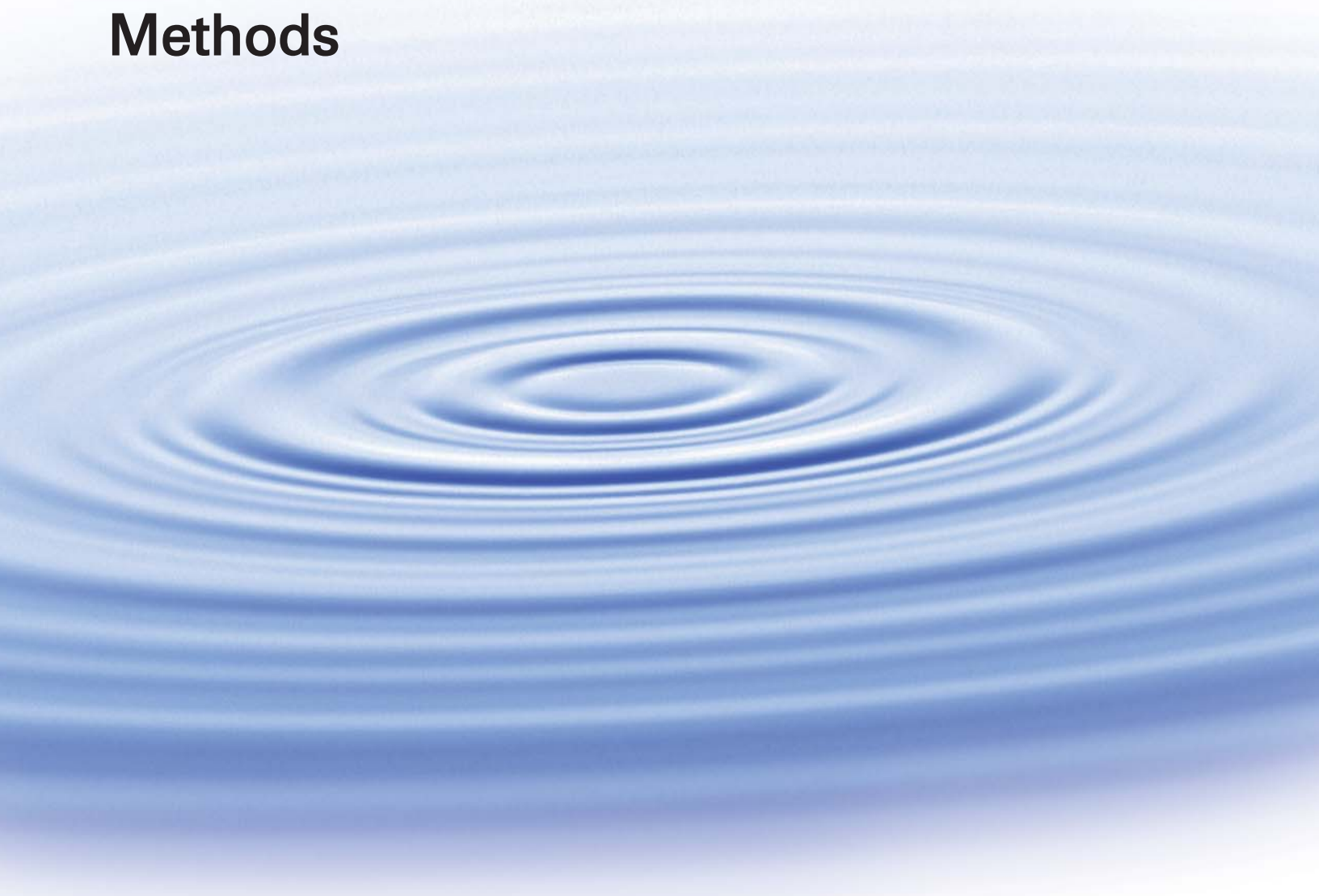
New Techniques for Real-Time Monitoring of Membrane Integrity for Virus Removal Using Submicron Particle Characterization Methods



WateReuse Research Foundation

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About the WateReuse Research Foundation

The mission of the WateReuse Research Foundation is to conduct and promote applied research on the reclamation, recycling, reuse, and desalination of water. The Foundation's research advances the science of water reuse and supports communities across the United States and abroad in their efforts to create new sources of high-quality water through reclamation, recycling, reuse, and desalination while protecting public health and the environment.

The Foundation sponsors research on all aspects of water reuse, including emerging chemical contaminants, microbiological agents, treatment technologies, salinity management and desalination, public perception and acceptance, economics, and marketing. The Foundation's research informs the public of the safety of reclaimed water and provides water professionals with the tools and knowledge to meet their commitment of increasing reliability and quality.

The Foundation's funding partners include the Bureau of Reclamation, the California State Water Resources Control Board, the California Energy Commission, and the California Department of Water Resources. Funding is also provided by the Foundation's subscribers, water and wastewater agencies, and other interested organizations.

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Acronyms

ASTM	American Society for Testing and Materials
CDPH	California Department of Public Health
CIP	clean-in-place
DLS	dynamic light scattering
EC	electrical conductivity
EFM	epifluorescence microscopy
gfd	gallons per square foot per day
gpm	gallons per minute
IC	inorganic carbon
LBWD	Long Beach Water Department
LBWRP	Long Beach Water Reclamation Plant
LVLWTF	Leo J. Vander Lans Water Treatment Facility
MF	microfiltration
mg/L	milligrams per Liter
mW	milliwatt
NF	nanofiltration
NTU	nephelometric turbidity units
mNTU	milli nephelometric turbidity units
PAC	project advisory committee
PCS	photon correlation spectroscopy
ppb	parts per billion
ppm	parts per million
psig	pounds per square inch gauge
RAC	research advisory committee
RO	reverse osmosis
RSD	relative standard deviation
TC	total carbon
TDS	total dissolved solids
TEM	transmission electron microscopy
TOC	total organic carbon
UCI	University of California Irvine
UF	ultrafiltration
WBMWD	West Basin Municipal Water District
WRD	Water Replenishment District of Southern California
WWTP	Wastewater Treatment Plant

Foreword

The WateReuse Research Foundation, a nonprofit corporation, sponsors research that advances the science of water reclamation, recycling, reuse, and desalination. The Foundation funds projects that meet the water reuse and desalination research needs of water and wastewater agencies and the public. The goal of the Foundation's research is to ensure that water reuse and desalination projects provide high-quality water, protect public health, and improve the environment.

An Operating Plan guides the Foundation's research program. Under the plan, a research agenda of high-priority topics is maintained. The agenda is developed in cooperation with the water reuse and desalination communities, including water professionals, academics, and Foundation subscribers. The Foundation's research focuses on a broad range of water reuse research topics including:

- Definition of and addressing emerging contaminants
- Public perceptions of the benefits and risks of water reuse
- Management practices related to indirect potable reuse
- Groundwater recharge and aquifer storage and recovery
- Evaluation and methods for managing salinity and desalination
- Economics and marketing of water reuse

The Operating Plan outlines the role of the Foundation's Research Advisory Committee (RAC), Project Advisory Committees (PACs), and Foundation staff. The RAC sets priorities, recommends projects for funding, and provides advice and recommendations on the Foundation's research agenda and other related efforts. PACs are convened for each project and provide technical review and oversight. The Foundation's RAC and PACs consist of experts in their fields and provide the Foundation with an independent review, which ensures the credibility of the Foundation's research results. The Foundation's Project Managers facilitate the efforts of the RAC and PACs and provide overall management of projects.

The Foundation's primary funding partners include the Bureau of Reclamation, California State Water Resources Control Board, the California Energy Commission, Foundation subscribers, water and wastewater agencies, and other interested organizations. The Foundation leverages its financial and intellectual capital through these partnerships and other funding relationships.

The priority in water reclamation is public safety. Microorganisms such as bacteria, viruses, and protozoa cysts are potential health hazards if not properly removed. The objectives of this report were to (a) identify feasible, reliable, and cost-effective monitoring devices for virus and/or submicron particle detection, (b) correlate results from submicron particle analyzers with epifluorescence direct viral counts, (c) validate the sensitivity of the test protocols, and (d) assess the cost of real-time integrity monitoring.

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Executive Summary

The priority in water reclamation is public safety. Microorganisms such as bacteria, viruses, and protozoa cysts are potential health hazards to the public if not properly removed. The typical advanced treatment systems today use membrane systems with various removal efficiencies depending on the pore size of the membrane. Verifying the integrity of these membranes requires real time virus monitoring to ensure public safety. Currently, there are no direct, online instruments or protocols available for membrane integrity assessments for compliance monitoring for virus removal.

The objectives of this study are to

(a) identify feasible, reliable, and cost-effective monitoring devices for virus and/or submicron particle detection for various types of membranes including microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO);

(b) correlate results from submicron particle analyzers with epifluorescence direct viral counts;

(c) conduct challenge tests by introducing defects to the membrane and validate the sensitivity of the test protocols; and

(d) assess cost of real-time integrity monitoring with a submicron particle analyzer as a surrogate for virus removal.

The study was conducted using bench- and pilot-scale testing at the Water Replenishment District of Southern California (WRD)'s Leo J. Vander Lans Water Treatment Facility (LVLWTF) and West Basin Municipal Water District (WBMWD)'s Edward C. Little Water Recycling facility.

A literature review was conducted to identify the various monitoring techniques. The review resulted with two feasible monitoring techniques: direct surrogate measurement (particle monitoring, turbidity, particle counting, membrane vessel probe, sulfate/phosphate, bi-selective salt test, fluorescent microsphere, American Society for Testing and Materials (ASTM) dye test, conductivity, dynamic light scattering, and laser diffraction) and indirect surrogate measurement (air pressure hold, sonic testing, bubble point, diffusive airflow, and vacuum hold test).

The bench-scale test was to develop sampling logistics and locations. The bench-scale tests were performed at the Leo J. Vander Lans plant and Edward C. Little plant. The instruments used for the bench tests were TOC analyzer, dynamic light scattering (DLS) instruments, electrical conductivity (EC) meter, turbidity meter, and epifluorescence microscopy (EFM). Results from the bench-scale tests demonstrated a linear correlation between EFM and DLS. However, based on the conditions tested, DLS does not provide information regarding the property of the nanoparticles, in spite of the higher sensitivity of detection. Among the DLS instruments tested, Nanotrac provided the most reliable measurements and lower detection limit; therefore, it was selected as the main instrument for pilot testing for membrane integrity assessment.

The pilot test was to verify the sensitivity of the instruments selected from the bench scale. The testing was carried out at WRD using a side-stream of MF filtration from the treatment plant. The RO pilot plant was monitored for total organic carbon (TOC), EC, and turbidity online instruments connected to Pilot RO permeate ports. Samples were also taken for nano particle analysis using Nanotrac DLS instrument at project team's facility and EFM at University of California Irvine (UCI). The five parameters collected in the pilot study including (1) TOC, (2) particle counts by Nanotrac (DLS system), (3) EC, (4) turbidity, and (5) virus counts by EFM were monitored to evaluate sensitivity and trend.

In order to evaluate the sensitivity of each test to detect potential virus breakthrough in RO permeate, RO connector O-rings were intentionally compromised to generate different levels of defect. The results of the pilot study showed that TOC and DLS measurements agreed with the EFM direct counts for virus breakthrough when the O-ring seal between permeate and concentrate was partially compromised.

Feasibility Analysis

A feasibility analysis was performed to consider the best options based on the annual cost, operational cost, sensitivity, maintenance, and online capability. The five instruments used in the pilot facility were included in the feasibility analysis.

The DLS system has the highest capital cost (approx. $40,000), but low operating cost and relatively high sensitivity to nanoparticle detection. TOC analyzers have a moderate capital cost (approx. $22,000) with a high annual cost and sensitivity to nanoparticle detection. TOC analyzers offer online continuous monitoring capability—thus, the implementation cost will be low; whereas DLS does not have the online monitoring capability at the time of this project. Both the DLS system and the TOC analyzers can be used as surrogates for virus break through, but the EFM method is the only method that directly detects viruses. The EFM method has a moderate capital cost (ranges from basic model of $8,000 to computer software-driven model of $20,000) and a high operation cost at $15 per sample. The turbidity meter (approx. $5,000) and EC meter (less than $1,000) have low capital and operating costs, but are less sensitive than the other monitoring methods for detecting leakage.

Conclusion and Recommendations

The DLS system is currently limited to grab samples only; therefore, the feasibility for an online monitoring DLS system requires further development. A full-scale design of DLS would also require a separate water line for test sample withdrawal. In addition, some DLS systems provide percent of particle size distribution; thus, the concentration of nanoparticles cannot be determined without referencing EFM virus count. Application of DLS to monitor membrane integrity requires establishment of baseline particle distribution. Because of the high variability of water quality in RO feed (i.e. the proportion of virus particles to other nanoparticles), the DLS data interpretation can be challenging. A more defined protocol including sampling, analysis, cleaning, maintenance, installation, and duplicate sample or other procedures is suggested to increase reliability to improve the usability of the system.

The new generation of online TOC analyzer tested during the pilot study provided comparable high sensitivity for detection of RO membrane system leakage during the controlled compromise test at the pilot scale. The results of the TOC analyzer also showed

positive correlation with results of EFM viral direct counts during bench-scale study. Thus, the TOC analyzer has the potential to be used as an online real time sensor to monitor the performance of the membrane system. The online capability of the TOC analyzer and the ease of use led the project team to conclude that it could be used for an alternative real-time monitoring of membrane integrity although it does not directly measure viruses or submicron particles. Future studies should focus on establishing a correlation among TOC, DLS, and EFM viral direct counts in full-scale water treatment plants of variable water quality.

In the interim, a possible solution from the public health perspective is to impose tighter controls on ammonia and turbidity with adequate disinfection to minimize health risks of the reuse water applications.

EFM remains the only method that can directly count the total number of viruses in the water sample. The pilot study confirmed the sensitivity of this method to detect the comprised O-ring seal between RO permeate and concentrate. Currently EFM is a labor-intensive and time-consuming testing method, and further development is needed for this to be applied as a real-time analysis instrument. Currently, the project team at UCI is developing a more efficient and user friendly virus detection method based on flow cytometry. Combining the brightness of new fluorescence dyes with the high sensitivity of a flow cytometry detection system, real-time virus monitoring is likely feasible in the near future.

Chapter 1

Introduction

1.1 Background

A large number of microorganisms including human viruses are present in secondary- or tertiary-treated wastewater effluent. They present potential health hazards if improperly treated prior to discharge. In order to protect public health, multiple barriers of treatment processes are necessary to ensure microbes and human pathogens are removed prior to discharge or reuse. Both MF and UF have demonstrated success for bacteria, viruses, and protozoa cysts removal. It is generally believed that MF can remove 1 to 4 log of bacteria and protozoa, and 0 to 2 log of viruses, whereas UF is shown to remove approximately 4 log of bacteria and perhaps equal amount of viruses (Asano et al., 2007). However, removal efficiencies vary with the membrane manufacturers, as well as the physical and chemical characteristics of the secondary or tertiary effluent.

Microbial removal rates may vary with different types of manufactured membranes, and virus monitoring is often needed to verify the integrity of the membrane. Several methods for membrane integrity exist including: TOC analyzers, DLS, laser diffraction, conductivity, fluorescent microsphere, American Society for Testing and Materials (ASTM) dye test, vacuum hold test, membrane vessel probe, sulfate or phosphate, and bi-selective salt test (Tiede, et al., 2008).

1.2 Overview

An RO membrane, in theory, removes microorganisms, including viruses, completely from permeate because it is designed for removal of dissolved solids. However, practical experience has shown that low levels of bacteria and viruses are found in the RO permeate. The Orange County Water District conducted two microbial studies at Water Factory 21 reclamation plant, in 1979 and 1981. These studies showed that, without disinfection, 5.3% of the samples from the RO effluent still contained viruses (NRC, 1982). Studies conducted by the City of San Diego during the Aqua II project also noted relatively high virus breakthrough in RO systems (Gagliardo et al. 1997a; Gagliardo et al. 1997b) and unpublished data by Jiang et al. recently detected viruses in RO permeate from a seawater desalination plant (Jiang et al., unpublished data). Leaks around the RO membrane seals and connectors are suspected as the cause of imperfect microbial removal (Asano et al., 2007). These results emphasize the importance of multiple barriers within a water reclamation facility and, more important, the need for a real-time, online monitoring method for membrane integrity testing for virus removal.

There are currently no direct, online instruments or protocols available for real-time membrane integrity assessment to support compliance monitoring for virus removal. There have been developments of direct monitoring methods for viral assays (EPA, 2005), but most of these methods still rely on off-site sample preparation, which makes these methods impractical as online methods (Di Giovanni et al., 2002; Higgins et al., 2003; Jiang et al., 2006).The goal of this project was to identify a method to monitor the removal of viruses in real-time, thus establishing stakeholder trust in the reliability of engineered systems for

protecting public health. The focus of this project was the evaluation of new instruments or methods that have potential to either directly or indirectly detect the breakthrough of viruses at water reuse treatment facilities.

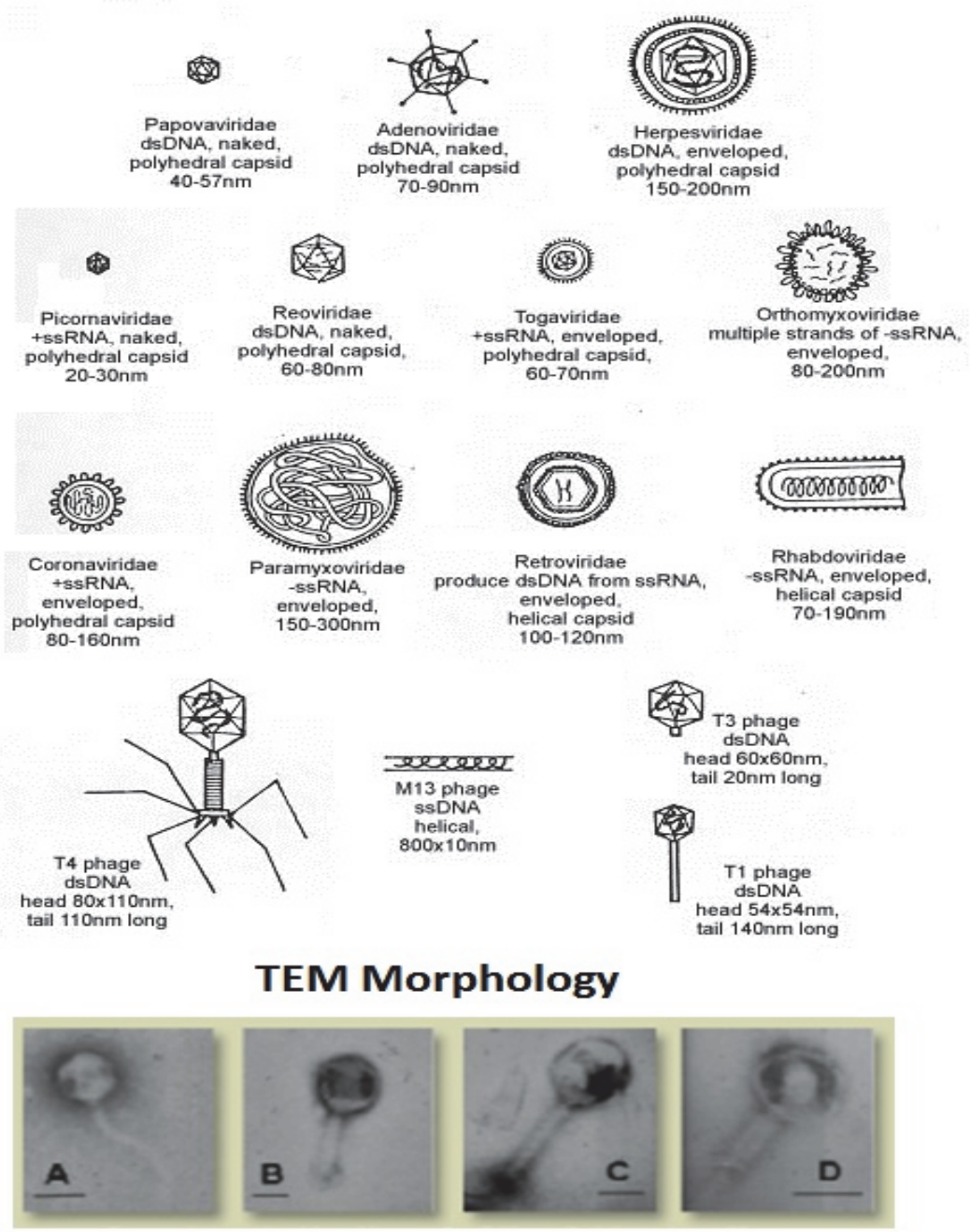


Figure 1.1. Size of various viruses and Transmission Electron Microscopy (TEM) Morphology of Bacteriophages isolated from Newport Bay, California. Scale bar indicates 50 nm (adapted from Kaiser, 2009; Jiang et al., 2007)

After performing a literature review, 46 methods were identified for virus monitoring; of the 46, the DLS system had the ability to analyze submicron particles as an indirect detection method. The focus of this study was to select and demonstrate the applicability of selected portable, online, real-time methods, such as DLS, that have been used for submicron particle characterization for monitoring viruses, which are typically in the range of 20 to 220 nm as shown in Figure 1.1. Although the DLS submicron-size particle analyzer does not characterize between viruses and the rest of the nanoparticles that may be present in reuse water systems, any increase in submicron particles from the baseline values in treated water (e.g., RO permeate) does indicate potential failure of the membrane and such monitoring can be used as an integrity monitoring method for virus removal.

1.3 Project Objectives

The objectives of this research were to:

- Identify feasible, reliable, and cost-effective monitoring devices for virus and/or submicron particle detection for various types of membrane including MF, UF, NF, and RO.
- Correlate results from submicron particle analyzers with epifluorescence direct viral counts.
- Conduct challenge tests by introducing defects to the membrane and validate the sensitivity of the test protocols.
- Assess the cost of real-time integrity monitoring with the submicron particle analyzer as a surrogate for virus removal.

1.4 Project Approach

Bench-scale testing was performed to develop sampling logistics and locations and to determine which instrumentation to use for the RO pilot-testing phase. Samples were taken from two locations: WRD and WBMWD. The samples were analyzed using the DLS system, EFM virus count, and a TOC analyzer. To verify the validity of using DLS submicron detection for virus removal, the results for DLS were compared to the EFM virus count results.

A RO pilot unit was installed on site at WRD's LVLWTF to enable online monitoring as well as collection of grab samples to assess the sensitivity of a number of analytical instruments for detecting leakage from the RO membrane under varying conditions. After flushing out the preservatives from the RO membranes in the first hour, a baseline of steady parameters was established.

There are a number of potential leakage points in a RO membrane element. One scenario is a physical failure where the O-rings can be flattened or cracked over time and, as a result, leaks may develop. RO pressure vessels may be probed to find faulty O-rings (Hydranautics, 2001). Probing also helps in determining the exact location of the compromised pressure vessel. The problem, however, may be either a poorly performing membrane element, an O-ring leak at an interconnector or end adapter, or possibly even a cracked adapter (Hydranautics, 1998). Accordingly, the connector O-ring on the pressure vessel adapter on the high pressure side that connects to the RO element was determined to be the most

vulnerable in terms of leakage. During the pilot testing, this specific O-ring was then progressively compromised to observe the sensitivities in the instrument response.

Response from DLS was compared with other online measurements, including TOC, turbidity, and total dissolved solids (TDS). Samples were also analyzed at UCI to confirm viral particle detection using EFM.

Chapter 2

Literature Review

A literature review was conducted to develop the foundation for virus monitoring methods. The three main components of this literature review were to:

- Understand viruses within water and water treatment,
- Identify virus monitoring methods in water, and
- Identify membrane integrity monitoring methods.

The aim of the literature review was to select potential real time virus monitoring methods to be used for the bench-scale test.

2.1 Viruses in Water and Water Treatment

Waterborne viral infections are some of the most important causes of human morbidity and mortality worldwide. There are hundreds of different types of human viruses present in municipal sewage that may potentially become the source of drinking and recreational water contamination if improperly treated. Table 2.1 shows important human viruses that are transmitted by water in the United States (Sinclair et al., 2009). Most of these waterborne viruses are enteric viruses, which cause infection of the gastroenteric system and are primarily transmitted via the fecal oral route through consumption of contaminated water and food.

Table 2.1. Enteric Viruses and Waterborne Viral Infections

Viral Agents	Disease Associated with Viral Infection
Norovirus	Gastroenteritis
Adenovirus	Conjunctivitis, gastroenteritis, respiratory disease, pharyngoconjunctival fever
Astrovirus	Gastroenteritis
Coxsackie viruses	Meningitis, pharyngitis, conjunctivitis, encephalitis
Echoviruses	Gastroenteritis, encephalitis, meningitis
Hepatitis A virus	Hepatitis
Enterovirus	Gastroenteritis

Enteric viruses are considerable more resistant to traditional water and wastewater treatment processes than are bacteria and protozoa. These are not easily removed by filtration and

sedimentation because of their small sizes. Noroviruses and adenoviruses are found in high concentrations, ranging from 10^2 to 10^6 genomes per liter, in secondary-treated effluent (Albinana-Gimenez et al., 2006; Bofill-Mas et al., 2006; Fong et al., 2010; Haramoto et al,. 2006; Haramoto et al., 2007; He and Jiang, 2005; Katayama et al., 2008; Nordgren et al., 2009; Pusch et al., 2005; Schlindwein et al., 2010; Victoria et al., 2010). Adenoviruses are also extremely resistant to UV radiation. UV doses of up to 200–300 mJ/cm^2 are required for a 4-$\log_{10}$ virus inactivation for a given UV reactor (Yates et al., 2006).

