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# Alternative removal methodologies for environmental waters

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**ALTERNATIVE REMOVAL METHODOLOGIES FOR  
ENVIRONMENTAL WATERS**

**BY**

**SHANNON HOGAN**

**B.A. University of New Hampshire, 1993**

**DISSERTATION**

**Submitted to the University of New Hampshire  
in Partial Fulfillment of  
the Requirements for the Degree of**

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**in**

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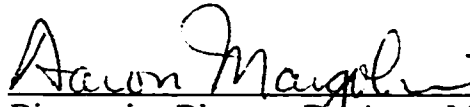
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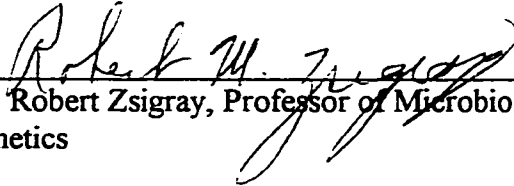
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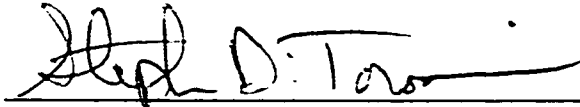
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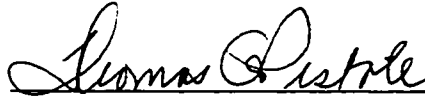
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**To my beloved Sarah**

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## ABSTRACT

### ALTERNATIVE REMOVAL METHODOLOGIES FOR ENVIRONMENTAL WATERS

BY

SHANNON HOGAN

University of New Hampshire, May, 1998

The Environmental Protection Agency (EPA) continues to establish criteria for the reduction of pathogens in environmental waters used for drinking water. The EPA also states criteria on the amount of chlorine by-products allowed in drinking water. Alternative removal methods that are used in conjunction with chlorine are needed to assure safe drinking water.

The Surface Water Treatment Rule (SWTR) states that a 99.99% reduction of viruses and a 99.9% reduction in *Giardia lamblia* cysts needs to be achieved by a treatment facility that uses surface water for the production of drinking water. The SWTR also states Maximum Contaminant Levels (MCL) on trihalomethane production. The Groundwater Disinfection Rule (GWDR) states that a 99.99% reduction of viruses must be achieved in treatment facilities using groundwater for the production of drinking water.

The ability of polysulfone hollow fiber (HF) ultrafiltration (UF) membranes to remove bacteriophage, MS2, *Giardia lamblia* cysts, and *Cryptosporidium parvum* oocysts from MilliQ water was examined. The membranes were placed in a Koch 5 bench scale disinfection unit. Operating conditions such as transmembrane pressure, pH, and

temperature were varied to assess their effects on removal capability. A 100,000D PMPW membrane consistently removed 99.99% of MS2 titer and 99.999% of *G. lamblia* cysts and *C. parvum* oocysts under different operating conditions. The PMPW membrane achieved a 99.99% removal of MS2 in trials where MS2 was continually added to the influent at a concentration of  $1.73 \times 10^4$  pfu/ml-min.

Ultraviolet (UV) irradiation was assessed for its ability to inactivate MS2, poliovirus LSc-1, hepatitis A virus HM-175, and rotavirus Wa in bench scale petri dish assays. Known concentrations of these viruses were added to MilliQ water and different groundwaters and UV irradiated. The least susceptible virus to UV irradiation was rotavirus Wa strain. In MilliQ water and groundwater, 99.99% reductions in titer occurred at 97.0 mWs/cm<sup>2</sup>. A 99.99% reduction in MS2 occurred at 80 mWs/cm<sup>2</sup> in MilliQ water and between 64.0 mWs/cm<sup>2</sup> and 93.0 mWs/cm<sup>2</sup> in groundwaters. Poliovirus and hepatitis A virus were considerably less resistant to UV inactivation. A cell culture-RT PCR assay was used to determine rotavirus Wa titers before and after UV irradiation.

Filtration was capable of meeting the pathogen removal criteria stated in the SWTR. UV irradiation met the virus inactivation criteria stated in the GWDR. Both methodologies could be used in full scale treatment plants under controlled conditions to determine their ability to remove pathogens from drinking water.

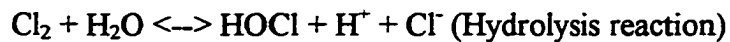
## OVERVIEW

The Environmental Protection Agency (EPA) has stated many rules and regulations on the removal of pathogens in drinking water. The Surface Water Treatment Rule (SWTR) states that treatment facilities using surface water or groundwater under the influence of surface water must meet the specific criteria for pathogens. Surface water is any environmental water that is found above ground level. This type of water includes river and lake waters. The regulation states that a four log (99.99%) reduction of all viruses and a three log (99.9%) reduction in *Giardia* cysts must be achieved for drinking water (USEPA, 1989). The proposed Enhanced Surface Water Treatment Rule (ESWTR) will include *Cryptosporidium* oocysts reduction in its regulations. A maximum contaminant level goal (MCLG) of zero for *Cryptosporidium* oocysts will be mandated. Log reductions required for *Giardia* cysts will be changed to three to six log removal depending on the quality of the water disinfected (USEPA, 1989).

The Groundwater Disinfection Rule (GWDR) is another proposed regulation which has not yet been instated. This regulation will state that a 99.99% inactivation of all viruses in groundwater used for drinking water must be achieved. Groundwater is water found below ground level. Groundwaters were originally deemed to be free of pathogenic organisms. The Disinfection/Disinfection By-Product (D/DBP) rule states a maximum contaminant level (MCL) on hazardous by-products from treatment facilities. By-products such as trichloroacetic acid and chloroform are suspected carcinogens formed from the interaction of residual chlorine and naturally occurring organics. The D/DBP rule makes it

impossible to simply increase chlorine concentration used for disinfection to meet the criteria set by the ESTWR or GWDR. Alternative methods must be found that will allow the reduction in pathogen concentrations required while simultaneously meeting the D/DBP regulations. Removal methods such as filtration and ultraviolet (UV) irradiation can be used in conjunction with chlorine in order to meet these goals.

The use of chlorine disinfection by itself has some shortcomings. The residual concentration provided to inactivate pathogens surviving initial treatment forms by-products. Chlorine disinfection is pH dependent. When chlorine is dissolved into water, it reaches an equilibrium (Bitton, 1980):



At a pH of less than 6, the concentration of hypochlorous acid is higher than hypochlorite ion from the ionization reaction. At a pH of 9.5 or greater, the hypochlorite ion is more abundant. Between pH 6 and pH 9.5, a mixture of the two forms is present (Bitton, 1980). Hypochlorous acid (HOCL) is more germicidal than the hypochlorite ion (OCl<sup>-</sup>). The hypochlorite ion is 20 times less effective as a disinfectant (White, 1992) Therefore, water pH needs to be altered to pH 6 or less in order to obtain the desired disinfection species. The handling of chlorine or chlorine derivatives is both hazardous and expensive due to the need of trained personnel for its transport. Protozoans, *Giardia* and

*Cryptosporidium*, are also somewhat resistant to chlorine concentrations typically used for disinfection.

Filtration methods, such as ultrafiltration (UF), are capable of removing pathogens based on size exclusion. This physical removal lacks the ability to create hazardous by-products while still serving as a potent barrier to pathogens. Filtration also removes organic solids which may decrease the inactivation capabilities of other disinfectants. The removal of these solids will decrease the by-products formed from chlorine disinfection. In Europe, ultrafiltration has been used to remove pathogens in both surface waters and groundwaters that are used for drinking water. In the United States, ultrafiltration is used in surface water disinfection.

UV irradiation is a potent virucidal agent. UV irradiation inactivates microorganisms by disrupting their nucleic acid structure. Like filtration, it fails to create carcinogens in water and may be used in conjunction with chlorine disinfection.

UV disinfection is already being used for surface water and groundwater treatment in Europe. Filtration is used prior to UV light disinfection in order to remove particulates in surface waters. Clays and other suspended solids absorb UV light. Particles also shield viruses, bacteria, and other microorganisms from UV light decreasing its capability to inactivate these pathogens (White, 1990).

This work describes two alternative methods to chlorine disinfection alone. One study involves using ultrafiltration to remove MS2 bacteriophage, *G. lamblia*, and *C. parvum* from MilliQ water. MilliQ water was used because it does not contain suspended solids or other particulate matter. These compounds can increase the removal capability of

ultrafiltration by forming a gel or cake layer which acts as a prefilter for ultrafiltration membranes.

The second study involves the inactivation of MS2, poliovirus, hepatitis A virus, and rotavirus in MilliQ water and groundwaters. UV doses which remove 99.99% of viruses in MilliQ water were determined. Viruses were suspended in various groundwaters and UV irradiated to assess the influence of naturally occurring particulates on UV inactivation. MS2 was used in these studies because it is typically used as a surrogate virus in other disinfection experimentation. The human viruses used in these studies are those pathogens which are most often associated with waterborne disease outbreaks.

## **Introduction**

### **Viruses in the Environment**

Enteric viruses are viruses which replicate in the intestines of an individual and are subsequently passed into the environment via feces (Horstmann, 1967). Infection by fecal-oral transmission occurs when fecally contaminated water is used as drinking water by an individual. Gastroenteritis and a host of other illnesses: myocarditis, encephalitis, and myelitis are the direct result of these types of infections (Belshe, 1991).

The ability of a virus to successfully infect an individual via environmental waters, such as groundwater used for drinking water, also depends on a number of factors. The type of virus and the portal of exit, where it is released from its human or animal reservoir, determines its initial entry into the environment. Viral transmissibility is affected by its ability to be carried by the fecal-oral route or via fomites. The susceptibility of the new host as well as its portal of entry into the host are other factors affecting the probability of a successful transmission of the virus and subsequent viral infection (Bitton, 1980).

The survivability of viruses in natural waters is determined by a number of physical influences: temperature, pH, suspended particle concentration, exposure to ultraviolet (UV) light, and moisture level, as well as enzymatic activity of naturally occurring bacteria (Cliver, 1972). Viruses, typically persist longer with colder temperatures and neutral to acidic pH values (Morris and Darlow, 1971).



Viruses are usually released from a cell in an aggregate. Aggregates are groups or clumps of viruses bound together and are often found in this form in the environment (Floyd and Sharp, 1979). Viruses within the core of the aggregate are less susceptible to inactivation by environmental stresses. Aggregation of viruses is dependent upon the concentration of virus, type of virus, cation concentration, and pH of the medium (Floyd and Sharp, 1979).

The higher the concentration of organic matter such as clays which typically have large surface areas, the more likely that viruses will adhere via van der Waals, electrostatic, or hydrophobic forces to this matter (Bitton, 1974). This decreases the susceptibility of the virus to becoming inactivated by other environmental stresses such as exposure to UV light from the sun (Bitton, 1980). Moisture may increase or decrease virus survivability in the environment. Decreased moisture promotes inactivation of the virus via desiccation (Mahl, 1967). However, a substantial increase in moisture level decreases the concentration of naturally occurring particulates. This dilution decreases the probability of viral adherence to particulates. A decrease in viral adherence leads to increased probability of viral inactivation by environmental factors. As with viral aggregation, divalent and trivalent cations may promote adherence or the dissociation of viruses depending on the type of virus, the pH of the suspension, and the type of cation present (Chang, 1967).

Infection rates for most enteric viruses are typically higher in the summer months in temperate climates (Abbaszadegan, 1993). Enteric virus outbreaks have been

documented as early as 1947, when a municipal drinking water system was responsible for 52 cases of acute hepatitis in U. S. Army soldiers in Germany (Moseley, 1965). A World Health Organization (WHO) report stated that in a five year period (1969-1973), 300 of the 1295 cases of cardiac disease reported were from the enterovirus, Coxsackie B virus (Goldfield, 1974). Rotavirus, classified within the *Reoviridae* family, is responsible for 5 to 18 million deaths from diarrheal disease annually worldwide (Belshe, 1991).

### **Surrogate Virus**

In order to understand how these animal viruses behave in water and in actual filtration and disinfection strategies, a coliphage, MS2, is often used as a surrogate virus. MS2 is a bacteriophage. It infects different strains of *Escherichia coli* and other bacteria which express an F pilus. *E. coli* is a bacterium that is naturally found in the intestinal tract of humans. Therefore, its presence in the water may indicate the presence of fecal material in that water. Since the primary host of MS2 is *E.coli*, its presence also indicates fecal contamination of environmental waters.

MS2 is similar to human enteric viruses in its morphology, type of genome, capsid diameter, stability under various environmental conditions, and inactivation by chemical disinfection (Bitton, 1980). Since MS2 is not a human pathogen, it can be used to determine the effectiveness of a disinfection strategy in a full scale drinking water treatment plant. Liters of MS2 at high titers ( $1 \times 10^{10}$  pfu/ml to  $1 \times 10^{12}$  pfu/ml) can be

produced to use in full scale treatment plant experimentation. The production of liters of human viruses at these titers is impractical. MS2 quantification assays benefit from the fact that they are relatively less expensive and less labor intensive than human virus quantification assays. It also requires less technical skill and is relatively inexpensive compared to assays used for human virus titer determination. MS2 is an icosahedral single stranded RNA phage, 25 nm to 27 nm in diameter, classified in the Leviviridae (DeBartolomeis, 1991).

