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Environmental Technology Verification Report

Removal of Microbial Contaminants in Drinking Water

Dow Chemical Company – Dow Water Solutions
SFD-2880 Ultrafiltration Module

Prepared by

NSF International

Under a Cooperative Agreement with
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Notice

The U.S. Environmental Protection Agency, through its Office of Research and Development, funded and managed, or partially funded and collaborated in, the research described herein. It has been subjected to the Agency's peer and administrative review and has been approved for publication. Any opinions expressed in this report are those of the author (s) and do not necessarily reflect the views of the Agency, therefore, no official endorsement should be inferred. Any mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Foreword

The U.S. Environmental Protection Agency (EPA) is charged by Congress with protecting the nation's air, water, and land resources. Under a mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, the EPA's Office of Research and Development provides data and science support that can be used to solve environmental problems and to build the scientific knowledge base needed to manage our ecological resources wisely, to understand how pollutants affect our health, and to prevent or reduce environmental risks.

The Environmental Technology Verification (ETV) Program has been established by EPA to verify the performance characteristics of innovative environmental technology across all media and to report this objective information to permitters, buyers, and users of the technology, thus substantially accelerating the entrance of new environmental technologies into the marketplace. Verification organizations oversee and report verification activities based on testing and quality assurance protocols developed with input from major stakeholders and customer groups associated with the technology area. ETV consists of six environmental technology centers. Information about each of these centers can be found on the internet at http://www.epa.gov/etv.

Under a cooperative agreement, NSF International has received EPA funding to plan, coordinate, and conduct technology verification studies for the ETV "Drinking Water Systems Center" and report the results to the community at large. The DWS Center has targeted drinking water concerns such as arsenic reduction, microbiological contaminants, particulate removal, disinfection by-products, radionuclides, and numerous chemical contaminants. Information concerning specific environmental technology areas can be found on the internet at http://www.epa.gov/nrmrl/std/etv/verifications.html.

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Abbreviations and Acronyms

ATCC	American Type Culture Collection
°C	degrees Celsius
CFU	colony forming units
cm	centimeter
EPM	Electrophoretic Mobility
ETV	Environmental Technology Verification
°F	degrees Fahrenheit
ft	foot(feet)
gfd	gallons per square foot per day
gpm	gallons per minute
in	inch(es)
L	liter
LRV	log removal value
LT2ESWTR	Long Term 2 Enhanced Surface Water Treatment Rule
m	meter
MFGM	Membrane Filtration Guidance Manual
mg	milligram
min	minute
mL	milliliter
mm	millimeter
mM	millimolar
MWCO	molecular weight cutoff
NRMRL	National Risk Management Research Laboratory
NSF	NSF International (formerly known as National Sanitation Foundation)
NTU	Nephelometric Turbidity Unit
ORD	Office of Research and Development
PFU	plaque forming unit
psig	pounds per square inch, gauge
PVDF	polyvinylidene fluoride
QA	quality assurance
QC	quality control
RPD	relative percent difference
SM	Standard Methods for the Examination of Water and Wastewater
TDS	total dissolved solids
TOC	total organic carbon
UF	ultrafiltration
µg	microgram
µm	microns
µS	microsiemens
USEPA	U. S. Environmental Protection Agency

Chapter 1
Introduction

1.1 ETV Program Purpose and Operation

The U.S. Environmental Protection Agency (USEPA) has created the Environmental Technology Verification (ETV) Program to facilitate the deployment of innovative or improved environmental technologies through performance verification and dissemination of information. The goal of the ETV Program is to further environmental protection by accelerating the acceptance and use of improved and more cost-effective technologies. ETV seeks to achieve this goal by providing high-quality, peer-reviewed data on technology performance to those involved in the design, distribution, permitting, purchase, and use of environmental technologies.

ETV works in partnership with recognized standards and testing organizations; with stakeholder groups consisting of buyers, vendor organizations, and permitters; and with the full participation of individual technology developers. The program evaluates the performance of innovative technologies by developing test plans that are responsive to the needs of stakeholders; conducting field or laboratory testing, collecting and analyzing data; and by preparing peer-reviewed reports. All evaluations are conducted in accordance with rigorous quality assurance protocols to ensure that data of known and adequate quality are generated and that the results are defensible.

The USEPA has partnered with NSF International (NSF) under the ETV Drinking Water Systems Center to verify performance of drinking water treatment systems that benefit the public and small communities. It is important to note that verification of the equipment does not mean the equipment is "certified" by NSF or "accepted" by USEPA. Rather, it recognizes that the performance of the equipment has been determined and verified by these organizations under conditions specified in ETV protocols and test plans.

1.2 Purpose of Verification

Testing of the Dow Chemical Company SFD-2880 Ultrafiltration (UF) membrane module was conducted to verify microbial reduction performance under the membrane challenge requirements of the USEPA Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR). This report meets the "Membrane Challenge Test Requirements" in section IV.D.11.a. of the LT2ESWTR. The report does not address the following section 11.a. LT2ESWTR requirements: *Membrane Direct Integrity Testing*; *Continuous Indirect Integrity Monitoring*, nor any non-testing requirements such as product modifications, or assuring that the membrane product sold conforms to the established quality control release value.

Please also note that this verification does not address long-term performance, or performance over the life of the membrane. This verification test did not evaluate cleaning of the membranes, nor any other maintenance and operation.

While the LT2ESWTR only addresses *Cryptosporidium*, the EPA Membrane Filtration Guidance Manual states that virus reduction can be tested under the same framework. Therefore, reduction of the coliphage virus MS2 was also evaluated during this study, using the same test protocol as that for *Cryptosporidium* reduction.

1.3 Testing Participants and Responsibilities

The following is a brief description of each of the ETV participants and their roles and responsibilities.

1.3.1 NSF International

NSF is an independent, not-for-profit organization dedicated to public health and safety, and to protection of the environment. Founded in 1944 and located in Ann Arbor, Michigan, NSF has been instrumental in the development of consensus standards for the protection of public health and the environment. The USEPA partnered with NSF to verify the performance of drinking water treatment systems through the USEPA's ETV Program.

NSF performed all verification testing activities at its Ann Arbor, MI location. NSF prepared the test/QA plan, performed all testing, managed, evaluated, interpreted, and reported on the data generated by the testing, and reported on the performance of the technology.

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1.3.2 U.S. Environmental Protection Agency

USEPA, through its Office of Research and Development (ORD), has financially supported and collaborated with NSF under Cooperative Agreement No. R-82833301. This verification effort was supported by the DWS Center operating under the ETV Program. This document has been peer-reviewed, reviewed by USEPA, and recommended for public release.

1.3.3 Dow Chemical Company

The Dow Chemical Company supplied the tested membrane modules, and also provided logistical and technical support, as needed.

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Chapter 2
Product Description

2.1 UF Membrane General Description

UF membranes remove contaminants from water through sieving based on the size of the membrane pores relative to the physical size of the contaminant. A common arrangement for the membranes is in hollow fibers, with the fibers "potted" in a resin. The flow of water through the fibers can be either "inside-out" or "outside-in". UF membranes can be classified by pore size or the molecular weight cutoff (MWCO) point. Pore sizes generally range from 0.01 to 0.05 microns (μm). Typical MWCO points are 10,000 to 500,000 Daltons, with 100,000 being a common MWCO rating for drinking water treatment. With these specifications, UF membranes can remove viruses, bacteria, and protozoan cysts, as well as large molecules such as proteins, and suspended solids.

2.2 SFD-2880 Membrane Module Description

The Dow SFD-2880 is a polyvinylidene fluoride (PVDF) hollow fiber ultrafiltration membrane module. The module specifications and operating parameters are listed in Table 2-1. The SFD-2880 is a pressure driven module, with the normal operating flow orientation from the outside to the inside of the fibers. The SFD-2880 is certified to NSF/ANSI Standard 61, which establishes minimum public health related requirements for drinking water system components.

Table 2-1. SFD-2880 Specifications

Parameter	Specification
Dimensions:	
Module outside diameter	8.9 inches (in) (225 millimeters (mm))
Module length	92.9 in (2360 mm)
Module volume	10.3 gallons (gal) (39 liters (L))
Nominal membrane pore size	0.03 μm
Maximum membrane pore size	0.05 μm
Average active membrane area (outer)	829 square feet (ft^2) (77 square meters (m^2))
Operating Limits:	
Filtrate flux range at 25°C	24-70 gallons per square foot per day (gfd) (40-120 $L/m^2/hr$)
Flow range	13.6-40.9 gallons per minute (gpm) (3.1-9.3 m^3/hr)
Operating temperature range	34-104 Fahrenheit (°F) (1-40 Celcius (°C))
Max. inlet module pressure	44 pounds per square inch (psi) (3.0 bar)
Max. transmembrane pressure (TMP)	30 psi (2.1 bar)
Operating pH range	2 – 11
Max. NaOCl	2,000 milligrams per L (mg/L)
Max. TSS	100 mg/L
Max. Turbidity	300 Nephelometric Turbidity Units (NTU)

A diagram of the SFD-2880 module is pictured in Figure 2-1. The module design allows for an optional reject line connection, but this port will be closed off for the challenge tests. The modules will be operated in dead-end mode.

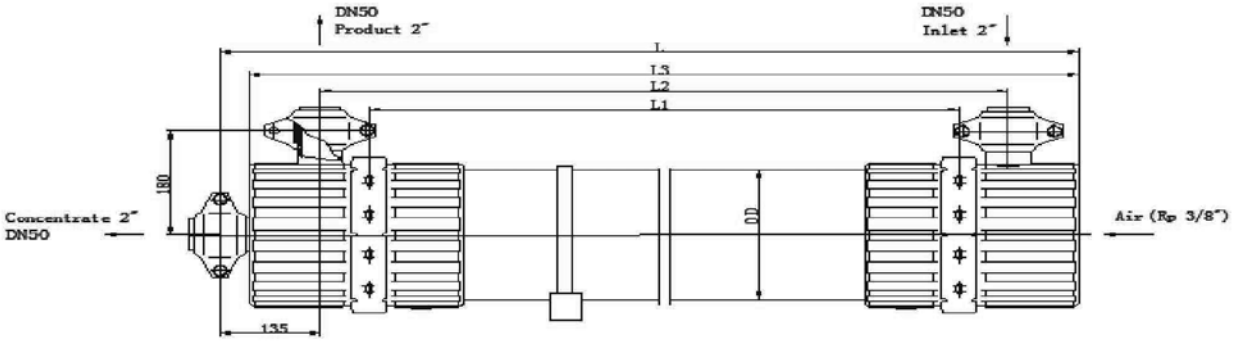

Figure 2-1. Diagram of the SFD-2880 UF module.

Dow supplied five new UF modules for testing. There was no seasoning period, other than that specified by Dow to sufficiently rinse out the membrane preservative and wet the membranes. See Section 3.5 for a description of the UF module conditioning procedure. The serial numbers of the tested modules are listed in Table 2-2. The first five modules submitted for testing were randomly selected by Dow personnel from existing inventory. For submission of the 6th module, Dow provided NSF with the serial numbers of three modules on hand in their Edina, MN warehouse, and NSF randomly selected a module for Dow to submit. The module numbers in the first column are the numbers used in Chapter 4 to identify each module.