The advances in membrane technologies and improvements in monitoring practices in the past decade have significantly enhanced the ability to remove viruses and other pathogens from water. However, there is still great uncertainty in the potential health risk associated with wastewater reuse because of the lack of an online monitoring system for membrane integrity that indicates viral breakthrough.

RO membranes, in theory, should remove all microbes, including viruses, from feed water, because they are designed for removal of very small molecules. However, practical experiences have shown that low levels of bacteria and viruses are frequently found in the RO permeate. Orange County Water District conducted two microbial studies at the Water Factory 21 reclamation plant in 1979 and 1981. These studies showed that without disinfection, 5.3%of the samples from RO effluent still contained viruses (NRC, 1982). Studies conducted by the San Diego County during the Aqua II project also noted relatively high virus breakthrough in the RO system. Gagliardo and colleagues (Gagliardo et al., 1997a; Gagliardo et al., 1997b) reported the removal of waterborne pathogens and surrogates with spiral-wound RO membrane elements in the Aqua II project and showed that although the relatively large *Giardia lamblia* cysts and *Cryptosporidium parvum* oocysts were always below detection in permeate samples, the surrogate virus MS2 was detected in the permeate from many of the membranes tested. A wide range of virus rejection was observed, which was dependent on the RO membrane type/manufacturer (Gagliardo et al., 1998). On the contrary, Iranpour (1998) reported successful removal of seeded viral surrogate MS2 at greater than 5 $\log_{10}$ by RO during testing at the Terminal Island Treatment Plant, operated by the City of Los Angeles Bureau of Sanitation. The viral surrogate was below detection in RO permeate for all runs. These results suggest that manufacture practice plays an important role in the variability of the membrane performance. Leaks around the seals and connectors were suspected as the cause of reduced microbial removal efficiency.

For small-scale applications of flat-sheet membranes, where seal and connectors were absent in the testing system, viral breakthroughs were also reported. Governal and Gerba (1999) tested a bench-scale RO system for production of ultrapure water and showed that the RO membrane was inferior to UV and ozone disinfection for removal of viral surrogates. The viral surrogates, MS2 and PRD1 bacteriophage, were detected in RO permeates in all test runs with a removal rate between 4 and 5 $\log_{10}$. Similarly, Adham et al.(1997; 1998) performed experiments in small, flat-leaf cells and concluded that viruses passed through imperfections in the RO membranes and not through defective product water tube seals or membrane leaf glue lines in the spiral-wound elements.

The presence or development of membrane element breaches of relatively large size might be detected by monitoring the product water conductivity. However, the development of smaller imperfections that may not produce measurable changes in conductivity could cause breakthrough of pathogens at undesirable levels. During the San Diego Aqua II project, it was shown that the virus removal efficiencies in the RO system did not correlate well with the

rejections of sodium chloride specified by the manufacturers of the various membranes tested (Gagliardo et al. 1997b). Consequently, there is a need for assessing the presence of such small imperfections in RO membrane elements and gaining a better understanding of the dynamics of membrane performance with respect to waterborne pathogen rejection. So far, only one study reported the assessment of a compromised membrane, which showed that small pinholes on the membrane did not have a significant impact on viral removal efficiency. The viral removal rate, in fact, improved after a period of membrane operation because of clogging of the pinholes by virus particles (Mi et al. 2004).

It is important to note that all previous studies of membrane integrity and viral rejection efficiencies are based on viral surrogates, which require seeding of bacteriophage at high concentration into the feed water. This type of practice may not provide the adequate indication of plant performance during normal operation. Seeding of bacteriophage is often introduced together with a portion of bacteriophage growth medium changing the water quality of the RO feed water. In addition, seeding has to be performed at the bacteriophage concentration several orders of magnitude greater than the indigenous viral concentration. The reported clogging of pinholes or blocking of cracked O-rings may not occur under normal operating conditions when lower concentrations of viral particles and organic nutrients are present in feed water. Most important, a seeding study will not provide an online monitoring result for the performance of the membrane during a normal operation.

2.2 Virus Monitoring in Water

Coliphage have traditionally been used as an indicator of the viral quality of water because their size and physiochemical characteristics are similar to human viruses. However, coliphage are only a small fraction of the total viral community in the sewage, and they are likely below the limit of detection after the first few steps of advanced water treatment. In general, they are not suited for monitoring membrane integrity because only low concentrations of coliphage are present in the RO feed water. In addition, the culture-based assay method requires the specific bacterial host for viral infection, which further limits the detection of coliphage in water. Alternative viral monitoring methods are not well known or practiced in water and wastewater treatment.

The epifluorescence viral direct counting method has been developed and applied in other areas of aquatic research since 1998. However, it has not been introduced to the water and wastewater treatment industry yet. This method is based on filtration of water samples onto a 0.02 μm pore-size filter followed by staining and visualization under an epifluorescence microscope. Counting the total number of viral particles in water samples has been made possible by the powerful nucleic acid stains. SYBR Green is the most commonly used nucleic acid stain that brightens the viral particles and allows their capture under the epifluorescence microscope (Noble and Fuhrman, 1998). Because the stain is nondiscriminative to double-stranded nucleic acid, any particle containing double stranded DNA will be captured. Viruses and other DNA-containing particles will be further differentiated by their size and intensity of fluorescent light emission. Bacteria in general do not interfere with virus counting because bacterial cells are 10 times less than viruses in number in the natural aquatic environment and sewage and 10 to 100 times larger in size than viruses. The background fluorescence resulting from staining the extracellular DNA in samples can be reduced using DNase digestion prior to the collection of viruses onto the filter membrane. However, in most cases, DNase digestion is not necessary unless the sample contains a large amount of broken cells such as those found in sludge and sediment samples. Total viral counts are routinely conducted together with total bacterial counts on the same filter (Jiang et al., 2004). The computer

software is capable of separating bacteria from viruses and other large eukaryotic cells based on the fluorescence area of the particle (Jiang et al,. 2004). Once a counting program is set up, the results can be generated semiautomatically. The results are typically available within 30 min of sample collection.

Total viral direct count is now routinely performed in aquatic microbial ecology research. The total numbers of viruses in lakes and oceans, as shown in Table 2.2, are in the range of 10^5 to 10^7 /mL (Fuhrman, 1999; Jiang et al., 2003; Jiang et al., 2004; Wommack and Colwell, 2000). Because of the high concentration of viral particles in water, only 1 mL to 10 mL of sample is sufficient for estimating viral concentration (Choi et al., 2009). Dilution is required for counting of viruses in sewage effluent. The only quantitative report of viruses in sewage samples using direct counting methods indicated that the total number of viruses is ~10^{10} /mL (Wu and Liu, 2009).

A flow cytometry-based method has been reported for counting viruses in ocean water. However, its application in other fields has not been explored because of the cost and availability of flow cytometer. Although the size of viruses is generally below the detection limit for most flow cytometry systems, Marie and colleagues reported enumeration of marine viruses stained with SYBR Green I by flow cytometry (Marie et al. 1999). Their results showed that flow cytometric analysis of virioplankton populations in water samples collected from different depths in the Mediterranean Ocean revealed two distinct virus populations based on size. Both the cost and capability of flow cytometer have improved significantly in recent years. The high sensitivity flow cytometer is more readily available. The flow cytometry-based method, if proven useful for sewage samples, can potentially be developed into an online monitoring system for viruses. Thus, this approach could dramatically change the current pathogen monitoring practice used by water utilities.

2.3 Membrane Integrity Monitoring

As previously noted, there are no direct, online instruments or protocols available for real-time membrane integrity assessment to support compliance monitoring for virus removal. Although epifluorescence provides the results in a relatively short time, the protocol for such testing does not allow the method to be implemented as an online method. There have been developments of direct monitoring methods for viral assays (EPA, 2005), but most of these methods still rely on off-site sample preparation similar to epifluorescence, which makes these methods impractical as online methods (Di Giovanni et al., 2002; Higgins et al., 2003; Jiang et al., 2006). The focus of this project is the evaluation of new instruments or methods that have potential either to directly or indirectly detect the breakthrough of viruses in all types of water treatment applications including potable and reclaimed water.

The detection of viruses, which are in nanometer scale, has benefitted from the development of nanotechnology and provides the key approach for this project. As nanotechnology has evolved in recent years, so has the monitoring of nano-scale particles. In fact, there have been significant improvements in submicron particle analyzers for process control and manufacturing in the pharmaceutical and vaccine manufacturing industries with an extensive summary of analytical methods to detect and characterize nanoparticles in the environment (Tiede et al., 2008). Approximately 46 analytical techniques ranging from simple DLS to sophisticated scanning transmission electron microscopy (TEM) were compared for nanoparticle analysis by Tiede and colleagues. The current project demonstrated the applicability of portable, online, real-time methods, such as DLS, that have been used for

submicron particle characterization as methods for monitoring viruses, which are typically in the range of 20 to 220 nm as previously discussed.

Table 2.2. Viral Abundance in the Aquatic Environment

Environments	Viral Particles (10^6/ml)
Ocean	
North Pacific	0.4–1.9
Equatorial Pacific	5.3
North Atlantic	14.9
Mediterranean Sea	2.3–6.5
Coastal Ocean	
North Adriatic Sea	1.2–87
Gulf of Mexico	0.3–79
Southern California	0.3–47
Freshwater	
Lakes, Quebec, Canada	41–250
Mono Lake, California	140–380
Danube River	12–61
Sewage	200–26,000

Source: Modified from Wommack and Colwell, 2000.

Laser turbidimeters are not sensitive enough to detect virus particle in the size range of 20 to 200 nm. On the basis of the review of membrane integrity monitoring methods and direct microbial detection methods, submicron size analysis, DLS, seems to be the most promising method in detecting virus-sized particles. DLS, also known as photon correlation spectroscopy (PCS), measures the intensity of light scattered by particles in the sample (Carr et al., 2009). DLS is an absolute measurement where knowledge of the particle composition is not required. It can measure particles ranging in size from 0.5 nm to 6 μm, and it works by measuring the intensity of light scattered in a particular direction. The intensity changes with time because of the Brownian motion of particles in suspension. DLS instruments obtain a correlation factor from the intensity versus time profile. The exponentially decaying correlation function is related to the diffusion coefficients. Using the Stokes-Einstein equation and these diffusion coefficients, the instrument calculates the particle radius.

Although the DLS submicron-size particle analyzer does not characterize between viruses and the rest of the nanoparticles that may be present in reuse water systems, any increase in submicron particles from the baseline values in treated water (e.g., RO permeate) does indicate potential failure of the membrane and such monitoring can be used as an integrity monitoring method for virus removal. Table 2.3 summarizes the available membrane integrity monitoring methods including potential online applicability of the methods.

Table 2.3. Summary of Available Membrane Integrity Monitoring Methods

Membrane Integrity Monitoring Methods	Applicable to Virus Size Particles	*Measure Type	Detectable Online	Potential Online Applicability	Cost to Implement
Particle Monitoring	No	DS	Yes	Yes	Low
Turbidity	No	DS	Yes	Yes	Low
Particle Counting	No	DS	Yes	Yes	Low
Air Pressure Hold	No	IS	No	No	Low
Sonic Testing	No	IS	No	No	Low
Bubble Point	No	IS	No	No	Low
Diffusive Air Flow	No	IS	No	No	Low
Vacuum Hold Test	Yes	IS	No	No	High
CorrTest	Yes	IS	No	No	High
Membrane Vessel Probe	Yes	DS	No	No	High
Sulfate or Phosphate	Yes	DS	+	+	Low
Bi-Selective Salt Test	Yes	DS	+	+	Mod
Fluorescent Microsphere	Yes	DS	+	+	Mod
ASTM Dye Test	Yes	DS	+	+	Mod
Conductivity	Yes	DS	Yes	Yes	Low
Dynamic Light Scattering	Yes	DS	Yes	Yes	Low
Laser Diffraction	Yes	DS	Yes	Yes	Low

Notes. DS=direct surrogate, IS=indirect surrogate, + = could not be confirmed.

The project team has tested various samples collected from a local water reclamation plant. Table 2.3 summarizes the size range of detection for each analyzer and the average and the range of particles detected in a MF feed sample (secondary effluent) for selected instruments. Three analyzers capable of measuring submicron particles online had similar results for submicron particles in terms of average size and the size ranges.

As mentioned, DLS does not differentiate viruses from other nanoparticles. The approach for implementing DLS is to use the instrument to detect nanoparticle breakthrough from the membrane. If higher than normal nanoparticles are present in RO permeate, it is likely that some of the nanoparticle may be viruses. Validation of such a hypothesis must be done using a direct method of measuring viruses, namely, epifluorescence microscopy as discussed previously.

Table 2.4. Size Range of Detection for DLS and Other Analyzers

Manufacturer	Model	Type	Size Range (nm)	Continuous Flow	MF Feed Average	MF Feed Range
NanoSight	LM20	DLS	10–2,000	Yes	233 nm	20–680 nm
Microtrac	Nanotrac	DLS	0.8–6,500	Yes	383 nm	36–815 nm
Brookhaven	NanoDLS	DLS	0.5–3,000	Yes	459 nm	92–820 nm
Horiba	LB550	DLS	1–6,000	Yes	10 nm	NA
	LA950V2	LD	10–3,000	Yes	NA	NA
	DT1201	EAS	5–100,000	No	NA	NA
	PSA300	IA	500–1,000,000	No		
JMAR	BioSentry	MALS	400–10,000	Yes	NA	NA
Particle Measuring	UltraChem			Yes	NA	NA
Beckman Coulter	Delsa Nanozeta		0.6–7,000	No	NA	NA
Pssnicomp	System 380		1–5,000	NA	NA	NA
Particle System		DLS		NA	NA	NA
Wyatt Technologies	WattQELS	QELS	1–500	Yes		
Malvern	ZetaSizer	DLS	0.6–6,000	Yes	NA	NA
Colloidal Dynamics	Acsousto Sizer IIs	EAS	20–10,000	Yes	NA	NA
Agilent	7030 Nicomp	DLS	0.5–6,000	No	NA	NA

Chapter 3

Bench-Scale Testing

Bench-scale testing using DLS was required for the validation of submicron particle analyzers as potential for virus monitoring. WRD's LVLWTF and WBMWD's Edward C. Little Plant were the sites for the testing. The following section discusses the details in the process and results of the bench-scale testing.

3.1 Sampling Protocol

Bench testing was conducted by collecting samples at various locations at different times and different days. Samples from WRD's LVLWTF were collected using sterile 1.5 liter whirlpool sampling bags and split for evaluation of the total submicron particles using DLS and viruses using EFM. Sampling locations were selected based on the water treatment processes. In cases of multiple lines of pretreatment, samples were collected from different locations for comparison.

Figure 3.1 shows the process schematic of WRD plant. In addition to RO feed and RO permeate, which are the main focus of the project, MF feed and MF filtrate samples were also collected. The sampling locations are indicated by star signs in the schematic.

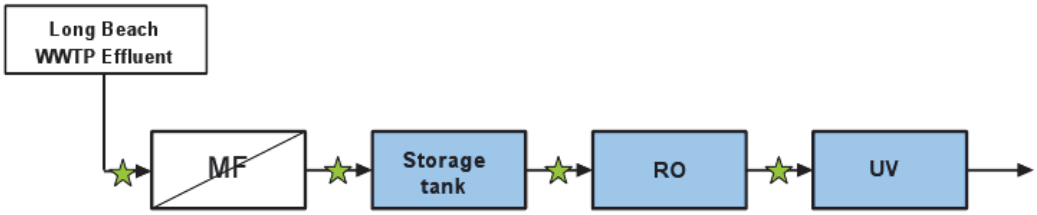


Figure 3.1. Sampling locations at WRD's LVLWTF in Long Beach, CA with plant influent from Long Beach's Wastewater Treatment Plant (WWTP).

Figure 3.2 shows the process schematic of the WBMWD plant. Two distinct types of microfiltration units were used for each specific process train. Samples were obtained from both trains: first from the pressure-MF side, marked RO-In(A) and RO-Out(A), and second from the submersible MF-side-marked RO-In(B) and RO-Out(B). MF feed, MF filtrate, and select UV effluent samples were also collected for comparison purpose.

The submersible MF-side, marked in the figure as Phase IV MF is followed by UV process and is similar to WRD train. This process train was given the sample priority, especially in situations where WRD was shut-down. The pressure MF-side was sampled only on specific days.

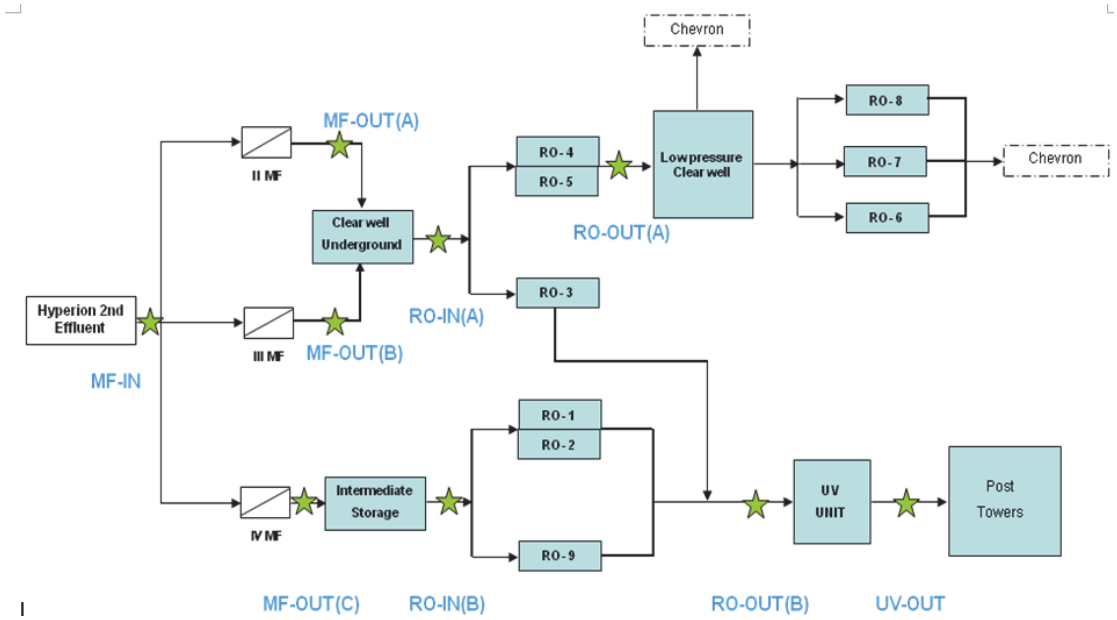


Figure 3.2. Sampling locations at WBMWD's Edward C. Little Plant in El Segundo, CA.

3.1.1. Bench Test

Two DLS instruments were available at the project team's facility, whereas the samples were also sent to the third DLS company's facility as the instrument was not available for onsite use. Although the DLS testing is similar to the conventional particle counter, the variability of each instrument and different parameters were studied. The testing strategy was slightly modified based on the plant schedule and sampling conditions (e.g., samples from select trains).

3.1.2. Sampling Procedure

The following procedure was used to collect samples from WRD. Special care was taken to avoid contamination while collecting, transporting, and storing these samples.

1. Powder-free gloves and lab coat were used at all locations. Gloves were disposed of after single use to minimize cross contamination onsite, and a new lab coat was used each sampling day.

2. Rigorous flushing by completely opening the sampling valve for 5 min was followed at each sampling location.

3. First set of samples was collected in 500 to 1,000 mL HDPE bottles for DLS analysis at the project team's facility.

4. A second set of 1,000 mL samples were collected in sterilized plastic sample containers for analysis at UCI. Into these samples, a 1 mL of 10% sodium thiosulfate was injected. A new syringe was used each time to avoid cross-contamination.

5. The samples were stored in an ice box during transportation. Blue ice packs were used to keep the temperature close to 4 °C.

3.2. Analytical Protocol

Upon receiving samples, the following procedure was followed for analyzing the samples:

1. Once delivered, the samples were analyzed on the same day.
2. All lab tests followed a definite sequence, starting with the DLS analysis on NanoSight followed by Nanotrac. Because the DLS instruments are sensitive to vibrations, the two DLS instruments were setup on different bench space.
3. Analysis on both DLS instruments took place simultaneously. The Nanotrac was operated while recording videos and processing the data on NanoSight.
4. The TOC analysis was conducted at the same time with DLS analysis.
5. Other parameters including turbidity, conductivity, and pH were measured after completing the DLS analysis.
6. On completion of all tests, samples were stored in a refrigerator for any subsequent analysis in the future.

The following three instruments were used in this study for the detection of submicron particles using optical microscopy.

- NanoSight LM20 by NanoSight LTD
- Nanotrac ULTRA by Microtrac Inc.
- Brookhaven NanoDLS by Brookhaven

Both the Nanotrac and the NanoDLS provide data on particle size and size distribution as a percentage by volume. These instruments also provide the percent number distribution, but this is relative to the volume distribution and is not the absolute particle count. The NanoSight, on the other hand, allows image analysis and enables the user to track individual particles. It can, thus, be used as a particle counter. The Zetaview from Microtrac provides image analysis similar to NanoSight. It uses a light scattering microscope with video camera to capture the movement of fine particles. The Zetaview instrument was not available during this study. NanoDLS from Brookhaven was not available for an onsite test, therefore only limited samples were analyzed by sending in samples. Table 3.1 provides a summary of the different features of each instrument.

Table 3.1. Overview of DLS Instruments Evaluated for Submicron Particle Analysis

Parameter	NanoSight LM20	Nanotrac ULTRA	Brookhaven NanoDLS
Technology	Image analysis of individual particles moving under Brownian motion	DLS analysis of Doppler shifted scattered light using patented technology	Hydrodynamic radii using DLS
Feature	Real-time dynamic nano-particle visualization, video clip capture	Flexible external probe option in combination with internal sample cell	Small volume flow cell, online and batch mode measurement
Measurement Range	10 to 1,000 nm	0.8 to 6,500 nm	0.5 to 3,000 nm
Measurement Output	Particle counting and sizing, size distributions displayed histograms	Particle size and size distribution	Particle size
Sample Volume	0.3 mL	3 mL	2.5 µL
Optical Component	Laser output 640 nm wavelength at 40 mW power	Laser output 80 nm wavelength at 3 mW power	Variable power laser at 638 nm, 35 mW power
Cut-off particle size determined from experiments	~ 25 nm	~ 2 nm	NA

3.2.1 Nanotrac ULTRA

Nanotrac Ultra is a product of Microtrac Inc. The company manufactures the instruments in York, PA and has a sales office in Largo, FL. The instrument provides the overall size distribution rather than providing an actual particle count. The distribution is displayed for both user-defined particle sizes as well as software-selected percentile points at a regular interval. The instrument offers analysis of particles of different shapes (irregular or spherical) and densities. All the particles are measured by the probe within a vicinity of 0.2 micron. It is therefore essential that the probe tip be completely submerged, the background clear of any impurities, and the sample cell free of minute air bubbles while testing. The design is flexible, and the probe can also be directly immersed in the sample container. The instrument has a measurement range of 0.8 to 6,500 nm. Figure 3.3 shows the assembly of the instrument.

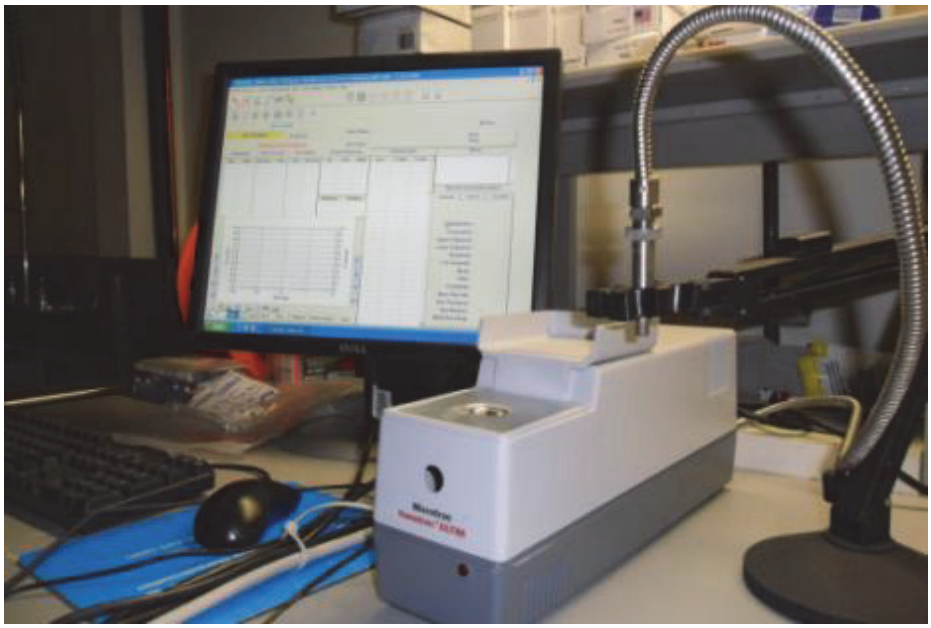


Figure 3.3. Nanotrac DLS assembly with an independent probe. The probe can also be connected to the sample cell.

