRT experiments were 25.9, 24.4, and 24.9°C, respectively. MBR temperature in the 2 day SRT was statistically greater than in either the 10 or 20 day SRT experiments. A t-test conducted on the MBR temperature data resulted in p-values of < 0.001, 0.011, 0.29 when comparing the 2 and 10 day SRT, 2 and 20 day SRT, and 10 and 20 day SRT experiments, respectively. However, the differences in temperature for the 3 experiments were small: between 0.5 and 1.5 degrees C difference on average. These small differences in temperature would not be expected to significantly affect either the microbial community or performance of the MBR. Ideally, the SRT experiments would have been conducted simultaneously to avoid these seasonal changes, particularly in temperature. This was not practical for several reasons. First, the lab work component of the project was too time consuming to run more than one experiment at a time. Second, there was insufficient power at the SWRP to supply to three MBRs and three RO units to operate them simultaneously. Third, there were not sufficient funds to set up 3 parallel systems of microfilter units, RO units, and pumps. However, experiments were run as close together temporally as possible. Another option to eliminate temperature as a variable would have been to control temperature in the MBRs with water heaters. The immersible water heaters proved unreliable over even short periods of time. A non-immersible water heater was available, but there was insufficient electrical power to allow temperature control.
The MLSS and MLVSS concentrations were measured before experiments were started and several times over the duration of each experiment to ensure steady state conditions. MLSS and MLVSS concentrations in MBRs prior to the start of the 2, 10 and 20 day SRT experiments are shown in Figure

Figure 10: Measured Water Temperature in the MBRs

Figure 11: Water Temperature in the MBRs during SRT Experiments
12, Figure 13, and Figure 14, respectively. MLSS and MLVSS concentrations during the 2, 10 and 20 day SRT experiments are shown in Table 6. The MLVSS to MLSS ratio for MBRs prior to experiments is shown in Figure 15. MLSS concentrations were nearly 5 times greater for the 10 day SRT experiment than the 2 day SRT experiment, and over 5.5 times greater for the 20 Day SRT experiment than the 2 day SRT. A greater difference in MLSS concentration was expected between the 10 and 20 day SRT conditions than was observed. The MLSS concentration had been as high as 14,000 mg/L in the MBR operating at a 20 day SRT, and had an average MLSS concentration of about 11,000 mg/L in the first few months of operation. The MLSS concentration remained somewhat steady between 4,500 mg/L and 6,500 mg/L thereafter. However, there was a small decline in MLSS concentration right before the start of the 20 day SRT experiment lasting half-way through the experiment. MLSS and MLVSS concentrations were much higher in February and March in both MBRs due to some issues in maintaining correct wasting flow rates. Periods where MLSS concentrations were lower than expected coincided with either MBR cleaning procedures or foaming events, both of which were infrequent for MBRs operated at the 10 and 20 day SRT values. Some mixed liquor was lost during MBR cleaning. Mixed liquor loss had a greater affect on MLSS concentration for MBRs with higher SRT. Foaming also led to some mixed liquor loss, though less than for MBR cleaning events.

Most of the suspended solids in the MBRs were volatile. Volatile solids accounted 87.4, 71, and 78.4 percent of the total solids for the 2, 10, and 20 day SRT experiments, respectively. The high MLVSS/MLSS ratio indicated that most of the suspended solids in the mixed liquor consisted of active biomass.
Figure 12: MLSS and MLVSS Concentrations reaching Equilibrium in MBR prior to 2 day SRT Experiment

Figure 13: MLSS and MLVSS Concentrations reaching Equilibrium in MBR before 10 day SRT Experiment
Figure 14: MLSS and MLVSS Concentrations reaching Equilibrium in MBR before 20 day SRT Experiment

Figure 15: Percent of MLSS that is Volatile in the MBRs prior to SRT Experiments
The MBR permeate pH values were near neutral for all SRT conditions, as shown in Figure 16. The average pH for the 2, 10 and 20 day SRT experiments was 7.0 ± 0.18, 6.8 ± 0.08, and 6.7 ± 0.22, respectively. T-tests were conducted to determine whether differences in pH values for different SRT conditions were significant. P-values when comparing the 2 day with the 10 day SRT experiment and the 2 day SRT and 20 day SRT were 0.014 and 0.0039, respectively. MBR permeate pH in the 2 day SRT was statistically greater than MBR permeate pH in the 10 and 20 day SRT. The p-value when comparing the 10 and 20 day SRT was 0.147. Observed differences in MBR permeate pH is likely due to nitrification reaction rates which are higher at the longer SRT values.
Electrical Conductivity (EC) is a measure of the ability of a sample to conduct an electric current. The EC of a water sample is proportional to the concentration of dissolved salts in that water sample. The dissolved salt concentration was not expected to increase in the mixed liquor in the MBR, as salts were neither rejected by the microfilters nor were they expected to accumulate significantly in or on the MBR biomass. Changes in MBR permeate EC would indicate a change in MBR feed EC. While some changes in EC may occur due to dilution or concentration of the MBR feed due to diurnal or seasonal changes, EC should not depend on MBR SRT. MBR permeate EC was, as expected, similar for all 3 tests, as shown in Figure 17. Average EC values for MBR permeate for the 2, 10 and 20 day SRT were 855 ± 33, 859 ± 31, and 856 ± 21 µS/cm, respectively. T-tests between the 2 and 10 day SRT, 2 and 20 day SRT, and 10 and 20 day SRT experiments generated p-values of 0.94, 0.89 and 0.82, respectively. There was no statistically significant difference in MBR permeate EC between the different SRT conditions.

Figure 17: MBR Permeate EC Values during SRT Experiments
Turbidity is a measure of suspended particles in a water sample. The microfiltration units in the MBR should remove essentially all particles, and particle removal should not depend on SRT. Well operated MF filters typically achieve turbidity less than 0.1 NTU. The MBR permeate turbidity was significantly higher, as shown in Figure 18. MBR permeate turbidity may have been due to biofilm growth on the permeate side of the Puron hollow fibers or in the tubing after the MBR. MBRs were periodically cleaned between experiments with sodium hypochlorite before the MBR permeate flow rate could drop below that necessary to maintain the 8.5 hour HRT. Cleaning would be expected to eliminate, or at least reduce, any biofilm within the microfiltration unit and tubing. MBRs were never cleaned during an experiment. Also, tubing was changed at the beginning of each experiment and as needed during experiments to limit biofilm growth.

![Figure 18: MBR Permeate Turbidity during SRT Experiments](image)

DOC concentrations in the MBR feed and permeate and percent removals in the MBR are shown in Table 7, Table 8, and Table 9, respectively. The DOC concentrations were highest by far in the MBR feed samples. Average MBR feed DOC concentrations for the 2, 10, and 20 day SRT conditions were 30.85, 37.58,
and 42.76 mg/L, respectively. The variability in MBR feed concentrations could be due to a number of factors including seasonal differences in the quality of feed water to the wastewater treatment plant or differences in operation of the primary clarifiers. MBR feed and permeate DOC concentrations and percent removals are shown in Table 7. DOC removal in MBRs operated at 2, 10 and 20 day SRTs was 83, 90, and 90, respectively. T-tests were run on the percent DOC removal data for the different SRT values to determine whether DOC removal at the different SRT conditions was statistically different. The p-value for DOC removal between the 10 and 20 day SRT experiments was 0.99, indicating that there was no statistical difference in DOC removal between the middle and high SRT conditions. DOC removal between the 2 and 10 day SRT conditions and the 2 and 20 day SRT values were found to be statistically significant, with p-values of <0.001 and <0.004. So, while the difference in DOC removal between the 10 and 20 day SRT experiments was not statistically significant, the difference between the 2 and 10 day SRT and the 2 and 20 day SRT experiments was statistically significant.

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Table 7: MBR Feed Water Quality Data
Table 8: MBR Permeate Water Quality Data

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Table 9: Removal of Organic Constituents from the MBR

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<td>81</td>
<td>1.2</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>10</td>
<td>1.0</td>
<td>18</td>
<td>2.3</td>
<td>67</td>
<td>3.6</td>
</tr>
</tbody>
</table>

Protein concentrations in the MBR feed and permeate and percent removals in the MBR are shown in Table 7, Table 8, and Table 9, respectively. The MBRs were efficient at degrading dissolved proteins: removals of 75, 76, and 81 percent were observed in the MBR for MBRs operated at a 2, 10, and 20 day SRT, respectively. Differences in protein removal between the 2 and 10 day SRT and the 10 and 20 day SRT experiments were not statistically significant, with p-values of 0.72 and 0.15, respectively. However, the difference in protein removal between the 2 and 20 day SRT experiments was statistically significant, with a p-value of 0.0070. Average protein concentrations in the MBR permeate from the bench scale MBR were 4.48, 3.47, and 4.30 mg/L for the 2, 10, and 20 day SRTs, respectively. MBR feed and permeate protein concentrations and percent removals are shown in Table 7. Fractional protein removal was less than the fractional DOC removal in the MBR.