The Safe Drinking Water Committee of the Environmental Protection Agency (EPA), 1977, stated that a useful enteric virus indicator must meet the following criteria: 1) be applicable to all types of water; 2) be present in sewage and polluted waters when viral pathogens are present and absent from unpolluted waters; 3) be present in numbers greater than the viral pathogens; 4) have greater survival times and greater resistance to environmental and engineered processes; 5) be easily detected by simple lab tests in the shortest time consistent with accurate results.

Phage are naturally present in human and animal fecal material (Furuse, 1985). Phage occur in large numbers in municipal wastewater. Their mean concentration in wastewater is 5000 pfu/ml (Bitton, 1980). Concentrations of  $1 \times 10^5$  pfu/ml to  $1 \times 10^7$  pfu/ml have been detected in domestic raw wastewater (Bell, 1976). In wastewater effluents, the average coliphage to enteric virus ratio is  $1 \times 10^3$  to  $1 \times 10^4$  (Bitton, 1980). Bacteriophages survive well at lower temperatures and pHs and are capable of binding to naturally occurring organic material in environmental waters (Havelaar, 1993). MS2 is

less sensitive to chlorine 20 mg/l to 40 mg/l at pH 6.0 in chlorinated oxidation ponds (Kott, 1974). MS2 is less sensitive to inactivation in tap water by ultraviolet (UV) light irradiation (Wiendermann, 1993). MS2 has similar inactivation rates as rotavirus SA-11 and Hepatitis A virus (HAV) in the presence of free chlorine (Sobsey, 1991). As previously stated, MS2 is nonpathogenic to humans and its enumeration assay is relatively easy to perform, rapid, and inexpensive.

MS2 is by not a perfect indicator of pathogen presence. In one study, MS2 was not detected in 63% of the raw water samples containing enteric viruses. (Kott, 1974). In another experiment, 99.9% of phage were removed in raw water by coagulation and sedimentation processes. Only 35% of enteroviruses and 25% of rotavirus SA11 were removed by the same processes (Gerba, 1984).

In filtration studies, two groups of coliphages may be employed as indicator organisms for human enteric viruses. These groups include somatic and FRNA, or male-specific, coliphages. Somatic coliphages are a heterogeneous group of phages with filamentous morphologies and varying capsid sizes (Havelaar, 1993). FRNA phages are a homogeneous group of phages which display similar physical properties to human enteric viruses (Havelaar, 1993). MS2 is a FRNA coliphage. Removal of viruses by ultrafiltration is based on size exclusion and both enteroviruses and MS2 share similar capsid diameters and morphologies.

## **Protozoans in the Environment**

*Giardia lamblia* and *Cryptosporidium parvum* are two ubiquitous protozoan pathogens responsible for causing diarrheal illness in all age groups. In one study, *Giardia* spp. and *Cryptosporidium* spp. were found in 81% and 87% of 66 raw water samples obtained from different areas of the U.S., respectively. That is, 97% of the raw untreated waters tested contained one or both protozoans (LeChevallier and Norton, 1991). Further analysis of 262 raw waters found *Giardia* present in 45.0% and *Cryptosporidium* in 51.5% of the raw waters (LeChevallier and Norton, 1991). On Christmas of 1986, 1400 cases of gastroenteritis caused by *Giardia* occurred in Sweden when 3000 people were exposed to water tainted with raw sewage (Ljungstrom, 1992). In the spring of 1993, 403,000 residents of Milwaukee, Wisconsin, experienced gastroenteritis due to contamination of drinking water with *Cryptosporidium parvum* oocysts (Cicerillo et al., 1997). Outbreaks typically occur in small community or non-community water systems due to inadequately treated groundwater (Craun, 1986)

Both protozoans are shed in the feces of man and up to 70 other animals (Char, 1991). Their passage in the environment is via the fecal-oral route. Their survivability is increased by their ability to resist the effects of desiccation, UV light, fluctuations in pH, and enzymatic degradation (Banedoch, 1990). Unlike the enteric viruses, their persistence is enhanced by alkaline environments (Campbell et al., 1982). Like the enteric viruses, they are capable of binding to organic material, such as clays, via electrostatic and hydrophobic forces, as well as salt bridging. Their resistance to chlorine disinfection far

exceeds that of the enteric viruses. It has been experimentally determined that *G. lamblia* cysts are 10 times more resistant to inactivation by chlorine disinfection than viruses. *C. parvum* oocysts have been determined to be 30 times more resistant to chlorine disinfection than *G. lamblia* cysts (Gerba, 1987) *Giardia* is capable of surviving in non-treated drinking water at 8 °C for up to two months. (APHA, 1989) *G. lamblia* is the most frequently identified protozoan pathogen in the U. S. It was found that 4% of all stools sampled are positive for *G. lamblia* ( Gerba, 1987). Although its prevalence and infectious potential is understood today, *C. parvum* was not recognized as a human pathogen until 1976 (Hayes et al., 1989).

*G. lamblia* is found as either an inactive ovoid cyst or actively feeding trophozoite. The infectious dose is said to be the ingestion of as little as one to 10 cysts (Ongerth et al., 1987). *Giardia's* life cycle involves the presence of both the trophozoite and the cyst stages. Cysts are more often found in environmental waters because they are more resistant to environmental factors over trophozoite forms. Cysts enters the stomach and duodenum, where they produce two binucleate trophozoites which later adhere to the crypts, the base of the intestinal epithelial microvilli, and cause a wide array of pathological activity (Farthing, 1990). Maladsorption of fats, decreased vitamin B<sub>12</sub> adsorption, destruction of intestinal microvilli, deconjugation of bile salts, and the blockage of intestinal mucosa all lead to diarrheal illness in an infected individual (Katelaris, 1992). These actions also allow for a higher probability of secondary bacterial infection (Katelaris, 1992). Symptoms of the illness occur in a 1 to 4 week period (Nash

et al., 1987). Sixty six percent of the individuals harboring the parasite are asymptomatic (Ljungstrom, 1992). This greatly influences the continued spreading of the pathogen to other locales. Treatment involves the administration of Flagil<sup>®</sup> quinacrine and metronidazole, for 1 week (Nash, 1987).

The cysts have an extremely durable and resistant outer layer composed of glycolipids which make it hydrophobic and relatively impermeable to ions. The inner plasma membrane is permeable to ions and can lead to salt-bridging effects which allow the organism to bind to organics in the environment and hinder disinfection strategies in inactivating these cysts (Upcroft et al., 1989). Identification of the cysts in environmental water samples is typically performed using indirect immunofluorescence (IIF) and Nomarski microscopy (ASTM, 1991). Structures typifying the presence of *G. lamblia* include median bodies, a membrane-bound nucleus, containing one to four nuclei, and a microtubule fibrous structure known as an axoneme (ASTM, 1991).

*C. parvum* is found in environmental waters as a small spherical double-membraned oocyst that is extremely resistant to environmental stresses (Rose, 1988). Two to four sporozoites are contained within the oocyst and are released, after the ingestion of the oocyst and its passage into the duodenum (Rose, 1988). After their release, they attach to the small intestine epithelial microvilli and transform into trophozoites. These forms divide asexually by a multiple fission process known as schizogony forming schizonts. Type I and II merozoites are then formed by further scizhogony with the later capable of sexual reproduction. A zygote is formed which develops into a mature oocyst and is then

shed in the feces of the infected individual (Rose, 1988) . However, 20% of the oocysts formed lack an intact oocyst wall and their sporozoites are released allowing an autoinfectious cycle to continue (AWWA, 1988). The diarrheal illness is considered self-limiting in nonimmunocompromised individuals and typically occurs for 5 to 11 days (Rose, 1988). In immunocompromised individuals, this illness can be life-threatening and permits the loss of up to 17 L of fluid per day per individual (Leng et al., 1996). *C. parvum* is Ziehl-Neelsen acid fast and is identified in human fecal samples via this type of analysis (Rose, 1988). In environmental samples, it is identified via IIF and Nemarski microscopy with the discerning structures being a suture line spanning the entire diameter of the oocyst wall and the presence of 2 to 4 sporozoites found internal to the oocyst wall (ASTM, 1991).

### **Drinking Water Regulations**

The SDWR (Safe Drinking Water Rule), promulgated by the EPA in 1974, initiated the increase in regulations for drinking water supplies. The number of drinking water supply systems subject to federal regulation increased from 700 to 60,000 (Gatson, 1996).

Following this, the SWTR (Surface Water Treatment Rule) stated removal and inactivation criteria for *Giardia* cysts and enteric viruses. A reduction or inactivation of 99.99% (4 log) of the viruses and 99.9% (3 log) of the *Giardia* cysts in surface water or



groundwater under the influence of surface water must be maintained by the water supply's treatment (USEPA, 1989). The 99.99% reduction criteria is said to mathematically ensure the risk of less than 1 infection per 10,000 people per year. Acceptable average concentrations for viruses would range from  $2.22 \times 10^{-7}/L$  to  $1.90 \times 10^{-3}/L$ , depending on the virus present in the raw water (Regli, 1989). The 99.9% removal in cyst concentration is based on the same mathematical derivation as stated for enteric viruses. The acceptable average concentration is  $6.75 \times 10^{-6}/L$  *G. lamblia* cysts (Regli, 1989). This goal translates into a lifetime risk per individual of less than 0.01 to 0.001 infections (Regli, 1989). Improvement in pathogen monitoring via more sensitive detection methodologies may alter the treatment goal. The proposed Enhanced Surface Water Treatment Rule (ESWTR) will include removal criteria for the protozoan pathogen, *Cryptosporidium parvum* (USEPA, 1989).

The Disinfection-Disinfection By-Products Rule (D-DBP) will state allowable maximum contaminant levels of trihalomethanes or halogenated compounds in drinking water effluent. These compounds are produced from the chemical reaction of chlorine and naturally occurring organics such as fulvic and humic acids (USEPA, 1989). These organic by-products are suspected to be carcinogenic to humans. It will become a necessity for some water facilities to find alternative methods of pathogen removal which may assist or replace chlorine disinfection. Because protozoans are more resistant to chlorine disinfection than enteric viruses, a reasonable solution would be to raise the chlorine concentration used for disinfection. However, increasing chlorine levels will

increase the carcinogen concentration in effluent which will violate the standards set by the D-DBP.

### **Membrane Filtration**

Filtration provides a physical removal of pathogens and suspended solids that may precede a chemical disinfection phase. A water treatment plant using filtration may be credited with a 0.0 to 3.0 log reduction in titer of viruses and 2.0 to 2.5 log reduction in titer of *Giardia* cysts. The difference in log removal credit is reflected in the type of filtration used by the treatment facility (USEPA, 1989). Water treatment plants that use filtration can use lower chlorine levels to inactivate the residual titers of viruses or other pathogens present after filtration. Decreased residual chlorine concentrations ensure a decreased production of harmful by-products such as trihalomethanes.

Filtration may be hindered by physical phenomena such as membrane fouling and the loss of membrane integrity. Membrane fouling is defined as the compression of membrane fibers and the adsorption or precipitation of material onto the filter surface (Cheryan, 1986). Adsorbed or precipitated material may form a gel or cake layer that impedes the membrane flux and subsequently decreases the volume of permeate produced. Performance is subsequently compromised and production cost is increased because increased pumping pressure is needed to produce a particular volume of permeate (AWWA, 1992). However, one advantage to fouling is that it may increase the membrane's filtration capability by providing a prefilter for the membrane (Taylor, 1995).

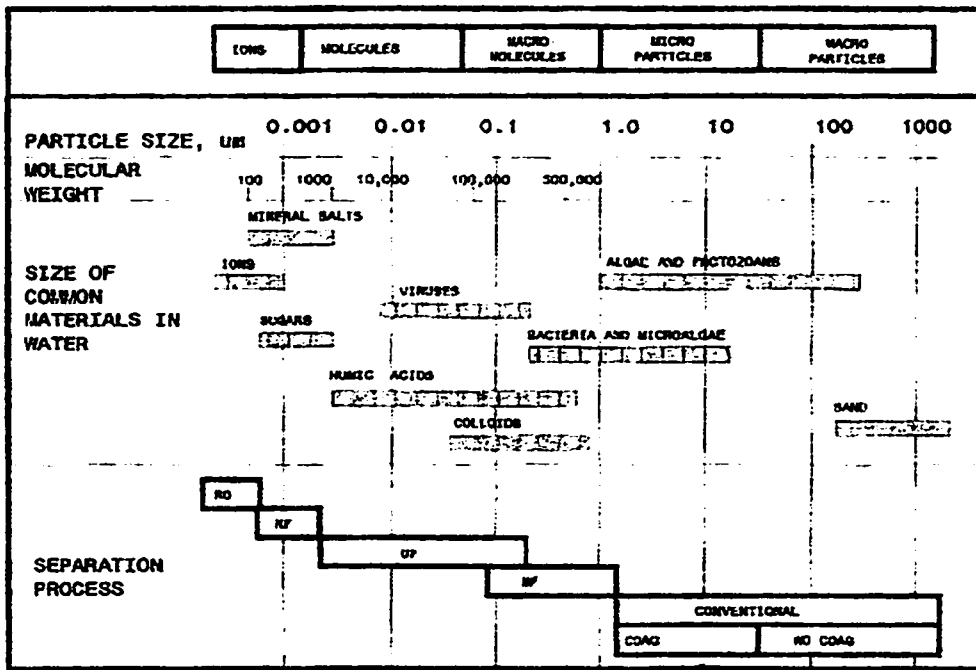
The rate of fouling is also dependent upon the concentration, composition, and size of particulate matter in the influent.