In the present study, the sample cell was used with the probe attached for measurements. The following procedure was used for analyzing the samples:

1. The measurement setup option of the software is used to apply appropriate parameters such as sample ID's, run time, fluid-particle characteristics, and so forth.
2. The sample cell is first flushed with 6 to 8 mL distilled water to remove any particles from previous test. It is then filled with 2 mL distilled water and the system is set at zero as a background level measurement.
3. Once the set-zero is performed, the sample cell is flushed by filling the cell with the most dilute sample.
4. The progress of the system is checked using a "sample loading" icon on the instrument.
5. The analysis can then be started with a push of a single key. Analysis time and other parameters can be adjusted using the measurement setup tab.
6. When the data collection is complete, the measurement report is calculated and displayed. This report can be printed or exported to an Excel file.

Figure 3.4 shows a screenshot of a typical measurement report displayed after the Nanotrac analysis is complete.

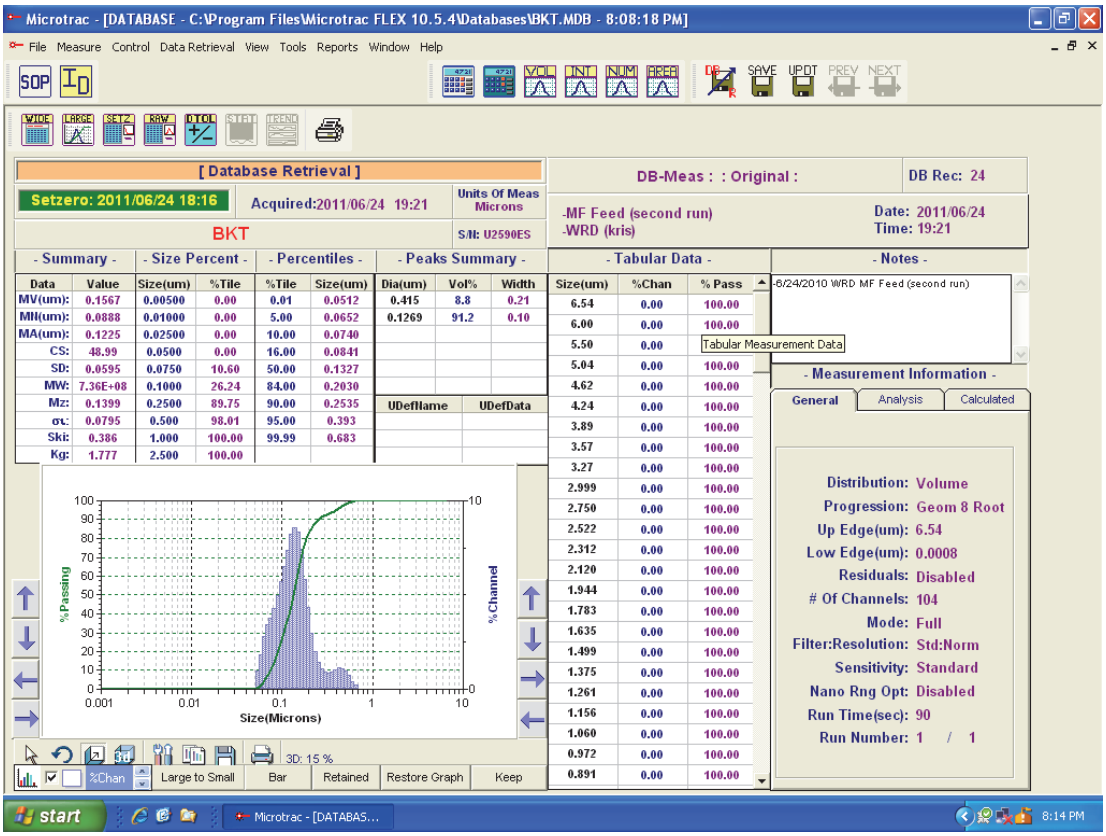


Figure 3.4. Example output from Nanotrac analysis. Graph shows particle distribution over volume of sample.

3.2.2. Brookhaven NanoDLS

The samples were sent out directly after collection to the Brookhaven instrument facility, located in Holtsville, NY for measurement. Samples, 50 mL each, were dispatched in sealed, compatible plastic bottles for analysis. The samples were shipped in an icebox.

The NanoDLS measures the scattered intensity of the total particles in the samples to provide particle size analysis. The size range for the instrument is 0.5 nm to 3 µm and has a sample cell volume of 2.5 µL. The sample is run through a 0.2 micron filter before the analysis. Because of limited availability of this instrument, NanoDLS was not used to critically evaluate submicron monitoring potential.

Figure 3.5 shows the general setup of the NanoDLS instrument.

Figure 3.5. Brookhaven NanoDLS assembly showing DLS unit on the right.

3.2.3. NanoSight LM20

The LM20 is a product from NanoSight Ltd. The corporate office is located in Wiltshire, United Kingdom. The office at Costa Mesa, CA, covers the west coast operations in the United States. The NanoSight LM20 DLS has the advantage of a real-time visual of particles. The particles in the field of view are enhanced by a near perfect black background. These particles appear individually as they scatter light under the Brownian motion. Figure 3.6 shows a typical NanoSight LM20 DLS assembly.

In order to quantify the real-time submicron-particle analysis, the software allows the user to automatically size and track particles on an individual basis. The instrument covers a wide range of particles from 10 nm to 1,000 nm, and the smallest of the organic particle detected by the software is close to 25 nm. For any particle type, the instrument uses Class 1 laser output of 40 mW at 640 nm.

The basic procedure of operation is as follows:

1. The sample cell is first cleaned with distilled water by flushing multiple times to remove any particles carried over from previous test.
2. Starting with the most dilute sample, 5 mL of sample is used for initial flushing using a disposable syringe. Next, 0.3 mL of sample is introduced in the field of view, ensuring that no air bubbles are trapped inside.
3. NanoSight first records the video to capture the reflection of particles followed by software analysis. A reference point is used as a starting point for video recording.

4. The focus of the lens is manually adjusted for the particles in the field of view. Proper adjustment of focus and brightness displays well-defined particles.
5. Video is recorded for a fixed period of time, typically 90 to 150 seconds.
6. Once the video is recorded, liquid from the sample cell is removed. The cell is flushed completely with distilled water, and the process is repeated.
7. In order to view the output, the saved video file has to be processed by adjusting user-defined parameters in the software. Some of the important parameters are the detection threshold adjustment and blur removal.
8. Particle count, size distribution, processing parameters, and other useful information are then summarized in a report. NanoSight has an option of exporting this report to an Excel spreadsheet. Figure 3.7 shows an example of a summarized report.

The concentration is available only on this DLS for the instruments tested and is expressed as particles/mL. This count was used for comparison with the EFM data from UCI.

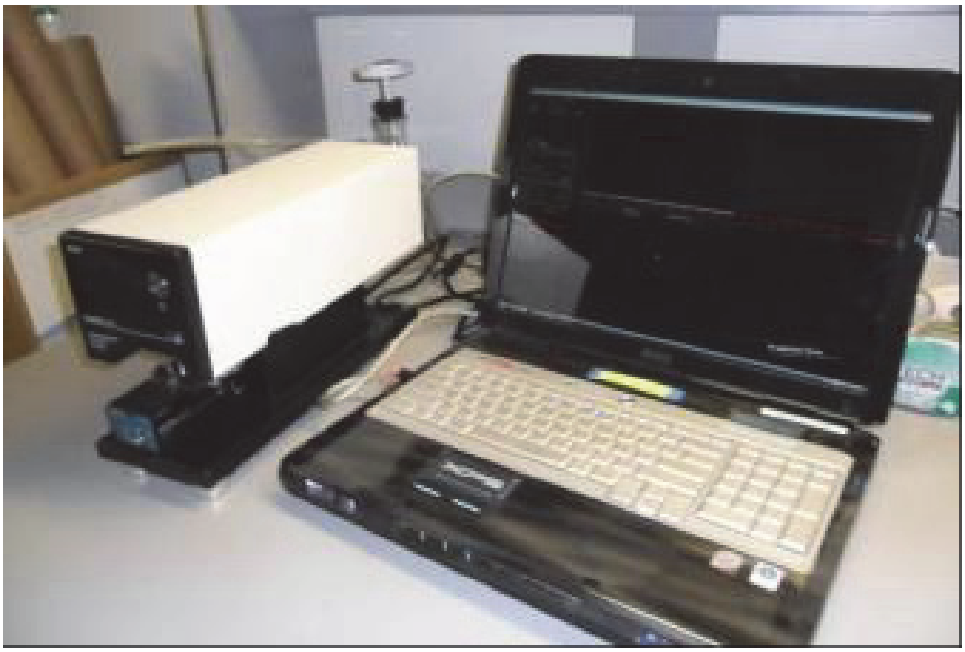


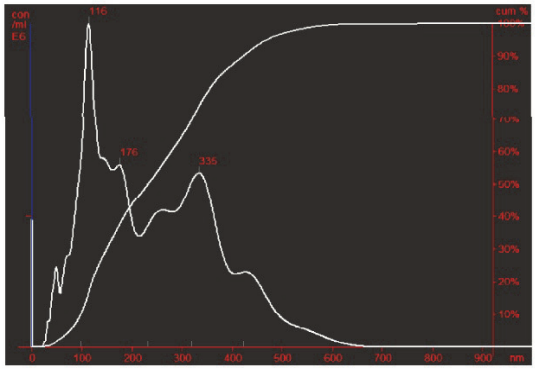
Figure 3.6. NanoSight DLS assembly with sample cell, blue laser and microscope. All particles can be observed on the screen.

NANOSIGHT

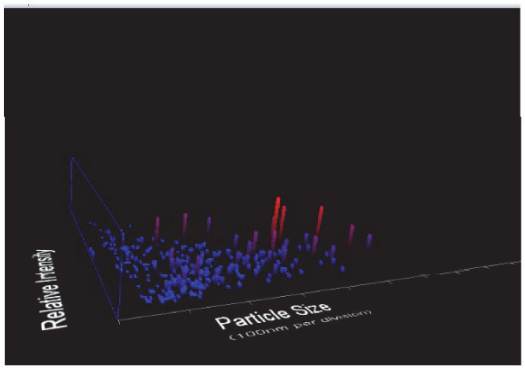
ANALYSIS REPORT

Nanoparticle Tracking Analysis (NTA) Version 2.2 Build 0366

Sample:
Video File: Carson plant MF Feed 02.avi; Analysis no. 002
Date/Time of Capture: 26 August 2011 13:58
Operator: k
Comments:



Particle Size / Concentration



Particle Size / Relative Intensity 3D plot

Bin Centre (nm)	Concentration E6 particles / ml	Percentile Undersize
10	0.982	0.21%
30	2.232	0.28%
50	9.891	1.83%
70	12.893	4.13%
90	22.190	7.70%
110	42.288	14.45%
130	35.758	23.78%
150	28.553	30.03%
170	27.747	35.89%
190	24.352	41.61%
210	17.852	45.84%
230	18.363	49.53%
250	20.640	53.71%
270	21.067	58.16%
290	21.239	62.59%
310	23.698	67.30%
330	26.497	72.69%
350	24.718	78.24%
370	18.313	82.78%
390	12.852	85.91%
410	11.428	88.37%
430	11.566	90.81%
450	10.299	93.16%
470	7.713	95.05%
490	5.355	96.38%
510	3.947	97.32%

Bin Centre (nm)	Concentration E6 particles / ml	Percentile Undersize
530	3.199	98.06%
550	2.636	98.67%
570	2.041	99.16%
590	1.428	99.52%
610	0.885	99.75%
630	0.479	99.89%
650	0.223	99.96%
670	0.087	99.99%
690	0.027	100.00%
710	0.006	100.00%
730	0.001	100.00%
750	0.000	100.00%
770	0.000	100.00%
790	0.000	100.00%
810	0.000	100.00%
830	0.000	100.00%
850	0.000	100.00%
870	0.000	100.00%
890	0.000	100.00%
910	0.000	100.00%
930	0.000	100.00%
950	0.000	100.00%
970	0.000	100.00%
990	0.000	100.00%
1000-2000	0.000	100.00%

Results
Mean: 245 nm
Mode:116 nm
SD: 127 nm
D10: 98 nm
D50: 232 nm
D90: 423 nm
User Lines: 0, 0 nm
Concentration: 4.73 x 10^8 particles/ml
Completed Tracks: 335

Measurement Conditions
Temperature: 22.00 °C
Viscosity: 0.95 cP
Frames Per Second: 30.00
Measurement Time: 60 of 60 seconds
Drift Velocity: 0 nm/s
Camera Shutter: 1449

Analysis Conditions
Blur: Auto
Detection Threshold: 12 Multi
Min Track Length: Auto
Min Expected Size: 80 nm

Figure 3.7. Example output from NanoSight analysis. NanoSight results relate to particle size.

3.2.4. GE Sievers 900 TOC Analyzer

The online TOC analyzer was provided by GE Analytical Division located in Boulder, CO. The TOC analyzer provided the trends in total organics concentration, rather than the individual particle count. The analyzer is based on the oxidation of organic compounds to form carbon dioxide using UV irradiation and a chemical oxidizing agent (ammonium persulfate). For each sample, the concentration of inorganic carbon species is determined and

the total organics concentration is expressed as a difference between the total carbon (TC) and the inorganic carbon (IC).For this instrument, the tests were performed as follows:

1. The test used 40 mL of sample in a sterile glass vial. A new glass vial is used for each sample.
2. The detection range for the instrument is 0.003 ppm to 50 ppm. No dilution was necessary for any of the samples under the concentration range used.
3. The equipment has the option of "Auto reagent," which selects the amount of acid and oxidizer for the reaction automatically. If the Auto reagent option is not selected, then these values need to be entered manually.
4. The TOC analyzer provides the organics concentration in ppm, once the analysis is complete.
5. The instrument is flushed with distilled water before subsequent tests and prior to system shutting down.

3.2.5. Hach FilterTrak 660sc Turbidimeter

The Hach FilterTrak 660sc is a product of HACH Company. The corporate office is located in Loveland, CO. The system uses a nephelometer laser sensor to detect turbidity levels, which has the ability to detect particles smaller than 0.1 μm. The FilterTrak 660sc offers online monitoring and can detect turbidity as low as 0.3 mNTU. The basic procedure of operation during pilot testing is as follows:

1 The sensor was cleaned before the testing with distilled water to ensure proper turbidity readings..

2 The sample inlet on the turbidimeter was connected to proper lines for analysis.

3 The sample drain on the turbidimeter was connected to the waste line for proper waste discharge.

4 The turbidimeter was flushed for 15 minutes to prevent contamination by previous samples.

5 After continuous readings, the data was exported from the turbidimeter to an Excel spreadsheet.

3.2.6. Additional Parameters

The samples were also analyzed for pH, conductivity, turbidity, and nitrate. Total nitrate was analyzed using the portable spectrophotometer for certain samples. Details regarding the instruments used for the additional parameters are found in Table 3.2.

Table 3.2. Additional Instrumentation Used for Bench Test Analysis

Name/ Designation	Description	Picture
Turbidity Meter Hach 2100Q	Range: 0 to 1000 NTU. Accuracy: (+/-) 2% of reading plus stray light from 0 to 1000 NTU. Repeatability: 1% of reading or 0.01 NTU, whichever is greater. Resolution: 0.01 NTU Stray Light: 0.02 NTU Operating Temperature: 0 to 50 °C	
Electrical Conductivity/ TDS meter	Conductivity Range: 0 to 199.9 mS/cm Resolution and Accuracy: ± 0.05% Full Scale ±1% Full Scale + 1 LSD Temperature Range: -10.0 to 110.0 °C Temp. Resolution and Accuracy: 0.1 °C	
Hach DR 2800 Spectrophotometer	Wavelength Range: 340 to 900 nm Wavelength Accuracy:(+/-) 1.5 nm Wavelength Reproducibility:< 0.1nm Wavelength Resolution: 1nm Operating Temperature:10 to 40 °C max. 80% relative humidity	

3.3. Results

Sampling schedule followed the test protocol. Plant conditions on different days and various physical factors while collecting the samples have been summarized in Table 3.3.

3.3.1. Test Parameters

Tests were performed to determine the number of particles, size distribution, and concentration. These tests were directed to determine the sensitivity of these instruments for different water samples.

3.3.2. Submicron Particles Analysis by DLS Systems

The details and results from the bench-scale testing by the DLS systems is discussed in the next subsections.

Table 3.3. Summary of Sampling Conditions at WRD and WBMWD

Date	Time	Location	Conditions
6/22/2011	1:30 p.m.	WRD	Plant resumed at 11 a.m. after shut down on 6/13/2011
6/24/2011	11:00 a.m.	WRD	
6/27/2011	12:30 p.m.	WRD	
6/28/2011	12:00 p.m.	WBMWD	
6/29/2011	11:00 a.m.	WRD	
6/30/3011	12:30 p.m.	WBMWD	RO Train 3 offline due to leakage
7/1/2011	12:30 p.m.	WRD	Plant shut down, restarted at 10 a.m.
7/5/2011	12:00 p.m.	WBMWD	RO Train 1 offline for cleaning schedule
7/6/2011	11:00 a.m.	WRD	
7/7/2011	10:30 a.m.	WBMWD	RO Train 2, 9 offline for maintenance
7/12/2011	10:30 a.m.	WRD	
7/20/2011	10:00 a.m.	WRD	
7/25/2011	10:30 a.m.	WRD	
7/26/2011	11:00 a.m.	WBMWD	

3.3.2.1. Nanotrac

Nanotrac provides the size distribution as percentage representation for different particle sizes. The procedure explained in the analytical protocol was followed to obtain trends of the samples. Analysis time of 90 seconds was used for all the samples unless otherwise noted. The instrument is designed to detect the presence of particles and their size rather than providing the absolute number of particles. The following figures show the size distribution of various samples obtained from Nanotrac. Percentage volume distribution is plotted for different particle sizes. Figure 3.8 shows the particle distribution of MF feed from WRD. As expected, this sample has relatively high concentration of particles of various sizes. Different peaks indicate the nonhomogenous nature of the wastewater.

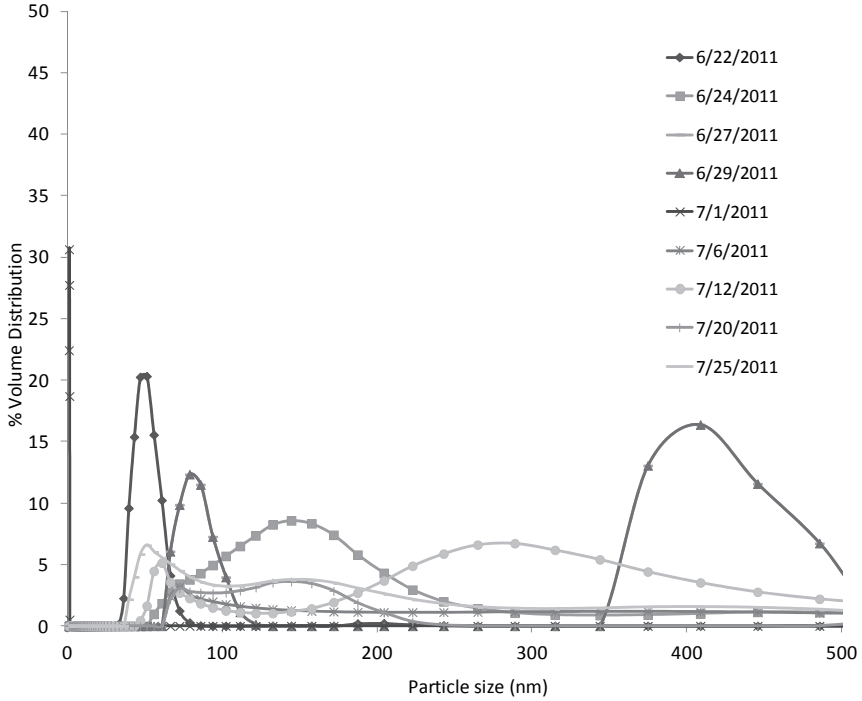


Figure 3.8. Size distribution of MF feed samples from WRD obtained from Nanotrac DLS.

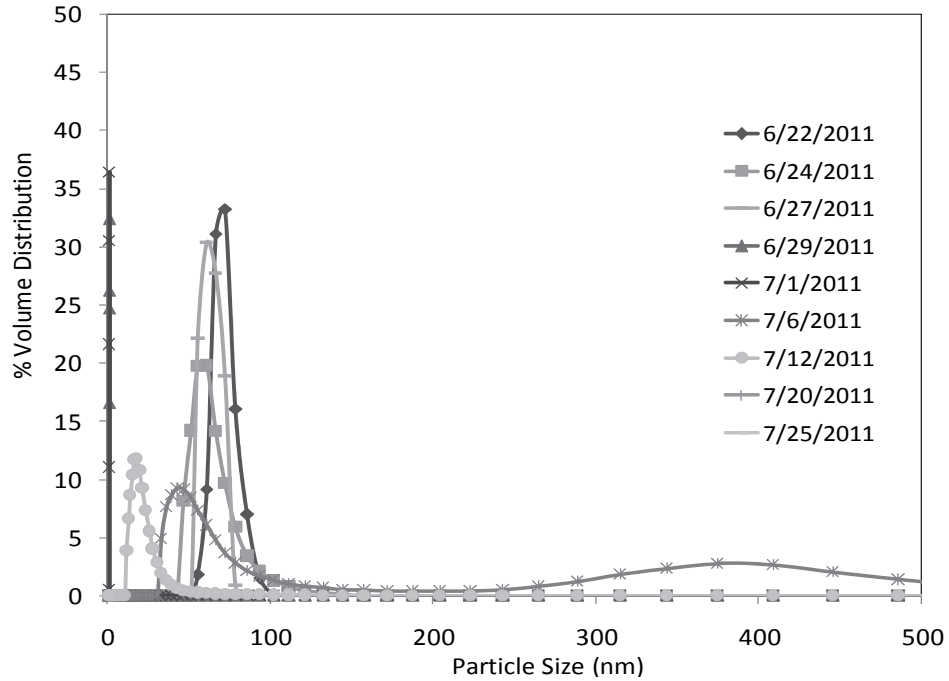


Figure 3.9. Size distribution of MF filtrate samples from WRD obtained from Nanotrac DLS.

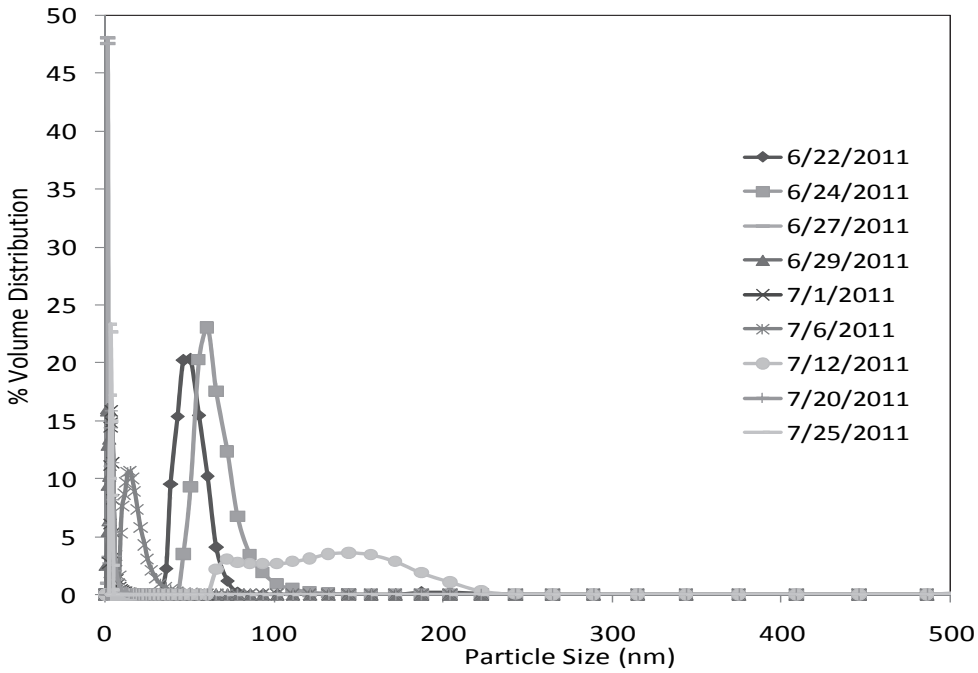


Figure 3.10. Size distribution of RO feed samples from WRD obtained from Nanotrac DLS.

Figures 3.9 and 3.10 are plotted for two similar samples—MF filtrate and RO feed from WRD. The MF membrane installed at WRD is the Pall Microza 0.1 micron PVDF membrane. Although the results indicate the presence of larger particles on certain days, a well-defined separation by the MF membrane for all particles greater than 100 nm can be observed. Compared to the MF feed distribution, the MF filtrate distribution is close to the membrane cut-off in Figure 3.9. Figure 3.10 shows a similar trend in the RO feed distribution. As expected, both the MF filtrate and the RO feed samples are seen to have similar particle distribution with no significant changes observed.

Figure 3.11 shows the distribution of RO permeate samples as a function of particle size where no distinct peaks are observed. The RO membrane installed at WRD is Hydranautics ESPA-2. Figure 3.12 presents a detailed view of the same RO permeate data. Distinct peaks are observed below 2 nm. These are much lower than the cutoff of 25 nm observed on NanoSight and can be related to noise, vibrations, or background interference according to the manufacturer.