Table 2-2. Serial Numbers of Tested Modules

Module	Serial Number
1	PEO9B00016
2	PEO9B00010
3	PEO9B00007
4	PEO9B00028
5	PEO9B00017
6	PEO9B00007

Chapter 3
Methods and Procedures

3.1 Introduction

The tests followed the procedures described in the *Test/QA Plan for the Microbial Seeding Challenge Study of the Dow Chemical Company SFD-2880 Ultrafiltration Module.* The challenge protocol was adapted from the *ETV Protocol for Equipment Verification Testing for Physical Removal of Microbiological and Particulate Contaminants*, and the USEPA *Membrane Filtration Guidance Manual* (MFGM). Note that the MFGM references the ETV protocol as an acceptable protocol for testing membrane products according the to the USEPA requirements. The test/QA plan is available from NSF upon request.

A total of six modules were submitted for testing. The test plan called for testing only five modules, but the module tested for *Cryptosporidium parvum* reduction developed an apparent membrane breach during the test. As a result, Dow chose to submit a sixth module for testing so they could have a five module data set demonstrating the performance of fully integral modules. The tests were conducted in October of 2009, February of 2010, and May of 2010. See Table 4-8 for the dates of each individual challenge test. In between tests, the modules were stored wet, and without any preservative, at NSF.

3.2 Organisms and Challenge Concentrations

The SFD-2880 modules were tested for removal of microorganisms using endospores of the bacteria *Bacillus atrophaeus* (American Type Culture Collection (ATCC) number 9372, deposited as *Bacillus subtilis* var. *niger*), and MS2 coliphage virus (ATCC 15597-Bl). In addition, one module was challenged with live *Cryptosporidium parvum* oocysts in order to experimentally establish the *B. atrophaeus* endospores as a surrogate for *Cryptosporidium*. *B. atrophaeus* was selected as a surrogate for *C. parvum*, due to the high cost and lack of availability of suitable numbers of *C. parvum* for challenging all five modules. The strain of *B. atrophaeus* used for testing yields orange colonies with a distinctive morphology on trypicase soy agar (TSA), so it can be distinguished from wild-type endospores that could be present as contamination. *B. atrophaeus* endospores are ellipsoidal (football shaped), with an average diameter of 0.8 µm, and an average length of 1.8 µm. See Appendix B for further discussion regarding the use of *Bacillus* endospores as a surrogate for *Cryptosporidium*.

Virus removal testing was conducted using MS2 for possible virus removal credits. MS2 is considered a suitable surrogate for pathogenic viruses because of its small size, at approximately 24 nanometers in diameter.

The following were the target challenge concentrations for each organism:

- MS2 – $5x10^5$ plaque forming units per milliliter (PFU/mL);
- *B. atrophaeus* – $1x10^7$ colony forming units (CFU) per 100 mL; and
- *C. parvum* – $5x10^5$ oocysts/L.

The LT2ESWTR calls for the maximum challenge concentration to be 6.5 $\log_{10}$ above the organism's detection limit. The detection limit of all challenge organisms was 1 per unit volume. The goal for the *B. atrophaeus* challenges was to be able to measure log reductions greater than six, so NSF elected to target $1x10^7$ CFU/100 mL in order to account for less than 100% recovery of spiked challenge organism concentration. After all six modules were tested, and the feed concentrations were found to be above 6.5 $\log_{10}$, NSF learned that the maximum 6.5 $\log_{10}$ challenge level is not just guidance, but rather the maximum allowed in the rule language in the Federal Register. Therefore, NSF decided to retest two modules with lower challenge levels to provide a data set that meets the requirements of the rule.

The MS2 stock suspension was purchased from Biological Consulting Services of North Florida, Inc. *B. atrophaeus* was purchased from Presque Isle Cultures. The *C. parvum* oocysts were purchased from Sterling Parasitology Lab.

3.3 Test Apparatus

The modules were tested in a test rig constructed specifically for these tests. The test rig construction conformed to the requirements of the MFGM. See Figure 3-1 for a schematic diagram of the test rig, and Figure 3-2 for a photo of the test rig.

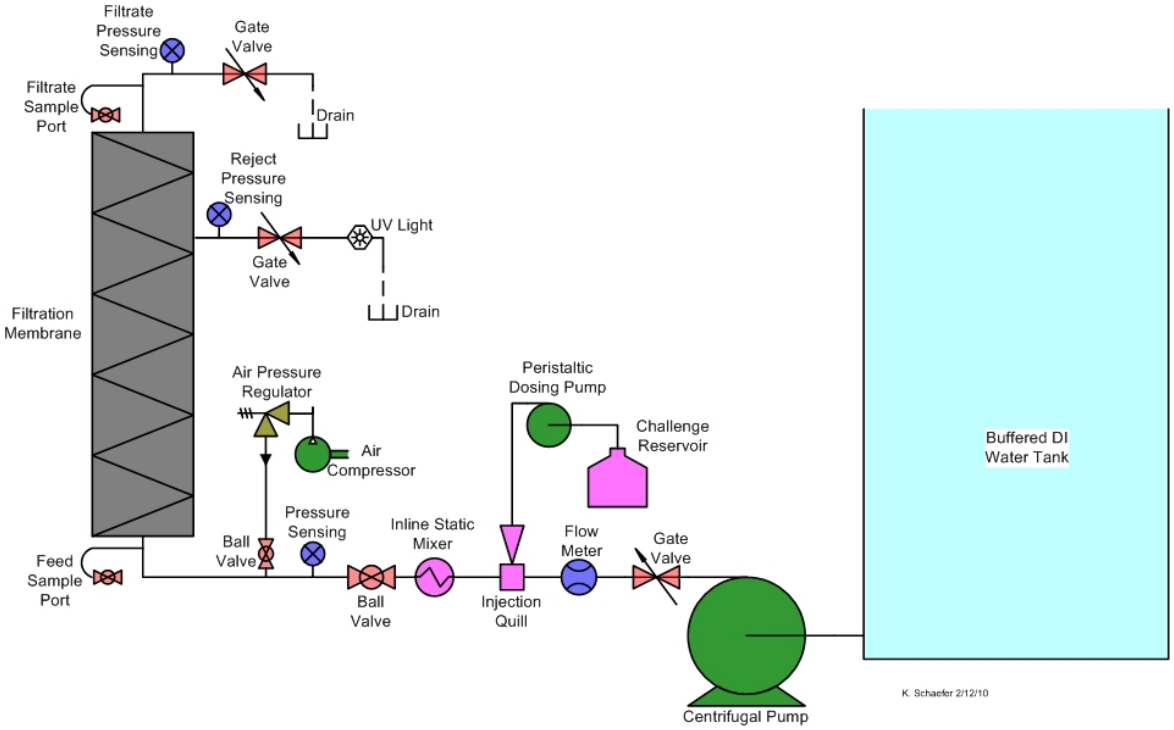

Figure 3-1. Schematic diagram of the test rig used for verification testing.

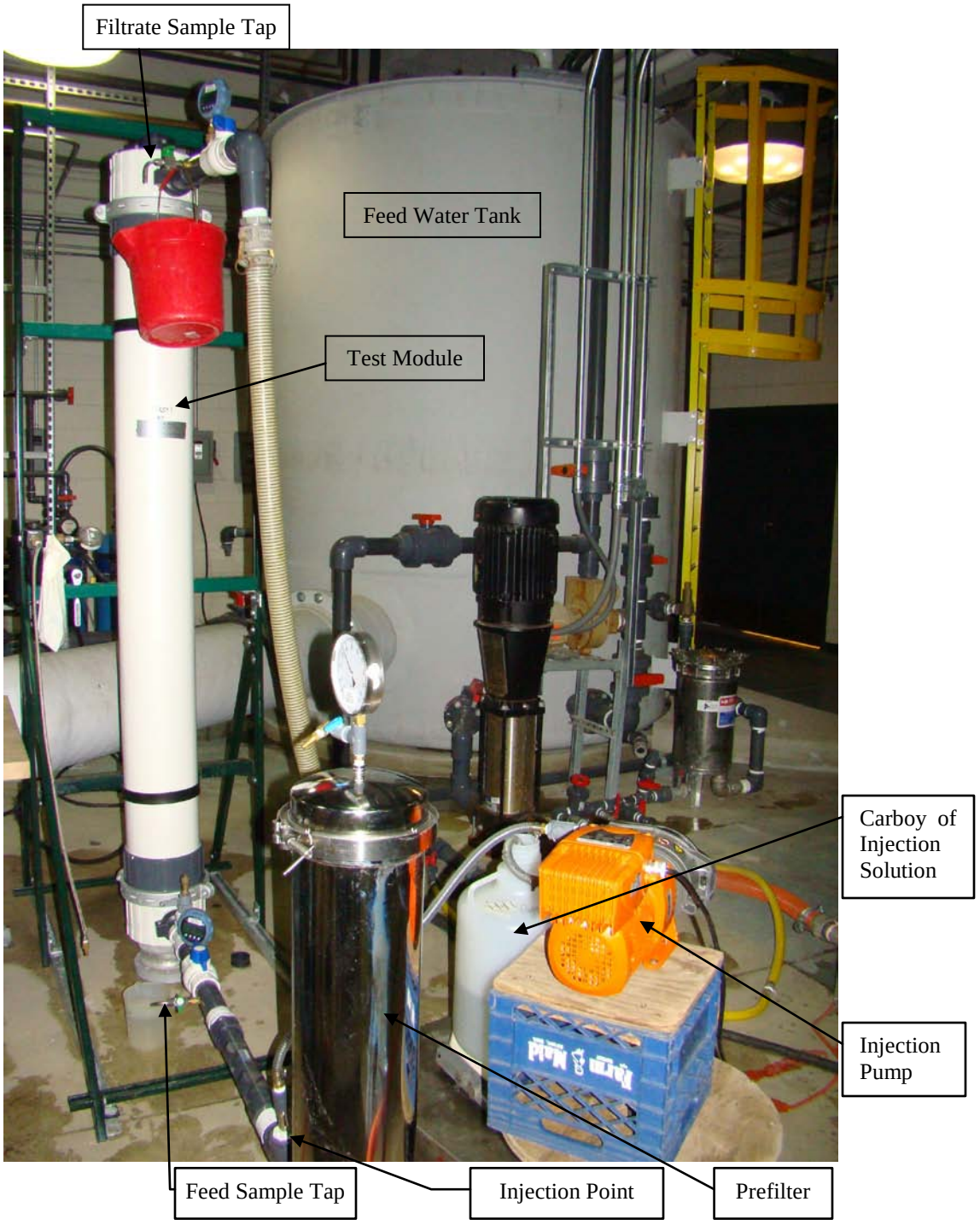

Figure 3-2. Photo of the test rig.