Carbohydrate concentrations in the MBR feed and permeate and percent removals in the MBR are shown in Table 7, Table 8, and Table 9, respectively. MBR feed and permeate carbohydrate concentrations and percent removals are
shown in Table 7. Carbohydrate concentrations were lower than protein concentrations in the MBR feed. Carbohydrate removal in the MBRs was also lower than the protein removal. Carbohydrate removals of 10, 18, and 67 percent were seen in the MBR for the 2, 10, and 20 day SRT experiments, respectively. Differences in MBR carbohydrate removal was not significant, with p-values of 0.72, 0.71, and 0.78 for the 2 and 10 day SRT, 2 and 20 day SRT, and 10 and 20 day SRT experiments, respectively. This increase in carbohydrate removal with increasing SRT is due to higher average carbohydrate concentrations in the MBR feed, and not lower MBR permeate carbohydrate concentrations. Average MBR feed carbohydrate concentrations were 5.06, 6.76, and 10.80 mg/L for the 2, 10, and 20 day SRT experiments, respectively. The MBR feed carbohydrate concentrations in 2 and 10 day SRT and the 10 and 20 day SRT experiments were not statistically different, with p-values of 0.43 and 0.11, respectively. The difference between the 2 and 20 day SRT experiments in terms of MBR feed concentrations was statistically significant, with a p-value of <0.0001. Average carbohydrate concentrations in the MBR permeate from the bench scale MBR were 4.54, 4.31, and 3.60 mg/L for the 2, 10, and 20 day SRTs, respectively. MBR permeate carbohydrate concentrations were not statistically significant, with p-values of 0.60, 0.42, and 0.46 for the 2 and 10 day SRT, 2 and 20 day SRT, and 10 and 20 day SRT experiments, respectively.

$UV_{254}$ absorbance values in the MBR feed and permeate and percent removals in the MBR are shown in Table 7, Table 8, and Table 9, respectively. $UV_{254}$ absorbance is a method for measuring the natural organic matter (NOM) in water, like DOC and is frequently attributed to the aromatic fraction of the constituents in the water. $UV_{254}$ absorbance removal was expected in the MBR process. $UV_{254}$ absorbance removal by the MBR was 62, 75, and 68 percent for the 2, 10, and 20 day SRTs, respectively. The 2 day SRT $UV_{254}$ absorbance percent removal was significantly different from the 10 day SRT experiment, with a p-value of 0.02. $UV_{254}$ absorbance removal was not statistically significant between the 2 and 20 day SRT and the 10 and 20 day SRT experiments, respectively. This means that while the $UV_{254}$ absorbance removal was lower for
the 2 day SRT experiment than for the 10 Day SRT experiment, there is no trend of \( \text{UV}_{254} \) absorbance removal changing with SRT.

SUVA is the ratio of the \( \text{UV}_{254} \) absorbance to the DOC concentration and measures how easily biodegradable the organic constituents are in the sample. The more easily biodegradable the water sample is, the higher the SUVA value. SUVA values in the MBR feed and permeate and percent removals in the MBR are shown in Table 7, Table 8, and Table 9, respectively. The MBR feed had the lowest SUVA value for all SRT conditions. The SUVA values in the MBR permeate increased compared to the MBR feed samples for all SRT conditions indicating an increase in \( \text{UV}_{254} \) absorbance values relative to the DOC concentrations. This suggests that the percentage of more complex, less readily biodegradable aromatic and double-bonded carbon compounds increased with MBR treatment. The more biodegradable compounds would have been consumed by the microorganisms in the MBR, so this result is expected. The MBR permeate SUVA values were 2.15, 2.52, and 2.60 for the 2, 10, and 20 day SRT, respectively. There was a significant difference in the SUVA values between low and high SRT experiments, with p-values of <0.0002 and <0.003 for the 2 and 10 day SRT and 2 and 20 day SRT experiments, respectively. Higher SUVA values indicate a higher fraction of hydrophobic constituents, which may contribute to higher RO fouling. Because the MBR permeate SUVA values were lowest for the 2 day SRT experiment, lower RO fouling would be expected. However, SUVA values for the 10 and 20 day SRT experiments were not significantly different, with a p-value of 0.42. Therefore, a trend of increasing MBR permeate SUVA values with increasing SRT can not be established.

The MBR yield was calculated in terms of the mass of VSS produced per mass of DOC removed. MBRs operated at higher SRTs were expected to have a lower yield because of increased endogenous decay of biomass. This trend was apparent from the yield data. The yield for the 2, 10, and 20 day SRTs was 5.22 ± 2.99, 3.46 ± 0.77, and 2.11 ± 0.97 g VSS/g DOC, respectively, as shown in Figure 19. The standard deviation of the yield for the 2 day SRT was much larger than the standard deviations for the 10 and 20 day SRT experiments. The
yield for the 10 and 20 day SRT experiments were significantly different with a p-value of 0.041. The yield for the 2 and 20 day SRT tests were less significantly different than the yield for the 10 and 20 day SRT experiments, with p-values of 0.078 and 0.041, respectively. The yield for the 2 and 10 day SRT experiments were not significantly different, with a p-value of 0.26. A typical chemical formula for bacterial composition is $C_5H_7NO_2$. Because bacteria are composed of approximately half carbon by mass, the mass of bacteria produced should be no greater than twice the mass of carbon removed or consumed in the MBR. However, DOC does not take into account carbon in particulate form, which could contribute significantly to the total carbon consumed by microorganisms in the MBR, leading to greater microbial biomass production.

The MBR fouled much faster when operated at a 2 day SRT than at a 10 or 20 day SRT. According to the literature, an increase in fouling rates is typically seen when MBRs are operated at lower SRTs. Faster MBR fouling rates result in more rapidly increasing transmembrane pressure and necessitate more frequent MBR cleanings. The MBR transmembrane pressure for both

![Figure 19: Average MBR Yields in Terms of g VSS Produced per g DOC Removed with 1 Standard Deviation](image-url)
MBRs is shown in Figure 20. Each large drop in MBR transmembrane pressure indicates an MBR cleaning procedure was performed. Over the same period of time, 7 MBR cleanings were performed on MBR #2, which operated at a 2 day SRT, whereas 4 MBR cleanings were performed on MBR #2, which operated at a 20 day SRT until the end of July, at which time it was operated at a 10 day SRT. The MBR specific flux was normalized to the initial specific flux at the beginning of each SRT experiment and is shown in Figure 22. A sharper decline in specific flux was seen in the MBR operating at a 2 day SRT than 10 or 20 day SRT over the duration of the experiments.

Figure 20: MBR #1 Transmembrane Pressure, the tick marks on top denote a MBR cleaning procedures
Figure 21: MBR #2 Transmembrane Pressure, the tick marks on top denote a MBR cleaning procedures.

Figure 22: MBR Normalized Specific Flux for each SRT Experiment.
Comparison between Bench scale and RDO and SWRP Performance

Inorganic and organic concentrations in the effluent of both the bench scale and full-scale systems were analyzed and compared to evaluate whether the bench-scale system operated in a comparable manner to similar full scale municipal wastewater treatment plants. A full-scale MBR (Rio del Oro (RDO)) plant and an activated sludge plant (SWRP) were used for this comparison. The bench scale MBR used the same primary treated wastewater as the SWRP. Grab samples of effluent from the RDO plant and the Southside Water Reclamation Plant SWRP were analyzed for various inorganic and organic parameters to compare with the bench scale MBR effluent inorganics.

Inorganics in the bench scale MBR were measured in the RO tank, but not in the MBR permeate. However, the MBR permeate was concentrated in the RO feed tank three fold by controlling flow MBR permeate flow rate to the RO tank and RO permeate flow, recycling RO concentrate completely, and maintaining constant volume in the RO tank. The MBR permeate was verified as being concentrated three fold in the RO tank by comparing the organic and EC content in the MBR permeate with the RO feed tank. Therefore, the bench scale MBR permeate inorganic concentrations were estimated by dividing the average inorganic concentrations in the RO feed tank by 3. The effluent inorganics for the bench scale MBR, the RDO plant, and the SWRP are shown in Figure 23.

Concentrations of inorganics for the bench scale MBR and the SWRP are similar, as expected, because they use the same primary treated effluent. The main differences are in the nitrite, nitrate, and phosphorus concentrations, which are lower for the SWRP effluent than the bench scale MBR permeate. This is likely due to the anaerobic/anoxic selectors at the SWRP which denitrify the water and have some capacity for phosphorus removal. Inorganic concentrations in the RDO plant effluent are lower than both the bench scale MBR permeate and the SWRP effluent, with the exception of potassium and magnesium, which had similar concentrations, and silicon, which is slightly higher in concentration.
DOC concentrations from the bench scale MBR, the RDO plant, and the SWRP are tabulated in Table 10. Average DOC concentrations in the MBR effluent from the bench scale MBR were 5.13, 3.86, and 4.19 mg/L for the 2, 10, and 20 day SRTs, respectively. The effluent DOC concentrations of the grab samples from the RDO plant were lower than in the SWRP at concentrations of 4.59 and 5.19, respectively. These DOC concentrations were near or within the range of concentrations measured in bench scale MBR. Thus, DOC concentrations in the grab samples from the RDO plant and the SWRP were similar to the MBR effluent from the bench scale MBR.