The properties that define a particular type of membrane used for filtration are the membrane's material composition, molecular weight cut-off (MWCO) or pore diameter, configuration, and its operating pressure (Dwyer, 1996). Membranes are composed of organic polymers, such as polyamides, cellulose acetate, polysulfone, ceramics or other material (Deanin, 1972). Adsorption of the particulate matter, with subsequent fouling of the membrane, is influenced by the membrane composition and the relative hydrophobicity of the organic polymer. (Taylor, 1995).

Pore size references the pore diameter of microfiltration (MF) membranes while MWCO indicates the minimum particulate size that a NF (nanofiltration), UF (ultrafiltration), or RO (reverse osmosis) membrane may separate from the permeate (Jacangelo, 1994). The pore size or MWCO of the membrane, influent type, configuration and maximum operating pressure of the membrane influence the flux that is achieved in a filtration system (Dwyer, 1996). Figure 1 shows the particulate removal capability of these membranes as well as conventional filtration removal capability.

Membrane configurations such as spiral wound (SW), tubular (T), plate and frame (PF), and hollow fiber (HF) are among the most common employed in water treatment facilities (Taylor, 1989). The mode in which water is received and discharged is determined by configuration. Knowledge of the configuration and maximum operating or transmembrane pressure aids in determining the flux and cost of potable water produced

Figure 1  
Size classification chart



(Dwyer, 1996). The operating or transmembrane pressure is the average pressure applied to the feed side less the pressure of the permeate. Operating pressures range from five to 30 psi for MF to 1200 psi for RO membranes (Jacangelo, 1994).

The membranes used in this study were hollow fiber polysulfone UF membranes with MWCOs of 500,000 dalton (D), 100,000 D, and 10,000 D. Polysulfone is hydrophobic and is negatively charged below pH 8 (Deanin, 1972). It does not adsorb ionic or inorganic solutes but may adsorb steroids or hydrophobic macromolecules (Laine, 1989). The membrane is composed of repeating phenylene rings which gives it high temperature stability, wide pH tolerance, and relatively high free chlorine resistance, up to 200 mg/l (Deanin, 1972). The primary limitation of polysulfone UF membranes in an HF configuration is their relatively limited maximum operating pressures, less than 100 psi (Cheryan, 1986).

Another drawback to polysulfone composition is its inability to bind proteins. The pH of the influent may alter the relative charge of some membranes and particulates, possibly increasing the electrostatic binding of protein to the membrane (Laine, 1989). Hydrophilic membranes perform better in certain raw waters because of the formation of a prefilter gel layer formed from organic binding of solutes. This layer allows for better particle separation and acts as a prefilter for the membrane (Laine, 1989). However, over time, fouling of hydrophilic membranes from this deposition of material may increase and subsequently retard membrane performance. HF membrane separation of particulates occurs at a membrane skin, which is a denser layer of material that is in direct contact with

the influent (Romicon, 1983). One major advantage of this membrane configuration is its relative stability to backflushing. Backflushing is used as a cleaning technique to reduce the potential of membrane fouling (Dwyer, 1986).

Like RO filtration, UF benefits from the production of superior quality water and the decreased need for the addition of chemicals into the disinfection process. Although RO filtration is capable of removing smaller particles, it has the disadvantage of having higher operating pressures and thus higher operating cost (Laine, 1989). UF is capable of removing most organic material found in groundwaters and surface waters due to its low MWCO. (Fiessenger, 1986).

If the MWCO of UF membrane is smaller than the capsid diameter of a virus than the virus is excluded on a size-only basis. *G. lamblia* cysts and *C. parvum* oocysts are at least two orders of magnitude larger than the size of material typically separated by UF (Weisner, 1993). Prior work illustrates that low pressure, 10 to 40 psi, HF UF reduces the concentration of viruses, bacteria and protozoans (Adham, 1994), as well as turbidity, and particle counts (Luitweiler, 1991) in raw waters. These reductions are in accordance with the limits set by the SWTR and proposed ESWTR. Previous work shows the reduction of six logs of MS2 virus and four logs of *G. muris* cysts were removed in raw water by low pressure HF UF (Jacangelo, 1989).

The design and construction of smaller scale systems which are easier to site and scale up is a practical advantages to UF (Jacangelo, 1989). It appears that HF UF serves as an effective barrier to pathogens and is capable of lowering free chlorine concentration

requirements in permeate. Together, with chlorine or other disinfection means, HF UF allows many water treatment plants to economically meet the pathogen removal criteria stated by existing and proposed disinfection rules. Filtration may also help facilities meet the D-DBP rule. Membrane UF removes organic particulate matter that reacts with chlorine to form carcinogenic DBP. The general public health is increased by the decrease carcinogen production as well as superior quality permeate.

## METHODOLOGY

### **Bench Scale Removal of MS2, *Giardia lamblia* Cysts, and *Cryptosporidium parvum* Oocysts By Polysulfone Hollow Fiber Ultrafiltration**

#### **Ultrafiltration Membranes**

The membranes utilized in this study were manufactured by Koch Membrane Systems, Inc. (Wilmington, Ma.). All membranes were hollow fiber polysulfone membranes. The PM500 membrane has an internal fiber length diameter of 43 mm, a pore diameter of 0.5  $\mu\text{m}$ , a MWCO of 500,000 D, and an overall surface area of 0.465  $\text{m}^2$ . The PMPW membrane internal fibers have a diameter of 30 mm, a pore diameter of 0.1  $\mu\text{m}$ , a MWCO of 100,000 D, and an overall surface area of 0.650  $\text{m}^2$ . The PM10 membrane has an interior fiber length diameter of 43 mm, a pore diameter of 0.01  $\mu\text{m}$ , a MWCO of 10,000 D, and an overall surface area of 0.465  $\text{m}^2$ . The membranes' operation occurs in the inside out mode (Figure 2). Separation of particulates occurs at the membrane surface on the inner circumference of the hollow fibers (Dwyer, 1996).

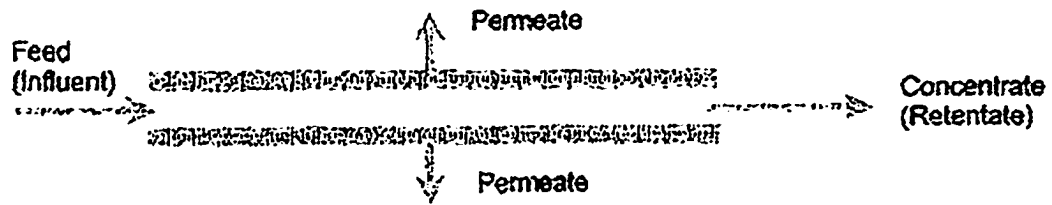
#### **Bench Scale Filtration System**

The Koch Lab 5 Ultrafiltration System with reverse flow was used for this experimentation. It was equipped with Romicon<sup>R</sup> ultrafiltration cartridges, 5.0 cm in diameter and 51.0 cm in length. The system also contained an OSS stainless steel



Figure 2

### Inside-out water flow for hollow fiber ultrafiltration

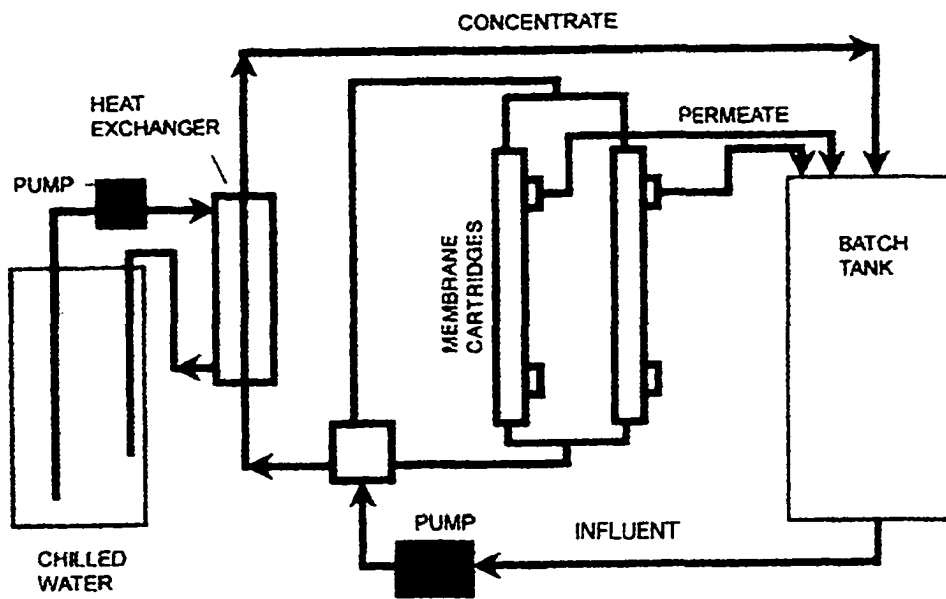


centrifugal pump responsible for circulating the distilled water used in each microbial challenge. A four way reversing valve, a pressure control valve, and an inlet pressure gauge were included on the bench scale apparatus. A second cartridge was set in parallel to the first when parallel design challenges took place (Figure 3). The 114 L stainless steel batch tank held the experimental waters prior to circulation. Water flows from the tank to the membrane housed within the cartridge and the permeate produced is circulated back to the batch tank. Water also flows from the four way valve to a heat exchanger and chiller to maintain water temperature. This concentrate is also recycled back to the steel tank. Sampling ports were located at the tank, to measure influent microbial concentration, before and after the membranes and in the permeate lines, to measure final microbe concentration at each sampling time (Figure 2). During a backflush cleaning procedure, the flow of water was simply reversed and microbes associated with the membranes were returned to the batch tank.

### **Membrane and Equipment Preparation**

Acid and caustic cleaning procedures were employed before and after each trial to remove metals and organic materials respectively. All solutions were prepared in room temperature deionized water and all parts of the bench scale apparatus including the membranes were subject to cleaning. The acid cleaning solution consisted of phosphoric acid and deionized water, pH 2.0 to 3.0, prepared according to the manufacturer's directions. A 1% sodium hydroxide solution, in deionized water, was prepared for the

Figure 3  
Bench Scale Filtration Apparatus



caustic cleaning procedure. Each cleaning solution, 76.0 L, was circulated throughout the system at a transmembrane pressure of 15.0 psi for 10 min. The permeate line was then closed and the cleaning solution was recirculated in reverse flow for 10 min and then this flow was reversed and circulation occurred for an additional 10 min. The transmembrane pressure was reduced to 12.5 psi and the system was then drained and rinsed with deionized water. Lastly, the apparatus, including the sampling ports, was disinfected with 76.0 L of 200 ppm of sodium hypochlorite solution at pH 10.25. The sodium hypochlorite solution was circulated and recirculated through the system using the same protocol described previously for the sodium hydroxide cleaning solution. To neutralize the chlorine disinfectant, a 1.0 N sodium thiosulfate solution was circulated and recirculated throughout the system. The system was drained and rinsed with deionized water.

### **MS2 Propagation**

MS2 was propagated using the host: *Escherichia coli* C3000. The host was grown at 37 °C to mid-exponential phase in 1L of tryptic soy broth (Fisher Scientific, Pittsburgh, PA) supplemented with 0.005 M CaCl<sub>2</sub> (Sigma, St. Louis, MO) in a 2 L Erlenmeyer flask (VWR Scientific, Bridgeport, NJ). The culture was agitated at 200 rpm in a gyrotory shaker (New Brunswick Scientific Co., New Brunswick, NJ) to provided aeration for maximal growth during the replication period. A total of  $1 \times 10^{13}$  pfu, an approximate multiplicity of infection (moi) of 1: 1000, of MS2 was added to the host and the

suspension was incubated for 150 minutes at 37 °C. The lysates of the infected cultures were chloroform treated and centrifuged at 10,000 x g for 10 min at 4 °C. The supernate recovered was then stored at 4 °C until use. Initial virus titers ranged from  $2.43 \times 10^{11}$  pfu/ml to  $3.75 \times 10^{11}$  pfu/ml.

### **MS2 Quantification**

The double agar overlay plaque assay (Debartolomeis et al., 1991) was used to enumerate MS2. Ten-fold dilutions of the virus,  $10^0$  to  $10^{-8}$ , were done in 1 X PBS. Approximately 0.2 ml of *E. coli* C3000 and 0.1 ml of virus dilution were added to 5.0 ml of 2X tryptic soy overlay and poured onto 100 x 15 mm<sup>2</sup> plastic petri dishes containing tryptic soy agar. Positive controls included 0.1 ml of virus of known concentration. The diluent, 1X PBS, was used for the negative control. Upon solidification of the overlay, plates were inverted and placed in a 37 °C incubator. All plaques were counted in 12 to 16 h. All experimental samples were titered using the same plaque assay methodology.

### **Protozoans**

*G. lamblia* cysts and *C. parvum* oocysts, fixed in 2.0% formalin were purchased from BioVir Laboratories, Benicia, Ca. Both protozoans were added at a concentration of  $1 \times 10^6$  particles/ml.

## Protozoan Quantification

Both protozoans were enumerated similarly to methods described by the American Society for Testing and Materials (ASTM) D-19 Proposal P 229 Test Method for *Giardia* Cysts and *Cryptosporidium* Oocysts. The protozoans were concentrated on disk filters instead of the spiral membrane filters mentioned in the ASTM protocol. Indirect immunofluorescent labeling of each organism was performed using the Meridian (Cincinnati, OH) Hydrofluor Combo Indirect Immunofluorescence (IIF) kit. All samples were processed utilizing a Hoefer (San Francisco, CA) Microanalysis Vacuum Manifold and all fluorescent and Nomarski microscopy analysis took place using an Olympus BH2 Epifluorescent microscope (Japan) and Sony CCD-IRIS videocamera (Japan).