Similar graphs were plotted for WBMWD samples. WBMWD receives water from the Hyperion wastewater treatment plant in Los Angeles. Figure 3.13 shows the particle distribution of MF feed water up to 6μm. The Siemens 90M10C MF units are installed at WBMWD. Each unit typically comprises of polypropylene hollow fiber membrane modules with an average pore size of 0.2 micron. Siemens Memcor CS submersible membranes are also installed at the plant. Samples were collected from both trains during this testing.

Figure 3.14 presents the MF filtrate results from Nanotrac for both trains. The pressure MF is marked Train-1 and submersible MF is marked Train-2. The distribution is seen to increase

close to the membrane cutoff and is an indicator of membrane separation. Similarly, Figure 3.15 provides a combined result of RO feed samples from both MF trains.

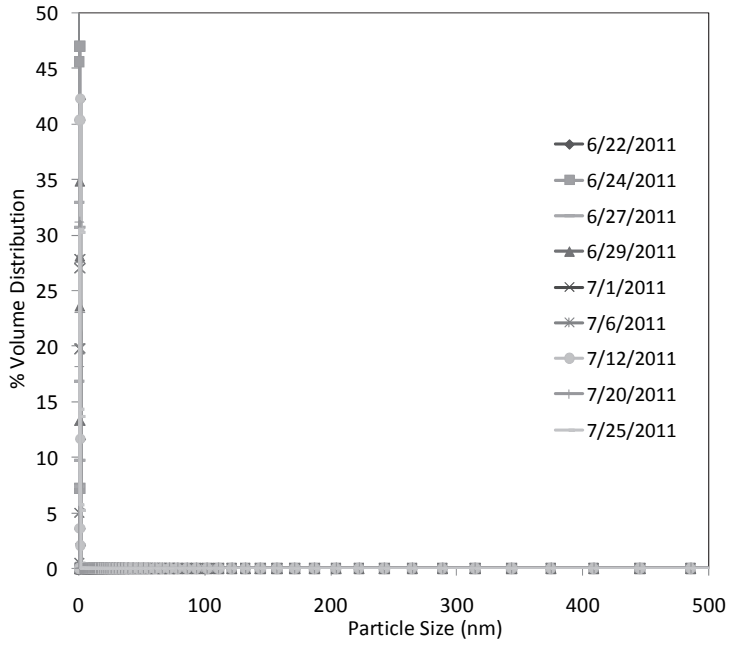


Figure 3.11. RO permeate samples from WRD obtained from Nanotrac.

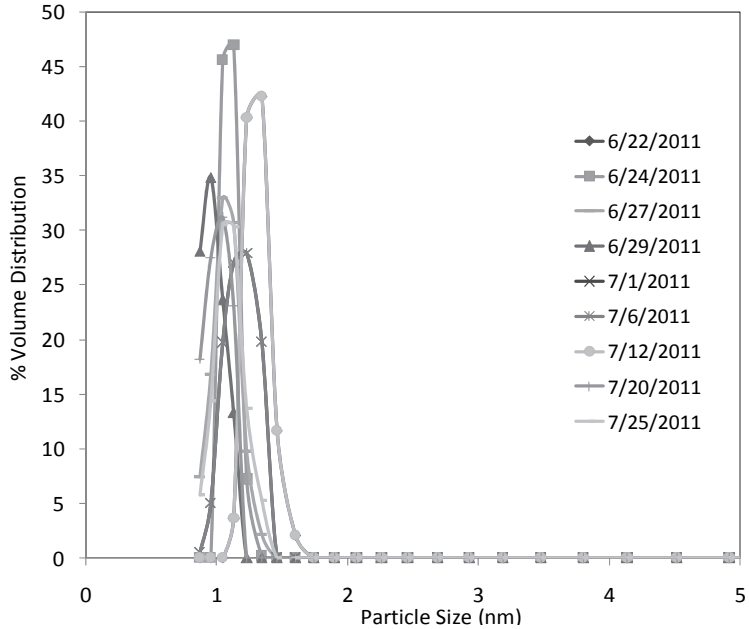


Figure 3.12. Reduced scale for RO permeate samples from WRD obtained from Nanotrac.

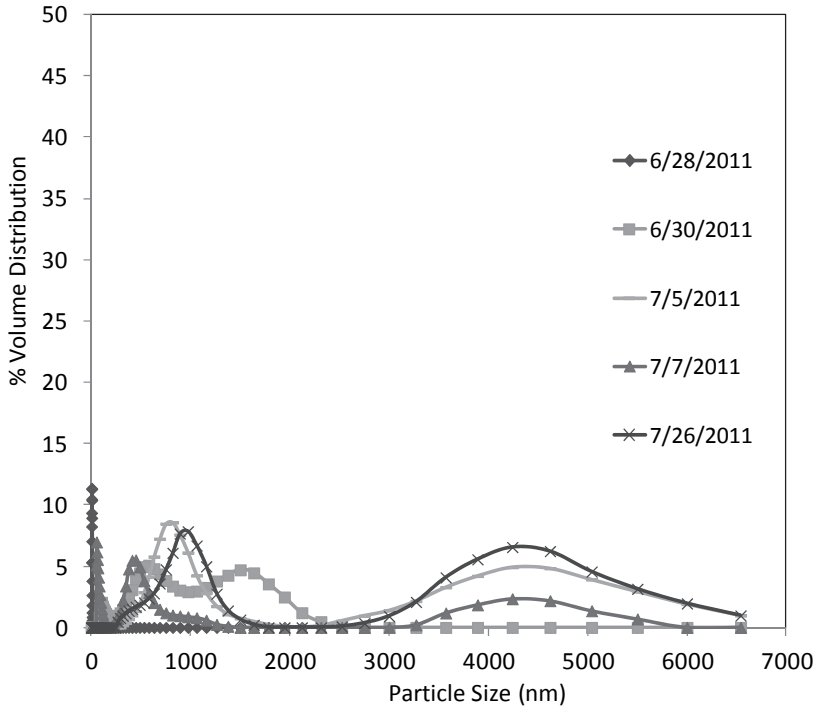


Figure 3.13. Size distribution of MF feed from WBMWD.

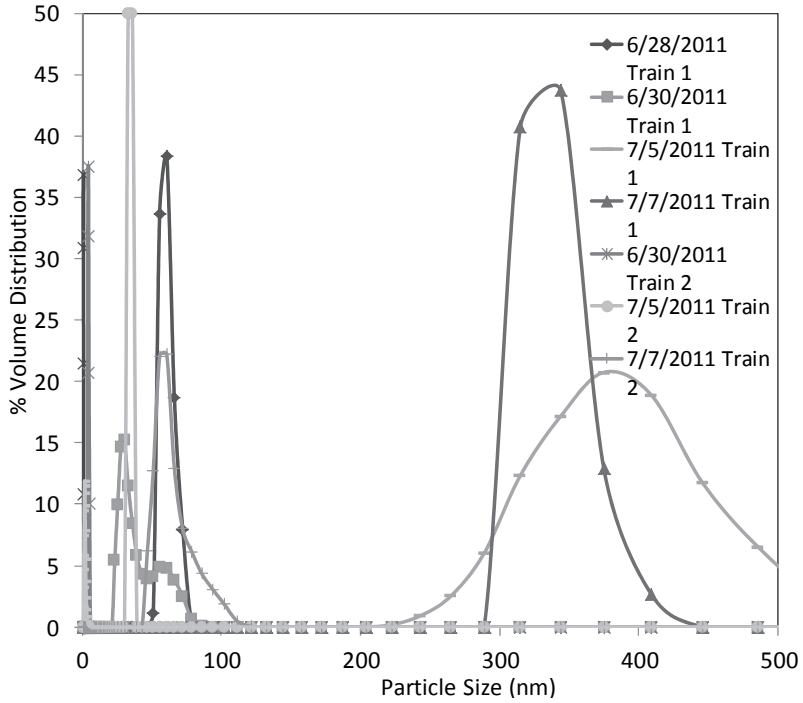


Figure 3.14. MF filtrate distribution of WBMWD samples obtained from Nanotrac.

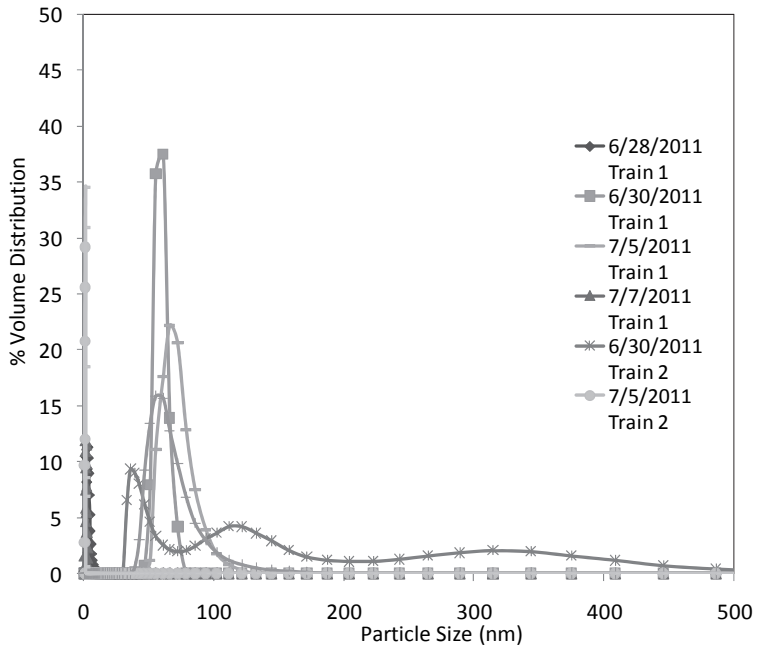


Figure 3.15. Distribution of RO feed samples from WBMWD.

Similar to WRD, the RO membrane installed at WBMWD is Hydranautics ESPA-2 for both trains. Figures 3.16 and 3.17 provide the RO permeate distribution obtained from WBMWD using Nanotrac. The results are comparable to the RO permeate obtained from WRD. Peaks can be seen at less than 2 nm on the detailed view graph likely caused by noise.

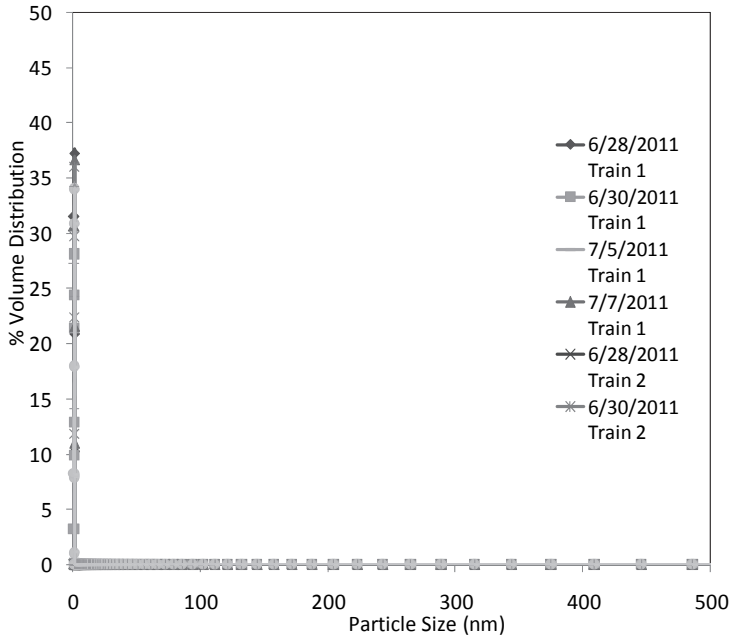


Figure 3.16. RO permeate samples from WBMWD obtained from Nanotrac.

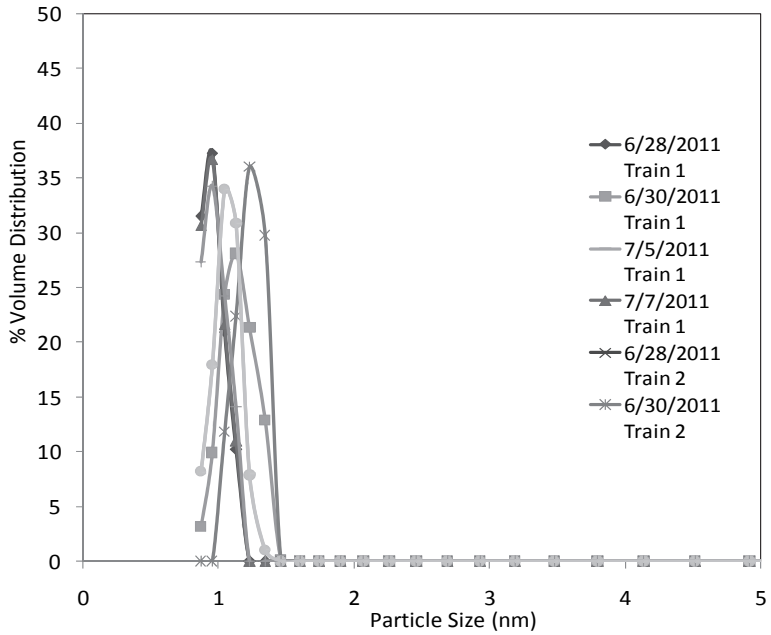
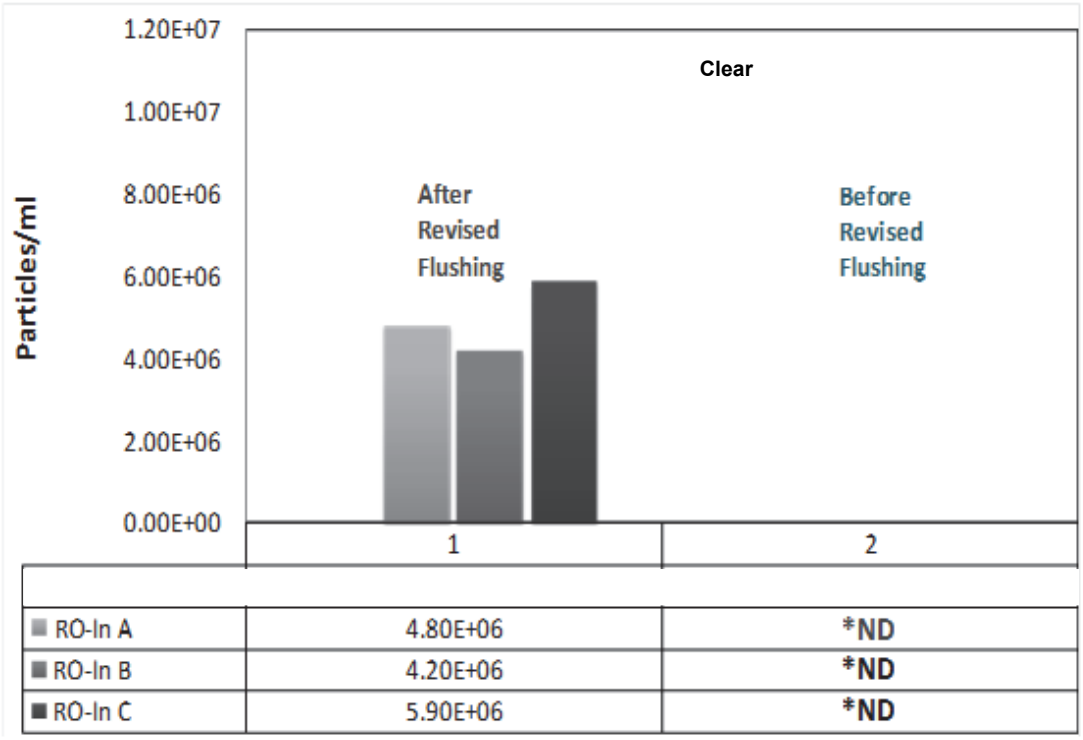


Figure 3.17. Reduced scale for RO permeate samples from WBMWD obtained from Nanotrac

3.3.2.2. NanoSight

The basic procedure described in the analytical protocol was followed. However, the initial samples provided inconsistent results with the NanoSight DLS. As a result, rigorous control tests were performed to determine the optimum time and procedure for operation. Control tests were aimed at determining suitable recording time, number of records for each sample, as well as processing parameters.

Early results signified the carryover of the slightest liquid from the previous test. Incorrect lens focus, short duration of recording, and potential cross contamination were some of the reasons behind such inconsistencies. The test procedure was revised to include extensive cleaning using distilled water before the start of a new set of samples with the right settings. Figure 3.18 shows the results before and after modifying the flushing procedure. No detections were observed in the RO feed before the new flushing procedure. Particles were observed only after using the new procedure.



*ND= Not Detected

Figure 3.18. WRD RO feed sample on 6-24-2011 before and after the flushing procedure.

The modified flushing procedure is as follows:

1. Open the sample cell and wash the glass with 5 mL of distilled water.
2. By observing the sample cell under the microscope, check the field of view on the screen for any residue particles left from the previous test, ensuring there are no trapped air bubbles.
3. Rinse and repeat steps 1 and 2 with distilled water until no particles are observed. Record the number of blank readings.
4. With a new syringe, flush the sample cell with 5 mL liquid starting with the most dilute sample. The sample cell capacity is 0.3 mL.
5. After flushing, inject the sample and observe the particles using DLS.
6. Record the video, followed by processing with the right settings and save on the computer.
7. Repeat steps 4 through 6 to obtain triplicate readings for each sample.
8. After testing the last sample, wash the sample cell with distilled water.

After adopting the new flushing procedure, no particles were found in the RO permeate. However, high fluctuations were observed in the analysis of the sample from the same location on different days. The samples were collected around the same time for each event, but that does not necessarily correlate to the backwash cycle because of the logistics in distributing the samples and the limited access to the site. Figure 3.19 shows the fluctuations

of particle concentration between the MF filtrate samples from WRD on different days. The particle counts for the same sample location changed by over two orders of magnitude on certain days.

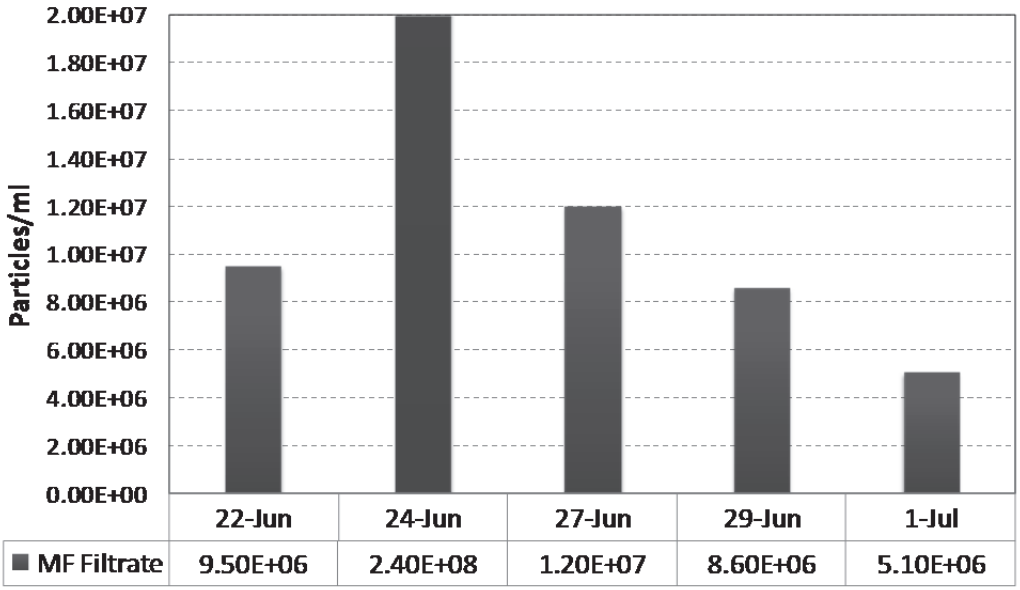


Figure 3.19. High fluctuations in results of virus size particle count for WRD MF filtrate sample on different days before revised flushing method.

Therefore, multiple readings were taken for the same sample to recognize the standard deviation between constant readings. Recording only three videos for 90 seconds showed a considerable fluctuation. Therefore, after developing the flushing procedure, rigorous control tests were performed to determine the optimum setting. For the control tests, differential time and constant number of readings were considered to determine the relative standard deviation (RSD).

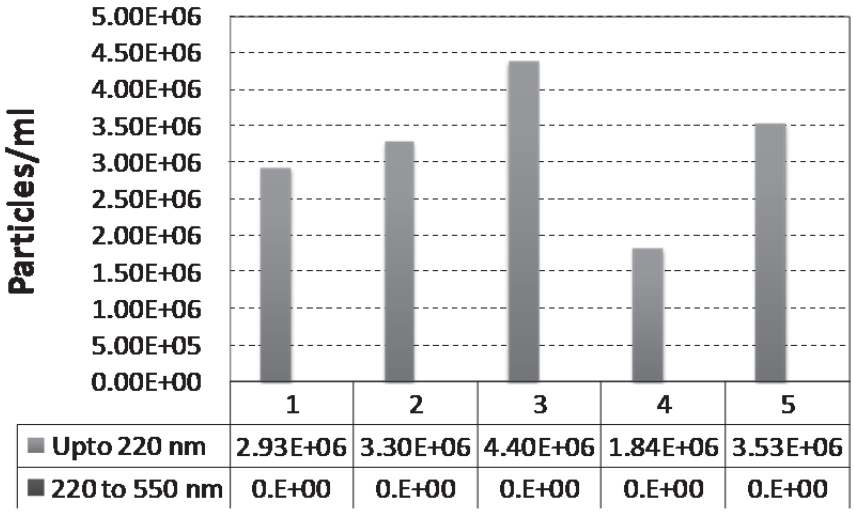
Determining the effect of different time interval:

The purpose of optimizing the setting was to determine if changing the recording time has any effect on the fluctuations observed in the results. Accordingly, consecutive videos were recorded to observe the variation in average particle count for each time interval. Two sets of readings were taken

- 5 consecutive readings of 90 seconds each for a total run time of 450 seconds
- 3 consecutive readings of 150 seconds each for a total run time of 450 seconds

A constant record duration was divided into larger intervals. Dividing the recording time into larger intervals showed only a marginal decrease in the RSD values. Five different measurements, each with a 90-second record are shown in Figure 3.20. The instrument responded to the nonhomogenous nature of the sample with certain deviation in each reading. The RSD value for this test was 27%. The deviation observed was due to an unsteady change

in the number of particles in the field of view. The duration of record step was then increased to improve the number of particles analyzed in the limited field of view. In Figure 3.21, the effect of increasing the recording time interval to 150 second is presented. The standard deviation decreased marginally to 21%. No detections were measured for 220 to 550 nm size particles.



	1	2	3	4	5
Upto 220 nm	2.93E+06	3.30E+06	4.40E+06	1.84E+06	3.53E+06
220 to 550 nm	0.E+00	0.E+00	0.E+00	0.E+00	0.E+00

Figure 3.20. Five consecutive readings of virus size particle count 90 second for RO feed from WRD on 7-1-2011, RSD 27%.

Determining the effect of number of consecutive readings for the same sample:

Five records of 150 seconds were compared to the previous 90-second records and a further decrease in the RSD was observed. Figure 3.22 shows the relatively consistent results for the 150-second record for the five records. The data reflects five separate readings from the same sample in the instrument. A longer duration of time increased the number of particles considered for the analysis for relative dilute samples. The total analysis time, however, increased from 450 seconds to 750 seconds in this case, which may be unrealistic in terms of on-time monitoring method.

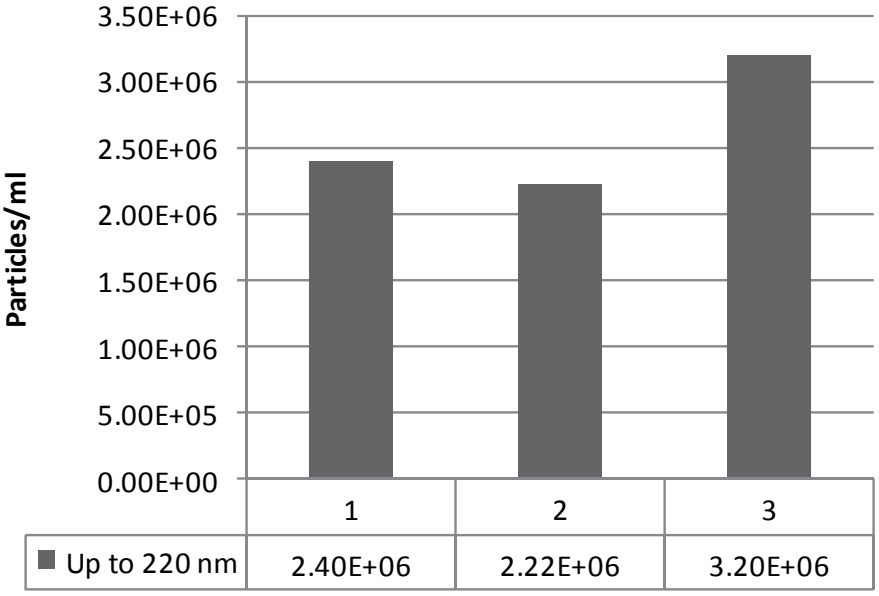


Figure 3.21. Three consecutive readings of virus size particle count 150 second for RO feed from WRD on 7-1-2011, RSD 21%.