Table 10: Comparison of Organic Constituents from the bench scale MBR to effluent from the RDO plant and SWRP

<table>
<thead>
<tr>
<th>Sample</th>
<th>DOC (mg/L)</th>
<th>Protein (mg/L)</th>
<th>Carbohydrate (mg/L)</th>
<th>UV$_{254}$ Absorbance</th>
<th>SUVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bench Scale MBR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 Day SRT</td>
<td>5.13</td>
<td>4.48</td>
<td>4.54</td>
<td>0.1100</td>
<td>2.15</td>
</tr>
<tr>
<td>10 Day SRT</td>
<td>3.86</td>
<td>3.47</td>
<td>4.31</td>
<td>0.0973</td>
<td>2.52</td>
</tr>
<tr>
<td>20 Day SRT</td>
<td>4.19</td>
<td>4.30</td>
<td>3.60</td>
<td>0.1085</td>
<td>2.59</td>
</tr>
<tr>
<td>RDO</td>
<td>4.59</td>
<td>5.00</td>
<td>1.15</td>
<td>0.1160</td>
<td>2.53</td>
</tr>
<tr>
<td>SWRP</td>
<td>5.19</td>
<td>4.60</td>
<td>3.55</td>
<td>0.1078</td>
<td>2.08</td>
</tr>
</tbody>
</table>
Protein concentrations from the bench scale MBR, the RDO plant, and the SWRP are tabulated in Table 10. Average protein concentrations in the MBR effluent from the bench scale MBR were 4.48, 3.47, and 4.30 mg/L for the 2, 10, and 20 day SRTs, respectively. Protein concentrations in the grab samples from the RDO plant and the SWRP were slightly larger than in the MBR effluent from the bench scale MBR, at concentrations of 5.00 and 4.60 mg/L, respectively.

Carbohydrate concentrations from the bench scale MBR, the RDO plant, and the SWRP are tabulated in Table 10. Average carbohydrate concentrations in the MBR effluent from the bench scale MBR were 4.54, 4.31, and 3.60 mg/L for the 2 day, 10 day, and 20 day SRT conditions, respectively. Carbohydrate concentrations in the grab samples from the RDO plant and the SWRP were both lower than in the MBR effluent from the bench scale MBR. Carbohydrate concentration of the grab samples from the RDO plant and the SWRP were 1.15 and 3.55, respectively.

The fraction of DOC in the form of carbohydrates, protein, and both protein and carbohydrates is shown for the bench scale MBR, RDO plant, and the SWRP effluent in Table 11. The average carbon content of protein is assumed to be 53 percent, because this is the average carbon content of the BSA standards used in the protein assay. The average carbon content of carbohydrates was assumed to be 40 percent, because this is the carbon content of the dextrose standard used in the carbohydrate assay. DOC concentrations at the SWRP were slightly larger than in the bench scale MBR effluent or the RDO plant effluent. Protein concentrations in the RDO plant and SWRP effluent were greater than in the bench scale MBR effluent. Carbohydrate concentrations were higher in the bench scale MBR effluent than in either the RDO plant or SWRP effluent. The protein fraction of the DOC was greater than the carbohydrate fraction in the bench scale MBR, RDO plant, and SWRP effluent. A greater percentage of DOC from the RDO plant and the SWRP effluent was unable to be classified as either protein or carbohydrates than in the bench scale MBR effluent.
UV$_{254}$ absorbance values from the bench scale MBR, the RDO plant, and the SWRP are tabulated in Table 10. UV$_{254}$ absorbance values were similar the effluent from the bench scale MBR, the RDO plant, and the SWRP. This indicates that all three plants had similar NOM content. The SUVA value for the bench scale MBR effluent was higher than for the SWRP effluent. The RDO plant effluent SUVA was between the 10 day SRT and the 20 day SRT SUVA values in the bench scale MBR.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Percent DOC as Protein</th>
<th>Percent DOC as Carbohydrates</th>
<th>Percent DOC as Protein or Carbohydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bench Scale MBR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 Day SRT</td>
<td>46</td>
<td>35</td>
<td>82</td>
</tr>
<tr>
<td>10 Day SRT</td>
<td>48</td>
<td>45</td>
<td>92</td>
</tr>
<tr>
<td>20 Day SRT</td>
<td>54</td>
<td>34</td>
<td>89</td>
</tr>
<tr>
<td>RDO</td>
<td>58</td>
<td>10</td>
<td>68</td>
</tr>
<tr>
<td>SWRP</td>
<td>47</td>
<td>27</td>
<td>74</td>
</tr>
</tbody>
</table>

Table 11: Comparison of Percent DOC as Protein and Carbohydrates between Bench Scale MBR Effluent and Effluent from the RDO Plant and SWRP

Samples collected in the effluent of the SWRP and RDO plant had measured turbidities of 1.9 and 0.13 NTU, respectively. The SWRP uses a traditional activated sludge process combined with secondary clarifiers. MBRs typically have lower effluent turbidity because water is filtered through the system instead of relying on sedimentation by gravity. Thus, lower turbidity in the effluent from the bench scale MBR than in effluent from the SWRP was expected. The RDO plant is an MBR plant like the bench scale MBR, except that it uses Kubota flat sheet microfiltration membranes instead of the Koch hollow fiber microfiltration membranes in the bench scale MBR. Turbidity of permeate from the RDO plant was 0.13 NTU. This was nearly three times lower than the measured average turbidity of the MBR permeate from the bench scale MBR, which was 0.31 NTU.
RO Performance

Rejection of Inorganics by the RO System

Reverse osmosis is expected to be very efficient in inorganic removal. This section presents the change in pH and rejection of electrical conductivity, cations (Ca, Fe, K, Mg, Na, Si), anions (Cl\(^-\), NO\(_3^-\), SO\(_4^{2-}\), F\(^-\), NO\(_2^-\), Br\(^-\), PO\(_4^{3-}\)), and alkalinity by the RO system. The average RO feed pH for the 2, 10, and 20 day SRT experiments was 7.41 ± 0.11, 7.22 ± 0.07, and 7.18 ± 0.17, respectively.

The average RO permeate pH for the 2, 10, and 20 day SRT experiments was 5.52 ± 0.29, 5.36 ± 0.12, and 5.91 ± 0.69, respectively. On average, RO permeate pH was lower than the RO feed pH by about 2 pH units for all SRT conditions. The drop in pH indicates a negative rejection of hydrogen ions across the RO membranes, as shown in Figure 24. Negative rejection of H\(^+\) is a common observation in RO systems and is caused by the high mobility of H\(^+\) and the need to maintain electroneutrality. There is greater variability in RO permeate pH in the 20 day SRT than in the lower SRT conditions. During the 20 day SRT, it took a little longer for the pH probe to reach the correct value, and sometimes the value was taken prematurely. This issue was resolved after cleaning the RO probe with mild detergent and alternately placing the pH probe in dilute acid and base solutions.

The average RO feed EC for the 2, 10, and 20 day SRT experiments was 2175 ± 521, 2528 ± 625, and 2560 ± 802 µS/cm, respectively. The average RO permeate EC for the 2, 10, and 20 day SRT experiments was 48.4 ± 25.6, 57.8 ± 23.8, and 120 ± 65.1 µS/cm, respectively. The EC percent rejection by the RO membranes was 97.8, 97.7 and 95.3 percent for the 2, 10, and 20 day SRT experiments, respectively. The RO permeate EC was consistently lower than the RO feed EC for all SRT conditions, indicating a significant salt rejection across the RO membranes. This is verified in the IC and ICP data, which measured specific ions in the RO feed and RO permeate samples. The EC of the RO feed and RO permeate were similar for the different SRT conditions, indicating that SRT did not affect the salt rejection across the RO membrane.
Significant changes in inorganic concentrations in the RO feed water were not expected over the duration of the experiments or for the different SRT conditions. Any differences in inorganic concentrations in the RO feed would be
due to changes in the inorganic concentrations in the MBR feed water. The lower EC values in the RO feed of the 2 day SRT were a result of the lower EC in the MBR permeate and may correspond with pH values in the MBR permeate for the 2 day SRT experiment being higher than in the 10 or 20 day SRT experiments. Some inorganics showed greater variability in the feed water than others, both over the duration of the experiments and for the different SRT conditions. RO feed cation concentrations from ICP analysis are shown in Figure 26. ICP analysis results showed that the predominant cations in the RO feed water were sodium and calcium at average concentrations of 262 and 114 mg/L, respectively. The high calcium concentration in the RO feed water for all three experiments was important because of calcium’s documented interaction with the carbohydrate portion of the EPS on the RO foulant layer. The RO feed water also contained potassium, magnesium, and silica at concentrations of 54, 19, and 63 mg/L, respectively. The iron concentration was near or below the detection limits. The 20 day SRT experiment had the largest variations in cation concentration in the RO feed over time. RO permeate cation concentrations from ICP analysis are shown in Figure 27. Percent cation removal by the RO unit is shown in Figure 28. Excellent removals of cations were seen for all SRTs. Percent removals of all cations were greater than 93 percent, except for iron in the 10 day SRT experiment, which had a 70 percent removal. This low percent removal is not significant because both the RO feed and RO permeate iron concentrations were very low (<0.1 mg/L).