Sartorius 0.22  $\mu\text{m}$  cellulose acetate disk filters (Fisher Scientific) were floated in 1X PBS prior to their placement on the vacuum manifold. Ten milliliters of the 50 ml experimental grab samples were adhered to the disk filters under a vacuum. The filters were washed with 10 ml of 1 X PBS and then washed with 50 ml of 1X PBS supplemented with 0.05% Tween-80 (Sigma). Primary murine anti-*Giardia* and anti-*Cryptosporidium* monoclonal antibody were diluted ten fold in 1X PBS containing bovine serum albumin (BSA) (Sigma) as a preservative and 0.5 ml of this solution was added to the filter for 25 min at room temperature. Each well was then washed with 5.0 ml of 1 X PBS three times at room temperature. Fluorescein isothiocyanate (FITC) anti-murine antibody was diluted ten fold in 1X PBS containing BSA and 0.5 ml was added to each filter at room temperature for 25 min in the absence of light. Each filter was washed three

times with 5.0 ml of 1X PBS after draining the labeling reagent from the well. The filters were then dehydrated with 2.0 ml of 10.0%, 20.0%, 40.0%, and 80.0% ethanol solutions containing 5.0 % of glycerol. A 2.0 ml aliquot of a 90.2% ethanol solution supplemented with 3.21 mg/ml of Eriochrome T Black (Fisher Scientific) stain was added to reduce background fluorescence. The 100.0 mm x 25.0 mm microscope slides and 25 mm x 25 mm coverslips were prewarmed at 37 °C for 30 min. Approximately 75.0 ml and 25.0 ml of 2.0% diazobicyclo [2.2.2] octane (DABCO) (Sigma) in glycerol were added to the slides and coverslips respectively. Each membrane was placed between a slide and coverslip and incubated at 37 °C for 15 to 20 minutes. Each slide was sealed with a mixture containing 50.0% paraffin and 50.0% petroleum jelly.

Fluorescent and Nomarski microscopy were used for enumeration of each protozoan under 10X and 40X magnification. The typical apple-green fluorescence was used to initially identify each organism. Diameter measurements, 8 to 12 µm, and visualization of internal structure such as axonemes, median bodies, and nuclei were used as presumptive criteria for *G. lamblia* cysts. Diameter measurements, 2 to 6 µm, and visualization of the suture line and internal sporozoites were used as a presumptive criteria for *C. parvum* oocysts. Confirmation of a positive identification of each organism was done by using Nomarski microscopy with the same identifying criteria, minus the typical green fluorescence used in the fluorescent microscopy phase. Positive controls and negative controls were processed similarly. *G. lamblia* and *C. parvum* oocysts (BioVir) were used for the positive controls and 1X PBS was used for the negative controls.

## **Nonspecific Viral Adsorption**

To determine if MS2 would adsorb to filter material under different pH conditions, a static experiment using individual polysulfone filter fibers and known concentrations of MS2 was performed. The virus and fibers were incubated together at 22 °C for 30 minutes at varying pH values: 3.5, 7.0, and 8.5. A similar trial to elucidate virus adherence to the system components was performed as well. Seventy six liters of RO water was added to the stainless steel tank. MS2 was added to produce a final concentration of  $3.0 \times 10^7$  pfu/ml. The water was recycled back to the tank in order to maintain a constant titer of phage and a constant volume of water. No UF membrane was placed in the cartridge. The virus was circulated for 60 min at pH 5.65, pH 7.00, and pH 8.10 and final titers were determined for each permeate.

## **MS2 Filtration Experiments**

A total of 28 MS2 experiments were performed using the PM500, PMPW, and PM10 membranes. Ten challenges were performed using the PM500 membrane, 13 challenges were performed using the PMPW membrane, and five challenges were performed using the PM10 membrane. The PMPW membrane was involved in the most experimentation since it was specifically developed for drinking water use. The PM500 and PM10 membranes were provided by the manufacturer to determine filtration capability of UF membranes whose MWCOs associate with the upper and lower limits of UF. A



synopsis of the experimentation performed using the PM500 and PM10 membranes is shown in Table 1. The synopsis of experiments performed with the PM100 membrane is shown in Table 2.

Grab samples of 50 ml were taken at 0, 5, 30, 60, 90, 120, and 180 min. The 0 minute sample represented the initial experimental titer and was taken from the steel tank before the challenge proceeded. Samples were taken in duplicate at the 0, 30, 120, and 180 min time points to show the reproducibility of the sampling. Samples were collected in 50 ml polypropylene conical tubes (Fisher Scientific). Samples were taken from the tank, the influent line before the membrane, and the permeate line. Before a challenge, samples were taken from the influent and permeate ports. No bacteriophage was detected prior to any of the challenges. Prior to sample collection, approximately 500.0 ml of deionized water was rinsed through the influent and permeate sampling ports. Temperature and pH were continually monitored with a thermometer and electronic pH meter (Fisher Scientific) placed within the steel tank. The permeate flow was measured at each sampling event and in duplicate at the 0, 30, 120, 180 min sampling points.

Log reductions of MS2 were calculated using the formula:  $\log N_T / N_0$  where  $N_0$  is equal to the titer of MS2 in the influent line and  $N_T$  is equal to the titer of virus in the permeate for that time point. Experimental titers were determined using the plaque assay methodology previously described. Plaque counts of between 30 and 300 are considered to be statistically significant for this assay. The plaque counts for the permeate samples were often below this accepted range because of the membranes' ability to remove nearly

Table 1

MS2 experiment parameters performed with PM10 and PM500 membranes

Membrane	Membrane #	Trial Length (min)	Trans-membrane Pressure (psi)	pH	Temperature (°C)	MS2 Titer <sup>1</sup> (pfu/ml)
PM10	3001	120	22.5	7	22	$3.00 \times 10^7$
PM10	3001	120	12.5	7	22	$3.00 \times 10^7$
PM10	3002	120	22.5	7	22	$3.50 \times 10^7$
PM10	3002	120	12.5	7	22	$3.50 \times 10^7$
PM10	3001	360	22.5	7	22	$2.43 \times 10^7$
PM500	3002	120	22.5	7	22	$3.75 \times 10^7$
PM500	3002	120	12.5	7	22	$3.75 \times 10^7$
PM500	3001	120	22.5	7	22	$3.50 \times 10^7$
PM500	3003	120	22.5	7	22	$3.50 \times 10^7$
PM500	3003	120	12.5	7	22	$2.43 \times 10^7$
PM500	3003	360	22.5	7	22	$2.43 \times 10^7$
PM500	3003	360	22.5	7	22	$2.43 \times 10^7$
PM500	3002,3003	180	22.5	7	22	$3.12 \times 10^7$
PM500	3002,3003	180	22.5	7	22	$3.12 \times 10^7$ & $1.73 \times 10^4$ <sup>2</sup>
PM500	3004,3005	180	22.5	7	22	$3.12 \times 10^7$

1- MS2 concentration in tank

2- Continuous addition (pfu/ml-min.)

Table 2

## MS2 experiment parameters performed with PMPW membrane

Membrane	Membrane #	Trial Length (min)	Trans-membrane Pressure (psi)	pH	Temperature (°C)	MS2 Titer <sup>1</sup> (pfu/ml)
PMPW	3001	360	22.5	7	22	$2.43 \times 10^7$
PMPW	3001	360	22.5	7	22	$2.43 \times 10^7$
PMPW	3001	360	22.5	5	22	$2.43 \times 10^7$
PMPW	3001	360	22.5	8	22	$2.43 \times 10^7$
PMPW	3001,3002	180	22.5	7	22	$3.12 \times 10^7$
PMPW	3001,3002	180	22.5	7	22	$3.12 \times 10^7$ & $1.73 \times 10^4$ <sup>2</sup>
PMPW	3001,3002	180	20.0	7	22	$3.12 \times 10^7$
PMPW	3001,3002	180	17.5	7	22	$3.12 \times 10^7$
PMPW	3001,3002	180	22.5	7	7	$3.12 \times 10^7$
PMPW	3001,3002	180	12.5	7	7	$3.12 \times 10^7$
PMPW	3001,3002	180	22.5	7	22	$3.12 \times 10^7$
PMPW	3001,3002	180	12.5	7	22	$3.12 \times 10^7$
PMPW	3001,3002	180	22.5	7	22	$3.12 \times 10^7$ & $6.24 \times 10^5$ <sup>2</sup>

1- MS2 concentration in tank

2- Continuous addition (pfu/ml-min.)

all of the phage added to the system. In this event, the final titer of phage is quantified as 0 pfu/ml.

### **Protozoan Experimentation**

Protozoan challenges were similar to the virus challenges in design. One challenge at a temperature of 22 °C and one challenge at 7 °C were performed for each protozoan using the PMPW membrane. All studies utilized a transmembrane pressure of 22.5 psi, an influent pH of 7.00, and were done for 120 min. The initial titers of *Giardia* and *Cryptosporidium* were 132 cysts/ml and 132 oocysts/ml respectively. Grab samples of 50 ml were taken from the influent and permeate in duplicate at 0, 2, 15, 60, 62, 75, 120 minutes.

A 30 s backflush step was conducted at the 60 min time point to release any *Giardia* or *Cryptosporidium* bound to the membrane. A second-60 min filtration experiment was then performed. Grab samples of 50 ml were taken to determine initial titer after the backflush step. Samples were taken at the 62, 75, and 120 min time points to determine log reduction of each organism at each time point. The backflush procedure was performed in order to determine if membrane filter integrity would be compromised by this procedure. This would cause a decreased log reduction in titer in the second 60 minute trial.

In the *Giardia* experiments, a nominal GF/F Whatman 1.5  $\mu\text{m}$  glass fiber filter (VWR Scientific) was attached to the permeate line after 120 minutes and the entire 76.0 L of water was filtered. The same procedure was performed with the *Cryptosporidium* experiments with the exception of the use of a nominal GF/F Whatman 0.7  $\mu\text{m}$  glass fiber filter (VWR Scientific).

Prior to visualization and enumeration of *Giardia* and *Cryptosporidium*, cysts and oocysts were first eluted from the glass filters, purified in an a continuous gradient, and then concentrated by centrifugation. To elute each organism from the glass filters, filters were cut into smaller pieces and placed in eluting solution which consisted of 800.0 ml of 1X PBS (Sigma), 100.0 ml of 10.0% SDS (Sigma), and 100.0 ml of 10% Tween-80 (Sigma). This solution was vortexed briefly every five minutes for 30 min at room temperature. The entire volume was then centrifuged in a swinging bucket centrifuge (IEC) at 1050 x g for 10 min. The supernatant fluid was then discarded and the pellet was suspended in 30.0 ml of Percoll (Sigma) -sucrose (Sigma) medium and centrifuged at 1050 x g for 10 min. The particulates located at the interface of the gradient were extracted and pipeted into another container and then washed two times with eluting solution. The pellet was then suspended in 2.0 ml of 1 X PBS and the entire volume was processed using the IIF procedure previously mentioned. Visualization of any cysts or oocysts was also performed in the same manner as previously described. The log reductions in protozoans were calculated using the same formula that was used in the MS2 experimentation:  $\log N_T/N_0$ .

## **RESULTS**

### **Polysulfone Hollow Fiber Ultrafiltration of MS2 Bacteriophage**

The removal of microbes in water via ultrafiltration has been previously performed. It appears that different membrane configurations, membrane compositions, membrane pore diameters, influent pHs, particulate types and sizes, water temperatures, and operating parameters (transmembrane pressures) have an effect on the quality and volume of permeate produced. Chemical and physical cleaning procedures may also have an effect on the membrane's performance in various waters. Here, three polysulfone hollow (HF) ultrafiltration (UF) membranes with different pore diameters or molecular weight cut-offs (MWCO) were assessed for their ability to remove 4 logs of MS2 bacteriophage under varying experimental conditions and cleaning procedures.

### **Adherence of MS2 to Polysulfone Fibers and Bench Scale Apparatus**

MS2 did not adsorb to the individual fibers at pH 3.5, pH 7.0, and pH 8.5. Titers of phage after the incubation period did not differ from initial titers. Any MS2 associated with the polysulfone membranes in the dynamic bench scale challenges would not be due to electrostatic binding as a result of influent pH.