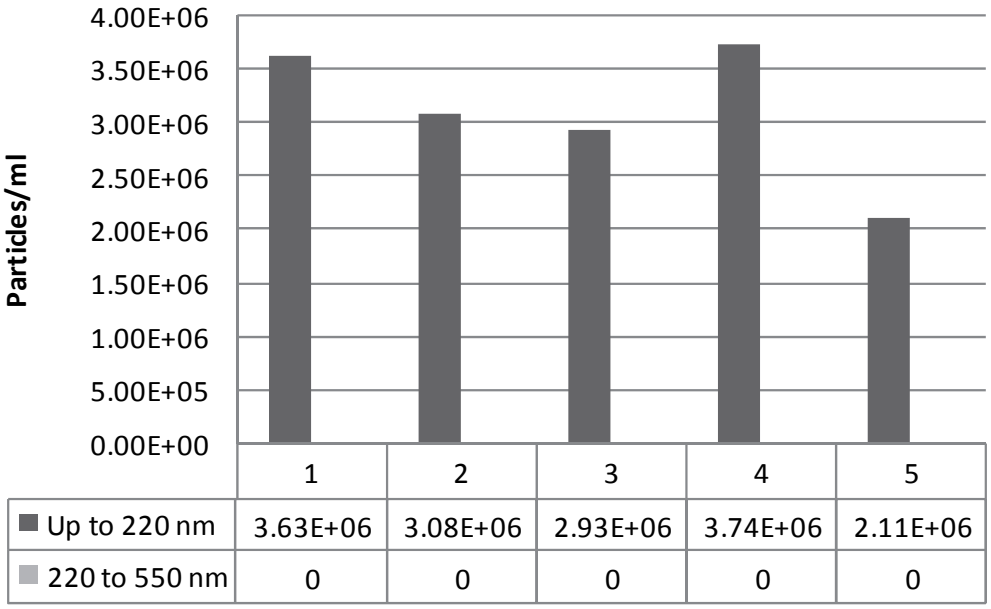
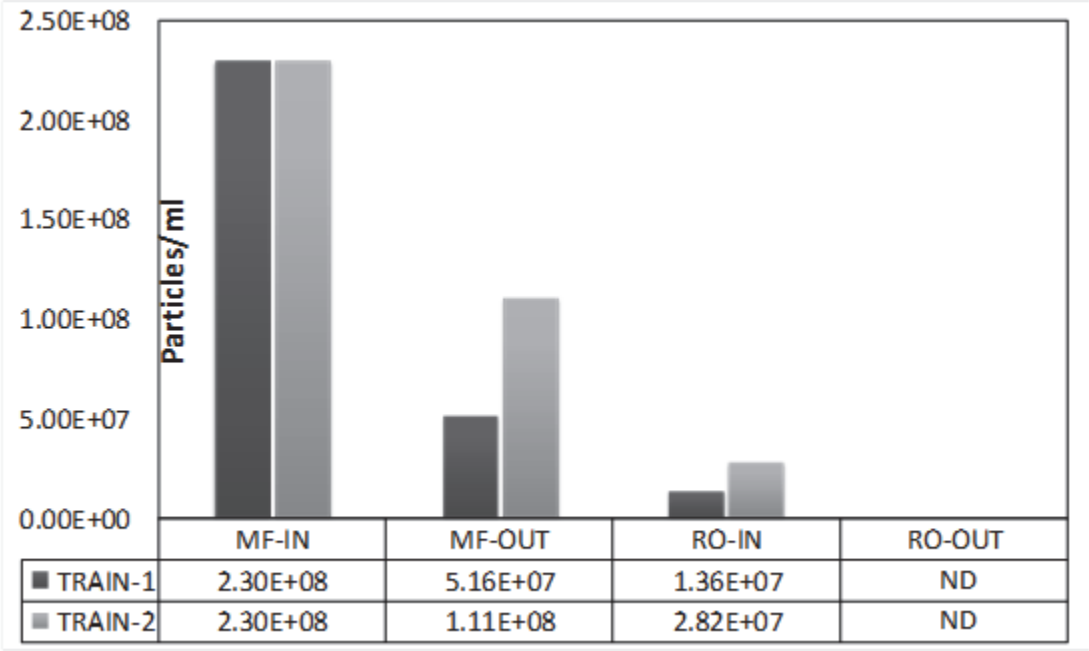


Figure 3.22. Five consecutive readings of virus size particle count 150 second for the same sample of RO feed from WRD on 7-1-2011, RSD 16%.

The procedure described in Section 3.2 was followed. For dilute samples, longer recording time was used to improve accuracy of the readings. Typically, 150-second videos were used for MF feed with high concentration, whereas RO feed was recorded for a total time as high as 450 seconds. As mentioned, longer recording time allows for averaging greater number of particles and reduces the effect of limited field of view. This may be essential for lower concentration samples, such as RO permeate. Figure 3.23 shows the spatial trend for the

samples collected from WBMWD. Two separate trains were considered for comparison and to assess how effectively the change in particle count is demonstrated by the instrument with change in the particle concentration in the sample. The dark columns indicate Train-1—Pressure MF, whereas the light columns show Train-2—Submersible MF at WBMWD. The samples were collected from different MF trains. No detections were measured in the RO permeate, as expected. The dark columns indicate first train from the pressure MF, whereas the light columns indicate second train from the submersible MF. Although there are differences in the MF filtrate from two different types of MF system, this data was based on a limited sampling event. More systematic sampling and analysis would be required to confirm and compare the differences between the two.



	MF-IN	MF-OUT	RO-IN	RO-OUT
TRAIN-1	2.30E+08	5.16E+07	1.36E+07	ND
TRAIN-2	2.30E+08	1.11E+08	2.82E+07	ND

Figure 3.23. Spatial trend of virus size particle count at WBMWD on 6-30-2011.

Figure 3.24 shows a similar trend for the WRD. No detection was measured for the RO permeate samples. MF feed and MF filtrate samples were analyzed for comparison. It was observed that for greater particle concentrations of MF feed (~10^8 particles/mL), the NanoSight provided accurate particle count with 10 to 15% RSD. However, for lower particle concentrations, the instrument cannot reliably reproduce the results for the same sample, with RSD decreasing to nearly 35% for comparatively dilute samples of MF filtrate and RO feed (approximately 10^6 particles/mL).The samples were collected from available sampling points at the participating plants as is—without any modification of sampling ports or in processing the sample, with an exception of flushing the sampling line. The instrument was found to provide fairly consistent results when multiple particles enter the field of view constantly over a longer period of time. However, for lower concentration, the statistical calculations of the software exhibited higher deviations as fewer particles start entering the field of view for a set time.

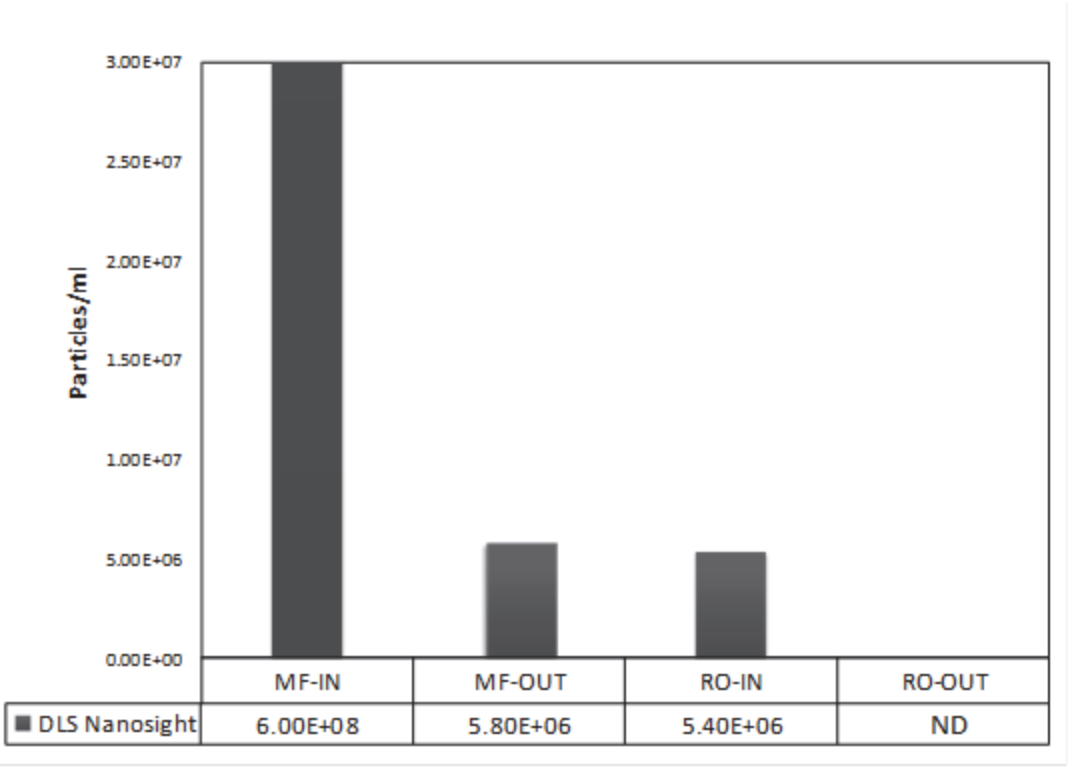


Figure 3.24. Trend displaying the spatial variation of NanoSight virus size particle count from WRD for 7-6-2011.

Figures 3.25, 3.26, and 3.27 show the temporal variation of the MF filtrate and RO feed samples from WRD and WBMWD respectively. The two vertical lines represent the days on which sample collection was resumed after the plant was shut down. The first shutdown took place on June 13 and the first sample was taken on June 22. There was another shutdown on the night of June 30 for 12 hours. Samples were collected immediately after start-up on July 1. The graph confirms the similar particle count for MF filtrate and RO feed samples on different days for both plants.

Table 3.4 summarizes the NanoSight particle count obtained for different samples obtained from WRD. These data were used to obtain spatial and temporal variation plots. Similarly, Table 3.5 provides the data for both MF trains from WBMWD. The samples marked "Not Available" from WBMWD were not collected.

Figures 3.28 and 3.29 present a summary of all the data obtained from WRD. A steady decrease in the particle count can be observed from MF feed to RO permeate. The MF filtrate and RO feed show similar counts. The MF filtrate shows a higher count on the day of plant shutdown.

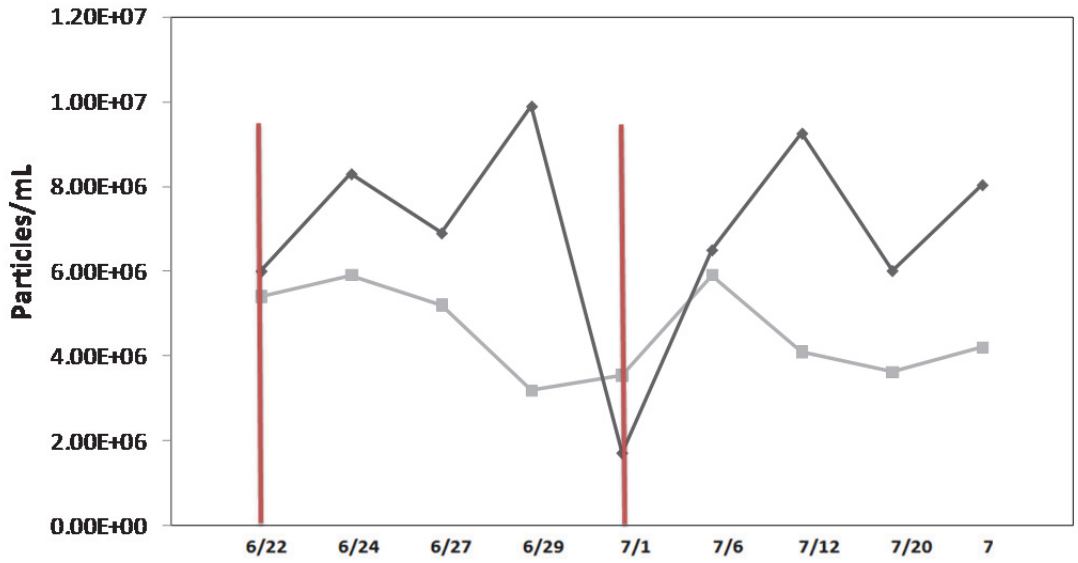


Figure 3.25. Temporal variations of virus size particle count for NanoSight results from WRD. Light line indicates RO feed, whereas the dark line indicates MF filtrate. No detection was observed for RO permeate

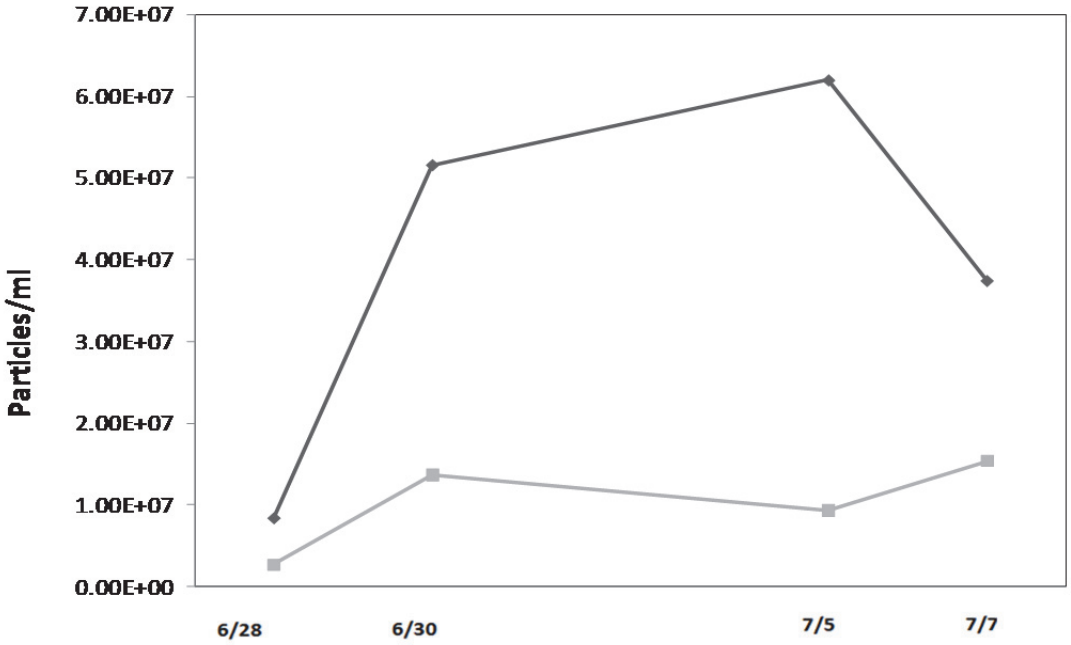


Figure 3.26. Temporal variations of virus size particle count for Train-1 from WBMWD. Light line indicates RO feed, whereas the dark line indicates MF filtrate. Train-1 samples were not collected on 7-26-2011. No detection was observed for RO permeate

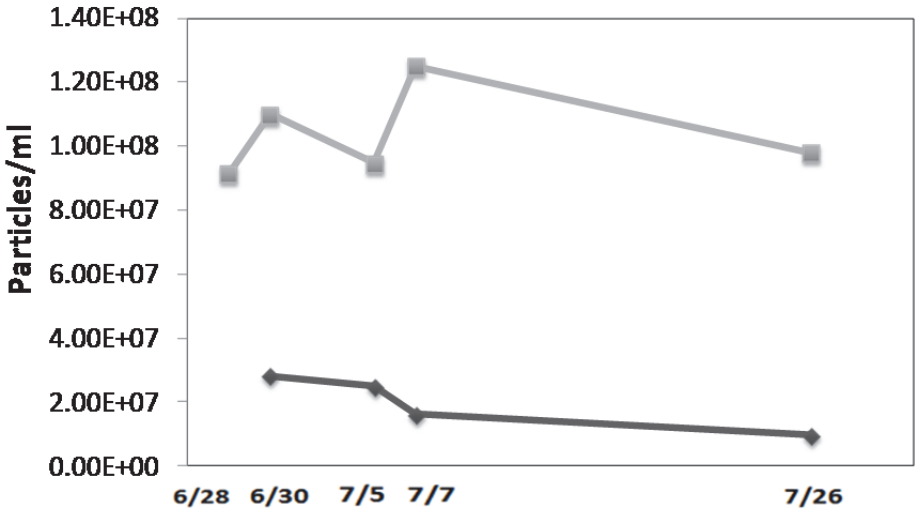


Figure 3.27. Temporal variations of virus size particle count for Train-2 from WBMWD. Dark line indicates RO feed, whereas the light line indicates MF filtrate. RO feed sample was not collected on 6-28-2011. No detection was observed for RO permeate

Table 3.4. Summary of Concentration in Virus Size Particles/mL on NanoSight for Samples from WRD

Date	MF Feed	MF Filtrate	RO Feed	RO Permeate
6/22/2011	7.35E+08	6.00E+06	5.40E+06	ND
6/24/2011	7.14E+08	8.30E+06	5.90E+06	ND
6/27/2011	5.50E+08	6.90E+06	5.20E+06	ND
6/29/2011	7.70E+08	9.90E+06	3.20E+06	ND
7/1/2011	6.30E+08	1.70E+06	3.56E+06	ND
7/6/2011	6.00E+08	6.50E+06	5.90E+06	ND
7/12/2011	6.16E+08	9.26E+06	4.10E+06	ND
7/20/2011	4.63E+08	6.01E+06	3.74E+06	ND
7/25/2011	5.31E+08	8.38E+06	4.21E+06	ND

Note. ND = not detected.

Table 3.5. Summary of Concentration in Virus Size Particles/mL on NanoSight for Samples from WBMWD

Date	MF feed	MF Filtrate Train-1	RO Feed Train-1	RO perm Train-1	MF Filtrate Train-2	RO feed Train-2	RO perm Train-2
6/28/2011	4.50E+08	8.30E+06	2.70E+06	ND	9.14E+07	NA	ND
6/30/2011	2.30E+08	5.16E+07	1.36E+07	ND	1.10E+08	2.82E+07	ND
7/5/2011	2.00E+08	6.20E+07	9.29E+06	ND	9.47E+07	2.48E+07	ND
7/7/2011	2.50E+08	3.74E+07	1.53E+07	ND	1.25E+08	1.62E+07	ND
7/26/2011	4.20E+08	NA	NA	NA	9.80E+07	9.60E+06	ND

Notes. NA = not available; ND = not detected.

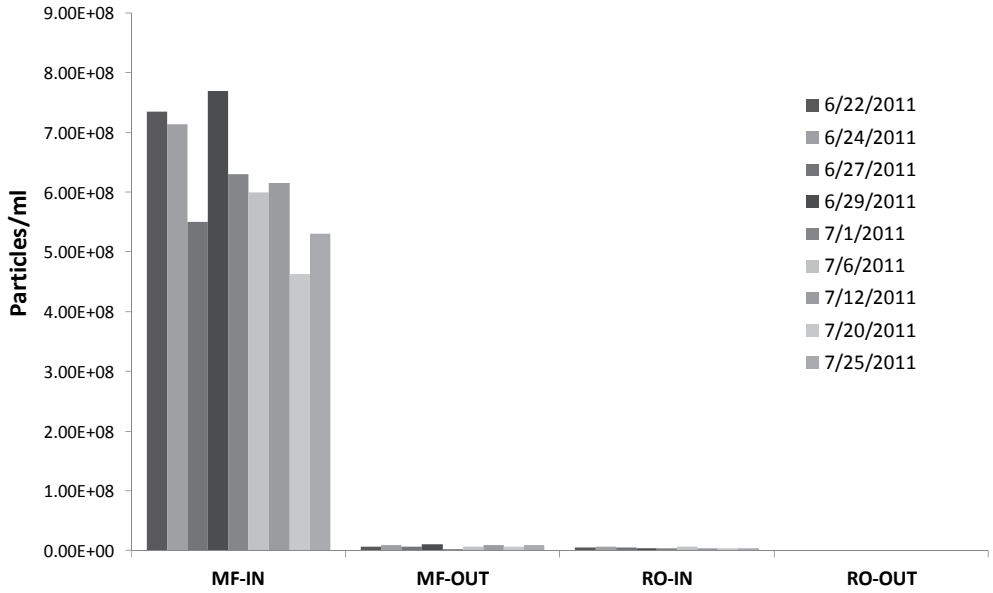


Figure 3.28. Comparison of virus size particle count of NanoSight results for all samples from WRD.

Figure 3.30 provides a similar summary of the Train-1 from WBMWD. This train has a pressure MF membrane. Figure 3.31 summarizes the results for the Train-2 from WBMWD. All three data sets confirm that MF filtrate and RO feed samples are similar and no particles are detected in RO permeate. Compared to WRD, RO feed samples at WBMWD have higher particle count. MF filtrate from Train 2 of WBMWD shows the highest particle count.

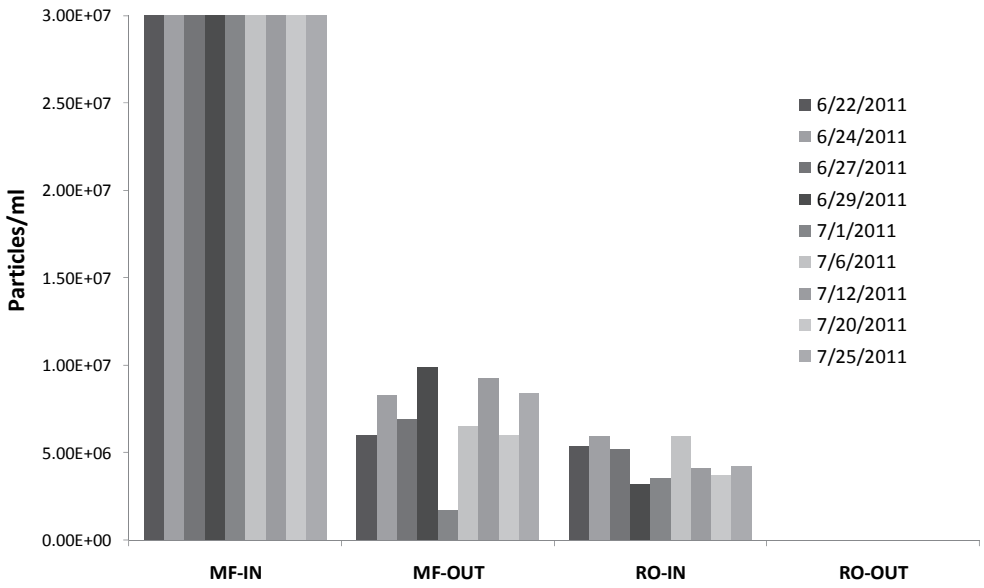


Figure 3.29. Expanded view comparison of virus size particle count of NanoSight results for all samples from WRD (full view is shown in Figure 3.28).

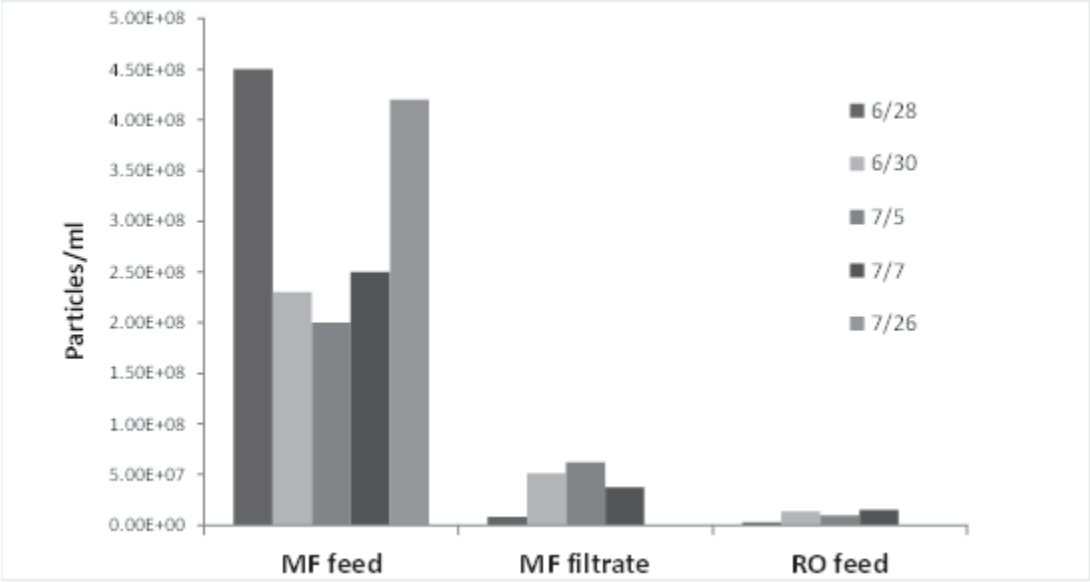


Figure 3.30. Comparison of virus size particle count of NanoSight data for Train-1 (Pressure MF) at WBMWD.