The percent cation removals were consistently lower and the RO permeate concentrations were higher for the 20 day SRT experiment than for the 2 or 10 day SRT experiments. This was due to the lower water flux across the RO membranes while salt flux remained the same. The solute flux across the RO membranes is [13]:

$$J_s = k_s(\Delta C)$$

where

- $J_s$ = the mass flux of the solute in mg/m$^2$·h
- $k_s$ = the mass transfer coefficient for solute flux in L/m$^2$·h
- $\Delta C$ = the concentration gradient across the membrane
The water flux across the RO membranes is expressed by the equation [13]:

\[ J_w = k_w (\Delta P - \Delta \pi) \]

where

- \( J_w \) = the volumetric flux of water in L/m\(^2\)·h
- \( k_w \) = the mass transfer coefficient for water flux in L/m\(^2\)·h·bar
- \( \Delta P - \Delta \pi \) = the net transmembrane pressure

The values of \( k_s \) and \( k_w \) of new RO membranes are determined by the membrane manufacturer, but change over the course of the experiment with RO membrane fouling. The RO permeate solute concentration is expressed by the equation:

\[ C_P = \frac{J_s}{J_w} \]

where

- \( C_P \) = RO permeate solute concentration

The net transmembrane pressure was much greater for the 20 day SRT experiment than the 2 or 10 day SRT experiments due to greater RO membrane fouling. However, the net applied pressure to the RO unit was adjusted to maintain constant water flux, so \( J_w \) remained over the course of the experiment. Thus, the RO permeate cation concentration was not expected to change unless \( k_s \) or \( \Delta C \) changed. There was a small difference in \( \Delta C \) for the 20 day SRT experiment because the RO permeate cation concentrations were slightly higher in the 20 day SRT experiment than the 2 or 20 day SRT experiments, but this effect was masked by the very high cation concentrations in the RO feed water relative to the RO permeate cation concentrations. The decrease in \( \Delta C \) would have led to a decrease in RO permeate cation concentration unless \( k_s \) increased. However, the RO permeate cation concentrations were higher in the 20 day SRT than the 2 or 10 day SRT, resulting in lower percent cation removals and higher RO permeate cation concentrations in the 20 day SRT experiment compared to the 2 or 10 day SRT experiments.

RO feed chloride, nitrate, and sulfate concentrations are shown for each SRT in Figure 29. Chloride, nitrate, and sulfate were the dominant anions in the RO feed water at average concentrations of 340, 243, and 316 mg/L,
respectively. RO feed fluoride, nitrite, bromide, and phosphate concentrations are shown in Figure 30. Fluoride, nitrite, bromide, and phosphate were also detected, but at significantly lower concentrations: 2.7, 17, 0.9, and 16 mg/L, respectively. Nitrite concentrations fluctuated significantly over the duration of each experiment. RO Permeate anion concentrations are shown in Figure 31. Percent anion removals for each SRT are shown in Figure 32. With the exception of fluoride, the 20 day SRT experiment exhibited the lowest anion removals and the RO permeate anion concentrations were higher for the 20 day SRT experiment than either the 2 or 10 day SRT experiments.
Figure 27: Concentration of Cations and Silica in the RO Permeate for SRT Experiments with Standard Deviation Bars

Figure 28: Percent Removal of Cations and Silica for SRT Experiments
Figure 29: Concentration of Chloride, Nitrate, and Sulfate in the RO Feed during SRT experiments with Standard Deviation Bars

Figure 30: Concentration of Fluoride, Nitrite, Bromide and Phosphate in the RO Feed during SRT Experiments with Standard Deviation Bars
Alkalinity is a measure of the capacity of a sample to neutralize acids. Weak bases and salts of weak acids contribute to the solution’s buffer capacity. In natural waters, carbonate or bicarbonate are significant contributors to alkalinity. Alkalinity of RO feed and RO permeate samples were reported in mg/L CaCO$_3$ and are shown in Figure 33. The average alkalinity of the RO feed water was 183 ± 26.6, 139 ± 34.5, and 151 ± 71.6 mg/L CaCO$_3$ for the 2, 10, and 20
day SRT experiments, respectively. The average alkalinity of the RO permeate water was 6.3 ± 1.9, 4.7 ± 1.2, and 5.8 ± 4.9 mg/L CaCO$_3$ for the 2, 10, and 20 day SRT experiments, respectively. The alkalinity of the RO Permeate water samples was very low for all SRT conditions. A decrease in alkalinity was expected due to an increase in nitrification reaction, which destroys alkalinity. However, the RO feed alkalinities were not statistically different, with p-values of 0.054, 0.39, and 0.75 when comparing the 2 and 10 day SRT, the 2 and 20 day SRT, and the 10 and 20 day SRT experiments, respectively. The RO permeate alkalinitities were also not statistically different, with p-values of 0.16, 0.86, and 0.65 when comparing the 2 and 10 day SRT, the 2 and 20 day SRT, and the 10 and 20 day SRT experiments, respectively.

![Figure 33: Alkalinity of RO Permeate and Feed Samples taken during SRT Experiments with Standard Deviation Bars](image)

**RO Physical Parameters**

The turbidity in the RO feed samples was due to particulate matter in the MBR permeate. Most of the visible particles in the MBR permeate were white, suggesting that they most likely originated from either biofilm growing on the tubing or from microfilter degradation. A small fraction of particles may have been introduced when the lid to the RO feed tank was knocked off accidently or
during sampling. The RO permeate turbidity was similar for all SRT conditions. RO permeate turbidity was near that of DI water. RO permeate turbidity was always below 0.15 NTU, and usually between 0.03 and 0.10 NTU. This is well below the allowable turbidity standard in the United States, which is 0.3 NTU for drinking water [28]. Thus, the RO system was effective at removing particles regardless of MBR SRT, as expected.

![RO Permeate Turbidity](image)

**Figure 34: RO Permeate Turbidity**

**Organics Constituents in Aqueous Samples**

RO feed DOC concentrations were 12.53 ± 2.89, 11.97 ± 1.48, and 13.56 ± 3.55 mg/L for the 2, 10, and 20 day SRT experiments, respectively. DOC concentrations were higher in the RO feed than the MBR permeate because the RO concentrate was completely recycled to the RO feed tank. RO permeate 0.09 ± 0.03, 0.10 ± 0.04, and 0.37 ± 0.28 mg/L, respectively. Very high DOC removal efficiencies were seen by the RO membranes, as expected. RO feed, RO feed <10 kDa, and permeate DOC concentrations are shown in Figure 35. DOC percent removals by the RO unit were between 97 and 99 percent for all SRT conditions. T-tests revealed no statistically significant difference in percent DOC removal by the RO unit for the SRT conditions tested. P-values were 0.57,
0.18, and 0.19 for the 2, 10, and 20 day SRT experiments, respectively. The average DOC concentrations for the <10 kDa fraction of the RO feed for the 2, 10, and 20 day SRT experiments were 13.61 ± 1.46, 11.61 ± 1.38, and 14.71 ± 6.18 mg/L, respectively. T-tests comparing DOC concentrations of the total and <10 kDa fraction generated p-values of 0.53, 0.73, and 0.82 for the 2, 10, and 20 day SRT experiments, respectively. Thus, no statistically significant difference existed between the DOC concentration total and <10 kDa fractions of the RO feed water for any of the SRT experiments. The DOC concentration in the RO feed was on average 2.9 times greater than in the MBR permeate for all SRT conditions. This is due to the concentrating of the MBR permeate in the RO tank by completely recycling the RO Concentrate to the RO tank. A four-fold increase was expected in DOC concentrations from the MBR permeate to the RO feed water samples with 75 percent recovery and 100 percent rejection. So the observed DOC concentration differences were lower than the expected differences between the MBR permeate and RO feed water.

Figure 35: DOC Concentration of Water Samples from SRT Experiments with Standard Deviations