### **Adherence of MS2 to the Bench Scale Apparatus**

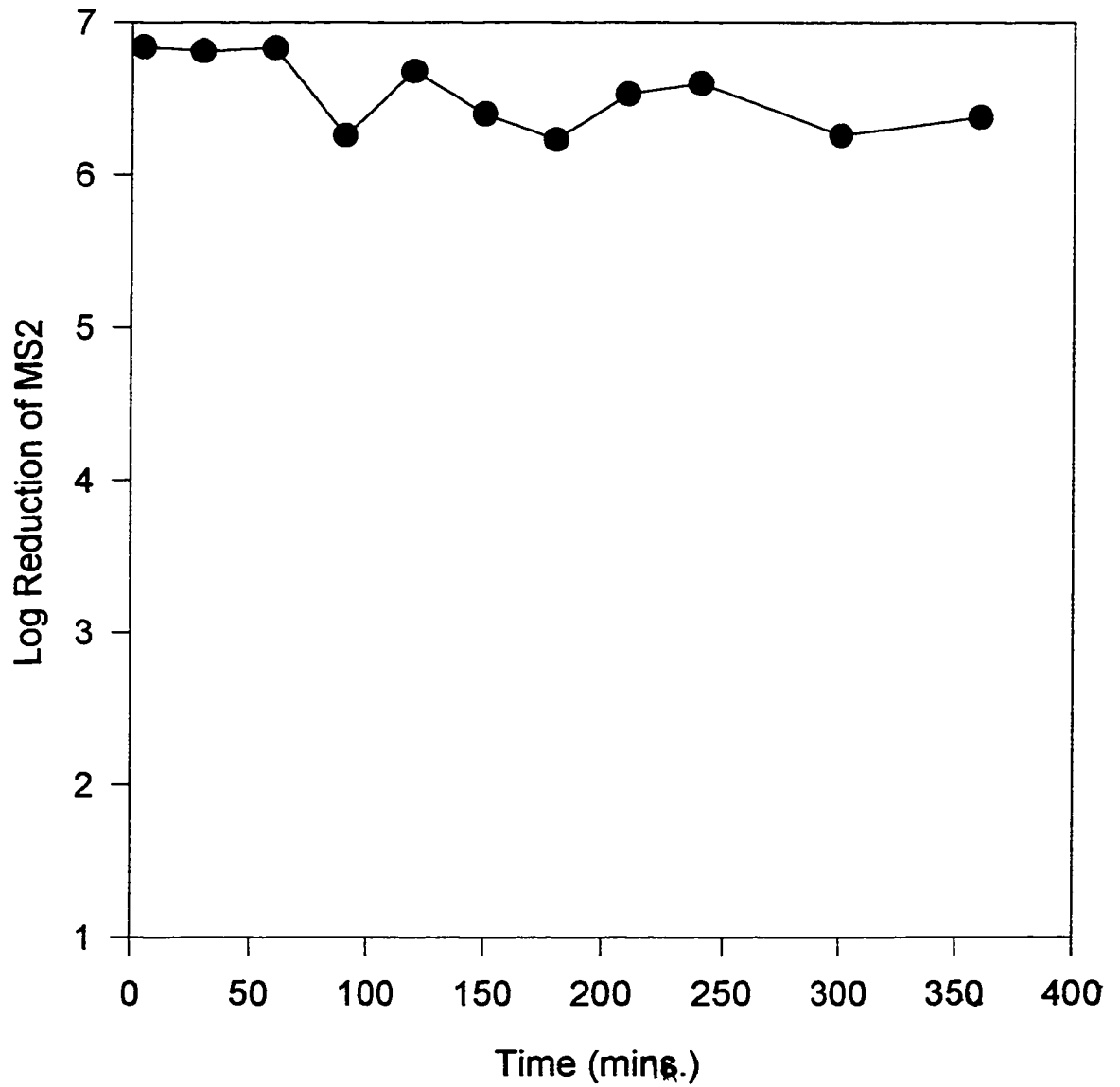
A one log reduction in virus titer due to viral adherence to the steel surface of the bench scale system occurred at pH 5.65, pH 7.00, and pH 8.10. A corrected initial titer, including this one log reduction, was used to determine log reductions for each time point. All metal influent and permeate sampling ports were replaced with polypropylene fittings to decrease the chance of viral adherence to the sampling ports.

The initial tank titer,  $3.00 \times 10^7$  pfu/ml, is representative of the initial titers used in all of the filtration studies. Log reductions were calculated using the influent and permeate concentrations of phage for each sampling time. Log reductions were calculated using the formula:  $\log N_0/N_t$ .

### **Ultrafiltration of MS2 (PM10 Membranes)**

The PM10 membrane was challenged in the single membrane configuration only. Maximum and minimum suggested operating transmembrane pressures were employed in two hour trials. No bacteriophage was detected in the permeate of any of these challenges. A six-hour challenge using one of the two PM10 membranes tested was performed at maximum operating pressure, 22 °C, and at pH 7.00 (Figure 4). Greater than 6 log removals were achieved over time with the PM10 membrane over time. MS2 did not adhere to the PM10 membrane. Therefore, the entire

Figure 4  
Log Reduction of MS2 Using PM 10 Membrane  
at pH 7.00, 22 °C, 22.5 psi





initial dose of MS2 remained in the circulating RO water. The membrane was challenged with greater than 6 logs of virus at each time point. The range of virus removal over time was 6.23 to 6.84 logs.

### **Ultrafiltration of MS2 (PM500 Membranes)**

The PM500 membranes showed variability in their ability to remove bacteriophage. The single membrane configuration was not consistently capable of achieving a 4 log reduction in virus titer at 22.5 psi and 12.5 psi, the minimum transmembrane pressure suggested by the manufacturer.

Two challenges were performed at a transmembrane pressure of 22.5 psi, pH 7.00, and a temperature of 22°C to determine if a 4 log reduction in virus titer could be sustained with two PM500 membranes set in parallel. Influent titers for both membranes remained above 5 logs the entire experiment duration (Figure 5). In the first parallel study, average log reductions for both membranes were at or above a 4 log reduction at the 5 and 30 min time points. Four log reductions in titer were not achieved at the 60, 90, 120, and 180 min time points (Table 3). In the second parallel study, a greater than 4 log reduction in virus titer was achieved at the 5 minute time point. At the 30, 60, 90, and 120 minute time points, a 4 log reduction was not achieved.

In the continuous seed study, a dose of phage at a concentration of  $1.73 \times 10^4$  pfu/ml-min. was added over time. A greater than 4 log reduction in titer occurred only

Figure 5

Influent MS2 Titers for PM500 Membranes in Parallel Challenge 1  
and Parallel Challenge 2 at pH 7.0, 22 °C, and 22.5 psi

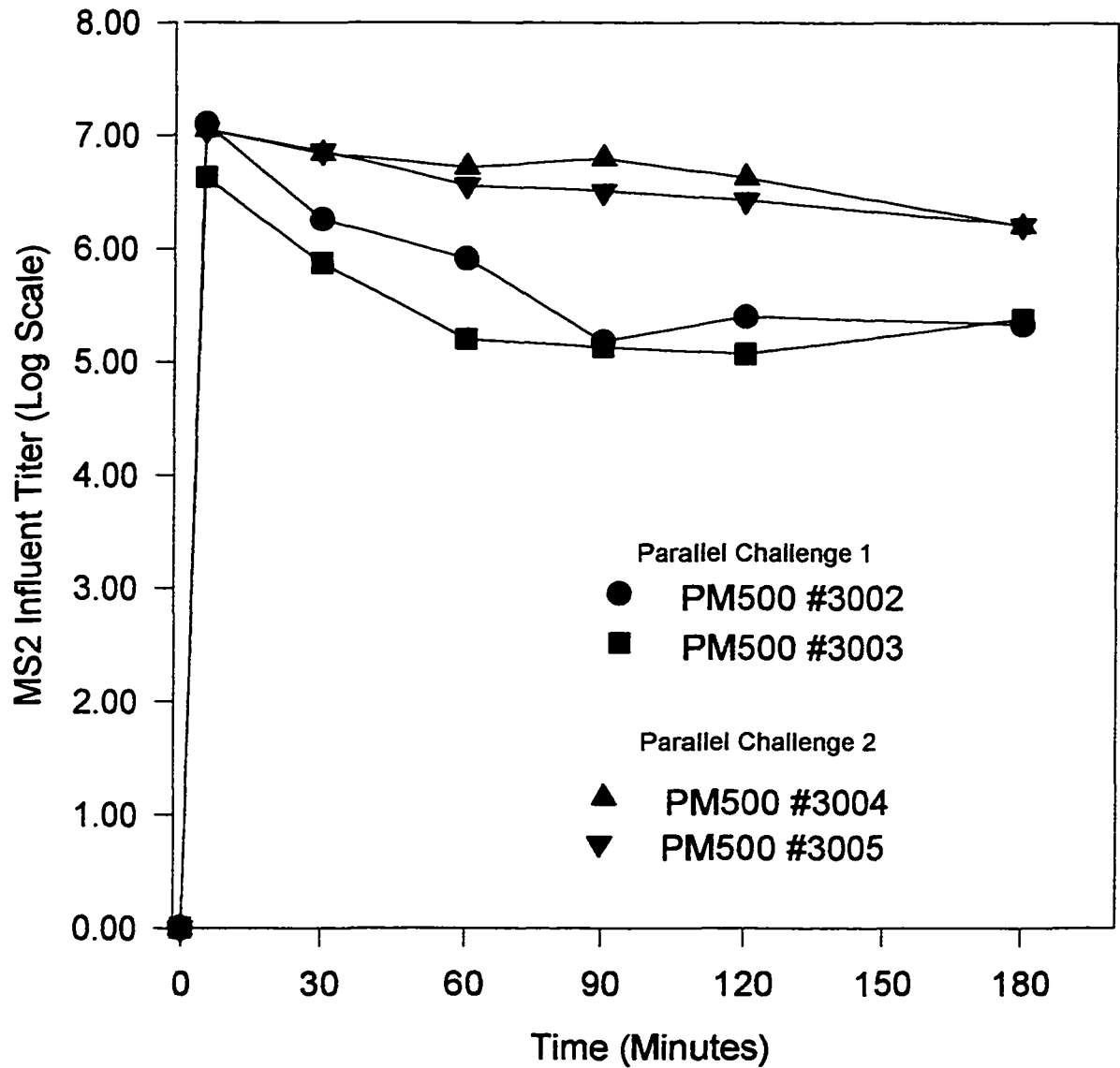


Table 3

Average log reductions of MS2 in 180 minute parallel PM500 membrane design challenges. Initial titer of  $3.12 \times 10^7$  pfu/ml, 22.5 psi, pH 7.00, and 22 °C.

Membrane #s	3002, 3003	3004, 3005	3002, 3003 <sup>1</sup>
Time (min)			
5	ND*	4.07	5.05
30	4.01	3.78	3.92
60	3.57	3.52	3.53
90	3.05	3.59	3.06
120	3.20	3.60	2.97
180	1.58	3.81	2.82

1- Continuous seed experiment,  $1.73 \times 10^4$  pfu/ml-min.

\* none detected

at the 5 min time point. These membranes were no longer studied due to their inability to achieve four log reductions in titer over time.

### **Ultrafiltration of MS2 (PMPW Membranes)**

The effects of pH, transmembrane pressure, temperature, and continual dosage of virus were ascertained for the PMPW membrane. The removals in single and parallel designs were between those found for the PM10 and PM500 membranes. Low counts, below 30 plaques, of MS2 were consistently found in the permeate samples of each trial. Although the PMPW membrane was not an absolute barrier to the virus, it consistently maintained 4 log reductions in virus titer over time for all experiments.

### **Effects of pH Variance (PMPW)**

It was shown that MS2 did not bind to the polysulfone membranes due to the effects of pH, see previous results on MS2 adherence to polysulfone membrane fibers. Six-hour single membrane experiments were conducted in order to determine any effect pH may have on filtration capability of the PMPW membrane. A 30 s backflush cleaning procedure was employed at 180 min and a second 180 min spiking interval similar to the first was conducted. Log removals of virus were not affected by pH (Figure 6). At pH 5.65, pH 7.00, and pH 8.10 log reductions were similar (Table 4). The titer of MS2 decreased over time for all three pH experiments as a result of viral