The challenge organisms were introduced into the feed water by intermittent injection during the challenge tests. Injection and mixing of the organisms followed the guidelines of the MFGM, except for the suggested distance between the injection point and feed sample tap. Specifically, the total stock solution volume injected into the feed stream during each challenge test was between 0.5 and 2 percent of the total spiked test solution volume, a chemical metering pump that delivered a steady flow of the challenge solution was used, and the injection port included a quill extending into the middle of the feed pipe. The MFGM also calls for a static mixer to be placed downstream of the injection point, and that the feed sample tap be located at least ten pipe diameters downstream of the static mixer. NSF misread this suggestion as ten pipe diameters including the static mixer. The inlet and outlet fittings on the SFD-2880 module are 2 inches (DN50), so the test rig plumbing was also 2 inches in diameter. For this test rig, the distance between the injection point and the feed sample tap, including the static mixer, was approximately 27 inches. The distance between the static mixer and the feed sample tap was not measured.

The filtrate grab samples were also collected from a sample tap with a quill extending into the middle of the pipe. Both the feed and filtrate sample taps were metal so they were able to be flame-sterilized prior to sample collection. The feed and filtrate sample ports were located immediately upstream and downstream of the membrane module.

3.4 Test Water Composition

Local tap water was further treated by carbon filtration, reverse osmosis, ultraviolet disinfection, and deionization at the NSF Laboratory to make the base water for the tests. The base water has the following quality control (QC) requirements for use in the NSF testing laboratory:

- Conductivity ≤2 microsiemens (μS) per centimeter (cm) at 25°C;
- Total organic carbon <100 micrograms (μg) per L;
- Total chlorine <0.05 mg/L; and
- Heterotrophic bacteria plate count <100 CFU/mL.

Of the above parameters, only total chlorine and total organic carbon were measured specifically for this verification. The other parameters are measured periodically by NSF as part of the internal quality assurance (QA)/QC program for test water quality.

A 4,000-gallon water supply tank was filled with the base water. For the first round of challenge tests in October 2009, sodium bicarbonate was added to the base water in sufficient quantity to provide alkalinity at a target of 100 ± 10 mg/L as calcium carbonate. The pH was then lowered with hydrochloric acid to a target range of 7.5 ± 0.5. For the retests in February 2010 and again in May 2010, NSF elected to switch to phosphate buffering at 0.1 milliMolar, because phosphate is called for in the ETV membrane challenge testing protocol.

Feed water samples were collected prior to each challenge period for analysis of total chlorine, alkalinity, pH, temperature, total dissolved solids, total organic carbon, and turbidity. These samples were collected prior to addition of the challenge organism.

3.5 UF Module Conditioning

Prior to testing, the modules were conditioned following a proprietary procedure supplied by Dow. Immediately prior to testing, each module was forward flushed at 40 gpm for one minute, then backflushed for one minute at 40 gpm.

3.6 Test Rig Sanitization

The Dow module conditioning procedure included an hour long flush with a bleach solution. This procedure was sufficient to sanitize the test rig prior to testing.

3.7 UF Module Integrity Tests

Before and after each challenge test, each module was subjected to a 20-minute pressure decay test to satisfy the non-destructive performance test requirement in Section 3.6 of the MFGM. The test procedure followed ASTM D6908-03 *Standard Practice for Integrity Testing of Water Filtration Membrane Systems*. The water was drained from the feed side of the membrane, but not the filtrate side. Approximately 20 psig of pressure was applied to the feed side and the remaining pressure was recorded every minute to chart the pressure decay. This applied pressure met the resolution requirement of Section 4.2.1 of the MFGM. The baseline decay rate of the pressurized portion of the test rig was also measured over 20 minutes immediately prior to each pre-challenge pressure decay test. This value was added to the expected UF module pressure decay rate to ensure that the final applied pressure at the end of the 20-minute test still met the applied pressure resolution requirement.

3.8 Microbial Challenge Test Procedure

Each of the SFD-2880 modules submitted for testing was challenged individually, as shown in the photo of the test rig (Figure 3-2). The target flux for membrane operation was Dow's maximum recommended value of 70 gfd at 25 °C, which equals a flow rate of approximately 40 gpm.

Separate tests were conducted for each challenge organism, so each module was tested twice over the course of the testing activities. In addition, two modules were tested a third time with live *C. parvum* oocysts. The modules chosen for the *C. parvum* challenges were the ones with the highest filtrate counts from the *Bacillus* endospores challenges. For most of the modules, both the MS2 and *B. atrophaeus* challenges were conducted on the same day.

At the end of the forward flush described in Section 3.5, a filtrate sample was collected to serve as the negative control flush sample.

Each challenge test was approximately 35 minutes in length. As discussed in Section 3.3, the challenge organisms were intermittently injected into the feed stream prior to, and during sample collection. Sections 3.10.2, 3.10.4, and 3.12.4 of the MFGM describe the requirements for the challenge test sampling plan. The MFGM requires that feed and filtrate samples not be collected until at least three hold-up volumes of water containing the challenge organism have passed through the membrane, to allow for establishment of equilibrium (equilibrium volume). The hold-up volume is defined as the "unfiltered test solution volume that would remain in the

system on the feed side of the membrane at the end of the test." Dow's specification sheet for the SFD-2880 gives the module volume as 10.3 gal. It is assumed that this volume is the total water holding volume of the module, not just the volume of the feed side of the membranes. As such, its use as the module hold-up volume added a safety factor to the holdup volume calculation.

The total hold-up volume also needs to include the pipe volume between the injection port and the module inlet. As discussed in Section 3.3, the test rig used 2-inch diameter pipe, and the injection port was approximately 45 inches upstream from the module inlet. Forty-five inches of 2-inch diameter pipe has a volume of approximately 141 in^3, which translates into 0.61 gal. The pipe volume plus the module volume gives a total hold-up volume of approximately 10.9 gal, which can be rounded up to 11 gal. If the hold-up volume is 11 gal, then the equilibrium volume is 33 gal. The challenge flow rate approximately 40 gpm, so the challenge organisms needed to be injected for about 1 minute prior to sampling to meet the requirement of passing the equilibrium volume. In practice, the injection times prior to sampling approximately two minutes, because the test engineer had to adjust the injection flow rate at the start of each injection period to ensure the proper challenge concentration.

The challenge organisms were injected into the feed stream at start-up, after 15 minutes of operation, and after 30 minutes of operation. After at least one minute of injection, grab samples were collected from the feed and filtrate sample taps. The sample taps were flame sterilized prior to sample collection. Also, at least 100 mL was collected and discarded prior to collection of each sample to flush the taps. After each round of sample collection, injection of the challenge organism suspension was turned off, and clean feed water was pumped through the modules at 40 gpm until the next sampling point.

3.9 MS2 Reduction vs. Flux

After the MS2 reduction data was shared with Dow, they requested that NSF conduct three more MS2 reduction challenges on one module at lower fluxes to identify whether MS2 reduction would increase as the flux was lowered, and to generate a curve of MS2 reduction vs. flux.

The challenge test procedure was the same as described above in Section 3.8. Module 5 was randomly chosen for testing by the laboratory engineer. The tests were conducted at the following target flow rates specified by Dow: 13.6 gpm, 25.4 gpm, and 35.6 gpm. These flow rates translate into fluxes of 23.6, 44.1, and 61.8 gfd, respectively.

3.10 Analytical Methods

A list of laboratory analytical methods can be found in Table 3-1. Single samples of adequate volume were collected for challenge organism enumeration, and were analyzed in triplicate.

Table 3-1. Analytical Methods for Laboratory Analyses			
Parameter	**Method**	**NSF Reporting Limit**	**Hold Time**
Alkalinity (total, as $CaCO_3$)	USEPA 310.2	5 mg/L	14 days
pH	SM[1] 4500-H^+	NA[2]	none[3]
Total Dissolved Solids (TDS)	SM 2540 C	5 mg/L	7 days
Total Chlorine	SM 4500-Cl G	0.05 mg/L	none[3]
Total Organic Carbon (mg/L)	SM 5310C	0.1 mg/L	28 days
Turbidity	SM 2130	0.1 NTU	none[3]
MS2	NSF 55[4]	1 PFU/mL	30 hours
B. atrophaeus Endospores	SM 9218[5]	1 CFU/100 mL	30 hours
Cryptosporidium Oocysts	USEPA 1623	1 oocyst/L	72 hours

(1) SM = Standard Methods
(2) Not Applicable
(3) Immediate analysis required
(4) Method published in NSF/ANSI Standard 55 – Ultraviolet Microbiological Water Treatment Systems. Method is similar to EPA Method 1601.
(5) TSA was substituted for nutrient agar in SM 9218 so that the challenge endospores could be distinguished from wild-type endospores. TSA gives orange colonies with a distinctive morphology.

Chapter 4
Results and Discussion

4.1 Introduction

For presentation of the challenge organism data in this chapter, the observed triplicate counts were averaged by calculating geometric means, as suggested for microbial enumeration data in SM 9020. Geometric means <1 were rounded up to 1, unless all three triplicate analyses had no organisms found. The mean counts were $\log_{10}$ transformed for the purpose of calculating log removal values (LRV). The triplicate counts for each sample are presented in Appendix C.

The LT2ESWTR and MFGM specify that an LRV for the test ($LRV_{C\text{-}TEST}$) be calculated for each module tested, and that the LRVs for each module are then combined to yield a single $LRV_{C\text{-}TEST}$ for the product. If fewer than 20 modules are tested, as was the case for this verification, the $LRV_{C\text{-}TEST}$ is simply the lowest LRV for the individual modules. However, the rule does not specify a method to calculate $LRV_{C\text{-}TEST}$ for each module. Suggested options in the MFGM include:

1. Calculate a LRV for each feed/filtrate sample pair, then calculate the average of the individual sample point LRVs;
2. Average all of the feed and filtrate counts, and then calculate a single LRV for the module; or
3. Calculate a LRV for each feed/filtrate sample pair, select the LRV for the module as the lowest (most conservative of the three options).

Options 1 and 2 give $LRV_{C\text{-}TEST}$ values that are either identical, or within a few hundredths of each other, so in this report, options 1 and 3 are used to calculate the LRV for each module. Since the triplicate counts were averaged by calculating geometric means, so too do the LRV calculations use geometric mean.

Each module was challenged with both *B. atrophaeus* and MS2 on the same day. After all of the modules were tested, the *B. atrophaeus* data was examined to choose the module to undergo the *C. parvum* challenge test. Modules 2 and 3 were the only ones with *B. atropheaus* CFU found in all three triplicate counts of a filtrate sample. For Module 2, 1 CFU was found in each of the triplicate measurements for the 2-minute filtrate sample. For Module 3, the 30-minute filtrate sample triplicate counts were 3, 1, and 1 CFU, so Module 3 was chosen over Module 2 for the *C.* parvum test. During the *C. parvum* test, there was a possible integrity breach that developed, because the post-test pressure decay rate was approximately double that measured immediately before the challenge test (See Table 4-5 for the pressure decay data). When the filtrate samples were analyzed, one *C. parvum* oocyst was found in one of the triplicate counts for the 30-minute filtrate sample. As a result, Dow decided to submit a sixth module for testing. This sixth module was first challenged with *B. atrophaeus* in order to compare its performance to the other modules. The *B. atrophaeus* data set was re-examined, omitting Module 3, and Module 2 was chosen for a second *C. parvum* challenge test.