RO feed, RO feed <10 kDa, and permeate protein concentrations are shown in Figure 36. The average RO feed protein concentrations were 12.3 ±
2.4, 13.0 ± 2.1, and 10.5 ± 1.2 mg/L for the 2, 10, and 20 day SRT experiments, respectively. The average RO permeate concentrations were 0.7 ± 0.2 mg/L for all SRT experiments. The average percent protein removal by RO membranes was 94, 95, and 93 percent for the 2, 10, and 20 day SRT, respectively. No correlation was evident between SRT and percent protein removal. The protein concentrations in the <10 kDa fraction of the RO feed were 12.2 ± 2.1, 12.9 ± 2.0, 13.2 ± 5.8 mg/L for the 2, 10, and 20 day SRT experiments, respectively. A t-test was run to compare the total protein fraction with the <10 kDa protein fraction with resulting p-values of 0.95, 0.94, and 0.64 for the 2, 10, and 20 day SRT experiments, respectively. So there was no significant difference between the total and the <10kDa fraction protein concentrations in the RO feed samples for any of the SRT conditions studied. Changing SRT did not change the percent of molecules greater than 10 kDa; most of the molecules in the RO feed had a low molecular weight. The RO membranes were very effective at removing protein from the RO feed water. The average RO permeate protein concentration was 0.7 mg/L for each of the three SRT experiments. The protein concentration was 3.0 times greater in the RO feed than the MBR permeate because the MBR permeate was concentrated about three times due to recycling of the RO Concentrate to the RO tank. This is similar to the results seen for the DOC analysis results.
Carbohydrate concentrations for the RO feed, RO feed <10 kDa, and RO permeate are shown in Figure 37. The RO feed carbohydrate concentrations were 11.9 ± 1.0, 13.7 ± 2.3, and 7.2 ± 3.6 mg/L for the 2, 10, and 20 day SRT experiments, respectively. The RO permeate carbohydrate concentrations were 0.9 ± 0.4, 0.7 ± 0.5, and 1.1 ± 0.05 mg/L for the 2, 10, and 20 day SRT experiments, respectively. The carbohydrate percent removal by the RO unit was 10, 18, and 67 percent for the 2, 10 and 20 day SRT experiments, respectively. T-tests conducted on the carbohydrate percent removal data revealed p-values of 0.38, 0.35, and 0.30 for the 2 and 10 day, 2 and 20 day, and 10 and 20 day SRT experiments, respectively. Therefore, differences between the SRT experiments with respect to percent carbohydrate removal were not statistically significant. The average carbohydrate concentrations for the RO feed <10 kDa fraction for the 2, 10, and 20 Day SRT experiments were 12.0 ± 1.3, 12.7 ± 1.6, and 7.1 ± 6.4 mg/L, respectively. T-tests comparing carbohydrate concentrations of the total and <10 kDa fractions of RO feed water generated p-values of 0.94, 0.44, and 0.98 for the 2, 10, and 20 day SRT experiments, respectively. Thus, no statistically significant difference existed between the total carbohydrate concentration and the <10 kDa fractions of RO feed.
feed water for any of the SRT experiments. The average RO feed carbohydrate concentration over the three SRT experiments was 2.6 times greater than the MBR permeate carbohydrate concentration. This is a smaller difference than expected, and is also less than the observed concentration factor for DOC and protein analysis. There are several possible explanations for this result. First, this could be an issue with the method used to collect samples. Water samples were collected as grab samples, which can lead to imprecision in the analysis results, as concentrations of various constituents vary over the course of the day. An effort was made to collect samples as close together temporally as possible (within an hour). Another issue could be the carbohydrate assay itself. While the phenol-sulfuric acid assay is quick, easy and commonly used, its downfall is that it is less precise than either the DOC or protein assays. There was always a greater difference in the replicate concentrations for the carbohydrate assays than for the DOC or protein assays.

![Figure 37: Carbohydrate Concentration in the Water Samples from SRT Experiment with Standard Deviations](image)

The RO process was expected to provide excellent removal of UV$_{254}$ absorbance. UV$_{254}$ absorbance values for the RO feed, RO feed <10 kDa, and
RO permeate are shown in Figure 38. The average RO feed $\text{UV}_{254}$ absorbance values were $0.2971 \pm 0.0293$, $0.3055 \pm 0.0489$, and $0.3491 \pm 0.0942$ for the 2, 10, and 20 day SRT experiments, respectively. The average RO permeate $\text{UV}_{254}$ absorbance values were $0.0017 \pm 0.0005$, $0.0019 \pm 0.0006$, and $0.0076 \pm 0.0085$ for the 2, 10, and 20 day SRT experiments, respectively. Excellent percent $\text{UV}_{254}$ absorbance removal was observed: 99, 99, and 97 percent for the 2, 10, and 20 day SRT conditions. The same percent removal values were seen for DOC removal in the SRT experiments. The p-values for percent $\text{UV}_{254}$ absorbance removal were 0.58, 0.27, and 0.29 for the 2, 10, and 20 day SRT experiments, respectively. Thus, no correlation can be made between SRT and percent $\text{UV}_{254}$ absorbance removal. The average RO feed $<10$ kDa $\text{UV}_{254}$ absorbance values were $0.2913 \pm 0.0274$, $0.3015 \pm 0.0475$, and $0.4032 \pm 0.1350$ for the 2, 10, and 20 day SRT experiments, respectively. T-tests indicate no difference between the total and $<10$ kDa fractions of the RO feed samples, with p-values of 0.78, 0.90, and 0.65 for the 2, 10, and 20 day SRT experiments, respectively. $\text{UV}_{254}$ absorbance increased by a factor of 3.0 between the MBR permeate and the RO feed water.

![Figure 38: UV$_{254}$ Absorbance of Water Samples with Standard Deviations](image)
SUVA values for the RO feed, RO feed <10 kDa, and RO permeate are shown in Figure 39. RO feed SUVA values were 2.46 ± 0.39, 2.55 ± 0.08, and 2.57 ± 0.14 for the 2, 10, and 20 day SRT experiments, respectively. This was expected because, even though all constituents were concentrated from the MBR permeate to the RO feed, the type and ratios of the constituents were expected to remain the same. The RO feed SUVA values were similar to the MBR permeate SUVA values. RO feed <10 kDa SUVA values were 2.14 ± 0.10, 2.59 ± 0.08, and 2.88 ± 0.43 for the 2, 10, and 20 day SRT experiments, respectively. RO permeate SUVA values were 1.86 ± 0.20, 2.27 ± 1.38, and 1.70 ± 0.70 for the 2, 10, and 20 day SRT experiments, respectively. The SUVA values for the RO permeate were a little lower than for the MBR permeate and RO feed water samples, but higher than the MBR feed samples. However, due to the very small values for the DOC and UV\textsubscript{254} absorbance for the RO permeate samples, small changes in these values led to large changes in SUVA values. Therefore, SUVA values for the RO permeate samples were not very important.

![Figure 39: SUVA Values of Water Samples with Standard Deviations](image-url)
**RO System Recovery**

The flow rates of the MBR permeate to the RO feed tank and RO permeate were adjusted to maintain a RO system recovery of approximately 75 percent. Solute rejection values were calculated from DOC, protein, carbohydrate, and EC concentrations in the MBR permeate, which fed the RO feed tank, and the RO permeate, using the equation [13]:

$$Rej = 1 - \left( \frac{C_P}{C_F} \right)$$

where $Rej$ = solute rejection by the RO membrane

$C_P$ = concentration in the RO permeate

$C_F$ = concentration in the MBR permeate, which feeds the RO tank

Recovery was calculated using the equation [13]:

$$C_C = C_F \left[ \frac{1 - (1 - Rej)^r}{1 - r} \right]$$

where $C_C$ = concentration in the RO feed tank

$r$ = the RO system recovery

The RO system recovery was calculated using EC concentrations in the RO feed tank and RO permeate and RO solute rejection values for each SRT experiment. Calculated RO system percent recoveries were 61, 66, and 67 percent for the 2, 10, and 20 day SRT experiments, respectively. T-tests on the RO percent recovery data resulted in p-values of 0.11, 0.056, and 0.71 for the 2 and 10 day, 2 and 20 day, and 10 and 20 day SRT experiments, respectively. The calculated RO system recoveries for the SRT experiments were lower than the desired RO system recovery of 75 percent by 10 to 20 percent. The concentration factor in the RO feed tank is expressed by the equation:

$$CF = \frac{C_{RF}}{C_{MP}}$$

where $CF$ = concentration factor in the RO feed tank

$C_{RF}$ = solute concentration in RO feed tank

$C_{MP}$ = solute concentration in the MBR permeate
Using EC concentrations, the concentration factors in the RO feed tank were 2.5, 2.9, and 3.0 for the 2, 10, and 20 day SRT experiments, respectively. Concentration factors were calculated using DOC, protein, and carbohydrate concentrations and were normalized to the concentration factors calculated using EC concentrations. These normalized concentration factors are shown in Figure 40. When the normalized concentration factor is close to 1, the closer the DOC, protein, or carbohydrate concentration factor is to the EC concentration factor. The normalized concentration factor for the 20 day SRT experiment calculated using average carbohydrate concentrations was low compared to the other SRT experiments. However, the standard deviation for the concentration factor using carbohydrate concentrations was high in the 20 day SRT experiment.

![Figure 40: Concentration Factors in the RO feed tank calculated using DOC, Protein, and Carbohydrate and Normalized to the EC Concentration Factor](image-url)
Membrane Autopsy

General Development of RO Foulant Layer

A visualization of the formation of the RO membrane foulant layer is shown in the time images from a stereoscope in Figure 41 for the 10 day SRT experiment. The RO membrane after 14 days is clearly more fouled than the RO membrane after 4 or 7 days. The autopsy of the RO membranes was complicated by variations in the foulant layer due to the RO feed spacer. The cleaned RO membrane for the 10 day SRT experiment is shown in Figure 42. Visually, the cleaned RO membrane does appear cleaner than the 4 and 7 day RO membranes. The remaining foulant layer is primarily composed of microorganisms, carbohydrates, protein, and some calcium, as shown in the membrane autopsy data, presented next.

Figure 41: Picture of Autopsied RO membranes for 10 Day SRT experiment. The first picture is after 4 days, the second after 7 days, and the last after 14 days.