Figure 6

Influent and permeate MS2 levels in pH study using PMPW membrane

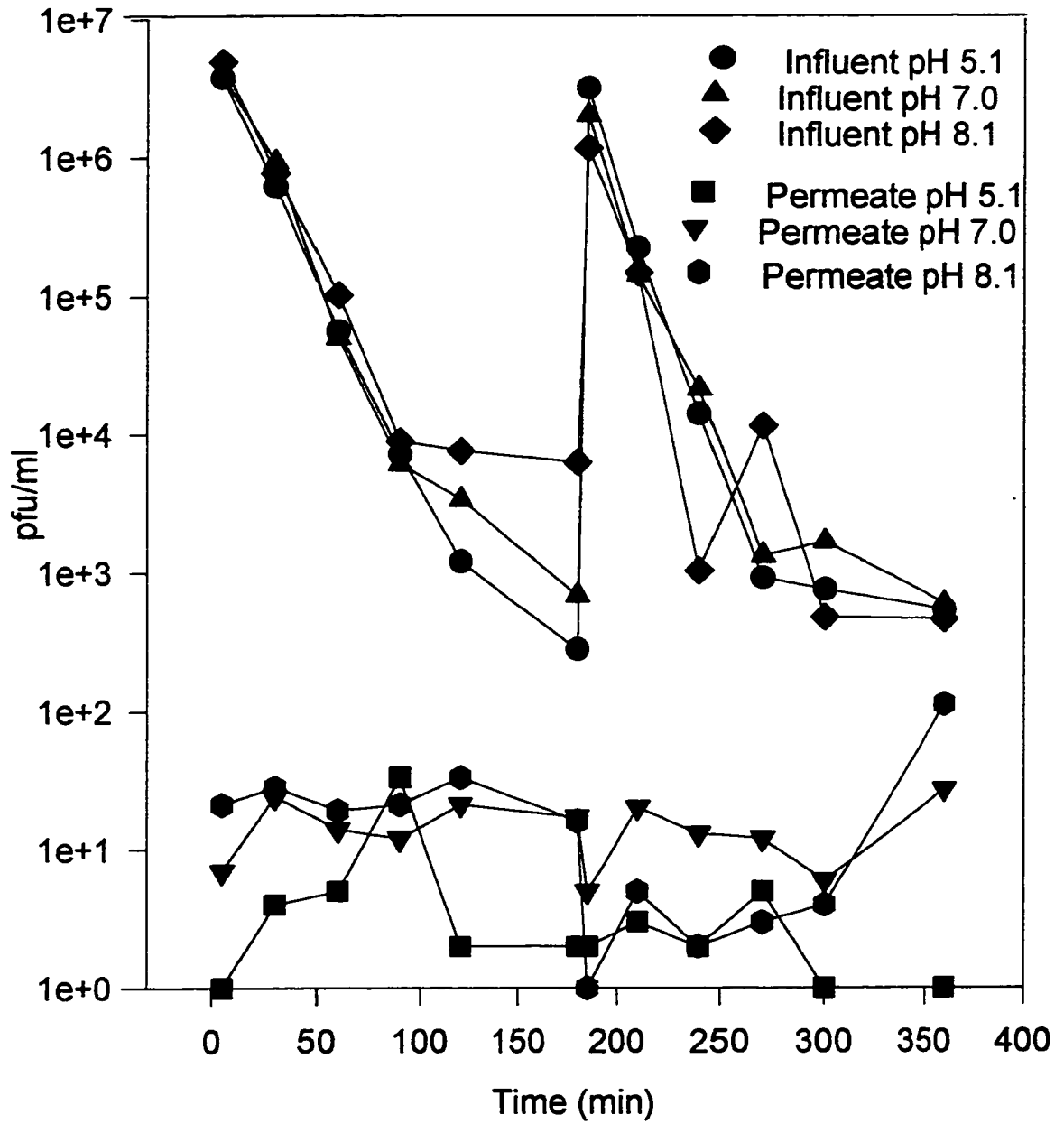


Table 4

Log reductions in MS2 titer in six hour trials using the PMPW membrane with a recycle backflush procedure at 180 min. Initial titer of  $2.43 \times 10^7$  pfu/ml, at 22.5 psi, 22 °C.

Time (min)	pH 5.65	pH 7.00	pH 8.10
5	5.98	5.76	5.38
30	5.09	4.59	4.44
60	4.15	3.55	3.73
90	3.38	2.71	2.63
120	2.61	2.21	2.36
180	2.15	1.61	2.60
185	6.20	5.61	6.07
210	4.87	3.85	4.47
240	3.85	3.22	2.72
270	2.26	2.04	3.58
300	2.88	2.44	2.08
360	2.73	1.35	0.61

removal with the PMPW membrane. The backflush procedure returned a concentration of virus,  $2.43 \times 10^7$  pfu/ml, to the tank and the second 180 min sampling periods duplicated the first 180 min period in each experiment.

### **Effect of Transmembrane Pressure Variance (PMPW)**

Parallel designed experiments involving two PMPW membranes, 3001 and 3002, were conducted at 22°C and pH 7.00 to determine the effect of transmembrane pressure on virus removal. Transmembrane pressures of 22.5 psi, 20.0 psi, 17.5 psi, and 12.5 psi were utilized to determine their effects on removal. Influent titer of MS2 for all 180-minute trials was  $3.12 \times 10^7$  pfu/ml for all challenges. Transmembrane pressure differences in the parallel system did not affect virus removal over 180 min, as shown by the average log reductions in titer for both membranes in parallel in Table 5. Interestingly, the 12.5 psi trial was the only trial that allowed between 30 pfu/ml and 300 pfu/ml, to be detected in the filter permeates at 22 °C. However, higher influent concentrations and lower membrane retention of virus at 12.5 psi were responsible for the similar log reductions of virus titer over time (Figure 7).

Table 5

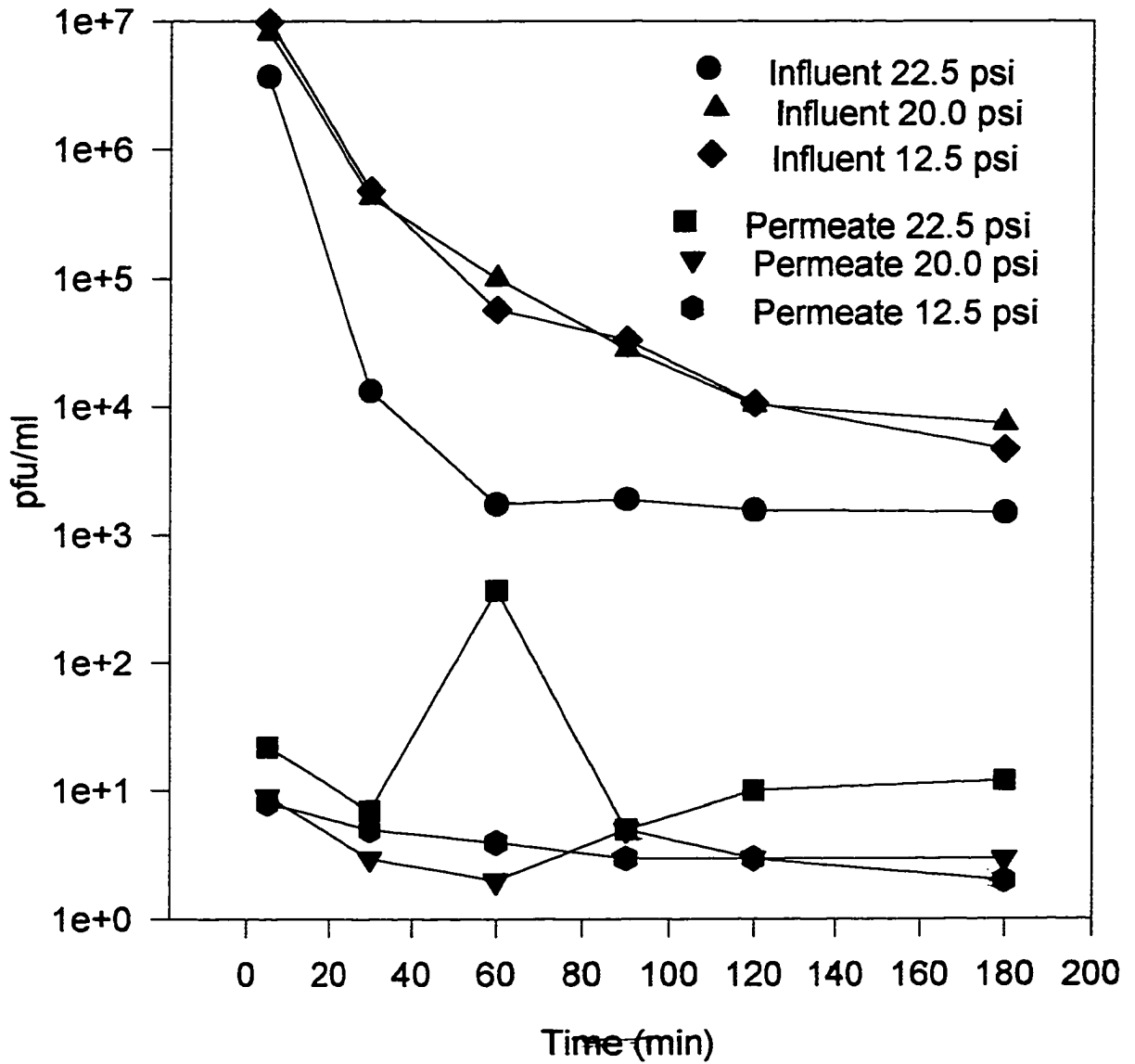
Average log reductions of MS2 virus in a parallel design experiments using PMPW membranes #3001 and #3002 at 22°C and pH 7.00. Initial titer of MS2 of  $3.12 \times 10^7$  pfu/ml.

Time (min)	22.5 psi	20.0 psi	17.5 psi	12.5 psi
5	5.37	6.18	5.90	5.85
30	3.81	4.76	4.72	4.30
60	3.08	4.54	4.08	3.66
90	2.10	3.86	3.81	3.02
120	1.76	3.70	3.49	2.49
180	1.89	3.35	3.24	2.86



Figure 7

Effect of varying transmembrane pressure on MS2 concentration of MS2 in parallel design at pH 7.0, 22 °C



### **Effect of Temperature Variance (PMPW)**

Parallel design experiments involving the PMPW membranes, 3001 and 3002, were performed at 22°C and 7 °C to assess the effect of temperature on virus removal. Initial virus titer for all experiments was  $3.12 \times 10^7$  pfu/ml. Log reductions in virus titer for each of the membranes in the parallel design were similar over time (Figure 8). Average log reductions of MS2 titer followed the same declining trend at both the 22 °C and 7 °C conditions independent of transmembrane pressure (Table 6). The transmembrane flux for the 7 °C conditions was reduced by 40% allowing for slightly higher log reductions at both transmembrane pressures.

### **Effect of Continual Addition of MS2 (PMPW)**

The average reduction in virus titer consistently exceeded four logs (99.99%) in the continuous addition parallel design experiments at pH 7.00 and 22 °C (Table 7). For individual membranes, log removals were below 4 logs in 4 of the 24 sampling time points (Table 7). The trials began with an initial titer of MS2 at  $3.12 \times 10^7$  pfu/ml. Trial one had a continuous dose of MS2 of  $1.73 \times 10^4$  pfu/ml-min. and the second trial,  $6.24 \times 10^5$  pfu/ml-min. The overall influent titers for the continuous addition studies were  $3.13 \times 10^7$  pfu/ml and  $3.49 \times 10^7$  pfu/ml respectively. The calculated theoretical influent titer was consistently maintained above  $1 \times 10^5$  pfu/ml at each time point. Log reductions declined

Figure 8

Effect of varying temperature with maximum and minimum transmembrane pressure on MS2 concentration

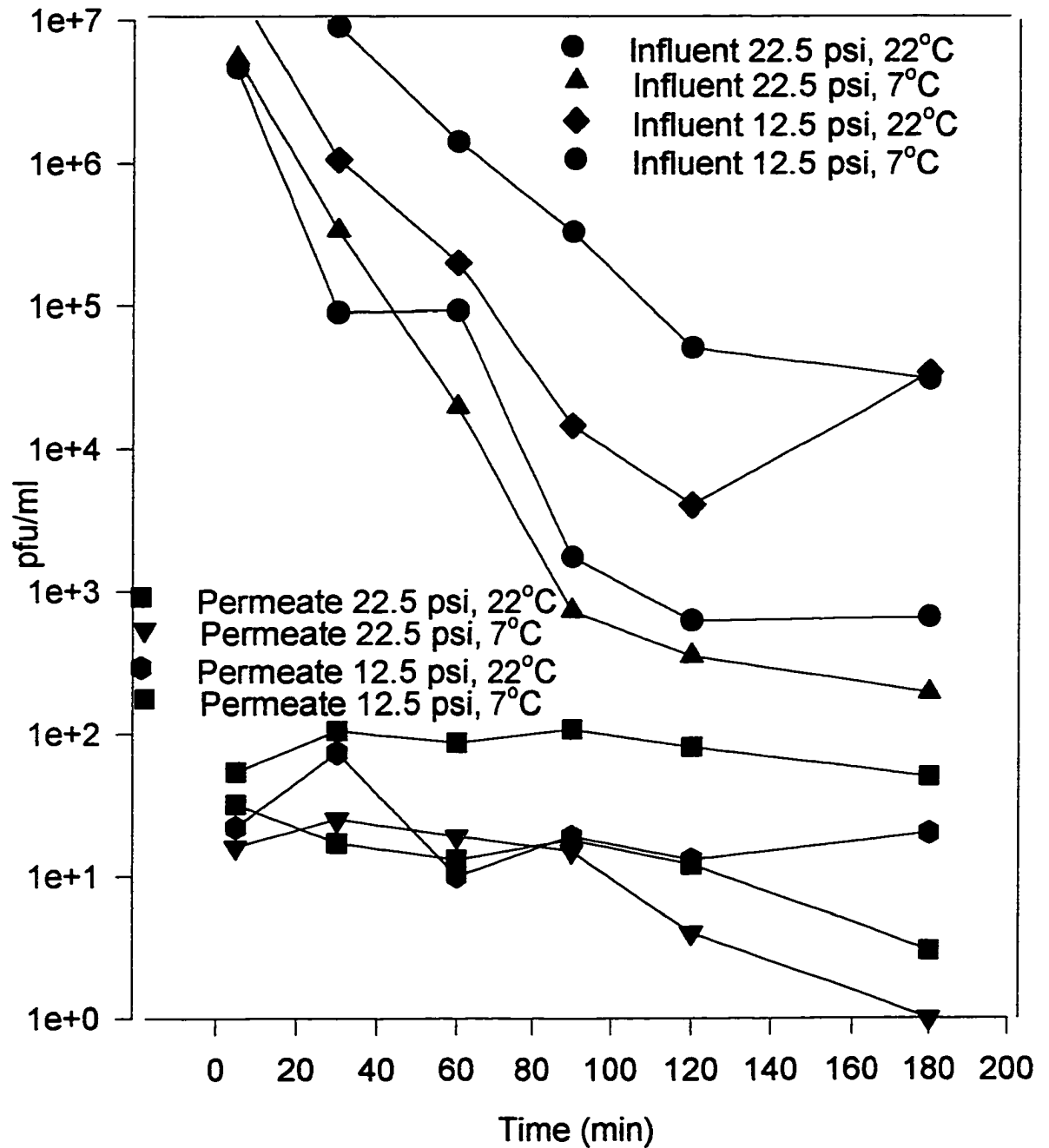


Table 6

Average log reductions of MS2 in parallel design, PMPW #3001, #3002, at 22.5 psi and 12.5 psi, at 22 °C and 7 °C, with initial titer of  $3.12 \times 10^7$  pfu/ml.