As discussed in Section 3.2, after the tests were conducted, NSF learned that the maximum 6.5 $\log_{10}$ challenge level is not just guidance, but rather the maximum allowed in the rule language in the Federal Register. Therefore, NSF decided to randomly pick two modules to retest with lower challenge levels to provide a data set that meets the requirements of the rule. NSF also learned from EPA that the States could accept the data from the high feed challenge tests, provided that the feed concentrations were capped at 6.5 $\log_{10}$ for the purpose of calculating the LRV. Therefore, two sets of LRV calculations are presented, one set using the observed feed counts, and a second set with the feed concentration set at 6.5 $\log_{10}$.

4.2 *C. parvum* Challenge Test

The *C. parvum* challenge data is presented in Table 4-1. As discussed in Section 4.1, based on a review of the *B. atrophaeus* challenge data, Module 3 was challenged with live *C. parvum* oocysts. One oocyst was found in one of the three triplicate analyses of the 30-minute filtrate sample from the Module 3 challenge test, so Dow requested that a second module be challenged with *C. parvum*, and they submitted a sixth module for testing. The sixth module was tested with *B. atrophaeus*, and the data set was reviewed again, excluding Module 3. NSF chose to test Module 2 for the second *C. parvum* challenge. No oocysts were found in any filtrate samples from the Module 2 challenge test.

In Table 4-1, the LRVs in the "Overall Mean" rows are the geometric means of the individual sample point LRVs. The $LRV_{C\text{-}TEST}$ using the overall means is 6.20. The $LRV_{C\text{-}TEST}$ based on the individual sample pairs is 5.97.

Table 4-1. *C. parvum* Challenge Results

Module Number	Sample Point	Feed		Filtrate		LRV
		Geometric Mean (Oocysts/L)	Log_{10}	Geometric Mean (Oocysts/L)	Log_{10}	
Module 3	Flush	—	—	<1	—	—
	2 Minutes	$1.74x10^6$	6.24	<1	0.0	6.24
	15 Minutes	$2.4x10^6$	6.38	<1	0.0	6.38
	30 Minutes	$9.4x10^5$	5.97	1	0.0	5.97
	Overall Mean	$1.6x10^6$	6.20	1	0.0	6.20
Module 2	Flush	—	—	<1	—	—
	2 Minutes	$2.02x10^6$	6.31	<1	0.0	6.31
	15 Minutes	$1.5x10^6$	6.18	<1	0.0	6.18
	30 Minutes	$1.92x10^6$	6.28	<1	0.0	6.28
	Overall Mean	$1.8x10^6$	6.26	<1	0.0	6.26

4.3 *B. atrophaeus* Endospores Challenge Tests

The *B. atrophaeus* endospore challenge results are displayed in Table 4-2. As discussed previously, the challenge concentrations for the first round of tests were above the allowable maximum of 6.5 $\log_{10}$, so two modules were retested with lower challenge concentrations. The results of these two retests are displayed at the bottom of Table 4-2. NSF has also learned from EPA that the States can accept the test data with the feed concentrations capped at 6.50 $\log_{10}$. Therefore, the LRV calculations with the feed concentrations capped at 6.50 $\log_{10}$ are presented in Table 4-3. Excluding the Module 2 and 4 lower challenge data, all modules had mean *B.*

atrophaeus LRVs of 6.50, except for Module 4, which was 6.40 due to one filtrate sample that was above 0.0. The $LRV_{C\text{-}TEST}$ from this subset would be 6.40 based on the overall mean LRVs, or 6.20 based on the lowest individual sample point LRVs. If the Module 2 and 4 lower challenge retest data is included in the dataset for determination of the $LRV_{C\text{-}TEST,}$ it is 5.90 based on the overall mean LRVs, or 5.77 based on the lowest individual sample point LRVs.

Table 4-2. *B. atrophaeus* Endospores Challenge Results

Challenge Test	Sample Point	Feed		Filtrate		LRV
		Geometric Mean (CFU/100 mL)	Log_{10}	Geometric Mean (CFU/100 mL)	Log_{10}	
Module 1	Flush	—	—	<1	—	—
	2 Minutes	$1.13x10^7$	7.05	<1	0.0	7.05
	15 Minutes	$1.1x10^7$	7.04	1	0.0	7.04
	30 Minutes	$1.17x10^7$	7.07	<1	0.0	7.05
	Overall Mean	$1.1x10^7$	7.05	1	0.0	7.05
Module 2	Flush	—	—	2	—	—
	2 Minutes	$1.36x10^7$	7.13	1	0.0	7.13
	15 Minutes	$1.20x10^7$	7.08	1	0.0	7.08
	30 Minutes	$1.22x10^7$	7.09	1	0.0	7.09
	Overall Mean	$1.26x10^7$	7.10	1	0.0	7.10
Module 3	Flush	—	—	<1	—	—
	2 Minutes	$1.07x10^7$	7.03	1	0.0	7.03
	15 Minutes	$1.0x10^7$	7.00	<1	0.0	7.00
	30 Minutes	$1.35x10^7$	7.13	1	0.0	7.13
	Overall Mean	$1.1x10^7$	7.05	1	0.0	7.05
Module 4	Flush	—	—	1	—	—
	2 Minutes	$1.16x10^7$	7.06	<1	0.0	7.06
	15 Minutes	$1.0x10^7$	7.00	2	0.3	6.70
	30 Minutes	$7.3x10^6$	6.86	<1	0.0	6.86
	Overall Mean	$9.5x10^6$	6.97	1	0.1	6.87
Module 5	Flush	—	—	1	—	—
	2 Minutes	$1.24x10^7$	7.09	<1	0.0	7.09
	15 Minutes	$1.28x10^7$	7.11	<1	0.0	7.11
	30 Minutes	$1.44x10^7$	7.16	<1	0.0	7.16
	Overall Mean	$1.32x10^7$	7.12	<1	0.0	7.12
Module 6	Flush	—	—	1	—	—
	2 Minutes	$1.46x10^7$	7.16	1	0.0	7.16
	15 Minutes	$1.63x10^7$	7.21	1	0.0	7.11
	30 Minutes	$1.43x10^7$	7.16	<1	0.0	7.16
	Overall Mean	$1.50x10^7$	7.18	1	0.0	7.18
Module 2 Retest with Lower Challenge	Flush	—	—	<1	—	—
	2 Minutes	$9.4x10^5$	5.97	1	0.0	5.97
	15 Minutes	$9.5x10^5$	5.98	<1	0.0	5.98
	30 Minutes	$1.0x10^6$	6.00	1	0.0	6.00
	Overall Mean	$9.6x10^5$	5.98	1	0.0	5.98
Module 4 Retest with Lower Challenge	Flush	—	—	1	—	—
	2 Minutes	$1.29x10^6$	6.11	2	0.3	5.81
	15 Minutes	$1.18x10^6$	6.07	2	0.3	5.77
	30 Minutes	$1.29x10^6$	6.11	1	0.0	6.11
	Overall Mean	$1.25x10^6$	6.10	2	0.2	5.90

While an oocyst was found in only one of the filtrate samples from the *C. parvum* challenges, *B. atrophaeus* endospores were found in many filtrate samples. This provides experimental evidence that endospores are indeed a conservative surrogate for *Cryptosporidium*.

Table 4-3. *B. atrophaeus* LRVs with the Feed Capped at 6.5 Log_{10}

Challenge Test	Sample Point	Log_{10} of Feed	Log_{10} of Filtrate	LRV
Module 1	2 Minutes	6.50	0.0	6.50
	15 Minutes	6.50	0.0	6.50
	30 Minutes	6.50	0.0	6.50
	Overall Mean	6.50	0.0	6.50
Module 2	2 Minutes	6.50	0.0	6.50
	15 Minutes	6.50	0.0	6.50
	30 Minutes	6.50	0.0	6.50
	Overall Mean	6.50	0.0	6.50
Module 3	2 Minutes	6.50	0.0	6.50
	15 Minutes	6.50	0.0	6.50
	30 Minutes	6.50	0.0	6.50
	Overall Mean	6.50	0.0	6.50
Module 4	2 Minutes	6.50	0.0	6.50
	15 Minutes	6.50	0.3	6.20
	30 Minutes	6.50	0.0	6.50
	Overall Mean	6.50	0.1	6.40
Module 5	2 Minutes	6.50	0.0	6.50
	15 Minutes	6.50	0.0	6.50
	30 Minutes	6.50	0.0	6.50
	Overall Mean	6.50	0.0	6.50
Module 6	2 Minutes	6.50	0.0	6.50
	15 Minutes	6.50	0.0	6.50
	30 Minutes	6.50	0.0	6.50
	Overall Mean	6.50	0.0	6.50

4.4 MS2 Challenge Tests

Table 4-4 displays the MS2 challenge data. As with the *B. atrophaeus* challenge levels, the MS2 challenges above 6.50 log_{10} were capped to calculate the LRVs. There was a wide range of MS2 reduction observed, from a mean LRV of 4.51 log_{10} for Module 1, down to 2.54 log_{10} for Module 6. Under both $LRV_{C\text{-}TEST}$ calculation methods, Module 6 gives the $LRV_{C\text{-}TEST}$ of 2.54 or 2.37 log_{10}.