Figure 42: Picture of Cleaned RO membrane from 10 Day SRT Experiment
Organic and Inorganic Content on Unused RO Membranes

To determine the baseline organic and inorganic content on the clean RO membranes, a study was conducted on a new RO membrane soaked in DI water for 24 hours and a new RO membrane placed in the RO unit with DI water circulated for 48 hours. The RO membranes were analyzed to determine the organic and inorganic constituents. There was greater variability in the measurements for the membrane in the RO unit than the DI water soaked membrane, but the values were similar. Protein and carbohydrates concentrations were measured from the membrane digests by sonicating the RO membranes in a 1 percent SDS solution and measuring the protein and carbohydrates in the extract. Cations were measured by digesting the RO membranes in aqua regia and measuring the cation concentrations in the filtered extract. Protein concentrations for the DI water soaked membrane and the RO Unit with DI water membrane were 2.6 and 1.9 µg/cm², respectively. Carbohydrate concentrations for the DI water soaked membrane and the RO Unit with DI water membrane were 17.4 and 18.3 mg/L, respectively. Protein and carbohydrate concentration data for the clean new membranes is presented in Figure 43. Cation concentrations on the clean new membranes were similar for both the DI water soaked membrane and the RO Unit with DI water membrane. The RO membrane digest primarily contained sodium, but also had measurable concentrations of calcium, potassium, and silicon. Little to no iron or magnesium was present in the RO membrane digests.
Inorganic Content on Fouled RO Membranes

The fouled RO membranes had greater concentrations of inorganics than baseline conditions. Until the end of the first week of the experiments, sodium was consistently the most abundant inorganic constituent on the RO membrane. However, the sodium concentration on the fouled RO membranes was never
much higher than the baseline, and did not increase significantly over the experiment. Calcium was consistently the most prevalent constituent on the RO membranes by the end of each experiment. With the exception of iron, the 20 day SRT condition had the highest inorganic concentrations of all SRTs.

Calcium, iron and magnesium were better removed from the RO membrane by the RO membrane cleaning procedure than potassium, sodium and silicon. Inorganics on the RO membranes for MBRs operating at 2 day and 20 day SRT conditions were better removed during cleaning than inorganics on the RO membranes for the MBR operating at a 10 day SRT.

Figure 45: Concentration of Cations and Silica on the 2 Day SRT Fouled Membranes
Figure 46: Concentrations of Cations and Silica on the 10 Day SRT Fouled RO Membranes

Figure 47: Concentrations of Cations and Silica on the 20 Day SRT Fouled Membranes
Organic Content on Fouled RO Membranes

The 2 day SRT condition tended to have the highest organic concentrations on the RO membrane, the 10 day SRT condition tended to have the lowest organic concentrations on the RO membrane, and the 20 day SRT condition usually had organic concentrations somewhere between the 2 day and 10 day SRT. Protein concentrations on the fouled RO membranes are shown in Figure 49 and the carbohydrate concentrations are shown in Figure 50. Both protein and carbohydrate concentrations on the RO membranes were larger than inorganic concentrations on the RO membranes.

Removal of organics by the RO membrane cleaning procedure was much less than removal of inorganics on the RO membranes. Percent removals from membrane cleaning are shown in Figure 48 for inorganics and in Table 12 for organic constituents. Protein was removed from the RO membranes between 2 and 6 times better than carbohydrates. Organics were removed better by cleaning in the 2 and 10 day SRT experiments than in the 20 day SRT experiment. The difference in cleaning effectiveness between the 2 lower SRT conditions and the highest SRT condition was more profound for carbohydrates.
than for protein. The 20 day SRT experiment had the greatest calcium concentration, followed closely by the 2 day SRT experiment, with much less in the 10 day SRT. Because calcium is involved in cementing the carbohydrates in the EPS of the RO foulant layer, the removal of carbohydrates would be expected to be inversely related to the calcium concentration on the RO membrane foulant layer. This was seen to be the case, as the percent removal of carbohydrates for the 20 day, 2 day, and 10 day SRT was 6, 13 and 19. This trend is not significant, because there is no statistical difference between the 2 and 10 day SRT and the 2 and 20 day SRT experiments with p-values of 0.30 and 0.21, respectively. Only the 10 and 20 day SRT experiments have statistical differences in carbohydrate removal, with a p-value of 0.042.

![Figure 49: Protein Concentration on Autopsied RO Membranes during SRT Experiments](image-url)
Figure 50: Carbohydrate Concentration on Autopsied RO Membranes during SRT Experiments

Table 12: Percent Removal of Protein and Carbohydrates from RO Membrane Cleaning Procedure

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Percent Removal from Membrane Cleaning</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Protein</td>
</tr>
<tr>
<td>2 Day SRT</td>
<td>43</td>
</tr>
<tr>
<td>10 Day SRT</td>
<td>42</td>
</tr>
<tr>
<td>20 Day SRT</td>
<td>32</td>
</tr>
</tbody>
</table>

Table 12: Percent Removal of Protein and Carbohydrates from RO Membrane Cleaning Procedure
Confocal Microscopy Results

The stained material on the RO membrane was more concentrated near the spacers than away from the spacers. This spatial heterogeneity in foulant layer distribution is shown previously in Figure 41. The microscopic heterogeneity can be seen in rotated and cross section views of the RO foulant layer stained with Syto 9 and propidium iodide in Figure 52 and Figure 53. The RO foulant layers were stained using Syto 9, ConA, FITC, and propidium iodide to image live and dead cells, proteins, and carbohydrates. CLSM images were taken at 50X magnification for a reference macroscopic view of the foulant layer and are not displayed in this paper. CLSM images taken at 630X magnification were used to characterize the morphology and distribution of the stained material in the foulant layer and to generate plots of the trends of the relative area of Syto 9, ConA, and FITC stained material to propidium iodide material over the depth of the foulant layer and the duration of the 10 day SRT experiment.

The foulant layer on the RO membranes changed in thickness and content over the duration of each experiment. The RO foulant layer thicknesses are

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**Figure 51: RO Membrane Foulant Layer Thickness with Standard Deviations as a Progression over Time for SRT Experiments**
shown for each SRT experiment in Figure 51. The foulant layer thickness increased between 3 or 4 days and 6 or 7 days for the 2 and 10 day SRT experiments. There was no data for RO foulant thickness before 9 days for the 20 day SRT. Over the last week of the SRT experiments, there was a small increase in RO foulant layer thickness for the 2 and 20 day SRT experiments, whereas there was a small decrease in RO foulant layer thickness for the 10 day SRT experiment, but these changes were minor. Changes also occurred in the distribution of stained material and morphology of the microorganisms on the foulant layer of the RO membranes. Initially, the Syto 9, propidium iodide, ConA, and FITC stained material was more uniformly distributed on the RO membrane. Figure 54 shows a picture of the RO foulant layer from the 10 day SRT experiment after 4 days. The microorganisms on the membrane were not very complex in shape, mostly simple rod and cocci shapes that were uniformly distributed in the foulant layer. As time progressed, the shapes of microorganisms became more complex and the microorganisms formed groups or communities instead of being evenly distributed as shown in Figure 55. Figure 55 shows the RO foulant layer from the 10 day SRT experiment after 7 days. After 14 days, the microbial community became more complex, with filamentous and other types of microorganisms present as seen in Figure 56. The groups of microorganisms seemed to stain more green than the surrounding dispersed microorganisms. This indicates that there was microbial growth on the RO membranes.

ConA stained material (carbohydrates) was initially more dispersed, but became more aggregated as time progressed. CLSM images of 10 day SRT fouled RO Membrane stained with ConA (green) and Propidium Iodide (red) after 4 and 14 days are shown in Figure 57 and Figure 58, respectively. There did not appear to be much difference in distribution or morphology of FITC stained material (proteins) between 4 and 14 days. CLSM images of 10 day SRT fouled RO Membrane stained with FITC (green) and Propidium Iodide (red) after 4 and 14 days are shown in Figure 59 and Figure 60, respectively.
Figure 52: Rotated View of a Syto 9 and Propidium Iodide Stained RO Foulant Layer

Figure 53: Cross Section of a Syto 9 and Propidium Iodide Stained RO Foulant Layer

Figure 54: CLSM Image of 10 Day SRT Fouled RO Membrane after 4 Days stained with Syto 9 (green) and Propidium Iodide (red). Microorganisms are simple and uniformly distributed.
Figure 55: CLSM image of 10 Day SRT Fouled RO Membrane after 7 Days Stained with Syto 9 (green) and Propidium Iodide (red). Microorganisms are more complex, often forming groups.

Figure 56: CLSM image of 10 Day SRT Fouled RO Membrane after 14 Days Stained with Syto 9 (green) and Propidium Iodide (red).
Figure 57: CLSM image of 10 Day SRT Fouled RO Membrane after 4 Days Stained with ConA (green) and Propidium Iodide (red). ConA stained material appears more dispersed.

Figure 58: CLSM image of 10 Day SRT Fouled RO Membrane after 14 Days Stained with ConA (green) and Propidium Iodide (red). ConA stained material seems to be more aggregated.
Figure 59: CLSM image of 10 Day SRT Fouled RO Membrane after 4 Days Stained with FITC (green) and Propidium Iodide (red).