Time (min)	22.5 psi, 22 °C	22.5 psi, 7 °C	12.5 psi, 22 °C	12.5 psi, 7 °C
5	5.37	5.58	5.85	5.91
30	3.81	4.23	4.30	4.51
60	3.08	3.11	3.66	4.26
90	2.10	2.21	3.02	3.61
120	1.76	2.21	2.49	2.87
180	1.89	2.01	2.86	2.53

Table 7

Log reductions over 180 minute sampling period for continuous addition challenges in parallel design system, PMPW #3001 and #3002, with initial titer of  $3.12 \times 10^7$  pfu/ml and continual addition titers of  $1.73 \times 10^4$  pfu/ml-min and  $6.24 \times 10^5$  pfu/ml-min.

Time (min)	$1.73 \times 10^4$ pfu/ml-min Average Log Reduction in Titer	Log Removal #3001	Log Removal #3002	$6.24 \times 10^5$ pfu/ml-min Average Log Reduction in Titer	Log Removal #3001	Log Removal #3002
5	5.44	5.46	5.43	5.76	5.39	6.14
30	5.00	4.71	5.29	5.38	5.33	5.42
60	4.69	5.22	4.17	4.68	4.91	4.46
90	4.14	4.02	4.27	4.37	4.89	3.84
120	4.28	4.67	3.90	4.57	4.86	4.27
180	4.23	4.48	3.97	4.26	4.95	3.58

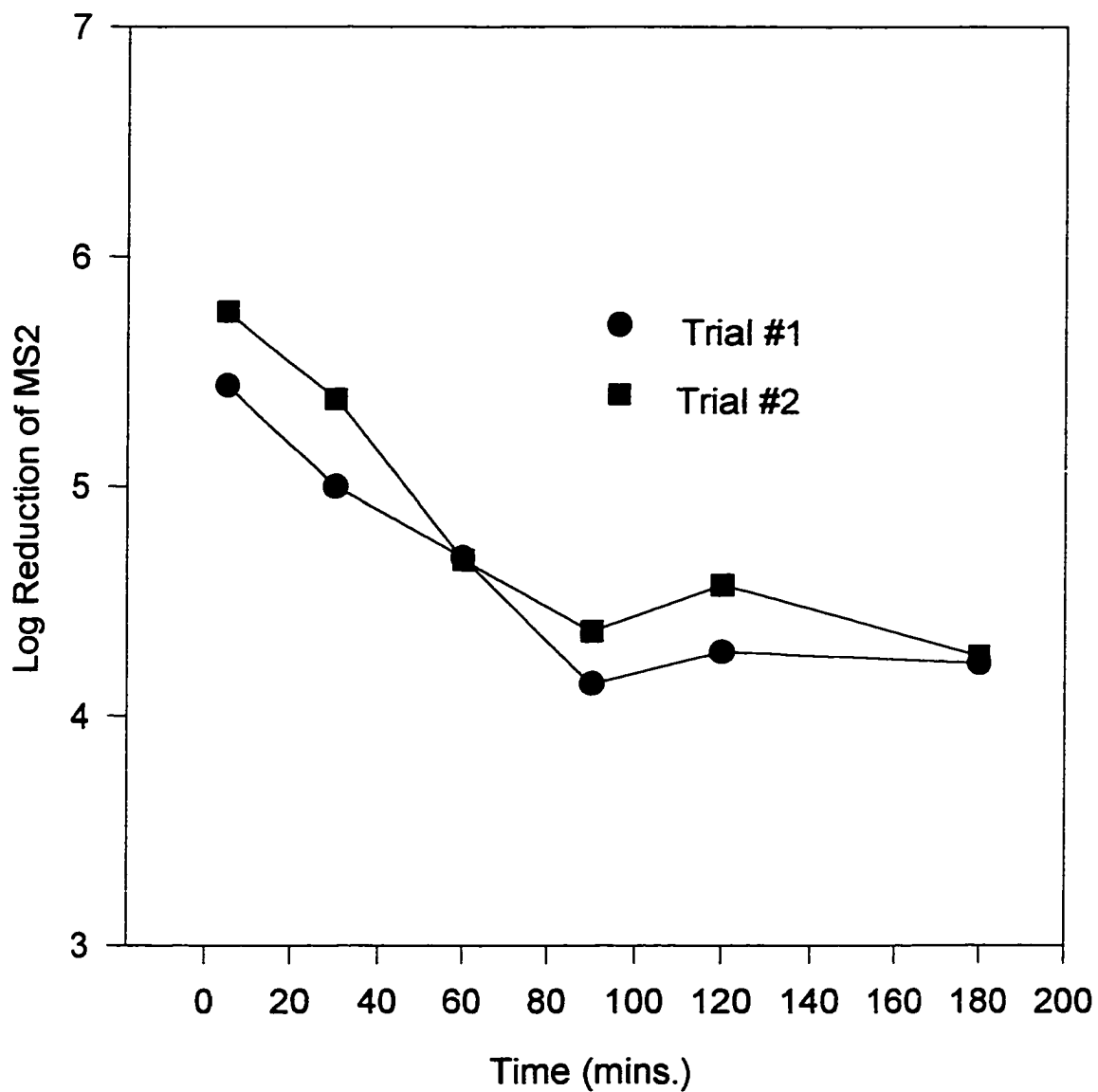
over time due to viral adherence on the membrane. Log reductions were initial greater due to a higher virus concentration in the influent. An initial dose of MS2,  $3.12 \times 10^7$  pfu/ml, was added to the tank at 0 min. Variability in log removals after 30 min were due to experimental variability of the enumeration assay. Permeate titer did not increase consistently as a function of time (Figure 9).

### ***Giardia and Cryptosporidium* Trials (PMPW)**

Removals of both protozoa at each temperature, 7 °C and 22 °C, were greater than 5.70 logs after two hours of operation using a singular PMPW membrane for each trial. No protozoans were found in the permeate in any of these studies. A transmembrane pressure of 22.5 psi, the maximum suggested operating pressure was used in all four challenges. All trials were 120 min in length with a recycle backflush employed at the 60 min time point. No protozoa were found in the permeate in any trials. Log reductions of both protozoa were calculated by using the formula  $\log N_0/N_t$ , where  $N_0$  represents the tank initial protozoan concentration and  $N_t$  represents the permeate titer at a given time point. The flux was reduced in the 7 °C condition similar to that found in the MS2 challenge experiments. Again, the greater log reductions of both protozoa in the 7 °C condition are a function of increased influent concentrations of each organism.

Figure 9

Average Log Reduction in MS2 Titer in Parallel  
Continuous Addition Challenge Experiments



## **Discussion**

### **Introduction to Polysulfone Ultrafiltration**

The removal of particulates by filtration is governed by many factors. These factors include membrane molecular weight cut-off (MWCO) and composition, influent pH, particulate size and concentration, membrane integrity, and the formation of a cake layer of naturally occurring material (Cheryan, 1986). Polysulfone UF membranes are tolerant of pH values as low as 1 and as high as 13 (Cheryan, 1986). Their stability to high influent and transmembrane pressures allow these filters to maintain membrane integrity during normal operation and physical cleaning procedures such as backflushing. Polysulfone membranes are tolerant of 200 ppm of sodium hypochlorite without loss of filter integrity. Therefore, their use in environmental water filtration appears efficacious as well as practical.

#### **PM10 Membranes**

The PM10 membrane was capable of providing an absolute barrier to MS2 in all of the challenges performed. Virus was not present in the permeates of any of the PM10 membrane studies. The capsid diameter of MS2 is 0.027  $\mu\text{m}$  which is representative of the enteroviruses. The absolute pore diameter of 0.001  $\mu\text{m}$  approaches the lower limit of



pore size for UF membranes. This allows UF membranes to remove viruses based on size exclusion of the particles. However, the use of this type of UF membrane for groundwater and surface water applications is impractical. The PM 10 produces the lowest flux of all the membranes tested. Lower flux is indicative of lower permeate production and a higher overall operating cost. A UF membrane with this pore diameter would require a greater number of membranes to produce an adequate volume of permeate.

### **PM 500 Membranes**

The PM500 membrane was not consistently capable of maintaining a 4 log reduction in virus titer at 22.5 psi, 22 °C, and pH 7.00. The continuous seed experiment using the parallel design showed that the PM500 membranes were incapable of removing a 4 log titer of phage over a 180 min time period. The PM500 membrane nominal pore diameter is 0.05  $\mu\text{m}$  which is larger than the capsid diameter of MS2. This explains why variant and lower log removal of virus were achieved by this membrane. Greater fluxes and increased virus removal were achieved with the PMPW membrane. The PMPW membrane would be a less expensive and a more useful membrane than the PM500 for virus removal at the conditions stated above. Therefore, the PM500 membrane was not viewed as a practical approach to UF in water treatment facilities.

### **PMPW Membranes (pH)**

The PMPW membranes were studied the most extensively. Influent pH did not affect viral removal by the PMPW membranes. For all three influent pHs tested, log reductions of virus decreased over time. This trend was most likely a function of membrane retention of the virus and not pH. Backflushing was employed between the two 180-min sampling periods. Backflushing was responsible for returning the entire initial virus concentration to the influent tank. The second sampling period showed the same trend of decreasing log reductions over time. Backflushing did not affect membrane integrity or permeate quality. A worst case scenario for UF removal of virus involves the operation of the system at maximum transmembrane pressure, the use of water that has an extremely low particulate concentration, and the use of a filter with no gel layer on it. The lack of particulates decreases the probability of the formation of a gel cake layer which may act as a prefilter and increase virus removal. Under these conditions, higher transmembrane pressures lead to higher fluxes and the greater possibility of a compromise in membrane integrity. At 22.5 psi, the maximum transmembrane pressure, the virus removal data stated agrees with other experiments using worst case scenario conditions (Adham, 1994).

### **PMPW Membranes (Transmembrane Pressure)**

Greater than 4 log reductions in virus titer over time were achieved at transmembrane pressures: 22.5 psi, 20.0 psi, 17.5 psi, and 12.5 psi. Greater than 4 log

reductions in titer were achieved independent of influent temperatures: 22 °C and 7 °C. Greater than 5 log reductions in phage titer occurred over the 180-min sampling period. Higher transmembrane pressures, producing higher fluxes, did not compromise filter integrity or permeate quality. In environmental water UF, higher fluxes allow for lower pumping pressures with a higher quantity of permeate produced for less cost (Crozes, et al., 1995). It was determined that lower transmembrane pressure was responsible for higher titers of phage in the recirculating influent samples. This can be explained by the fact that lower transmembrane pressures will yield lower fluxes. At lower flux conditions, less viruses are associated and held to the membrane. Therefore, a higher concentration of viruses is found in the tank where initial titer samples are taken. When determining log reduction for a particular sampling point, the initial titer will be higher and thus a greater log reduction will be calculated.

Lower operating temperatures increased the viscosity of the water which decreased the flux. Here, the flux at 7 °C was 40% less than the flux observed at 22°C. Greater log reductions were observed at 7 °C independent of the operating transmembrane pressure due to the decrease in flux and increase in influent virus titer. The decrease in log reductions over time occurred due to the membranes' removal of phage in the recirculating influent and not the physical compromise of the UF membrane.

### **PMPW Membranes (Continuous Addition)**

The continuous addition experiments demonstrated the ability of the parallel design

to consistently remove 99.99% of the influent virus titer over 180 min at pH 7.00, 22 °C, and at a transmembrane pressure of 22.5 psi. At four sampling points, reductions for the singular membrane were lower than 99.99%. Throughout the entire trial, influent titers for each membrane remained within a one log variance. Based on the quantities of phage added over time, total influent concentrations of  $3.13 \times 10^7$  pfu/ml and  $3.49 \times 10^7$  pfu/ml were calculated. This level on microbial influence is more representative of concentrations of virus found in wastewater effluent and not those of groundwaters.

### **PMPW Membranes (Protozoans)**

Both protozoans were removed from the permeate by physical separation. The pore diameter of the PMPW membranes is three orders of magnitude smaller than the diameter of the protozoans. No *G. lamblia* cysts and *C. parvum* oocysts were found in the permeates in any of the trials. Temperature had no effect on the membranes' capacity to remove cysts and oocysts from the permeate. It is apparent that UF would easily meet 99.9% removals of *Giardia* stated in the SWTR.

Polysulfone hollow fiber UF demonstrated consistent removal of greater than 99.99% of MS2, *G. lamblia* cysts and *C. parvum* oocysts over time. Transmembrane pressure, pH, and temperature differences all had minimal effect on the 0.005  $\mu$ m filters ability to physically remove virus and protozoans from worst case scenario, distilled water and maximum suggested operating transmembrane pressure conditions.

## **Practical Application of PMPW in Treatment Facilities**

HF UF far exceeds the standard 2 log reduction in virus titer normally granted to water facilities using filtration (USEPA, 1989). The SWTR and proposed ESTWR state that water treatment facilities must achieve a 4 log reduction in initial virus titer and a three log reduction in initial *Giardia* cyst titer. In these studies, the PMPW membranes were consistently capable of producing a 4 log reduction in MS2 titer and a greater than five log reduction in *Giardia* and *Cryptosporidium* cyst and oocyst titers.