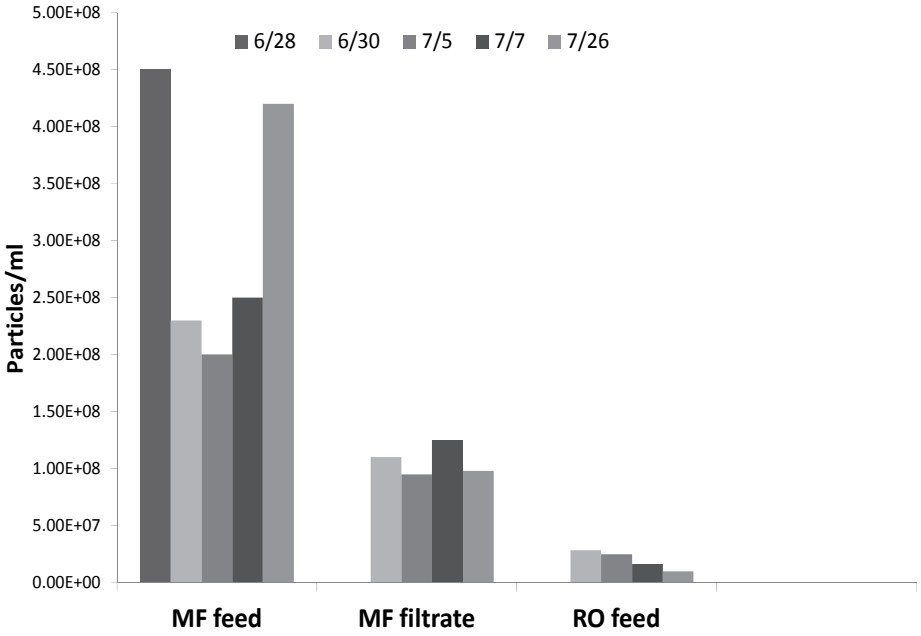


Figure 3.31. Comparison of virus size particle count of NanoSight data for Train-2 (submerged MF) at WBMWD.

The results obtained from NanoSight were compared with the EFM results from UCI. The second set of samples from the recycled water treatment plant was delivered to the UCI laboratory within 2 hours of sampling. Testing on the EFM started immediately.

3.3.3. Submicron Particles Analysis for Epifluorescence Microscopy

Epifluorescence microscopy (EFM) is used to obtain direct viral counts to compare results with the DLS systems in order to validate DLS as a potential technique for real time virus monitoring. The setup of the EFM system is shown in Figure 3.32.

The basic procedure for determining the EFM count is as follows:

1. Sample fixation: 25% glutaraldehyde is added to the water sample to the final concentration of 0.5% in a 4-inch chamber for 15 min.
2. Sample filtration: The sample is then filtered at room temperature onto a 0.02 µm pore-size membrane (Anodisc) with vacuum pressure no greater than 20 kPa. Then 0.8 mL of sample is filtered for MF feed, MF filtrate, and RO feed, and the volume of RO permeate filtered is 10 mL.
3. Sample staining: After the particles are collected onto the filter membrane, the Anodisc filter is laid on 50 µL staining solution (25X SYBR Green) for 15 min in the dark.
4. Slide preparation: After staining, the Anodisc filter is mounted onto a glass slide with 30 µL anti fading solution. A clean cover slip with 5–10 µL anti-fading solution is used to cover the sample filter.
5. Slide Counting: Slides are counted immediately with Epifluorescence microscope under blue-green light excitation .The Olympus Microfire image system is employed

to take a picture of each slide. At least 15 random view fields are recorded for each slide.

Figure 3.32. Epifluorescence Microscope (EFM) System.

Figures 3.33 through 3.36 present the comparison of the NanoSight and EFM results for the samples collected from WRD. The DLS result showed a trend very similar to the EFM result. In general, the NanoSight count is higher than the EFM count, as the DLS detects both organic and inorganic particles. For the MF feed sample, the large variation on July 1may be due to the shutdown of the plant before sampling.

Figure 3.34 presents the comparison of the virus-sized particles in the MF effluent from WRD. The first two days after the first shutdown (June 22 and June 24), the particles were below the detection limit of EFM. The detection limit was computed on the basis of the volume of water collected onto the 0.02µm anodisc filter. Normally, about 10 to 15 ml of water sample was used because an increase in water volume will increase the filtration time significantly by slowing the filtration rate. The detection limit computation considers the view field on the filter. If no virus particles are observed on any of the 10 randomly selected fields, it is considered that the concentration is less than 1 per volume filtered (the detection limit). The EFM counts also showed a decrease after the second shutdown on July 1.

Although particles were detected in the preliminary test in May, the RO feed samples were below the detection limit of EFM. Figure 3.35 shows the difference in the detections of EFM and NanoSight DLS. The viral-size particles detected by the DLS could be inorganic, which cannot be detected by EFM.

Figure 3.36 shows the comparison of RO permeate samples on different days with no detections on either instrument.

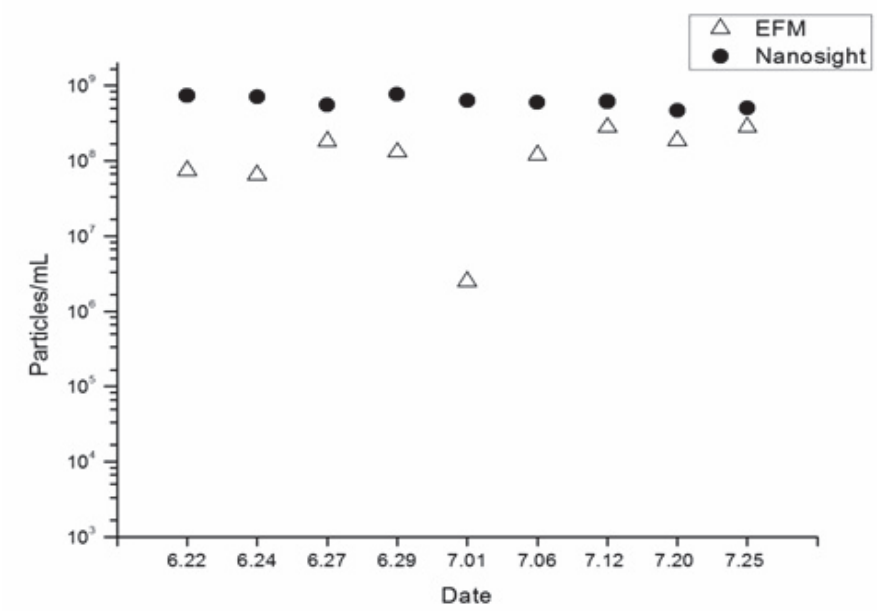


Figure 3.33. Viral-size particles—comparison of the EFM and NanoSight for MF feed samples from WRD.

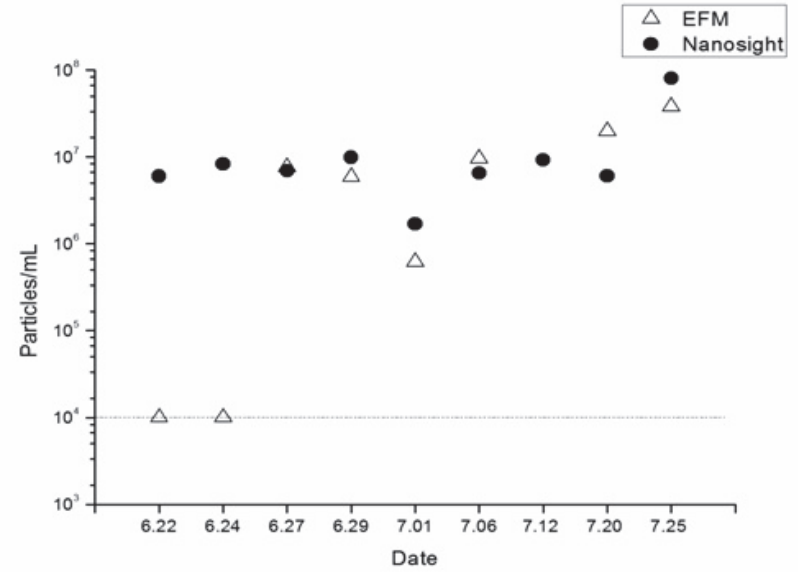


Figure 3.34. Viral-size particles—comparison of the EFM and NanoSight for MF filtrate samples from WRD.

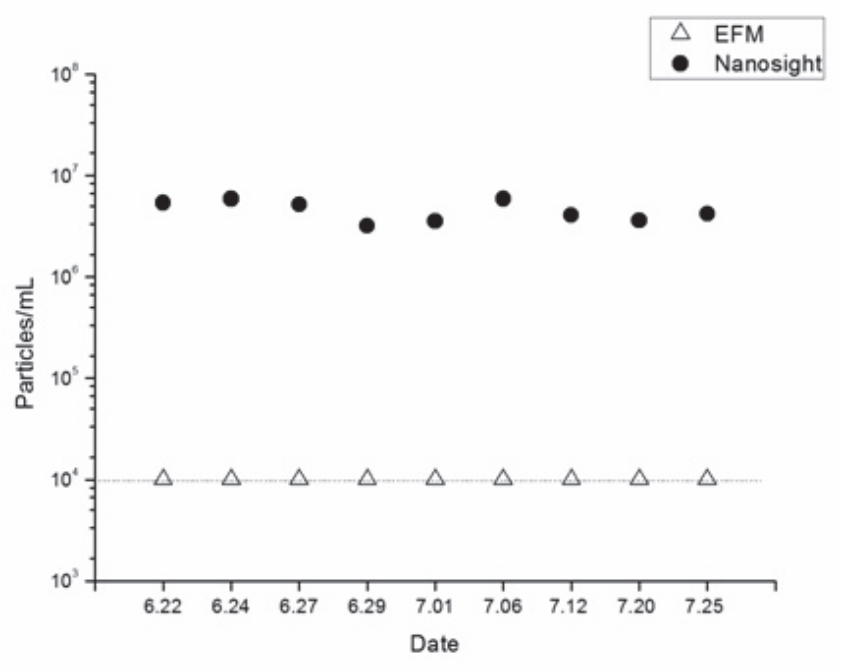


Figure 3.35. Viral-size particles—RO feed samples from WRD.

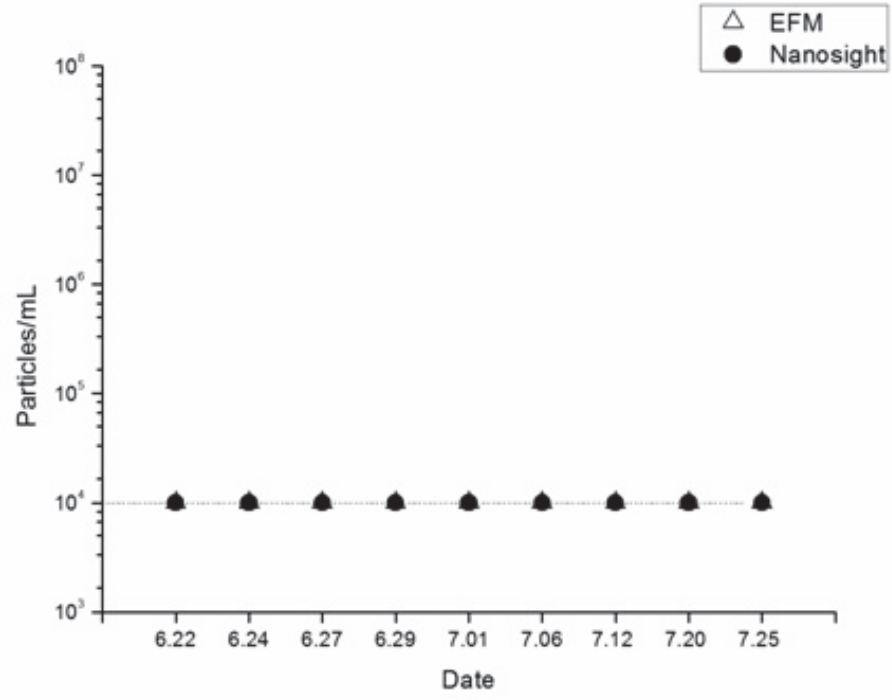


Figure 3.36. Viral-size particles—RO permeate samples from WRD.

Based on the results, a correlation was developed to compare the particle count results obtained from the two methods of analysis: EFM and DLS. Because the data consisted of a wide range of counts from about10^6 particles/mL for RO feed to about10^8 particles/mL for MF feed, the EFM data was plotted against the NanoSight data on a log scale. Figure 3.37 shows the line with a linear regression with an R^2 value of 0.91. A linear relationship

indicates consistent particle count between the two instruments. The correlation was developed on the basis of the virus-size particles from WRD on different days.

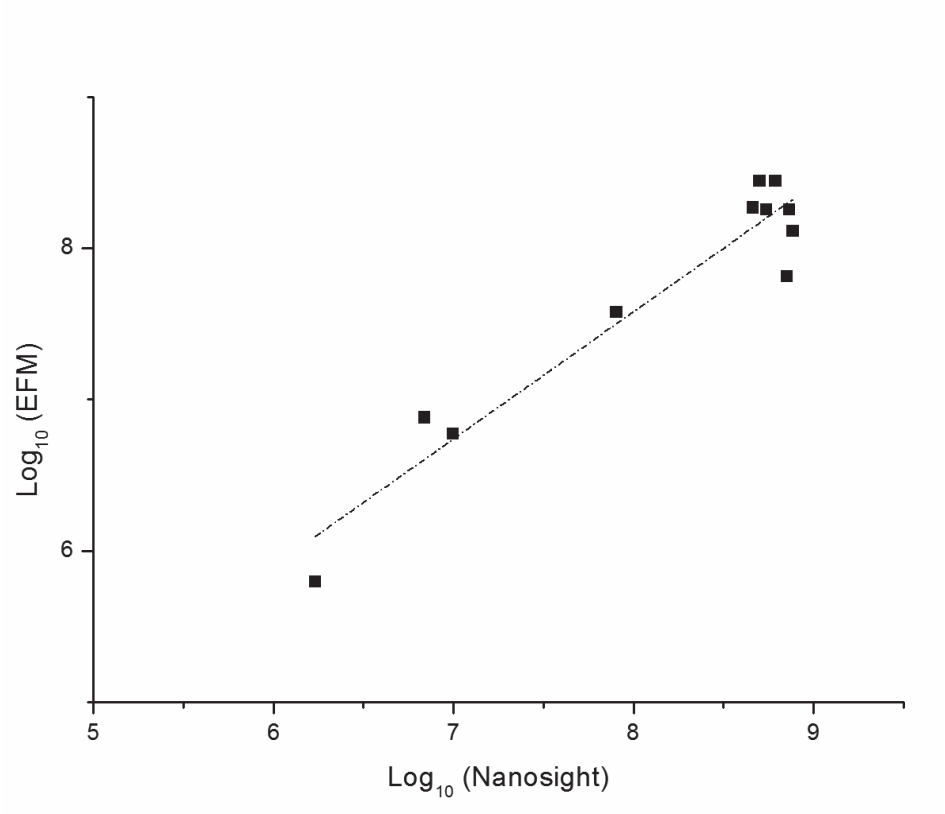


Figure 3.37. Correlation of virus-size particles between EFM and DLS NanoSight.

Table 3.6 summarizes the EFM particle count obtained for different samples from WRD. Similarly, Table 3.7 provides the EFM data for both the trains from WBMWD. The samples marked "Not Available" from WBMWD were either not collected or testing was not complete.

Table 3.6. Summary of Virus Count Using EFM for All Samples Obtained from WRD

Date	MF Feed	MF Filtrate	RO Feed	RO Permeate
6/22/2011	7.40E+07	ND	ND	ND
6/24/2011	6.50E+07	ND	ND	ND
6/27/2011	1.80E+08	7.60E+06	ND	ND
6/29/2011	1.30E+08	5.90E+06	ND	ND
7/1/2011	2.50E+06	6.20E+05	ND	ND
7/6/2011	1.20E+08	9.60E+06	ND	ND
7/12/2011	2.79E+08	NA	ND	ND
7/20/2011	1.86E+08	1.99E+07	ND	ND
7/25/2011	2.80E+08	3.80E+07	ND	ND

Notes. NA= not available; ND = not detected.

Table 3.7. Summary of Virus Count Using EFM for All Samples Obtained from WBMWD

Date	MF Feed	MF Filtrate Train-1	RO Feed Train-1	RO Perm Train-1	MF Filtrate Train-2	RO Feed Train-2	RO Perm Train-2
6/28/2011	9.20E+08	NA	1.00E+06	ND	NA	4.20E+06	ND
6/30/2011	6.60E+07	7.00E+05	2.90E+05	ND	ND	ND	ND
7/5/2011	6.70E+08	3.00E+06	2.20E+05	ND	NA	2.00E+05	ND
7/7/2011	4.70E+08	NA	NA	ND	1.80E+05	1.40E+05	ND
7/26/2011	5.55E+09	NA	NA	NA	3.03E+07	6.46E+06	ND

Notes. NA = not available; ND = not detected.

3.3.4. Additional Parameters

3.3.4.1. TOC Analysis

Tests on the TOC analyzer followed the particle-size analysis. As mentioned in Section 3.2, the instrument requires a fixed amount of acid and oxidizer reagent for the oxidation of organic compounds. TOC results are displayed as an average of four results, the typical analysis time being 30 min. Samples were first analyzed using the Auto reagent mode. During this operation, the instrument determines the acid and oxidizer amount for the specific sample, which is unique to that sample. These values can be generalized to analyze the same

sample on different days using the manual mode, where acid and oxidizer values are defined by the user.

Figures 3.38 and 3.39 show the trends in the TOC (ppm) and pH of RO feed and RO permeate samples from WRD and WBMWD on different days. On the basis of the data, WBMWD samples have higher organics. The RO feed from WRD can be seen to be close to 6 ppm on different days. The decrease in the organics after RO permeate is clearly reflected as consistent values at less than 0.3ppm. Immediately after the first shutdown at WRD, the organics content was high but remained steady thereafter. No significant change was observed after the second shutdown. Both the TOC and pH at WBMWD have been steady.

The spatial variation in TOC and pH values for the samples is presented in Figures 3.40 and 3.41. At WBMWD where the particle count of MF feed was observed to be much higher, there has been about a 30% decrease in the TOC across the MF membrane. The TOC content in the RO permeate is less than 0.3 ppm. The decrease in the TOC shows the effectiveness of the membrane in removing organics. The relatively similar TOC measure across the MF at WRD suggests that the water has higher dissolved organics.

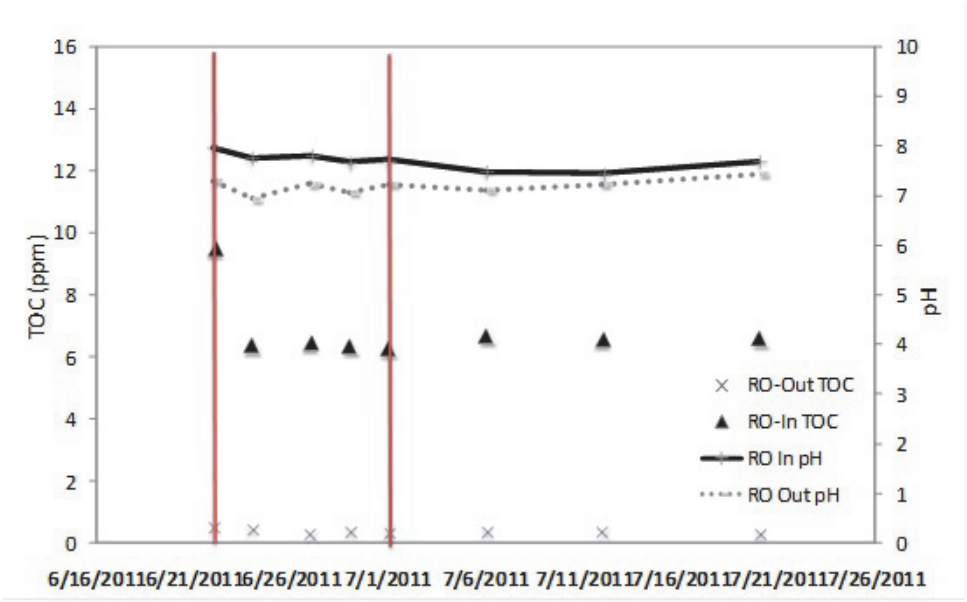


Figure 3.38. Temporal variation in TOC and pH results for RO feed and RO permeate samples from WRD.

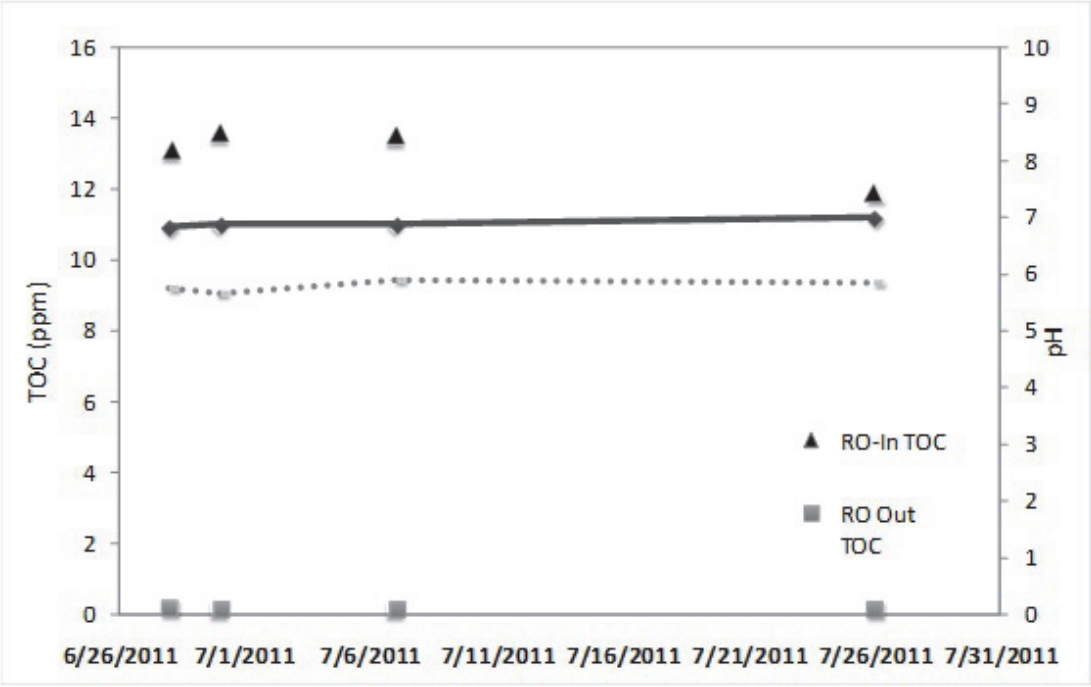
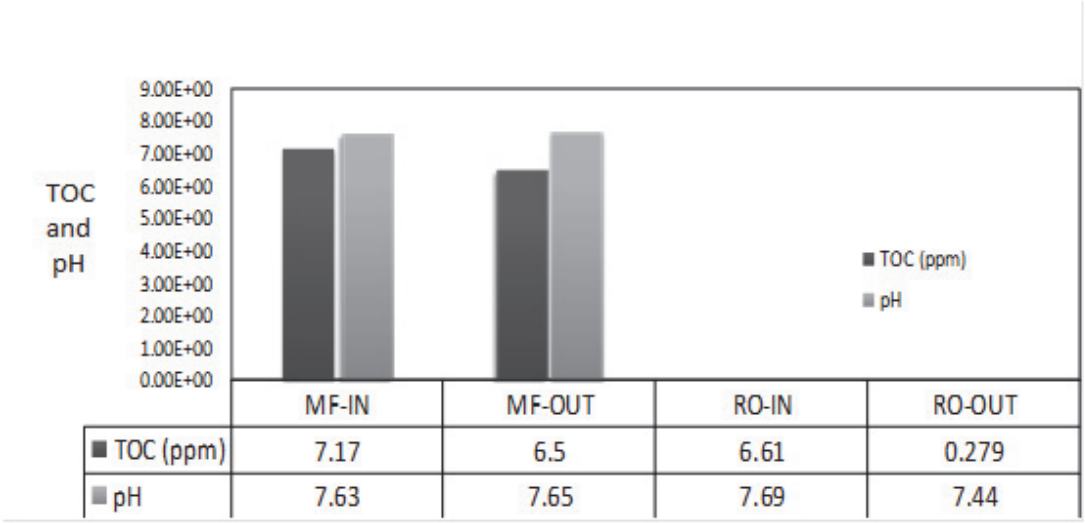


Figure 3.39. Temporal variation in TOC and pH results for RO feed and RO permeate samples from WBMWD.



	MF-IN	MF-OUT	RO-IN	RO-OUT
TOC (ppm)	7.17	6.5	6.61	0.279
pH	7.63	7.65	7.69	7.44

Figure 3.40. Spatial variation in TOC and pH results for RO feed and RO permeate samples.

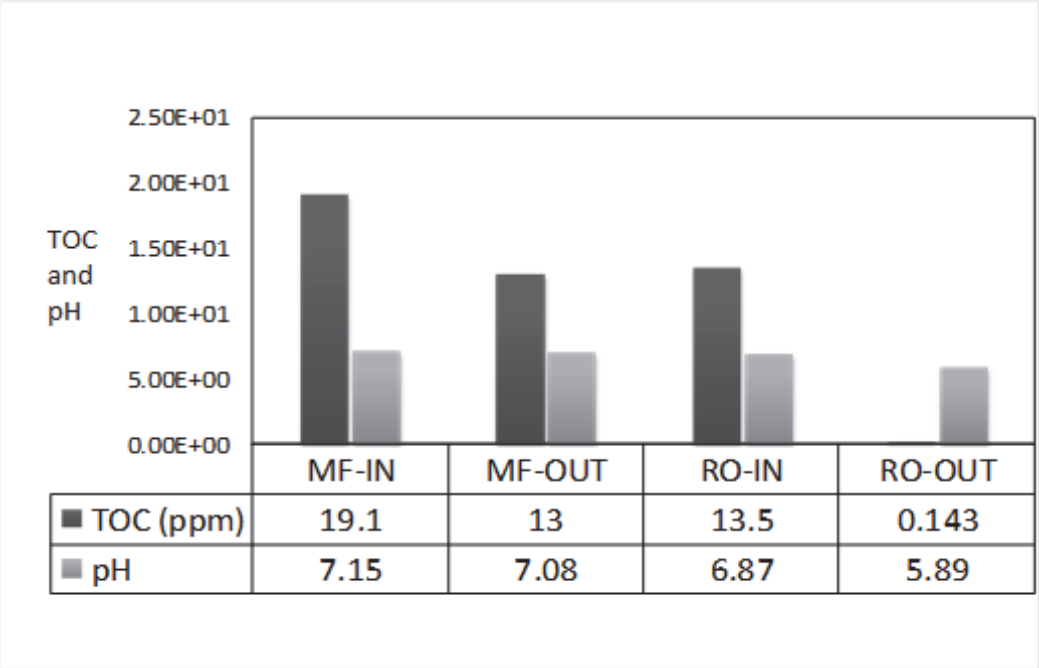


Figure 3.41. Spatial variation in TOC and pH results for RO feed and RO permeate samples.