Figure 60: CLSM image of 10 Day SRT Fouled RO Membrane after 14 Days stained with FITC(green) and Propidium Iodide (red).
An image-processing program, Image J from the National Institute of Health (NIH), was used to convert each image from a stack of images to black and white images and determine the area of stained material. Because stains could not all be used simultaneously on a single RO membrane piece, the area of each stained material (using Syto 9, ConA, and FITC) was normalized to the area of propidium iodide stained material. The area of each stain was graphed over the thickness of each RO foulant layer from the top foulant layer to the membrane-foulant interface. An example is shown in Figure 61 of the foulant layer from the 10 day SRT experiment after 14 days of operation. A1 and A2 refer to two different spots on one piece of membrane cut from the RO membrane sheet at one end of the feed channel (see Figure 6). B1 and B2 refer to two different spots on another piece of membrane cut from the other end of the membrane along the flow axis. The four sample points were considered replicates. Comparing the replicates was complicated by the fact that the thickness of the foulant layer was different at each point that was imaged. Therefore, the ratio of stained areas as a function of depth was normalized by plotting again using percent depth as the independent variable instead of the foulant layer depth. An example is shown in Figure 62. The results were easier to interpret, but did not allow data from different time points during the same experiment to be compared. The replicates needed to be averaged. The difficulty with this was that data points did not have the same percent depth. So the data points were averaged over 10 percent intervals. Ratios of Syto9, ConA, and FITC to propidium iodide were plotted in this way and are shown for the 10 day SRT experiment in Figure 63, Figure 64, and Figure 65, respectively. Data from the top and bottom 10 percent of the foulant layer were least reliable because there was very little material at the top of the RO foulant layer, and there was some autofluorescence from the membrane at the bottom of the RO foulant layer. The ratio of Syto 9 (live cells) to propidium iodide (dead cells) is similar over the RO foulant depth except for near the membrane surface, where an increase was observed in the Syto 9 to propidium iodide ratio. After the RO membrane cleaning procedure, the Syto 9 to propidium iodide ratio decreased,
indicating that the cleaning procedure was ineffective at reducing the RO foulant layer thickness. The ConA (carbohydrates) to propidium iodide ratio seemed to be higher at the surface of the RO foulant layer, and decline slowly over the depth of the RO foulant layer to the membrane surface. This trend was apparent for all autopsied membranes from the 10 day SRT experiment, including the membrane autopsied after the RO cleaning procedure. The ConA to propidium iodide ratio was lower after RO cleaning than before, indicating that the cleaning procedure was able to reduce the carbohydrates relative to the microorganisms on the foulant layer. An unexpected result of the CLSM analysis was that the Syto9 and ConA to propidium iodide ratios were highest in the middle of the experiment for the 10 day SRT experiment. The FITC (protein) to propidium iodide ratio did not change over the depth of the RO foulant layer or over the duration of the 10 day SRT experiment, even after the RO cleaning procedure.

Figure 61: Ratio of Syto9 to Propidium Iodide on the Foulant layer for 10 Day SRT after 14 Days over the Depth of the Foulant Layer Starting from the Top of the Foulant Layer to the Foulant Layer-RO Membrane Interface
Figure 62: Ratio of Syto 9 to Propidium Iodide on the Foulant Layer for 10 Day SRT after 14 Days over the Percent Depth of the Foulant Layer starting from the Top of the Foulant Layer to the Foulant Layer-RO Membrane Interface

Figure 63: Ratio of the Relative Stained Areas of Syto 9 to Propidium Iodide for the 10 Day SRT Experiment
Figure 64: Ratio of the Relative Stained Areas of ConA to Propidium Iodide for the 10 Day SRT Experiment

Figure 65: Ratio of the Relative Stained Areas of FITC to Propidium Iodide for the 10 Day SRT Experiment

Figure 66 shows the syto 9 (live cells) to propidium iodide (dead cells) ratio over the course of the 2 day SRT experiment. The ratio of syto 9 to propidium iodide (dead cells) decreased over the course of the 2 day SRT experiment and after RO membrane cleaning. After 3 days of operation, the syto
9 to propidium iodide ratio decreased from the top of the foulant layer to the RO membrane surface. After 6 days of operation, the syto 9 to propidium iodide ratio increased from the top of the foulant layer to the RO membrane surface. These results indicate prevalence of live cells at the surface early in the experiment, but prevalence of live cells deeper in the biofilm as the experiment progressed.

The ratio of ConA (carbohydrates) to propidium iodide (dead cells), shown in Figure 67, decreased between 3 and 6 days, but was highest after 14 days for the 2 day SRT experiment. Carbohydrates present at the beginning of the experiment could be more due to deposition from the RO feed water, whereas the higher carbohydrate concentrations at the end of the experiment could be due to EPS formation from microbial growth on the RO membrane. The ConA to propidium iodide ratio decreased after the RO cleaning procedure. The ConA to propidium iodide ratio decreased from the top of the foulant layer to the RO membrane surface for all autopsied RO membranes in the 2 day SRT experiment.

The ratio FITC (protein) to propidium iodide (dead cells) is shown in Figure 68 for the 2 day SRT experiment. The FITC to propidium iodide ratio was similar over the foulant layer for all autopsied RO membranes in the 2 day SRT experiment, as in the 10 day SRT experiment.
Figure 66: Ratio of the Relative Stained Areas of Syto 9 to Propidium Iodide for the 2 Day SRT Experiment

Figure 67: Ratio of the Relative Stained Areas of ConA to Propidium Iodide for the 2 Day SRT Experiment
The syto 9 (live cells) to propidium iodide (dead cells) ratio is shown after 14 days of operation for the SRT experiments in Figure 69. After 14 days of operation, the syto 9 to propidium iodide ratio was greatest for the 20 day SRT experiment, decreasing from the top of the foulant layer to the RO membrane surface. The syto 9 to propidium iodide ratio was similar for the 2 and 10 day SRT experiments, and did not change from the top of the RO foulant layer to the RO membrane surface.

The ratio of ConA (carbohydrates) to propidium iodide (dead cells) after 14 days for the SRT experiments is shown in Figure 70. The ConA to propidium iodide ratio was greatest for the 20 day SRT experiment, but was lowest for the 10 day SRT experiment after 14 days of operation. The ratio of ConA to propidium iodide decreased from the top of the RO foulant layer to the RO membrane surface for all SRT experiments. The higher ratio of carbohydrates to dead cells in the 20 day SRT experiment could be due to the increase in active microorganisms, which would be expected to lead to higher EPS concentrations on the RO membrane.
Figure 71 shows the ratio of FITC (protein) to propidium iodide (dead cells) after 14 days for the SRT experiments. The FITC to propidium iodide ratio increased in the 2 and 10 day SRT experiments, but remained constant in the 20 day SRT experiment from the top of the RO foulant layer to the surface of the RO membrane. There was no significant difference or trend in FITC to propidium iodide ratio with respect to MBR SRT. Overall, protein staining showed the least variability as a function of SRT, depth in the RO foulant layer, or duration of the experiment. As a result, protein staining is the least effective of the stains used in this project for identifying variability in RO foulant layer development.

Figure 69: Ratio of the Relative Stained Areas of Syto 9 to Propidium Iodide after 14 Days for the SRT Experiments
Figure 70: Ratio of the Relative Stained Areas of ConA to Propidium Iodide after 14 Days for the SRT Experiments

Figure 71: Ratio of the Relative Stained Areas of FITC to Propidium Iodide after 14 Days for the SRT Experiments
Specific Flux

The specific flux through the RO membrane for each test was normalized against the initial clean water flux averaged over a 10-hour period at the beginning of the test. There was no DI flux data in the 10 day SRT, so the first 10 hours of the test were used to normalize the 10 day SRT specific flux. Normalizing the flux against the initial clean water flux is commonly done in membrane research because each small piece of membrane material might have a different permeability due to variations in fabrication, so it is difficult to compare the fouling unless the specific flux is normalized to a known starting point.

Specific flux was normalized to 25 degrees C. Specific flux for the three SRT experiments is shown in Figure 72. The data used for the specific flux graph was collected using Labview, except for the 2 day SRT experiment. There were problems with the electronic RO permeate flow meter after a few days of starting the 2 day SRT experiment, so manual flow measurements were collected by measuring the volume of a graduated cylinder over a timed interval and were used to calculate the specific flux for the remainder of the experiment. Manual flow measurements were more accurate than those collected by Labview due to drift in the electronic flow meter. However, this drift in the electronic flow meter was very gradual. When a linear regression was plotted between the RO permeate flow rates taken manually and the RO permeate flow rates taken using the electronic flow meter, high coefficients of determination were calculated. The correlation between manual and electronic flow measurements can be seen in the Appendix. Using the linear regression equations, Labview flow rates were converted to measured flow rates to determine RO specific fluxes. RO specific flux declined more sharply when MBRs were operated at a higher SRT. This was unexpected, as MBR fouling rates were observed to be inversely proportional to SRT both in this project and in the literature. Differences in specific flux decline between the SRT experiments were expected to be due to differences in the amount of inorganic and organic constituents in the MBR permeate feeding the RO unit. However, differences in the inorganic and organic constituents in the MBR permeate were relatively small. Even small differences
in calcium could have changed the decline RO specific flux rates. However, calcium, known for cementing carbohydrates together in EPS of foulant layers, was present at similar concentrations in the RO feed water for all SRT conditions. While there was an increasing calcium concentration in the RO feed with increasing SRT, only the 2 and 20 day SRTs were statistically different. The MBR feed samples were grab samples, so this trend of increasing calcium concentration may not have been representative of actual average calcium concentrations. No trends in protein concentrations were seen in the RO feed over the different SRT conditions. There was less DOC removal and higher DOC concentrations in the MBR permeate for the 2 day SRT, which had the least specific flux decline of all experiments, so DOC concentrations in the MBR permeate does not appear to be responsible for the change in specific flux decline. DOC concentrations in the RO feed between the different SRT conditions were not statistically different, so DOC can not be implicated as the cause for the difference in specific flux. The RO feed water was filtered by a 10 kDa filter and analyzed for DOC, UV$_{254}$ absorbance, protein and carbohydrate to see if higher molecular weight fractions which have been implicated in microfiltration membrane fouling in previous studies would change with MBR SRT. However, constituents in the RO feed water were less than 10 kDa for all SRT values. So differences in specific flux decline were unlikely due to differences in inorganic or carbohydrate concentrations.