Table 4-4. MS2 Challenge Results							
		Feed			Filtrate		
	Sample Point	Geometric Mean (PFU/mL)	Log_{10}	Capped Log_{10}	Geometric Mean (PFU/mL)	Log_{10}	LRV
Module 1	Flush	—	—	—	1	—	—
	2 Minutes	$3.0x10^6$	6.48	—	80	1.90	4.58
	15 Minutes	$3.0x10^6$	6.48	—	93	1.97	4.51
	30 Minutes	$3.2x10^6$	6.51	6.50	$1.1x10^2$	2.04	4.47
	Overall Mean	$3.07x10^6$	6.49	—	94	1.97	4.52
Module 2	Flush	—	—	—	<1	—	—
	2 Minutes	$2.3x10^6$	6.36	—	$5.8x10^2$	2.76	3.60
	15 Minutes	$3.1x10^6$	6.49	—	$5.3x10^2$	2.72	3.77
	30 Minutes	$3.4x10^6$	6.53	6.50	$4.4x10^2$	2.64	3.89
	Overall Mean	$2.9x10^6$	6.46	—	$5.1x10^2$	2.71	3.75
Module 3	Flush	—	—	—	<1	—	—
	2 Minutes	$2.41x10^6$	6.38	—	$4.8x10^2$	2.68	3.70
	15 Minutes	$1.34x10^6$	6.13	—	$5.7x10^2$	2.76	3.37
	30 Minutes	$1.14x10^6$	6.06	—	$4.7x10^2$	2.67	3.39
	Overall Mean	$1.54x10^6$	6.19	—	$5.0x10^2$	2.70	3.48
Module 4	Flush	—	—	—	<1	—	—
	2 Minutes	$1.31x10^6$	6.12	—	$1.1x10^3$	3.04	3.08
	15 Minutes	$1.22x10^6$	6.09	—	$5.0x10^2$	2.70	3.39
	30 Minutes	$1.1x10^6$	6.04	—	$3.0x10^2$	2.48	3.56
	Overall Mean	$1.2x10^6$	6.08	—	$5.5x10^2$	2.74	3.34
Module 5	Flush	—	—	—	<1	—	—
	2 Minutes	$7.6x10^5$	5.88	—	$7.7x10^2$	2.89	2.99
	15 Minutes	$9.3x10^5$	5.97	—	$4.6x10^2$	2.66	3.31
	30 Minutes	$9.3x10^5$	5.97	—	$3.3x10^2$	2.52	3.45
	Overall Mean	$8.7x10^5$	5.94	—	$4.9x10^2$	2.69	3.25
Module 6	Flush	—	—	—	<1	—	—
	2 Minutes	$1.15x10^7$	7.06	6.50	$5.2x10^3$	3.72	2.78
	15 Minutes	$1.2x10^7$	7.08	6.50	$1.1x10^4$	4.04	2.46
	30 Minutes	$1.40x10^7$	7.15	6.50	$1.35x10^4$	4.13	2.37
	Overall Mean	$1.2x10^7$	7.10	6.50	$9.2x10^3$	3.96	2.54

4.5 Pressure Decay Test Results

The pre-test and post-test pressure decay test results are displayed in Table 4-5. Immediately prior to each pre-test pressure decay measurement, the background pressure decay rate of the pressurized test rig plumbing was measured, and the observed background decay rate, if any, was recorded. The background pressure decay rates were subtracted from the measured decay rates, and the corrected pressure decay rates are displayed in the last column of Table 4-5. For most challenge tests, the post-test pressure decay rate was lower than the pre-test decay rate. The membranes were not backwashed prior to measuring the post-test pressure decay rate, so the lower post-test decay rates could be a result of accumulation of particulate matter, including the challenge particulates, on the membrane surface. However, the challenge test results do not indicate that any accumulation of particulates improved membrane performance over the test periods.

Table 4-5. Pressure Decay Data								
Module	**Test**	**Date**	**Starting Pressure (psig)**	**Final Pressure (psig)**	**Elapsed Time (min)**	**Decay Rate (psig/min)**	**Background Decay Rate (psig/min)**	**Corrected Decay Rate (psig/min)**
#1	MS2 Pre-test	10/05/2009	16.86	15.97	20.67	0.0431	0.0000	0.0431
	MS2 Post-test	10/05/2009	16.40	15.68	20.00	0.0360	0.0000	0.0360
	Bac. Pre-test	10/05/2009	16.96	16.19	20.00	0.0385	0.0000	0.0385
	Bac. Post-test	10/05/2009	16.76	16.63	20.00	0.0065	0.0000	0.0065
#2	MS2 Pre-test	10/14/2009	20.88	19.55	20.00	0.0665	0.0085	0.0580
	MS2 Post-test	10/14/2009	20.70	20.58	20.00	0.0060	0.0085	-0.0025
	Bac. Pre-test	10/14/2009	20.90	19.83	20.00	0.0535	0.0085	0.0450
	Bac. Post-test	10/14/2009	20.26	20.15	20.00	0.0055	0.0085	-0.0030
	C.parvum Pre-test	02/26/2010	20.91	20.32	20.00	0.0295	0.0000	0.0295
	C. parvum Post-test	02/26/2010	20.59	19.83	20.00	0.0380	0.0000	0.0380
	Bac. Pre-retest	05/14/2010	21.42	20.54	20.00	0.0440	0.0110	0.0330
	Bac. Post-retest	05/14/2010	21.39	20.17	20.00	0.0610	0.0110	0.0500
#3	MS2 Pre-test	10/02/2009	15.62	14.57	20.00	0.0525	0.0000	0.0525
	MS2 Post-test	10/02/2009	17.09	15.82	20.13	0.0631	0.0000	0.0631
	Bac. Pre-test	10/02/2009	16.29	15.26	20.00	0.0515	0.0000	0.0515
	Bac. Post-test	10/02/2009	16.70	15.46	20.00	0.0620	0.0000	0.0620
	C. parvum Pre-test	10/23/2009	20.85	19.50	20.00	0.0675	0.0180	0.0495
	C. parvum Post-test	10/23/2009	22.40	19.78	20.66	0.1268	0.0180	0.1088
#4	MS2 Pre-test	10/07/2009	21.27	19.84	21.33	0.0670	0.0255	0.0415
	MS2 Post-test	10/07/2009	20.76	20.60	20.00	0.0080	0.0255	-0.0175
	Bac. Pre-test	10/07/2009	20.55	19.29	20.00	0.0630	0.0255	0.0375
	Bac. Post-test	10/07/2009	20.55	19.13	20.00	0.0710	0.0255	0.0455
	Bac. Pre-retest	05/14/2010	21.46	20.39	20.00	0.0535	0.0700	-0.0165
	Bac. Post-retest	05/14/2010	21.68	20.12	22.00	0.0709	0.0700	0.0009
#5	MS2 Pre-test	10/09/2009	20.27	19.17	20.00	0.0550	0.0170	0.0380
	MS2 Post-test	10/09/2009	20.07	19.32	20.00	0.0375	0.0170	0.0205
	Bac. Pre-test	10/09/2009	20.19	18.98	20.00	0.0605	0.0170	0.0435
	Bac. Post-test	10/09/2009	21.17	20.21	20.00	0.0480	0.0170	0.0310
#6	MS2 Pre-test	02/11/2010	20.26	19.17	20.00	0.0545	0.0095	0.0450
	MS2 Post-test	02/11/2010	20.39	19.53	20.00	0.0430	0.0095	0.0335
	Bac. Pre-test	02/11/2010	20.31	19.60	20.00	0.0355	0.0095	0.0260
	Bac. Post-test	02/11/2010	20.29	19.58	20.00	0.0355	0.0095	0.0260

4.6 MS2 Reduction vs. Flux

Dow requested that NSF conduct three extra MS2 challenge tests at lower flows to determine whether MS2 reduction increased as the flux decreased. Module #5 was chosen for these tests because it was the worst performing module of the five that had been tested at the time.

The data for these tests is displayed in Table 4-6. The LRV numbers for each challenge test are also displayed graphically in Figure 4-1. The data does indicate that MS2 reduction is inversely proportional to the flux, but the observed LRVs for the lower flow rate tests are all within the range of LRVs from the maximum flux tests, except for the first sampling point from the 13.6 gpm test. The feed concentrations for these challenges are not capped at 6.5 $\log_{10}$ because the

intent of this study was not to provide regulatory compliance data, but rather only to supply comparative data on membrane performance at lower fluxes.

Table 4-6. MS2 vs. Flux Results

Test Flow	Sample Point	Feed		Filtrate		LRV
		Geometric Mean (PFU/mL)	Log_{10}	Geometric Mean (PFU/mL)	Log_{10}	
13.6 gpm	Flush	—	—	<1	—	—
	2 Minutes	$1.31x10^7$	7.12	223	2.35	4.77
	15 Minutes	$1.1x10^7$	7.04	690	2.84	4.20
	30 Minutes	$1.1x10^7$	7.04	$1.35x10^3$	3.13	3.91
	Overall Mean	$1.2x10^7$	7.07	590	2.77	4.29
25.4 gpm	Flush	—	—	44	—	—
	2 Minutes	$1.0x10^7$	7.00	$1.52x10^3$	3.18	3.82
	15 Minutes	$1.1x10^7$	7.04	$3.4x10^3$	3.53	3.51
	30 Minutes	$1.2x10^7$	7.08	$5.4x10^3$	3.73	3.35
	Overall Mean	$1.1x10^7$	7.04	$3.0x10^3$	3.48	3.56
35.6 gpm	Flush	—	—	50	—	—
	2 Minutes	$1.1x10^7$	7.04	$2.9x10^3$	3.46	3.58
	15 Minutes	$1.0x10^7$	7.00	$6.5x10^3$	3.81	3.19
	30 Minutes	$8.5x10^6$	6.93	$1.41x10^4$	4.15	2.78
	Overall Mean	$1.0x10^7$	6.99	$6.5x10^3$	3.81	3.18

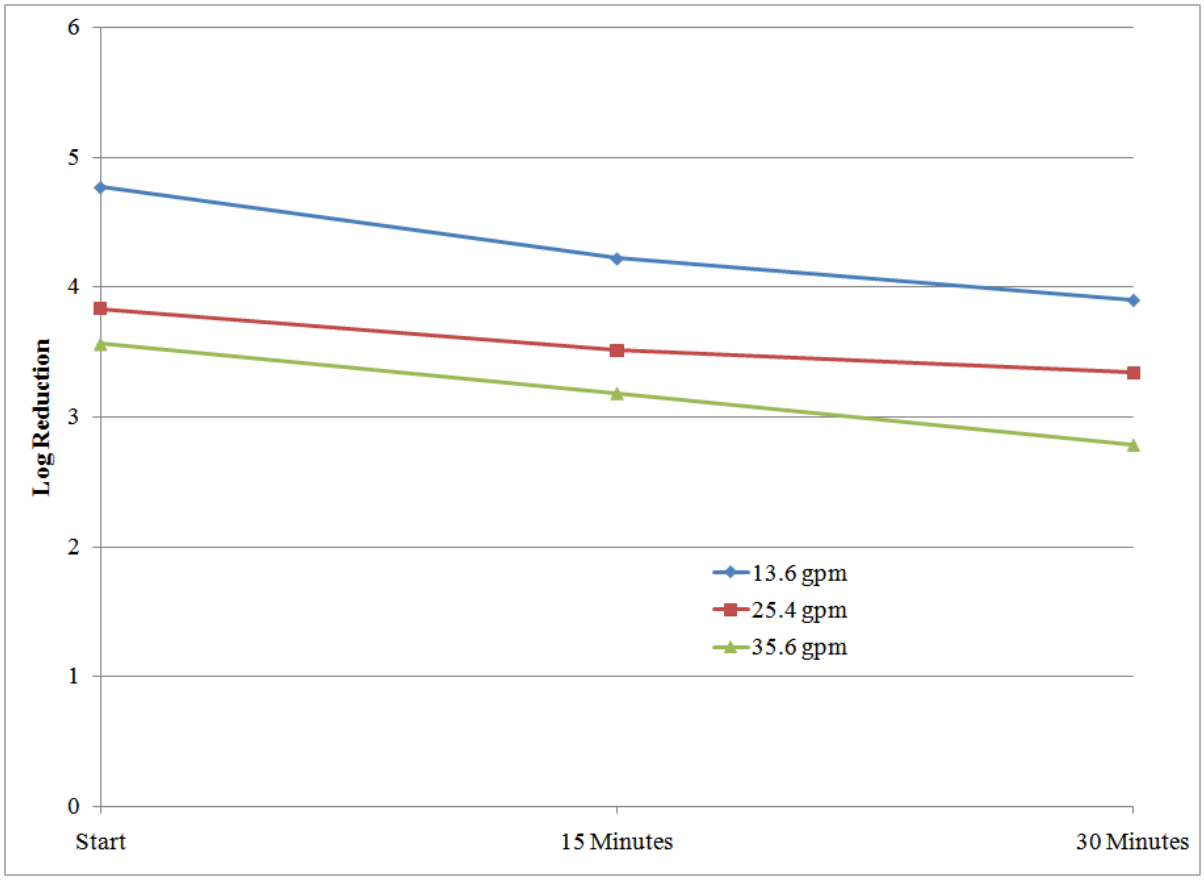

Figure 4-1. MS2 LRVs at lower flow rates.