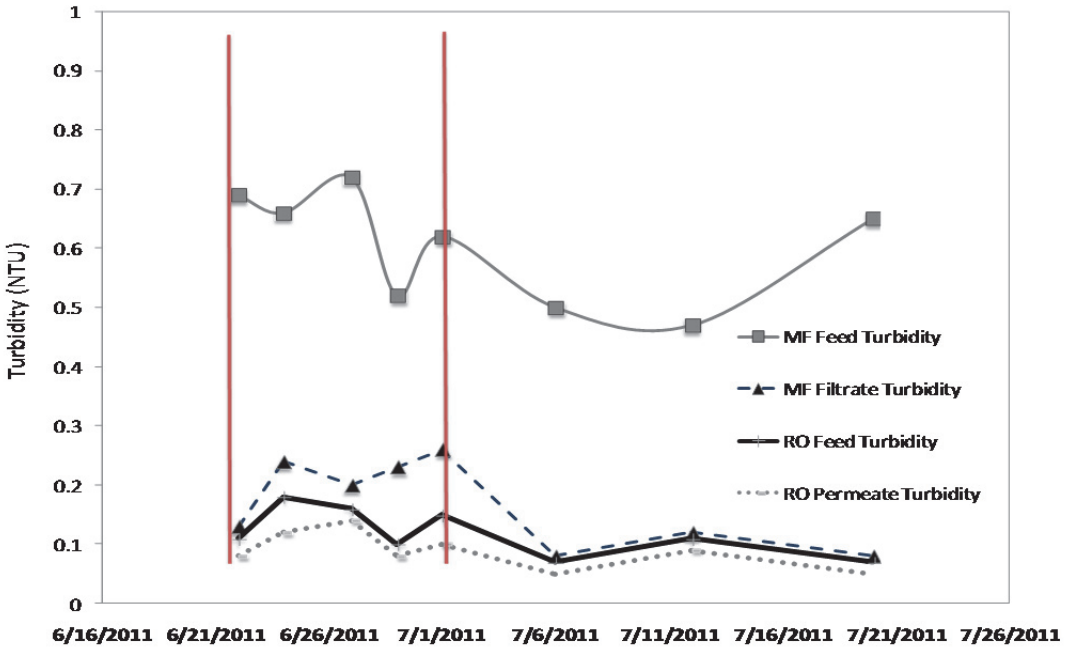


Figure 3.42. Plot of turbidity from WRD on different sampling dates.

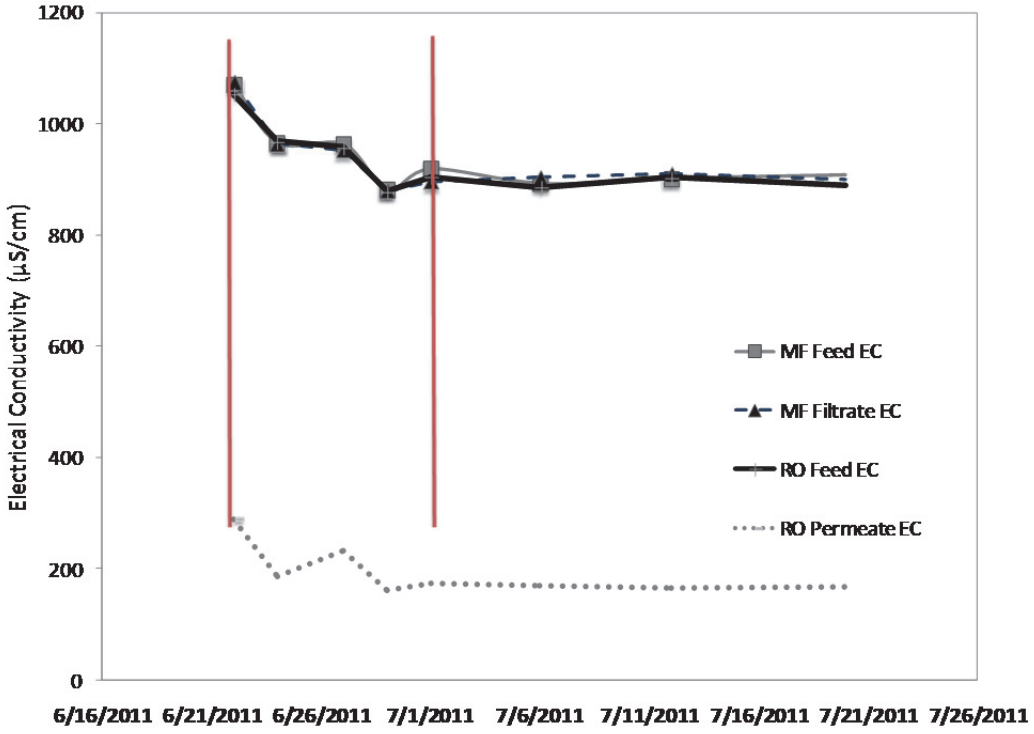


Figure 3.43. Plot of conductivity from WRD on different sampling dates.

3.3.4.2. Turbidity and Conductivity

The turbidity and conductivity of all samples were also measured. The turbidity and conductivity data from WRD for all the samples on different sampling days have been plotted in Figures 3.42 and 3.43 respectively. The MF feed is seen to vary between 0.5 NTU and 0.7 NTU. After the initial fluctuation in the conductivity, the values seem to stabilize after the second shutdown. The conductivity of the MF feed, MF filtrate, and RO feed is between 800 and 1200 (µS/cm). A clear reduction in the conductivity can be seen across the RO membrane.

Chapter 4

RO Pilot Testing

4.1. RO Pilot Scope

The onsite RO pilot test used a combination of real-time RO feed and the online instruments sensitive toward any small change in this phase. Nanotrac DLS instrument, which provides the particle size distribution, was selected on the basis of its sensitivity, stability, and ability to detect a wider range of fine particles. However, the instrument currently available for the study was limited for operation in the grab analysis mode only. This particular DLS instrument has a probe-based sensor. A possible online analysis using this instrument would require a system with solenoid valves for a semicontinuous sampling, similar to certain online instruments.

In order to confirm detections from the DLS instrument as well as continuous monitoring of the plant conditions, online analyzers that can respond to the slightest change were incorporated in the analytical protocols, including the GE TOC analyzer and HACH laser turbidity meter. Additional data were also collected using a conductivity meter installed on the RO pilot system.

4.1.1. RO Pilot Specifications

Figure 4.1 shows the pilot scale RO system used to accommodate the project requirements. The pilot system had a two-stage array where the concentrate from the first-stage membranes was treated again by the second-stage membrane. The pilot system includes built-in permeate and concentrate flow meters, a feed and system pressure gauge, and a conductivity meter.

After installation of the online monitors, the RO pilot system was continuously operated, except on days of plant shut down. The system specifications are summarized in Table 4.1.

Table 4.1. Pilot RO System Specification

Model	**Customized 4-Membrane System with Codeline Pressure Vessels**
Dimensions (L x W x H)	32” x 25” x 58”
Weight (approx.)	450 lbs
Motor HP	5
Voltage	220
Amperage	13.8
Frequency	50/60 Hz
Feed Pressure Range	20 to 270 psi
Feed Flow Rate Range	4 to 12 GPM

Figure 4.1. RO pilot system used for this study at WRD.

4.1.2. Membrane Specifications

RO membranes were provided by CSM (Anaheim, CA) for the pilot study. This membrane is currently used at a similar recycled water facility at the WBMWD located in El Segundo, CA, a participating utility for this project. The specifications of the membranes used are summarized in Table 4.2.

Table 4.2. Specifications of the Reverse Osmosis Membranes

Model	RE 4040 FEN, CSM Membranes
Element Configuration	Spiral wound, Polyamide membrane
Permeate Flux	24.7 GFD
Effective Membrane Area	85 ft^2
Minimum Salt Rejection	99.5%
Dimensions	40.0” x 4.0” x 0.75”
Max. Operating Pressure	600 psi
Max. Operating Temperature	45 ^oC
Operating pH Range	3.0–10.0
CIP pH Range	2.0–11.0

4.1.3. Pilot Operating Conditions

With the CSM membranes installed, the RO pilot was operated for a total duration of two weeks. Table 4.3 lists the operating conditions at WRD for the pilot operation. The objective of the pilot run was to assess the sensitivity of the analytical methods. The condition for the pilot testing was slightly different than the actual operating condition of the plant at the time of sampling, but the pilot system offered an opportunity to provide a controlled condition for testing the methods during the challenge tests. The flux and recovery rate of the operating conditions are significantly higher than the plant operational scale because new CSM membranes were used. New membranes produce higher flux and recovery because of miniscule scale formation and fouling compared to old membranes. The WRD plant has an MF outlet pressure of 30 to 40 psi. In order to have a better controlled testing environment, the pilot was operated with an inlet pressure between 26 to 30 psi.

Table 4.3. Summary of Pilot Operating Conditions

Parameters	Values
Pilot Feed Pressure	26 to 30 psi
Temperature	22 to 29 ^{o}C
Recovery	80%
Permeate Flux	23.3 GFD
Permeate Flow Rate	5.5 GPM
Concentrate Flow Rate	1.5 GPM

4.1.4. RO Pilot Layout

Figure 4.2 shows the schematics of the RO pilot layout. The T-connectors were installed to bypass a part of permeate to the online monitors. A valve installed at the end of the permeate line was used to throttle the flow to the instruments. The feed line to the instruments consisted of semirigid Teflon tubes, whereas the discharge was led directly to the sewer. Figure 4.3 shows the actual setup at WRD showing instrument locations connected.

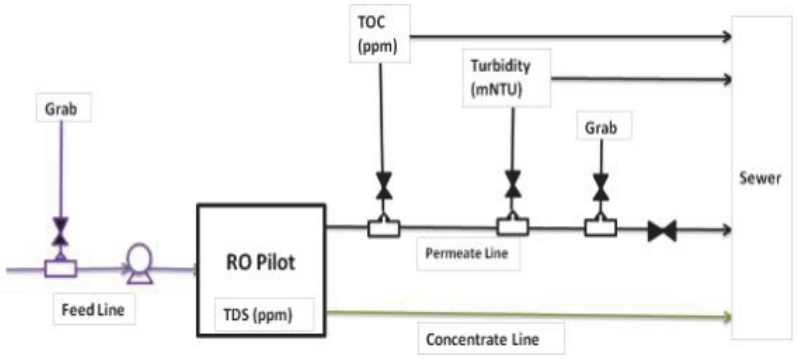


Figure 4.2. Pilot process layout.

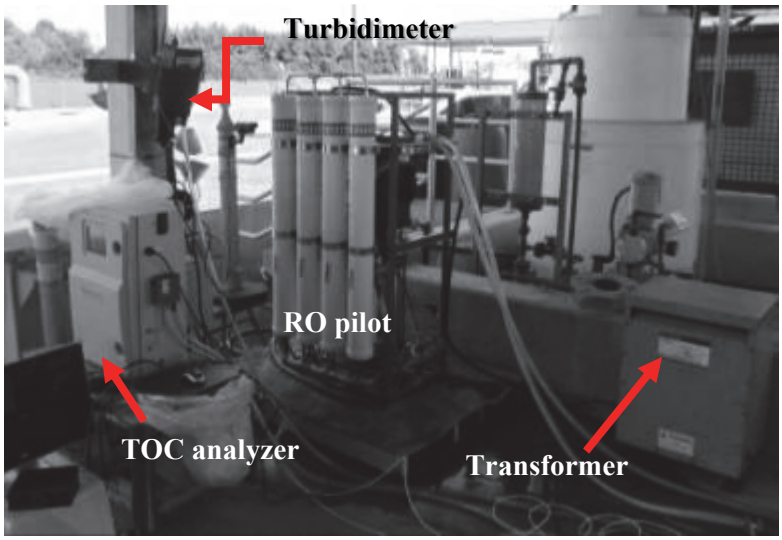


Figure 4.3. RO pilot setup with online monitors.

4.1.5. Instrument Requirements and Specifications

The following is a short summary of the instruments used for the pilot-test analysis.

1. **GE Sievers 900 TOC Analyzer**

   The high sensitivity GE instrument measures concentration of TOC, expressed as a difference of TC and IC. The instrument can be used to monitor high-purity water containing less than 0.3 parts per billion (ppb) TOC and requires a sample flow rate of 50 to 300 mL/min with an analysis time of 4 min.

2. **HACH FilterTrak 660sc**

   The lowest expected reading for the HACH turbidimeter is 7 mNTU with a resolution of 0.1 mNTU during operation. It requires an uninterrupted flow of 100 to 750 mL/minute for operation. It was installed away from any vibrations with a semirigid Teflon tube at the inlet. Samples were measured at an interval of every 5 min.

3. **Conductivity Meter**

   A conductivity meter installed on the RO pilot system was used to record TDS results in parts per million (ppm) continuously at an interval of 60 min.

4. **DLS**

   Grab samples were analyzed off-site at the project team's facility using DLS Nanotrac. The sample volume for the instrument was 2 mL. A bench surface free of vibrations was used to install the instrument.

5. **EFM**

   In addition, grab samples were sent to UCI for viral particle analysis by EFM. The particle count was obtained as concentration in particles/mL.

4.2. Leakage Test

A leakage test was performed after completing all the installations to ensure full functionality of the pilot setup and the online analyzers. Several conditions can cause leakage in membrane systems such as membrane damage, holes in the membrane, compromised O-ring, and so forth. Compromised O-rings were used to simulate controlled leakage conditions for the pilot test. Baseline test conditions were established by obtaining stable results with complete analytical measurements. After the baseline conditions were established, the pilot system was tested with a completely cut, open O-ring to check the maximum possible detection. A partially compromised O-ring was then introduced to test the sensitivity of the DLS and EFM monitoring. The online instruments were also used to monitor the change on a continuous basis. Progressively increasing damage was tested by introducing additional cuts of the O-ring. Different methods of O-ring compromise have been summarized in the following section.

4.2.1. Baseline

In order to obtain the plant operating conditions and the performance of the RO pilot, a baseline was developed for different parameters. Any compromise with the O-ring was compared to these results. A permeate was obtained on start-up, and the output of the DLS and EFM was below detection limit. Because of noise and vibration, the 95 percentile cut-off for the DLS instrument below detection limit corresponds to a value of 1.23 nm. Online instruments were checked continuously for consistent readings as baseline results.

Figure 4.4 shows the TOC trend of the pilot unit in RO permeate before the WRD plant was shut down for a scheduled maintenance. The TOC can be seen to decrease in the first 24 hours after which the water quality starts stabilizing. The fluctuations in the first hour of operation correspond to the time required for flushing out the preservative solution (sodium bisulfite). After this initial 12-hour period, the average of all values provided a baseline of 44.2 ppb. A similar trend in the turbidity after the initial start-up of the pilot system is shown in Figure 4.5. The turbidity shows a slight dip initially and confirms the initial system fluctuations. The turbidity data was taken every 15 min for this period. The average turbidity after 12 hours of operation was 36.2 mNTU.

After this short period of baseline operation, the WRD plant was shut down for a period of 5 days. A new baseline was established using the same method after the plant start-up. The RO pilot feed turbidity decreased from 0.137 NTU before shutdown to 0.05 NTU after shutdown.

Figure 4.6 shows the variation in RO pilot feed turbidity. The baseline results are summarized in Table 4.4.

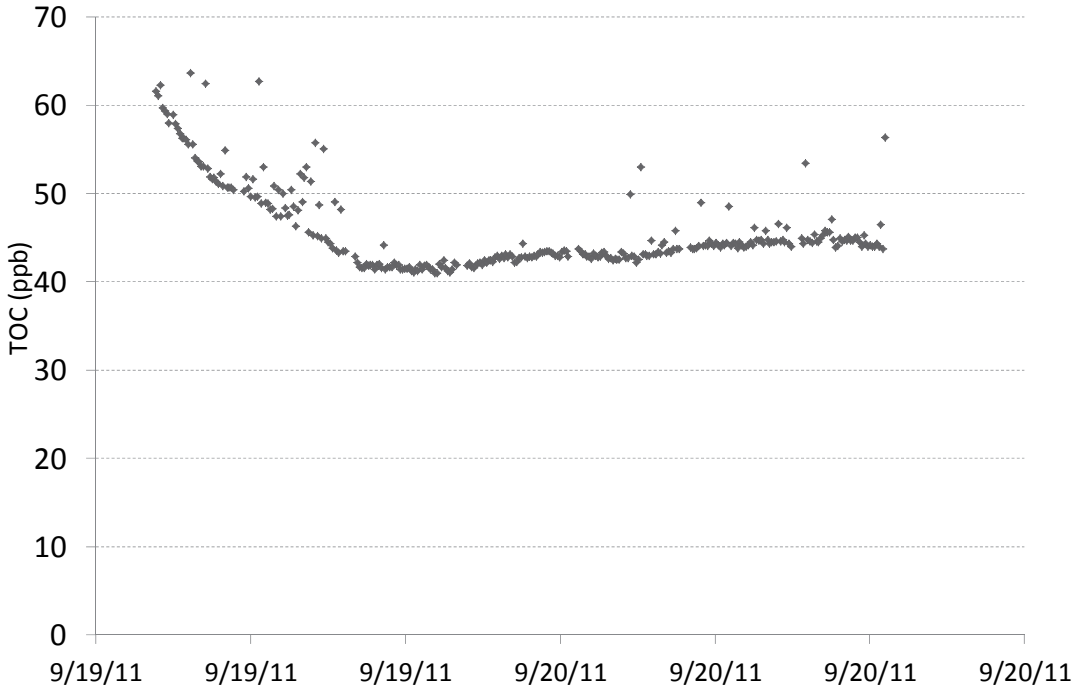


Figure 4.4. Trend in TOC of RO pilot permeate before shutdown.

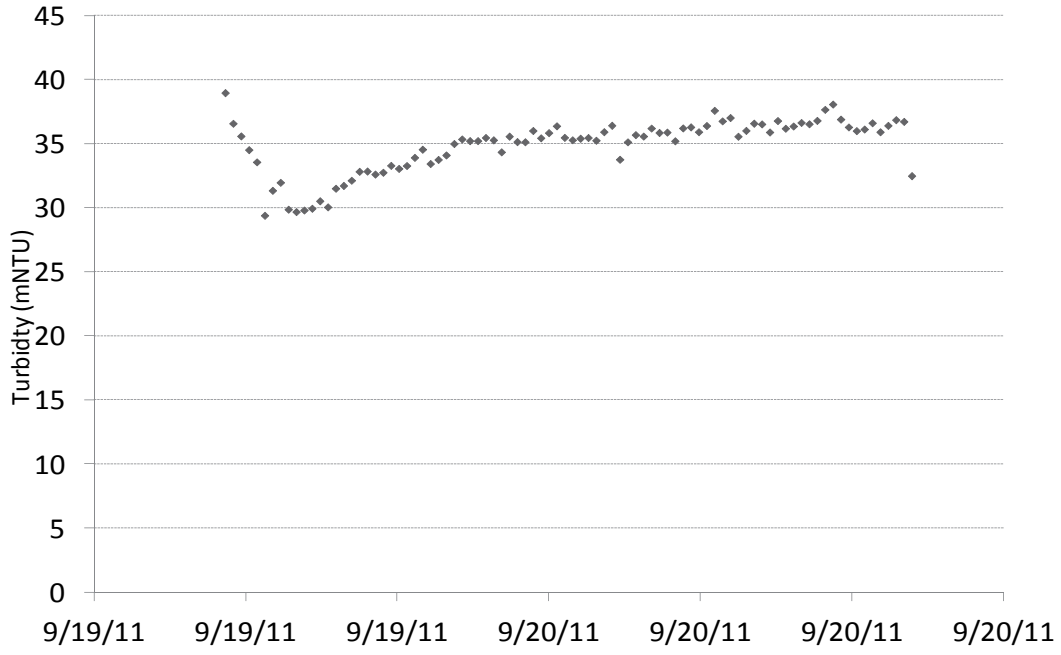


Figure 4.5. Trend in turbidity of RO pilot permeate before shutdown.

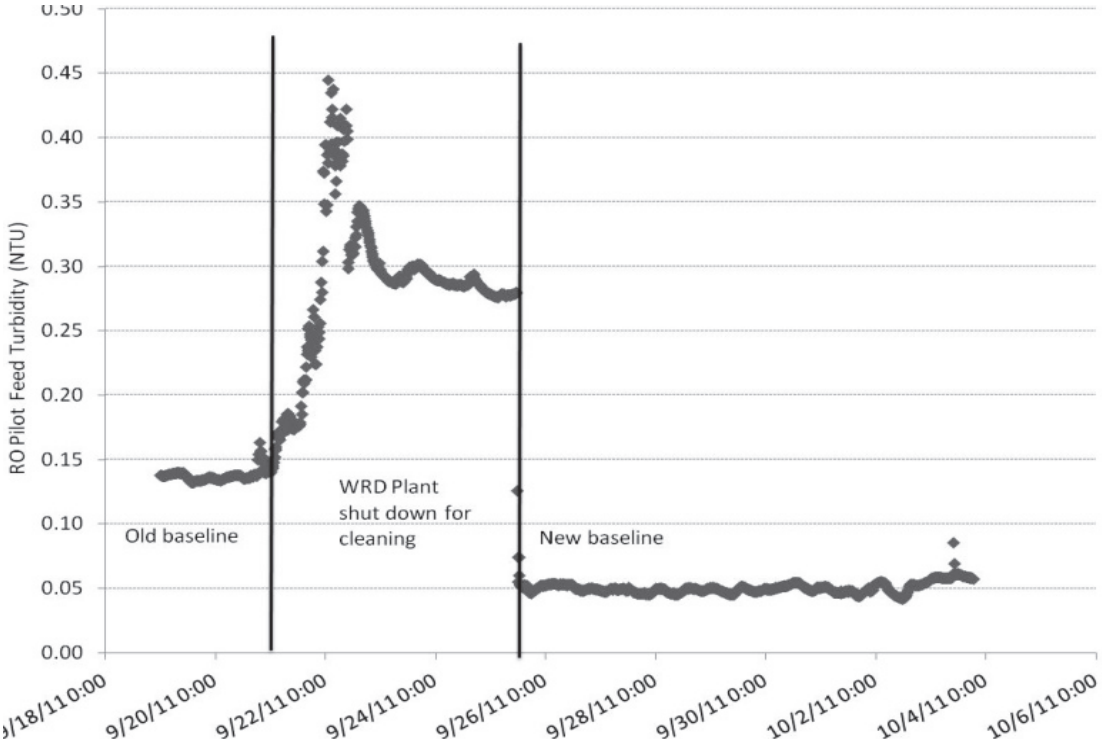


Figure 4.6. RO pilot feed turbidity during pilot operation at WRD.

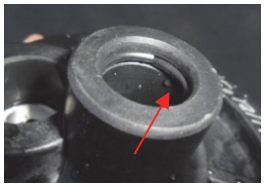
Table 4.4. Baseline Conditions Before and After Shutdown for Maintenance at WRD

Parameter	Old Baseline Before Plant Shutdown	New Baseline After Plant Shutdown
TOC (ppb)	44.2	39.8
Turbidity (mNTU)	36.2	12
TDS (ppm)	5	5
DLS (95 percentile, nm)	1.23 (Below detection limit)	1.23 (Below detection limit)
EFM (particles/mL)	Below detection limit	Below detection limit

4.2.2. O-Ring Compromise

Because the typical point of compromise is the connector O-rings on the head of a single membrane housing pressure vessel, the O-ring on the high pressure end (feed inlet) of the second pass pressure vessel was used for different compromise on this O-ring, as summarized in Table 4.5. The arrows denote points of compromise. Any leakage in this O-ring causes concentrate to enter the permeate stream.

Table 4.5. Progressive Compromise on the Connector O-ring of the Pressure Vessel

	O-ring subjected to stretch. This shape deformation was used to check for any leakage.
	Open O-ring: The O-ring was cut all the way to obtain two separate ends before installing in the groove.
	A small incision was made on a new O-ring to form a notch on the surface.
	A second indentation was made on the same O-ring on the opposite end

A third indentation was made to facilitate additional leakage.

Additional compromise was exhibited by a fourth indentation on the same O-ring

4.3. RO Pilot Results

The real-time data from online instruments display the variations in the values and provide a good understanding of the pilot operating conditions. TOC concentrations (ppb) and turbidity (mNTU) are shown in Figure 4.7 and Figure 4.8.

The TOC values were recorded every 4 min, whereas the turbidimeter recorded values at an interval of every 5 min. The data from the final two days could not be retrieved from the turbidimeter because of a loss of data from the HACH controller. Turbidity values measured from grab samples at the plant site were used for those two days.