The RO foulant layer thickness was determined using confocal microscopy and is shown in Figure 51. The RO foulant layer thickness seems to be consistently greater in the 2 day SRT experiment compared to the 10 day SRT experiment. The 20 day SRT experiment has a decreasing RO foulant layer thickness over time. The foulant layer thickness after 14 days of RO operation decreased with increasing MBR SRT. This may be a coincidence, or could mean the foulant layer is more compressed with a lower porosity when the MBR is operated at higher SRTs.

Differences in specific flux decline for the different SRT conditions could also be a function of the type of organic matter rather than concentration. The
type of organic matter that affects the specific flux decline may not be able to be classified simply as DOC, protein or carbohydrate or even be classified by molecular size fractions. Some constituents in the RO feed water may be more attracted to the RO membranes than others. The EPS generated by the microorganisms may be more sticky as the SRT increases.

Figure 72: Specific Flux over Time for SRT Experiments

Specific flux before and after the RO membrane cleaning and specific flux recovery is shown for the different SRT conditions in Table 13. The specific flux recovery increased with increasing SRT. The absence of specific flux recovery for the 2 day SRT experiment was due to the limited specific flux decline during the 2 day SRT experiment and thus high specific flux prior to cleaning. There was a larger specific flux recovery for the 20 day SRT experiment than the 10 day SRT experiment, but the specific flux for the 10 day SRT was higher than the 20 day SRT experiment prior to cleaning.
Conclusions and Future Work

Conclusions

A bench scale MBR-RO was operated at a 2, 10, and 20 day SRT to treat settled municipal water. The MBR treated permeate was fed to a series of RO membranes and the RO process was evaluated for inorganic and organic removal and fouling. The primary object of this research was to evaluate RO membrane fouling by wastewater treated by the MBR process operated at different Solids Retention Times (SRTs). The main conclusion of this research is that higher MBR SRT values led to low MBR fouling, but more rapid RO membrane fouling.

While MLSS and yield in the MBR did vary, no statistically significant trends between MBR effluent quality and SRT were found. SRT was positively correlated with MLSS and MLVSS concentrations and negatively correlated with MBR biomass yield, defined as the amount of MLVSS produced per amount of DOC consumed in the MBR. High removals of organic constituents were seen in the MBR at all SRT conditions, with the exception of carbohydrates. No consistent trends were evident between SRT and DOC, protein, or carbohydrate concentrations in the MBR permeate. DOC removal was highest at the 10 and 20 day SRT, though no statistically significant difference in DOC removal was seen between the 10 and 20 day SRT. Average protein removals increased with increasing SRT, but this was only statistically significant between the 2 and 20 day SRT experiments. Average carbohydrate removals also increased with increasing SRT, but no statistically significant differences were found between

<table>
<thead>
<tr>
<th>MBR SRT</th>
<th>Flux before Membrane Cleaning (LMH/bar)</th>
<th>Flux after Membrane Cleaning (LMH/bar)</th>
<th>Percent Flux Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 Days</td>
<td>3.04</td>
<td>3.04</td>
<td>0</td>
</tr>
<tr>
<td>10 Days</td>
<td>1.48</td>
<td>2.55</td>
<td>42</td>
</tr>
<tr>
<td>20 Days</td>
<td>1.23</td>
<td>2.26</td>
<td>46</td>
</tr>
</tbody>
</table>

Table 13: Percent Flux Recovery
the SRT experiments. No trend existed in average \( \text{UV}_{254} \) absorbance or SUVA over the different SRT conditions. However, SUVA values did increase with increasing SRT, but only the 2 and 10 day and 2 and 20 day SRT experiments had statistically different SUVA values.

MBR effluent concentrations of organic and inorganic constituents were typical of effluents from full scale MBR and full scale activated sludge plants. However, some notable differences were observed. Inorganic concentrations were most similar between the bench scale MBR and full scale activated sludge plant, due to the fact that both the bench scale MBR and full scale activated sludge plant were using the same primary effluent. The bench scale MBR effluent had higher concentrations of nitrate, nitrite, and phosphate, because the system used in this study was not designed for biological nitrogen removal.

High molecular weight compounds have previously been attributed to contributing to RO fouling. The RO feed samples were divided into total and less than 10 kDa fractions and both fractions were analyzed for DOC, protein, carbohydrate and \( \text{UV}_{254} \) absorbance. The > 10 kDa fraction was calculated as the total minus the <10 kDa fraction. The DOC content with molecular weight less than 10 kDa in the RO feed samples was not statistically different than the total DOC at all SRT values as were protein and carbohydrate concentrations and \( \text{UV}_{254} \) absorbance values. Thus, the MBR completely removed all organic constituents with molecular weight greater than 10 kDa, regardless of SRT value. The increase in RO fouling rates over SRT can not be attributed to the high molecular weight fraction. While increased fouling rates were found to be related to increasing MBR SRT, this does not appear to be related to a change in high molecular weight compounds.

Examination of the fouled RO membranes by confocal microscopy allowed the spatial and temporal characterization of the RO foulant layer. After 3 days, a somewhat uniform distribution of Syto 9 stained (live) and propidium iodide stained (dead cells), ConA stained (carbohydrates), and FITC stained (protein) material were observed in the RO foulant layer. Microorganisms initially had simple rod and cocci morphology. After 7 days, the live microorganisms in the
RO foulant layer became more organized and grouped. By the end of the experiment, filamentous microorganisms were more prevalent, the ConA stained material became more aggregated, but the distribution and morphology of the FITC stained material remained relatively constant. The Syto 9 to propidium and ConA to propidium ratios were highest in the middle of the experiment than at the beginning or end. The Syto 9 to propidium iodide ratio was higher near the RO membrane surface and decreased after RO membrane cleaning across the RO foulant layer depth. The ConA to propidium iodide ratio decreased from the top of the foulant layer to the RO membrane surface and decreased slightly after RO membrane cleaning. The FITC to propidium iodide ratio remained constant over the depth of the RO foulant layer and the duration of the experiment, and was not significantly affected by RO membrane cleaning.

MBR fouling decreased with increasing SRT, as was seen in previous studies. This same trend was expected for RO fouling rates as well. However, the opposite trend was found in this project. Permeate from the MBR process with the longest SRT values produced the smallest decline in specific flux through the RO membranes. Flux recovery after RO cleaning increased with increasing SRT. This may not be due to the effectiveness of the RO cleaning procedure as much as the value of specific flux before RO cleaning. The 2 day SRT had the lowest percent flux recovery, but it also had the highest specific flux before RO cleaning of any of the experiments.

**Future Work**

Future work would include operating an MBR-RO pilot plant at a 10 day SRT. The 10 day SRT would be the recommended operating SRT for an MBR-RO plant based on the fact that MBR fouling was less at the 10 day SRT than the 2 day SRT, and the 10 day SRT resulted in less RO membrane fouling than the 20 day SRT. The foulant layer could be extracted and characterized in terms of its hydrophobicity, aromaticity, solubility and carboxylate/acid/base nature. The RO feed water could be divided into total and <10 kDa fractions, with both fractions analyzed for hydrophobicity, aromaticity, solubility and
carboxylate/acid/base nature. Another study would involve comparing RO fouling of MBR effluent operated at a high SRT with and without a GAC column. MBRs operating at high SRTs have less MBR fouling but generate more RO fouling. The hydrophobic fraction of the RO feed water has been attributed to RO fouling in previous studies. Therefore, use of a GAC column to remove the hydrophobic constituents from MBR effluent would allow the MBR to operate at high SRT without negatively impacting the RO specific flux. All future MBR work would include an anoxic selector for improved nutrient removal. Ammonia would also be measured to allow for total nitrogen removal to be calculated.
References


Appendix

Figure 73: Labview RO Permeate Flow Rate is Related to Measured Flow Rate for 2 Day SRT Experiment

\[ y = 0.9175x - 0.041 \]
\[ R^2 = 0.9849 \]

Figure 74: Labview RO Permeate Flow Rate is Related to Measured Flow Rate for 10 Day SRT Experiment

\[ y = 0.6977x + 2.6134 \]
\[ R^2 = 0.9874 \]
Figure 75: Labview RO Permeate Flow Rate is Related to Measured Flow Rate for 20 Day SRT Experiment

\[ y = 1.0506x + 0.9057 \]

\[ R^2 = 0.9705 \]