The pressure decay data for the lower flux tests is displayed in Table 4-7. The data does not indicate that there were any membrane integrity issues during these challenge tests.

Table 4-7. MS2 vs. Flux Study Pressure Decay Data

Module	Test	Date	Starting Pressure (psig)	Final Pressure (psig)	Elapsed Time (min)	Decay Rate (psig/min)	Background Decay Rate (psig/min)	Corrected Decay Rate (psig/min)
#5	13.6 gpm Pre-test	02/24/2010	20.26	19.55	20.00	0.0355	0.0005	0.0350
	13.6 gpm Post-test	02/24/2010	21.03	20.33	20.00	0.0350	0.0005	0.0345
	25.4 gpm Pre-test	02/24/2010	20.96	20.04	20.00	0.0460	0.0005	0.0455
	25.4 gpm Post-test	02/24/2010	20.97	20.17	20.00	0.0400	0.0005	0.0395
	35.6 gpm Pre-test	02/25/2010	21.14	20.34	20.00	0.0400	0.0305	0.0095
	35.6 gpm Post-test	02/25/2010	21.02	20.15	20.00	0.0435	0.0305	0.0130

4.7 Operational Data and Water Quality Data for All Challenges

The pilot unit operational data is presented in Table 4-8. The filtrate flows, and feed and filtrate pressure readings were recorded onto bench sheets. The fluxes were calculated from the flow data. The target flux for the tests was 70.0 gfd. The recorded flows translated into fluxes ranging from 69.5 to 71.9 gfd. Except for the 41.4 gpm filtrate flow measurement, all recorded flows were within 0.5 gpm or less of the target flow of 40.3 gpm for the tests.

Table 4-8. Operation Data

Module #	Date	Filtrate Flow Rate (gpm)		Flux (gfd)		Feed Pressure (psig)		Filtrate Pressure (psig)	
		0 Min.	30 Min.	0 Min	30 Min	0 Min.	30 Min.	0 Min.	30 Min.
MS2 Challenges									
Module 1	10/05/09	40.0	40.1	69.5	69.7	25.75	25.05	2.10	2.02
Module 2	10/14/09	40.3	40.1	70.0	69.7	21.62	20.90	0.55	0.51
Module 3	10/02/09	40.2	40.0	69.8	69.5	21.90	20.97	1.06	1.05
Module 4	10/07/09	41.4	40.7	71.9	70.7	24.29	23.31	2.52	2.32
Module 5	10/09/09	40.0	40.0	69.5	69.5	25.06	24.38	2.16	2.19
Module 6	02/11/10	40.7	40.4	70.7	70.2	24.95	23.80	0.84	0.74
Mod. 5 13.6 gpm	02/24/10	13.7	13.7	23.8	23.8	13.08	12.65	2.07	2.01
Mod. 5 25.4 gpm	02/24/10	25.7	25.4	44.6	44.1	18.52	18.02	1.40	1.43
Mod. 5 35.6 gpm	02/25/10	35.7	35.7	62.0	62.0	24.86	24.20	2.68	2.72
***B. atrophaeus* Challenges**									
Module 1	10/05/09	40.3	40.0	70.0	69.5	25.88	25.35	1.97	2.01
Module 2	10/14/09	40.1	40.0	69.7	69.5	21.83	21.29	0.25	0.32
Module 3	10/02/09	40.1	40.1	69.7	69.7	22.17	21.42	2.08	2.13
Module 4	10/07/09	40.1	40.1	69.7	69.7	23.65	22.97	1.93	1.95
Module 5	10/09/09	40.4	40.0	70.2	69.5	25.38	24.33	2.09	2.01
Module 6	02/11/10	40.3	40.3	70.0	70.0	25.92	25.12	1.86	1.92
Mod. 2 Retest	05/14/10	40.0	40.4	69.5	70.2	27.42	27.01	6.72	6.86
Mod. 4 Retest	05/13/10	40.8	40.4	70.9	70.2	27.78	26.80	6.04	5.82
***C. parvum* Challenge**									
Module 3	10/23/09	40.3	40.1	70.0	69.7	21.83	20.96	1.39	1.36
Module 2	02/26/10	40.1	40.3	69.7	70.0	26.69	24.85	4.05	3.16

The feed water chemistry data is displayed in Table 4-9. As discussed in Section 3.4, the water recipe was changed between the first round of the tests in October 2009, and the second and third rounds of tests in February and May of 2010. The phosphate buffered water had very low alkalinity and TDS compared to the calcium carbonate buffered water. The phosphate buffered water also had a lower pH. In fact, the calcium carbonate buffered water slightly exceeded the target pH range of 7.0 to 8.0 for three of the eight tests with that water.

Note that the measured alkalinity for the Module 3 *C. parvum* challenge was <5 mg/L, when it should have been around 100 mg/L. There was a preliminary alkalinity of 99 mg/L recorded on the bench sheet for this test, but the pH was measured at 6.70, which is similar to the other pH measurements for the phosphate buffered waters with no carbonate alkalinity. Therefore, NSF believes that the water did not contain any calcium carbonate. NSF does not think this is a significant issue though, since the Module 3 *C. parvum* challenge water was similar to the phosphate buffered water with respect to alkalinity and pH.

Table 4-9. Feed Water Chemistry Data

Module #	Alkalinity (mg/L $CaCO_3$)	pH	Temp. (°C)	Total Chlorine (mg/L)	TDS (mg/L)	TOC (mg/L)	Turbidity (NTU)
MS2 Challenges							
Module 1	83	7.98	22.0	<0.05	86	<0.1	0.10
Module 2	99	7.97	21.9	<0.05	120	<0.1	0.11
Module 3	99	7.95	22.2	<0.05	100	<0.1	0.07
Module 4	100	7.96	22.5	<0.05	110	<0.1	0.09
Module 5	99	8.00	21.9	<0.05	110	<0.1	0.07
Module 6	ND(5)	7.30	19.7	<0.05	16	<0.1	0.20
Mod. 5 13.6 gpm	6	6.73	18.5	<0.05	28	<0.1	0.15
Mod. 5 25.4 gpm	6	6.72	18.9	<0.05	22	<0.1	0.25
Mod. 5 35.6 gpm	6	6.83	17.5	<0.05	20	<0.1	0.09
***B. atrophaeus* Challenges**							
Module 1	97	8.08	22.4	<0.05	100	<0.1	0.11
Module 2	100	8.01	22.0	<0.05	120	<0.1	0.14
Module 3	92	7.96	22.1	<0.05	92	<0.1	0.21
Module 4	100	7.96	19.9	<0.05	120	<0.1	0.10
Module 5	97	8.05	22.2	<0.05	110	<0.1	0.08
Module 6	5	7.36	20.0	<0.05	12	<0.1	0.15
Mod. 2 Retest	7	7.26	19.7	<0.05	23	<0.1	0.11
Mod. 4 Retest	8	7.41	19.0	<0.05	27	<0.1	0.28
***C. parvum* Challenge**							
Module 3	<5	6.70	21.5	<0.05	<5	0.3	0.14
Module 2	6	7.13	17.4	<0.05	11	<0.1	0.18

Chapter 5
Quality Assurance/Quality Control

5.1 Introduction

An important aspect of verification testing is the QA/QC procedures and requirements. Careful adherence to the procedures ensured that the data presented in this report was of sound quality, defensible, and representative of the equipment performance. The primary areas of evaluation were representativeness, accuracy, precision, and completeness.

Because this ETV was conducted at the NSF testing lab, all laboratory activities were conducted in accordance with the provisions of the NSF International Laboratories Quality Assurance Manual.

5.2 Test Procedure QA/QC

NSF testing laboratory staff conducted the tests by following a USEPA-approved test/QA plan created specifically for this verification. NSF QA Department staff performed an audit during testing to ensure the proper procedures were followed. The audit yielded no significant findings.

5.3 Sample Handling

All samples analyzed by the NSF Chemistry and Microbiology Laboratories were labeled with unique identification numbers. All samples were analyzed within allowable holding times.

5.4 Chemistry Laboratory QA/QC

The calibrations of all analytical instruments and the analyses of all parameters complied with the QA/QC provisions of the NSF International Laboratories Quality Assurance Manual.

The NSF QA/QC requirements are all compliant with those given in the USEPA method or Standard Method for the parameter. Also, every analytical method has an NSF standard operating procedure.

5.5 Microbiology Laboratory QA/QC

5.5.1 Growth Media Positive Controls

All media were checked for sterility and positive growth response when prepared and when used for microorganism enumeration. The media was discarded if growth occurred on the sterility check media, or if there was an absence of growth in the positive response check.

5.5.2 Negative Controls

For each sample batch processed, an unused membrane filter and a blank with 100 mL of buffered, sterilized dilution water was filtered through the membrane were also placed onto the appropriate media and incubated with the samples as negative controls. No growth was observed on any blanks.

5.6 Documentation

All laboratory activities were documented using specially prepared laboratory bench sheets and NSF laboratory reports. Data from the bench sheets and laboratory reports were entered into Microsoft™ Excel® spreadsheets. These spreadsheets were used to calculate the geometric means and $\log_{10}$ reductions. One hundred percent of the data entered into the spreadsheets was checked by a reviewer to confirm all data and calculations were correct.

5.7 Data Review

NSF QA/QC staff reviewed the raw data records for compliance with QA/QC requirements. As required in the ETV Quality Management Plan, NSF ETV staff checked at least 10% of the data in the NSF laboratory reports against the lab bench sheets.

5.8 Data Quality Indicators

The quality of data generated for this ETV is established through four indicators of data quality: representativeness, accuracy, precision, and completeness.

5.8.1 Representativeness

Representativeness is a qualitative term that expresses "the degree to which data accurately and precisely represent a characteristic of a population, parameter variations at a sampling point, a process condition, or an environmental condition." Representativeness was ensured by consistent execution of the test protocol for each challenge, including timing of sample collection, sampling procedures, and sample preservation. Representativeness was also ensured by using each analytical method at its optimum capability to provide results that represent the most accurate and precise measurement it is capable of achieving.

5.8.2 Accuracy

Accuracy was quantified as the percent recovery of the parameter in a sample of known quantity. Accuracy was measured through use of both matrix spikes of a known quantity and certified standards during calibration of an instrument.