Initially, the brine seal O-ring was tested to determine any leakage, followed by a stretched O-ring. These two conditions did not change any of the baseline parameters. The O-ring on the pressure vessel head was confirmed to be the most vulnerable on the basis of the test results. A significant increase of value was shown by both TOC and turbidimeter for a completely open O-ring. After achieving high detection, further experiments were performed by only slightly damaging this O-ring to different degrees to evaluate how low of a sensitivity each instrument could detect. Progressively higher detections were observed as the number of notches on the O-ring increased. The facility was shut down on the final day because of a power failure, and data from the final days were not available.

A similar trend has been plotted for the TDS in Figure 4.9. The TDS probe installed on the RO skid was set to record every hour to obtain a trend. The relatively stable values can be attributed to the relative high detection limit of the instrument at 1 ppm. The TDS result reiterates the TOC and turbidity data in Figures 4.7 and 4.8 by displaying maximum detection for the open O-ring.

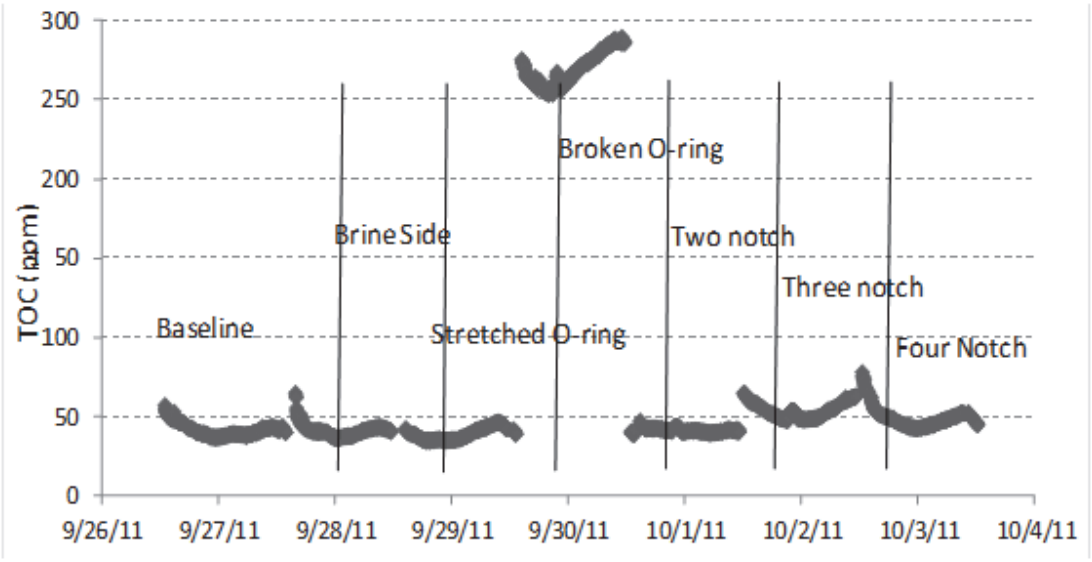


Figure 4.7. Trend in TOC concentrations with various O-ring damage.

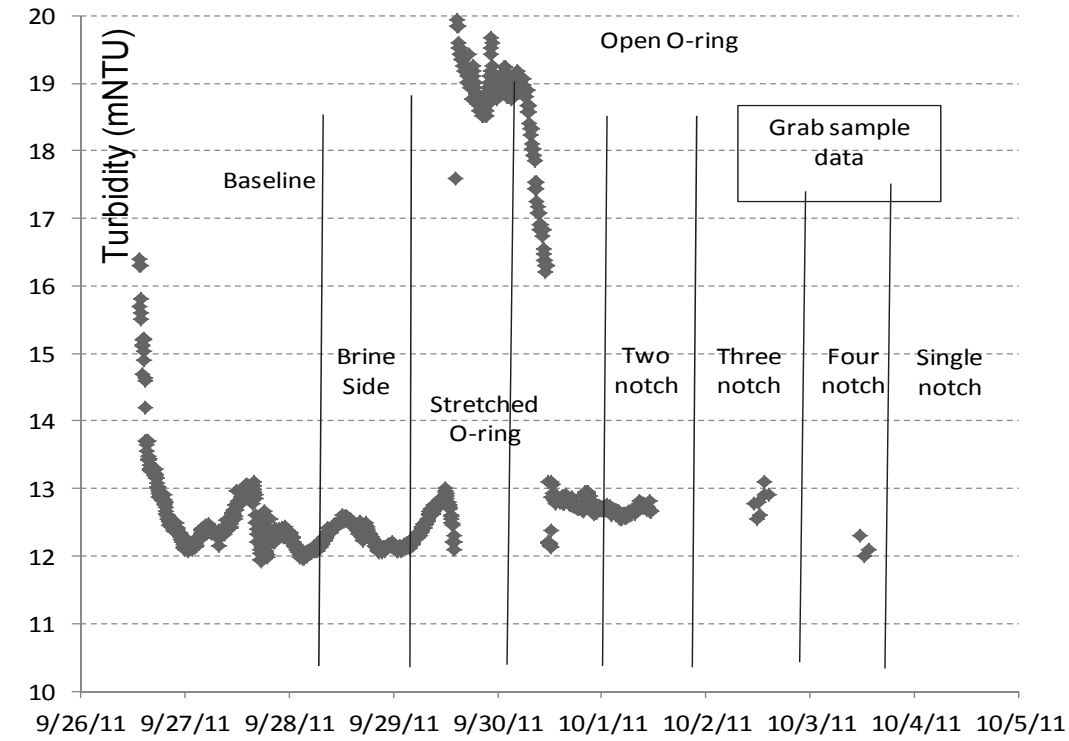


Figure 4.8. Trend in turbidity with various O-ring damage.

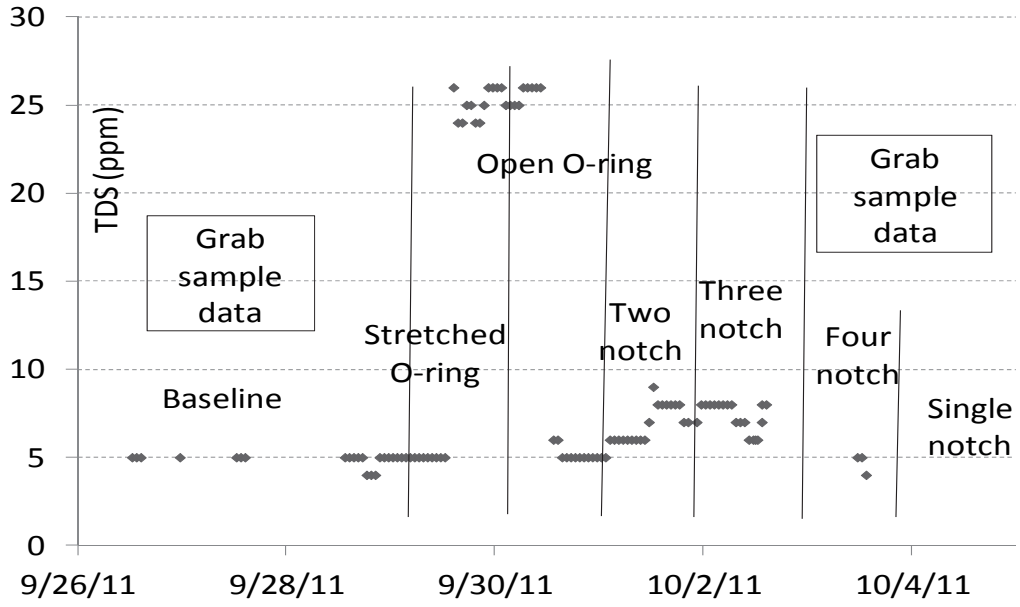


Figure 4.9. Trend in TDS with various O-ring damage.

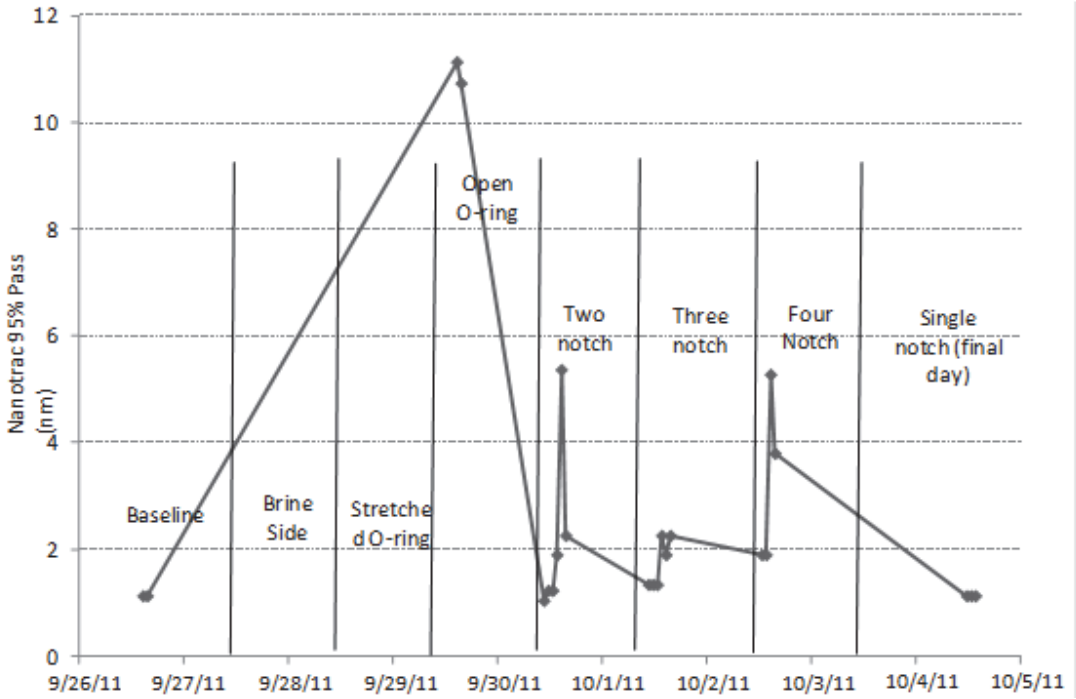


Figure 4.10. Trend in particle size for samples collected at different intervals on various days.

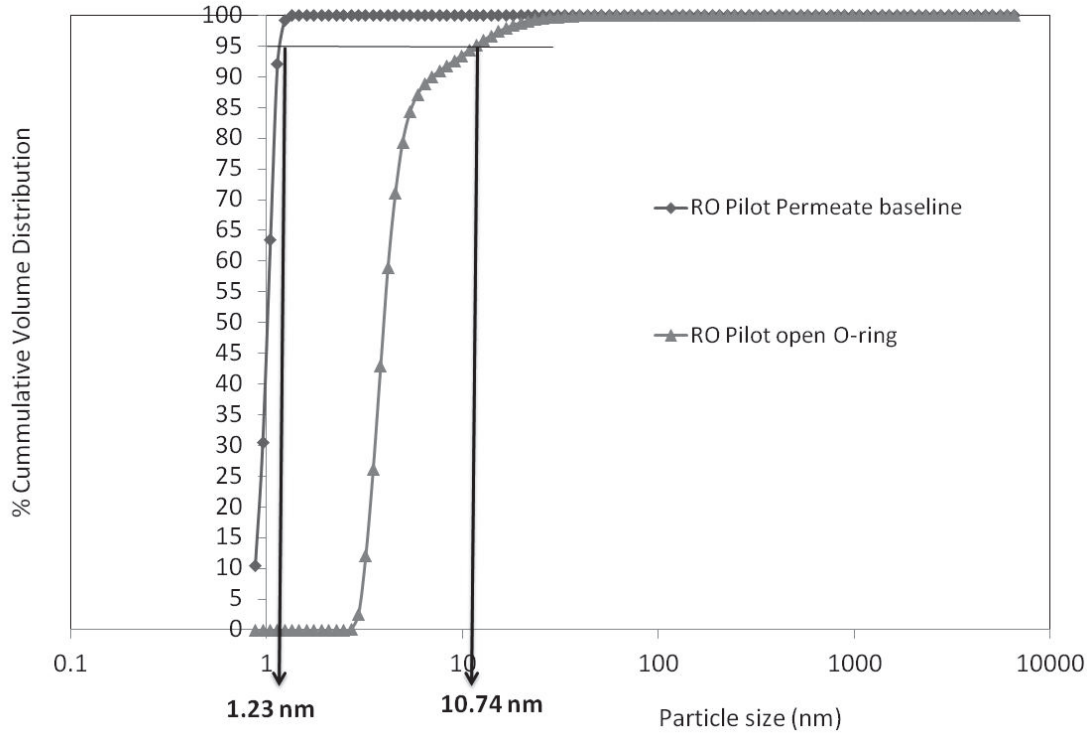


Figure 4.11. Size distribution as % cumulative volume distribution with 95 percentile cut-off.

Figure 4.10 shows the trend in the DLS data. The DLS instrument detects Brownian motion of the particle and thus can only be monitored in a stationary sample cell. The graph is based on grab samples collected every hour of operation. Multiple permeate samples were also obtained to confirm the varying extent of O-ring compromise. The graph represents the trend in particle analysis correlating to the continuous online monitoring instruments. Sharp peaks correspond to the particle size of these samples, for which 95% of the particles are smaller (95th percentile) than the size noted.

The Nanotrac DLS instrument reports the size distribution as percentage of volume distribution. This distribution cannot be used easily as a monitoring value. Figure 4.11 presents the percentage of cumulative volume distribution for the open O-ring relative to the baseline result.

Once detection was confirmed, the DLS instrument produced slightly different signals for the varying extent of O-ring compromise. Although the DLS instrument showed good sensitivity for change in the water quality, a further study may be required to correlate the effectiveness of the DLS instrument in detecting its sensitivity to water quality changes.

The changes in water quality of RO permeate that was due to O-ring compromise has been detected by all instruments used. Discreet, noteworthy increase in the values for each case of O-ring compromise was demonstrated. Open O-ring resulted in maximum deviation from baseline and, as expected, a single notch exhibited the least deviation for all the instruments tested.

The increase in the values relative to the baseline were also expressed as a percentage change. Figure 4.12 presents this percentile increase in the instrument response after one hour of

operation for each case of O-ring compromise. The graph compares directly the sensitivity of instruments. All the instruments used to test permeate quality were able to detect changes at different degrees. The values show a good separation in the sensitivity of the individual instruments.

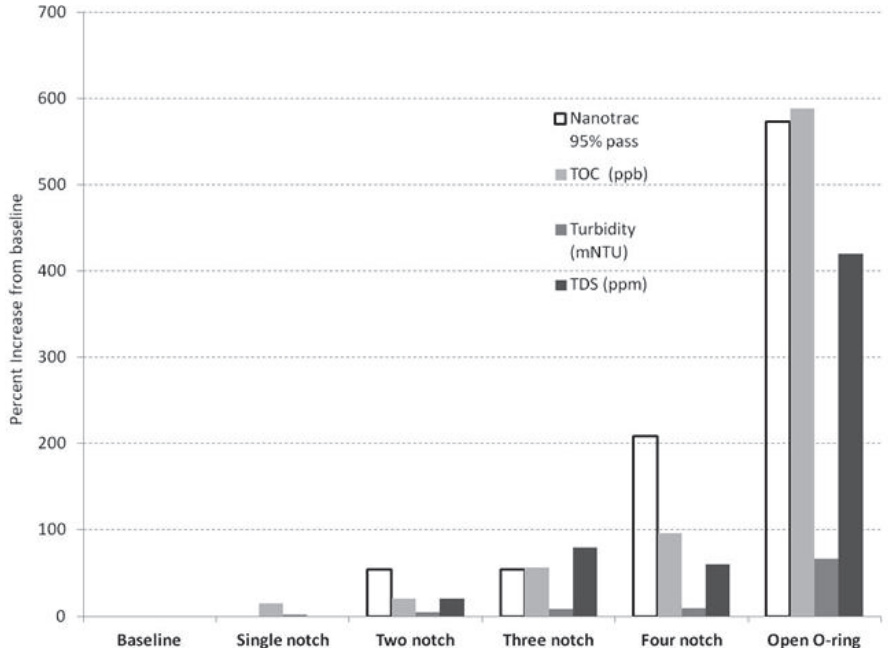


Figure 4.12. Percentage increase in response from each instrument for different cases.

The DLS exhibits a sharp rise in the signal as the O-ring is progressively damaged, and the DLS instrument shows corresponding percentage change. However, the TOC analyzer and the turbidimeter were able to detect signals for a single cut on the O-ring as well. The percentage change by the DLS and the TOC analyzer are comparable for a completely cut, open O-ring. A more sensitive conductivity meter with a lower detection limit is required to detect smaller changes in water quality.

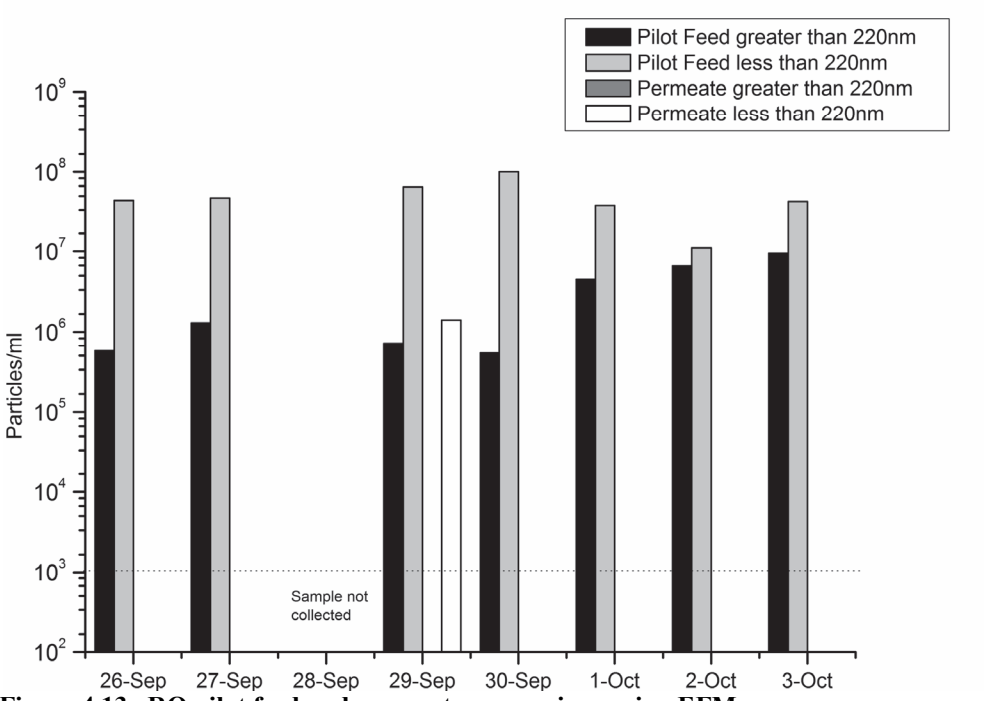


Figure 4.13. RO pilot feed and permeate comparison using EFM.

Table 4.6. Summary of Particle Analysis Using EFM for RO Pilot Feed and Permeate

	Particles /mL	26-Sep	27-Sep	28-Sep	29-Sep	30-Sep	1-Oct	2-Oct	3-Oct
Pilot Feed	Greater than 220 nm	5.80E +05	5.80E +05	No sample	7.20E +05	5.40E +05	4.50E +06	6.60E +06	9.40E +06
	Less than 220 nm	4.40E +07	4.40E +07	No sample	6.50E +07	1.00E +08	3.80E +07	1.10E +07	4.30E +07
Perm -eate	Greater than 220 nm	ND	ND	ND	ND	ND	ND	ND	ND
	Less than 220 nm	ND	ND	ND	1.40E +06	ND	ND	ND	ND

Note. ND = not detected.

Figure 4.13 shows the EFM analysis for pilot feed and permeate samples. The EFM provides direct DNA containing particle count for the RO feed samples. Greater than 220nm fluorescence particles are considered bacteria and less than 220 nm particles are considered viruses. The results showed that virus particles are consistently present in the RO feed at the concentration range between $4X10^7$ to $1X10^8$ per mL. However, most of the permeate samples were free of virus particles. Viruses were only detected in the permeate sample when the O-ring was completely cut open. Table 4.6 summarizes the EFM analysis.

Chapter 5

Conclusion

5.1. Summary

After conducting the literature review, the core method chosen to be evaluated for virus detection was EFM. The core technology evaluated as a potential indirect surrogate for virus monitoring was the DLS technology for measuring nanoparticles, which is the size range for viruses. After the literature review phase was completed, steps were taken toward a bench-scale testing phase.

Samples from WRD and WBMWD were analyzed using DLS during the bench-scale phase. Various parameters including TOC were measured for all the samples. Two different DLS nanoparticle analyzers were tested for ease of operation, sensitivity, and reproducibility. The particle count was compared with the EFM results to evaluate if DLS is a feasible surrogate method for virus monitoring by comparing to a direct virus monitoring method (EFM). The correlation shows a good match for the particle count with a R^2 value of 0.91. The NanoSight DLS has the advantage of displaying a visual image of particles. It can also be used as a single particle analyzer as it provides an absolute concentration in particles/mL.

The output from the two DLS instruments was not directly comparable as the Nanotrac provided particle distribution and not the absolute particle count as NanoSight. Nanotrac was more reliable and sensitive to detect particles less than 10 nm, much less than the cut off of 25 nm for the NanoSight. Nanotrac also has the advantage of an independent, sensitive probe that can be easily used to detect particles, along with the much larger sample cell volume to mitigate the sensitivity issue. Because Nanotrac provides more reliable measurement and lower detection limit, it was used as a primary instrument during the pilot phase. In addition to DLS, the TOC analyzer, EC meter turbidimeter, was considered for the pilot phase to detect small changes in the fluctuation because these instruments are sensitive to small changes. It would be worth noting that in water reclamation applications, the instruments will be operated in a chloramine residual and that long-term operation of DLS and a flow through EFM would need to have impacts of low chloramine on their accuracy considered similar to the turbidity meters, which are affected by low levels of chloramines.

5.2. Feasibility Analysis

Based on the instrument sensitivity, basic cost of analysis, maintenance, and operation, a feasibility analysis in terms of full-scale installation for each instrument is presented in Table 5.1.The instruments were rated high, moderate, or low relative to the listed options. The approximate cost is based on the capital cost, operating cost, installation cost, and overhead expenses provided by the manufacturers.

Table 5.1. Overall Performance and Feasibility Analysis of Instruments

Instrument	Approx. Capital Cost	Operating Cost	Sensitivity	Maintenance	Online Option	Install. Cost
DLS	High (~$40,000)	Low	High	Operator required	Maybe	High
EFM	Moderate ($8,000 to $20,000)	High (~$15/sample)	Low	Operator required	No	NA
TOC analyzer	Moderate (~$22,000)	Moderate (Annual Service fees + Reagent cost ~$3,000)	High	Periodic replacement of components	Yes	Low
Turbidimeter	Low (~$2,500)	Low	Moderate	None/Low	Yes	Low
Conductivity meter	Very Low (<$1,000)	Low	Low	None/Low	Yes	Low

Turbidimeter and conductivity meter are not feasible options for virus monitoring because of the sensitivity limitation, although they provide the membrane system integrity. These options have moderate to lower costs, and because these are already widely used as online monitors, using these instruments in conjunction with the higher sensitivity instruments for virus detection is recommended for better perspective and analysis of data. The three instruments of particular consideration for real-time monitoring of virus removal are DLS, EFM, and TOC.

The DLS instrument has a high capital cost because of the equipment associated with running the instrument. DLS also currently has high installation cost that are due to specific customization requirements for an online system; two parameters of concern are effects of vibrations and isolating sample for measurement. Because DLS is dependent on Brownian motion, the system is susceptible to vibrations that compromise the analysis results. In order to prevent vibrations from influent flow and pressure from the pipe, vibration absorbing connections would be required. Because DLS requires an isolated, noncontinuous sample, a solenoid valve would have to be installed for sampling. These parameters would require customized installation and therefore a higher loop cost. DLS is a feasible real-time monitoring option as it only takes between 20 to 30 minutes to run the analysis of the samples. One significant limitation is that the method cannot directly detect viruses, but can serve as only a surrogate measurement device.

The TOC analyzer has moderate costs and provides online monitoring options. The TOC analyzer performed well in sensitivity during the pilot phase; TOC seems to be a feasible method for membrane integrity monitoring, although it is not a direct method for virus monitoring.

Although EFM provides a direct viral count, it requires staining of the samples, which accounts for the higher operating cost. Although online monitoring does not exist, there is potential development for the automation of the system to extract and stain samples and to take viral counts. The technology to do this currently exists. Real-time monitoring does not necessarily entail an online instrument. Real-time monitoring pertains to the time frame window of when the water sample is drawn and analysis is complete, so EFM can be considered a real-time virus monitoring method. However, EFM has economical limitations as a continuous method of monitoring because it is expensive to continually operate.

Integrating the TOC, DLS, and EFM methods may provide better feasibility of real-time monitoring of virus removal. TOC and DLS do not provide direct virus detection, but can be a strong indicator of when to implement and analyze the samples using the EFM method to confirm the virus leakage in the treated water. When the TOC or DLS detects compromise within the membranes, the EFM system could be signaled to operate and sample the system. An integrated system would be more economical than an EFM system alone and more reliable than a standalone TOC or DLS system. The integration of these methods and performance as a combined monitoring method would require further study.

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