The following equation was used to calculate percent recovery:

$$\text{Percent Recovery} = 100 \times [(X_{known} - X_{measured})/X_{known}]$$

where: X_{known} = known concentration of the measured parameter
$X_{measured}$ = measured concentration of parameter

Accuracy of the benchtop chlorine, pH, and turbidity meters was checked daily during the calibration procedures using certified check standards. Alkalinity and TDS were analyzed in batches. Certified QC standards and/or matrix spikes were run with each batch.

The NSF Laboratory Quality Assurance Manual establishes the frequency of spike sample analyses at 10% of the samples analyzed for chemical analyses. Laboratory control samples are also run at a frequency of 10%. The recovery limits specified for the parameters in this

verification, excluding microbiological analyses, were 70-130% for laboratory-fortified samples and 85-115% for laboratory control samples. The NSF QA department reviewed the laboratory records and found that all recoveries were within the prescribed QC requirements. Calibration requirements were also achieved for all analyses.

5.8.3 Precision

Precision refers to the degree of mutual agreement among individual measurements and provides an estimate of random error. One sample per batch was analyzed in duplicate for the TDS measurements. At least one out of every ten samples for alkalinity was analyzed in duplicate. Duplicate municipal drinking water samples were analyzed for pH, total chlorine, and turbidity as part of the daily calibration process. Precision of duplicate analyses was measured by use of the following equation to calculate RPD:

$$RPD = \left| \frac{S_1 - S_2}{S_1 + S_2} \right| \times 200$$

where:

S_1 = sample analysis result; and

S_2 = sample duplicate analysis result.

Acceptable analytical precision for the verification test was set at an RPD of 30%. Field duplicates were collected at a frequency of 1 out of every 10 samples for each parameter, to incorporate both sampling and analytical variation to measure overall precision against this objective. In addition, the NSF Laboratory also conducted laboratory duplicate measurements at 10% frequency of samples analyzed. The laboratory precision for the methods selected was tighter than the 30% overall requirement, generally set at 20% based on the standard NSF Chemistry Laboratory method performance.

All RPD were within NSF's established allowable limits for each parameter. Please note that samples from this evaluation for alkalinity and TDS were batched with other non-ETV samples. The duplicate analysis requirements apply to the whole batch, not just the samples from this ETV.

5.8.4 Completeness

Completeness is the proportion of valid, acceptable data generated using each method as compared to the requirements of the test/QA plan. The completeness objective for data generated during verification testing is based on the number of samples collected and analyzed for each parameter and/or method, as presented in Table 5-1.

Table 5-1. Completeness Requirements

Number of Samples per Parameter and/or Method	Percent Completeness
0-10	80%
11-50	90%
> 50	95%

Completeness is defined as follows for all measurements:

$$\%C = (V/T) \times 100$$

where:

%C = percent completeness;
V = number of measurements judged valid; and
T = total number of measurements.

One hundred percent completeness was achieved for all aspects of this verification. All planned testing activities were conducted as scheduled, and all planned samples were collected for challenge organism and water chemistry analysis.

Chapter 6
References

APHA, AWWA, and WEF (1999). *Standard Methods for the Examination of Water and Wastewater*, 20th Edition.

NSF International (2007). *NSF/ANSI Standard 55 – Ultraviolet Microbiological Water Treatment Systems*.

USEPA (2005). *Membrane Filtration Guidance Manual* (EPA 815-R-06-009).

USEPA and NSF International (2005). *ETV Protocol for Equipment Verification Testing for Physical Removal of Microbiological and Particulate Contaminants*

Appendix A
Test/Quality Assurance Project Plan

Contact Mr. Bruce Bartley at 734-769-5148 or bartley@nsf.org for a copy of this document.

Appendix B
Bacillus Endospores as a Surrogate for *C. parvum* Oocysts

The EPA LT2ESWTR allows the use of a surrogate for *C. parvum*, provided the surrogate is conservative. The EPA MFGM specifically discusses *Bacillus subtilis* as a surrogate, but states "Because there is limited data currently available regarding the use of *Bacillus subtilis* in membrane challenge studies, a characterization of this organism would be necessary to determine whether it could be used as a *Cryptosporidium* surrogate…" The MFGM also states "Based on the size…*Bacillus subtilis* could potentially be considered a conservative surrogate…pending a comparison of other characteristics (e.g., shape, surface charge, etc.)…"

1. Organism Size and Shape

C. parvum is spherical in shape, while *Bacillus* endospores are ellipsoidal in shape (football shaped). *C. parvum* has a diameter of 4-6 µm. *Bacillus* endospores are approximately 0.8 µm in diameter, and 1.8 µm in length. Therefore, *Bacillus* endospores are a conservative surrogate for *C. parvum*, no matter what the orientation of the endospore is when it impacts the test membrane.

Baltus et. al. (2008) studied membrane rejection of bacteria and viruses with different length vs. diameter aspect ratios. They theorized, based on a transport model for rod-shaped particles, that rejection would improve as the aspect ratio (length vs. diameter) increased for a fixed particle volume. However, their experimental results contradicted this, with similar rejection rates for particles with a range of aspect ratios. The model assumed that particles would impact the membrane with equal frequency for all particle orientations. They theorize that instead, an end-on orientation was favored for transport of the particles in the water stream. They concluded that microorganism removal by membranes could be conservatively estimated using only the rod diameter in transport models. These findings add an additional safety factor to using *Bacillus* endospores as a surrogate for *C. parvum*.

2. Electrophoretic Mobility and Isoelectric Point

A suitable surrogate should have a surface charge similar to *C. parvum*, as measured through the isoelectric point and electrophoretic mobility (EPM). The isoelectric point is the pH at which the particle has a neutral surface charge in an aqueous environment. Below this point the particle has a net positive charge, above it a net negative charge. Many studies have pegged the isoelectric point of *C. parvum* between pH values of 2 and 4, thus it would have a negative surface charge in the neutral pH range. The isoelectric point can be found by measuring the EPM of the particle at various pH values. The pH where the EPM is zero is classified as the isoelectric point.

Lytle et. al. (2002) measured the EPM of both *C. parvum* and *B. subtilis* endospores in solutions of increasing buffer concentration (0.915 millimolar, mM, 9.15 mM, and 91.5 mM KH_2PO_4). They found that increasing the buffer concentration also increases the EPM toward a positive value. The buffer concentration of the test water for the Dow verification tests was approximately 1 mM for the carbonate buffered test water, and 0.1 mM for the phosphate

buffered test water. Therefore, the 0.915 mM data from this study should be the most accurate representation of the *C. parvum* and *B. subtilis* EPM for the ETV tests. In 0.915 mM solutions at pH values between 7 and 8, they observed EPM of approximately -2.2 to -2.6 µm cm V^{-1} s^{-1} for *C. parvum*, and -1.9 to -2.2 µm cm $V^{-1}s^{-1}$ for *B. subtilis*. For *B. subtilis*, the researchers did not measure an isoelectric point at any buffer concentration. For *C. parvum*, they did find an isoelectric point at a pH around 2.5, but only for the 9.15 mM solution. For both organisms, the 0.915 mM solution generally gave lower (more negative) EPM values than the solutions with higher buffering capacity.

3. Aggregation

The NSF Microbiology Laboratory microscopically examined a sample of the *B. atrophaeus* stock solutions purchased for the tests. The sample was suspended in sterile, buffered, deionized water and stirred at moderate speed for 15 minutes. The estimated cell density was $1x10^9$ CFU/100 mL, which is approximately 100 times higher than the suspensions injected into the pilot units to challenge the UF membranes. Figure B-1 is a photograph of the *B. atrophaeus* endospores in the sample. The magnification is 1000x oil immersion with differential interference contrast microscopy. No evidence of endospore aggregation was found.

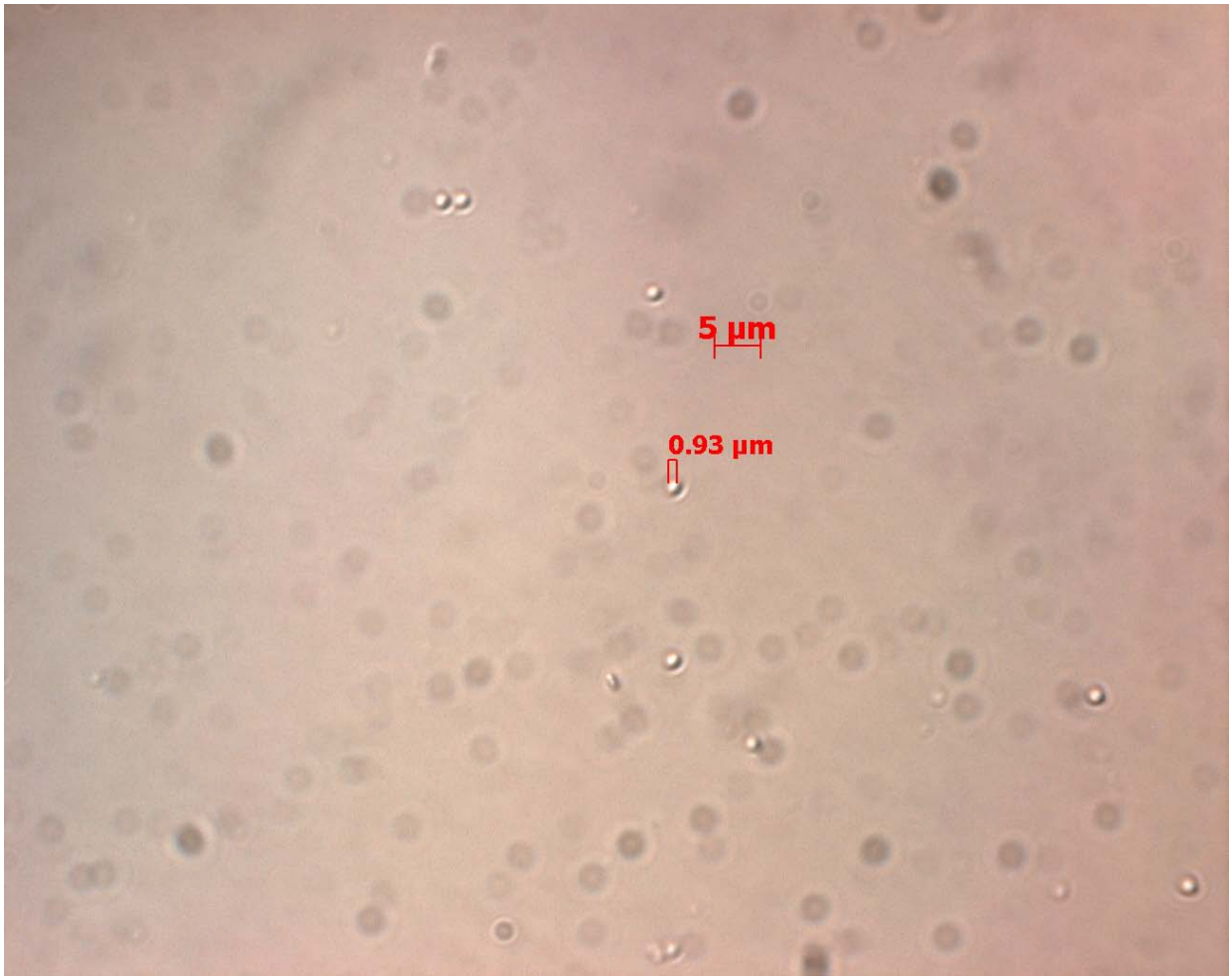

Figure B-1. Mono-dispersed *B. atrophaeus* endospores used for challenge tests.

References

Baltus, R. E., A. R. Badireddy, W. Xu, and S. Chellam (2009). Analysis of Configurational Effects on Hindered Convection of Nonspherical Bacteria and Viruses across Microfiltration Membranes. *Industrial and Engineering Chemistry Research.* In press.

Brush, C. F., M. F. Walter, L. J. Anguish, and W. C. Ghiorse (1998). Influence of Pretreatment and Experimental Conditions on Electrophoretic Mobility and Hydrophobicity of *Cryptosporidium parvum* Oocysts. *Applied and Environmental Microbiology.* 64: 4439-4445.

Butkus, M. A., J. T. Bays, and M. P. Labare (2003). Influence of Surface Characteristics on the Stability of *Cryptosporidium parvum* Oocysts. *Applied and Environmental Microbiology.* 69: 3819-3825.

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Appendix C
Challenge Organism Triplicate Counts

Table C-1. MS2 Triplicate Count Data

		Feed (PFU/mL)			Filtrate (PFU/mL)		
Module	**Sample**	**Count 1**	**Count 2**	**Count 3**	**Count 1**	**Count 2**	**Count 3**
Module 1	Flush	—	—	—	<1	<1	1
	2 Minutes	$3.4x10^6$	$2.77x10^6$	$2.97x10^6$	82	81	77
	15 Minutes	$2.8x10^6$	$3.1x10^6$	$3.0x10^6$	$1.11x10^2$	92	79
	30 Minutes	$3.4x10^6$	$2.9x10^6$	$3.4x10^6$	$1.06x10^2$	93	$1.42x10^2$
Module 2	Flush	—	—	—	<1	<1	<1
	2 Minutes	$3.0x10^6$	$2.5x10^6$	$1.64x10^6$	$6.1x10^2$	$5.5x10^2$	$5.8x10^2$
	15 Minutes	$2.8x10^6$	$4.2x10^6$	$2.5x10^6$	$3.0x10^2$	$6.6x10^2$	$7.6x10^2$
	30 Minutes	$4.7x10^6$	$2.6x10^6$	$3.2x10^6$	$4.6x10^2$	$4.5x10^2$	$4.1x10^2$
Module 3	Flush	—	—	—	<1	<1	<1
	2 Minutes	$2.37x10^6$	$2.44x10^6$	$2.42x10^6$	$5.6x10^2$	$4.1x10^2$	$4.7x10^2$
	15 Minutes	$1.49x10^6$	$1.18x10^6$	$1.37x10^6$	$5.4x10^2$	$5.6x10^2$	$6.2x10^2$
	30 Minutes	$1.08x10^6$	$1.21x10^6$	$1.13x10^6$	$5.3x10^2$	$4.7x10^2$	$4.3x10^2$
Module 4	Flush	—	—	—	<1	<1	<1
	2 Minutes	$1.28x10^6$	$1.53x10^6$	$1.14x10^6$	$1.34x10^3$	$1.22x10^3$	$8.1x10^2$
	15 Minutes	$1.22x10^6$	$1.35x10^6$	$1.09x10^6$	$5.8x10^2$	$4.8x10^2$	$4.5x10^2$
	30 Minutes	$9.7x10^5$	$1.33x10^6$	$1.11x10^6$	$2.8x10^2$	$3.3x10^2$	$3.0x10^2$
Module 5	Flush	—	—	—	<1	<1	<1
	2 Minutes	$7.9x10^5$	$7.6x10^5$	$7.2x10^5$	$8.8x10^2$	$8.0x10^2$	$6.5x10^2$
	15 Minutes	$1.19x10^6$	$8.0x10^5$	$8.5x10^5$	$4.6x10^2$	$5.1x10^2$	$4.2x10^2$
	30 Minutes	$9.4x10^5$	$9.1x10^5$	$9.5x10^5$	$2.9x10^2$	$3.6x10^2$	$3.3x10^2$
Module 6	Flush	—	—	—	<1	<1	<1
	2 Minutes	$1.26x10^7$	$1.13x10^7$	$1.06x10^7$	$5.6x10^3$	$5.3x10^3$	$4.8x10^3$
	15 Minutes	$1.48x10^7$	$1.34x10^6$	$9.1x10^6$	$9.8x10^3$	$1.11x10^4$	$1.24x10^4$
	30 Minutes	$1.60x10^7$	$1.37x10^7$	$1.25x10^7$	$1.50x10^4$	$1.17x10^4$	$1.40x10^4$
Module 5 at 13.6 gpm	Flush	—	—	—	<1	<1	<1
	2 Minutes	$1.42x10^7$	$1.36x10^7$	$1.16x10^7$	$2.00x10^2$	$2.05x10^2$	$2.71x10^2$
	15 Minutes	$1.58x10^7$	$8.5x10^6$	$1.13x10^7$	$6.8x10^2$	$6.2x10^2$	$7.8x10^2$
	30 Minutes	$1.45x10^7$	$1.04x10^7$	$8.1x10^6$	$1.39x10^2$	$1.24x10^2$	$1.42x10^2$
Module 5 at 25.4 gpm	Flush	—	—	—	58	34	43
	2 Minutes	$1.08x10^7$	$9.8x10^6$	$9.9x10^6$	$1.35x10^3$	$1.66x10^3$	$1.56x10^3$
	15 Minutes	$1.28x10^7$	$9.8x10^6$	$1.06x10^7$	$3.7x10^3$	$2.9x10^3$	$3.7x10^3$
	30 Minutes	$1.49x10^7$	$9.6x10^6$	$1.10x10^7$	$6.8x10^3$	$4.1x10^3$	$5.5x10^3$
Module 5 at 35.6 gpm	Flush	—	—	—	51	42	59
	2 Minutes	$1.33x10^7$	$9.7x10^6$	$9.5x10^6$	$3.2x10^3$	$2.39x10^3$	$3.3x10^3$
	15 Minutes	$1.39x10^7$	$7.9x10^6$	$8.8x10^6$	$6.4x10^3$	$6.5x10^3$	$6.5x10^3$
	30 Minutes	$9.9x10^6$	$7.7x10^6$	$8.0x10^6$	$1.46x10^4$	$1.52x10^4$	$1.27x10^4$

Table C-2. *B. atrophaeus* Triplicate Count Data							
		Feed (CFU/mL)			Filtrate (CFU/100mL)		
Module	**Sample**	**Count 1**	**Count 2**	**Count 3**	**Count 1**	**Count 2**	**Count 3**
Module 1	Flush	—	—	—	<1	<1	<1
	2 Minutes	$1.04x10^5$	$1.01x10^5$	$1.39x10^5$	<1	<1	<1
	15 Minutes	$9.9x10^4$	$1.14x10^5$	$1.20x10^5$	2	1	<1
	30 Minutes	$1.23x10^5$	$1.00x10^5$	$1.30x10^5$	<1	<1	<1
Module 2	Flush	—	—	—	2	4	2
	2 Minutes	$1.18x10^5$	$1.53x10^5$	$1.40x10^5$	1	1	1
	15 Minutes	$1.15x10^5$	$1.16x10^5$	$1.28x10^5$	1	2	<1
	30 Minutes	$1.26x10^5$	$1.14x10^5$	$1.25x10^5$	1	<1	<1
Module 3	Flush	—	—	—	<1	<1	<1
	2 Minutes	$1.06x10^5$	$1.15x10^5$	$1.00x10^5$	1	<1	1
	15 Minutes	$1.07x10^5$	$1.06x10^5$	$9.8x10^4$	<1	<1	<1
	30 Minutes	$1.29x10^5$	$1.40x10^5$	$1.36x10^5$	3	1	1
Module 4	Flush	—	—	—	2	1	1
	2 Minutes	$1.20x10^5$	$1.23x10^5$	$1.07x10^5$	<1	<1	<1
	15 Minutes	$9.9x10^4$	$1.03x10^5$	$1.05x10^5$	11	<1	<1
	30 Minutes	$6.3x10^4$	$7.1x10^4$	$8.6x10^4$	<1	<1	<1
Module 5	Flush	—	—	—	<1	<1	<1
	2 Minutes	$1.21x10^5$	$1.23x10^5$	$1.29x10^5$	<1	<1	<1
	15 Minutes	$1.30x10^5$	$1.28x10^5$	$1.26x10^5$	<1	<1	<1
	30 Minutes	$1.46x10^5$	$1.45x10^5$	$1.40x10^5$	<1	<1	<1
Module 6	Flush	—	—	—	1	1	2
	2 Minutes	$1.36x10^5$	$1.53x10^5$	$1.50x10^5$	1	<1	<1
	15 Minutes	$1.64x10^5$	$1.58x10^5$	$1.66x10^5$	2	<1	<1
	30 Minutes	$1.33x10^5$	$1.59x10^5$	$1.38x10^5$	<1	<1	<1
Module 2 Retest	Flush	—	—	—	<1	<1	<1
	2 Minutes	$8.5x10^3$	$9.7x10^3$	$1.02x10^4$	1	<1	<1
	15 Minutes	$8.9x10^3$	$1.05x10^4$	$9.2x10^3$	<1	<1	<1
	30 Minutes	$1.06x10^4$	$9.8x10^3$	$1.10x10^4$	1	1	<1
Module 4 Retest	Flush	—	—	—	<1	<1	1
	2 Minutes	$1.26x10^4$	$1.32x10^4$	$1.29x10^4$	2	1	2
	15 Minutes	$1.18x10^4$	$1.21x10^4$	$1.16x10^4$	2	<1	2
	30 Minutes	$1.26x10^4$	$1.23x10^4$	$1.37x10^4$	1	<1	1

Table C-3. *C. parvum* Triplicate Count Data							
		Feed (oocysts/L)			Filtrate (oocysts/L)		
Module	**Sample**	**Count 1**	**Count 2**	**Count 3**	**Count 1**	**Count 2**	**Count 3**
Module 3	Flush	—	—	—	<1	<1	<1
	2 Minutes	$2.63x10^6$	$1.38x10^6$	$1.46x10^6$	<1	<1	<1
	15 Minutes	$1.39x10^6$	$6.9x10^6$	$1.38x10^6$	<1	<1	<1
	30 Minutes	$1.74x10^6$	$6.1x10^5$	$7.8x10^5$	1	<1	<1
Module 2	Flush	—	—	—	<1	<1	<1
	2 Minutes	$1.49x10^6$	$2.14x10^6$	$2.58x10^6$	<1	<1	<1
	15 Minutes	$8.4x10^5$	$2.05x10^6$	$2.06x10^6$	<1	<1	<1
	30 Minutes	$2.02x10^6$	$1.78x10^6$	$1.97x10^6$	<1	<1	<1