HF UF used in conjunction with another primary disinfection strategy such as chlorine, ozone or UV light would allow a facility to easily meet the requirements of the SWTR and ESWTR. Water treatment facilities using chlorine as their main disinfectant may fail to meet the requirements of the DBP/P. Chlorine reacts with naturally occurring organic material to produce carcinogenic byproducts. The DBP/P will state a maximum contaminant level (MCL) on byproducts produced by a treatment plant. The addition of filtration into the existing disinfection scheme will allow plant operators to decrease the concentration of chlorine used for disinfection. UF removes viruses and protozoans from environmental waters. If filtration is used prior to chlorine disinfection, less chlorine is needed to remove the decreased concentration of pathogens in the water. UF is capable of removing most of the organics found in environmental waters. This will decrease the concentration of residual byproducts without sacrificing the quality of the permeate produced.

In order to assess HF UF membrane pathogen removal capability, these studies were performed using RO water to simulate worst case conditions for membrane filtration. Without naturally occurring particulates such as clays, soil, etc., the formation of a gel or cake layer on the membrane is not possible. This layer serves as a prefilter and can increase the potential reduction in pathogens by the membrane. However, this build up of materials may also serve to foul the membrane.

Fouling is the decrease in permeate volume due to the binding and aggregation of material on the membrane. This material may also compromise or destroy membrane integrity. The aggregation of materials onto the membrane is due to the particulate type and concentration in the water, pH of the water, and material composition of the membrane. A decrease in water pH increases the probability that humic and fulvic acids will bind to some membranes. Polysulfone membranes bind organic substances such as clays and proteins very poorly over a wide pH range. Therefore, membranes composed of polysulfone have low fouling potential in natural waters (Adham et al., 1995). Polysulfone UF membranes are also extremely resistant to membrane degradation due to physical and chemical cleaning procedures which remove substances from the membrane (Adham, 1991).

Polysulfone UF membrane integrity is not compromised under high transmembrane pressures (100 psi). Higher transmembrane pressures are often used in the physical cleaning of the membrane by backflushing. Membrane integrity may be assessed by using MS2 studies like those presented in this paper or by using other particulates.

Polysulfone membranes are tolerant of temperatures up to 75 °C (Cheryan, 1986). In the temperature studies performed here, there was no difference in reduction of MS2 at 4 °C and 22 °C. The 4 °C condition did lower permeate produced by 40%. However, this occurred due to the increased viscosity of the water. Continuous addition studies show that PMPW membranes were capable of removing titers of  $6.24 \times 10^4$  pfu/ml-min over time. These studies assure the membrane's ability to remove viruses at titers normally found in groundwaters and surface waters. HF UF membranes are used currently worldwide for pathogen removal in water treatment plants (Adham et al., 1995).

Polysulfone membranes have the advantages of low solute binding, wide pH range tolerance, high transmembrane pressure tolerance and serve as effective barrier to pathogens. These membranes are a cost effective alternative to other membranes because they are extremely durable and survive cleaning procedures which remove aggregated material which may compromise filter integrity.

The pore diameters of HF UF membranes like the PMPW are more effective at removing viruses than microfiltration (MF) membranes which have a larger pore diameter. Nanofiltration (NF) membranes of smaller pore diameter are more apt to foul when filtering groundwaters and surface waters (Dwyer, 1996). UF produces four to 60 times the permeate that NF produces under lower transmembrane pressures (Jacangelo, 1989). Fouling causes a loss of filter integrity and a decreased potential to remove pathogenic organisms from environmental waters.

Future studies to determine if these membranes remove the same titers of

pathogens after extensive use should be performed. Use in full scale water treatment plants to determine MS2 reduction capabilities in natural waters should be assessed. Here, membrane integrity can also be evaluated over time. Lastly, the membranes' permeate output using environmental waters under a variety of different transmembrane pressures should be assessed. These data will determine if these membranes serve as an economically practical barrier to waterborne disease.



## APPENDIX A

### PREPARATION OF MEDIA

#### Agar overlay (2X)

30 g of tryptic soy broth powder (Difco)

5 g of yeast extract (Difco)

7.5 g Bacto-agar (Difco)

0.075 g CaCl<sub>2</sub> (Sigma)

*Bring up to 500 ml with MilliQ water. Mix with a magnetic stir plate with heat. Autoclave 15 minutes. Store at 57°C until use.*

#### Tryptic soy agar

40 g tryptic soy agar powder (Difco)

*Bring up to 1000 ml with MilliQ water. Mix with a magnetic stir plate with heat for 20 minutes. Autoclave for 15 minutes. Leave at 57°C until use.*

#### Tryptic soy broth

30 g tryptic soy broth powder (Difco, Detroit, MI)

*Bring up to 1000 ml with MilliQ water. Autoclave 15 minutes. Leave at 4°C or room temperature until use.*

## APPENDIX B

### BUFFERS AND SOLUTIONS

#### Alcohol/glycerol solutions

<u>Final</u> <u>Ethanol</u>	<u>95%</u> <u>Ethanol</u>	<u>Glycerol</u>	<u>MilliQ</u> <u>Water</u>	<u>Final</u> <u>Volume</u>
10%	10ml	5ml	80ml	95ml
20%	20ml	5ml	70ml	95ml
40%	40ml	5ml	50ml	95ml
80%	80ml	5ml	10ml	95ml
90.2%	95ml	5ml	0ml	100ml

#### Bovine serum albumin (BSA) (1%)

1g BSA crystals (Sigma)

100ml 1X PBS

*Sprinkle crystals into PBS, pH 6.9 on a magnetic stir plate with heat. Completely dissolve before use. Store at 40C up to 7 days.*

#### DABCO mounting medium (2%)

2g diazobicyclo [2.2.2] octane (Sigma)

100ml glycerol

*Mix with heat on a magnetic stir plate. Store in dark at room temperature.*

#### Eluting solution

800 ml 1X PBS

100 ml 1% sodium dodecyl sulfate (SDS)

100 ml 1% Tween 80

#### Eriochrome T black

0.05g Eriochrome T black (Fisher)

5ml glycerol (Sigma)

95ml 95% ethanol

#### Eriochrome T black counter stain

1ml Eriochrome T black stock solution

75ml 95% ethanol

4ml glycerol

#### Percoll-sucrose gradient

45ml Percoll (Sigma)

45ml MilliQ water

10ml 2.5 M sucrose

**Phosphate Buffered Saline (PBS) (1X)**

9.785g of PBS powder (Sigma)

*Bring up to 1000 ml with MilliQ water. Autoclave for 15 minutes. Store at 4°C or room temperature. Final pH 6.9.*

**SDS (1%)**

1g SDS powder (Sigma)

*Bring up to 100 ml with MilliQ water*

**Tween 80 (1%)**

1 ml Tween 80 (Sigma)

*Bring up to 100 ml with MilliQ water*

## **Introduction**

### **Waterborne disease**

Waterborne disease outbreaks are often due to the consumption of virally contaminated drinking water (Sobsey, 1989). The positive identification of viruses in the environment has been documented for over five decades (Paul and Trask, 1941). In 1966, viruses were first isolated in drinking water which had undergone only marginal chlorination (Coin, 1966). This result challenged and correctly disproved the original idea that viruses were incapable of surviving in the environment for a long enough period of time to remain intact and infectious. Of the over 700 viruses that cause infection in man, 120 have been isolated in various types of water (Havelaar, et al., 1993).

Enteric viruses are viruses which cause infection by entering the human host via the fecal-oral route (Horstmann, 1967). Person-to-person contact, aerosols, and fomites are alternative routes used to initiate infection by many enteric viruses (Sattar, 1986). There are over 100 known enteric viruses (Bitton, 1980). Some enteric viruses include poliovirus, coxsackie virus, echovirus, hepatitis A virus, norwalk virus, enteric adenovirus, and rotavirus. Enteric viruses are one of the most common causes of waterborne disease outbreaks, typically inducing gastroenteritis in infected individuals (Bitton, 1980). Myelitis, encephalitis, meningitis, the common cold, and a wide range of other illnesses are also produced by enteric viruses (Gerba et al., 1985). Infectious dose, that is, the amount of viruses needed to cause infection in an individual is theoretically one infectious viral

particle (Berg et al., 1976). Typically, bacteria require between 10 and  $1 \times 10^8$  organisms to cause infection (Bitton, 1980)

Fecal material containing up to one billion viral particles per gram may enter drinking water via inadequately disinfected or filtered sewage (Davidson et al., 1977). In rapidly expanding communities, the volume of human waste may exceed the limitations of a wastewater facility and routinely escape into raw waters furthering the chance of communal enteric infection (Buras, 1974). In smaller communities, improperly maintained septic systems discharge fecal material into the environment. Nearby shallow drinking water wells are often infiltrated by this waste (Margolin et al., 1991).

Rotavirus is responsible for 744 million to one billion diarrheal episodes (Sattar et al., 1994) and 10 to 18 million deaths worldwide per year (Kapikian and Chanock, 1985). It is also estimated that in U. S. alone, rotavirus is responsible for nearly 250 million dollars spent in medical and hospitalization expenses as well as missed wages (Glass et al., 1985).

Hepatitis A virus is responsible for up to 60,000 cases of acute hepatitis in the U.S. every year (Belshe, 1991). Hepatitis A virus is found in both contaminated water and shellfish (Belshe, 1991). Enteric viruses also possess the ability to act as possible cofactors in other etiologies such as diabetes mellitus (Belshe, 1991).

### **Chlorine Disinfection**

Routine chlorination has been used since 1854. The Royal Sewage Commission used chloride of lime in London sewage to reduce odor. (White, 1986). Later it was

recognized for its microbiocidal capabilities. In 1902, chlorine was first used as a routine disinfectant for source water to be used as potable water in Middelkerke, Belgium (White, 1986). Chlorine is the most widely employed treatment of disinfection because it is inexpensive, requires little technical ability for its application, and remains a very effective disinfectant against bacteria, viruses, and some protozoan parasites (Jolley et al., 1986).

However, chlorine can be harmful to the general public health as well as plant and animal life. Together, chlorine and naturally present organics form trihalomethanes and other halogenated compounds that are carcinogenic to humans. The Disinfection-Disinfection By-Product (D-DBP) rule was proposed in order to define maximum contaminant levels (MCLs) of harmful byproducts allowed in drinking water produced by treatment facilities. Water treatment suppliers not in compliance with the D-DBP rule will be required to alter their disinfection methodology. It has been previously stated that filtration is one disinfection strategy that may aid in lowering DBP production in drinking water (USEPA, 1989).

### **Ultraviolet (UV) Irradiation Disinfection**

UV irradiation is capable of inactivating viruses, bacteria, and pathogenic protozoa in environmental waters (Sobsey, 1989). Wastewater and water treatment facilities use UV irradiation which is generated via an electron flow in a low pressure mercury vapor germicidal lamp (Wilson et al., 1992). UV dose is measured in  $\mu\text{Ws}/\text{cm}^2$ . The germicidal spectrum for UV light is between 240.0 nm and 280.0 nm. Up to 90.0% of a UV lamp's peak output is between 253.7 nm and 254.3 nm (Zinnbauer and Conacher, 1987).

UV light inactivates organisms by creating thymine-thymine, thymine-cytosine, and/or cytosine-cytosine dimers in organisms whose genome is single-stranded (ss) or double-stranded (ds) DNA (Bitton, 1980). UV irradiation creates uracil-uracil, uracil-cytosine, and cytosine-cytosine dimers in ss or ds RNA (Bitton, 1980). More specifically, DNA or RNA is altered by the formation of covalent linkages of successive bases in their strands. The covalent linkage of bases that are physically near one another because of tertiary structure and the irreversible covalent linkage of proteins to the nucleic acid are two more ways in which UV light inactivates microbes. These bonds are formed due to the absorption of the energy by the hydrogen bonds in the nitrogenous bases in the nucleic acid. Covalent bonds physically inhibit enzymes used for nucleic acid synthesis rendering the organism inactive (Freifelder, 1987). In organisms possessing ds genomes, strand replacement may counteract the effects of UV irradiation and render the organism potentially infectious (Battigelli et al., 1993). Thymine is affected more readily than uracil. One study (Jagger, 1967) shows that UV irradiation is more effective creating dimers in DNA viruses over RNA viruses. It is posited that RNA viruses are better models for determining an optimum UV dose for pathogenic virus inactivation.

Enteric viruses are more resistant to UV irradiation than vegetative bacteria due to the nature of their nucleic acid (Sobsey, 1989). In bench scale experimentation, rotavirus is the most UV resistant human enteric RNA virus to date (Battigelli, 1993). In UV irradiation studies, the simian rotavirus strain, SA-11, is used to determine rotavirus susceptibility to UV disinfection. However, it would be more pertinent to use the Wa strain of rotavirus. The Wa strain shows longer survivability over the SA-11 strain in

varying types of raw waters (Pancorbo et al., 1987). It is possible that it has a decreased susceptibility to UV irradiation over the SA-11 strain due to a difference in outer glycoprotein composition or RNA sequence diversity. The Wa strain is also pathogenic to humans and its UV susceptibility data would be more useful in determining optimum UV dose for inactivation of human enteric viruses in environmental waters. The drawback to using the Wa strain in these types of experimental designs is that the assays used to titer this virus are more expensive, laborious, and require an understanding of current molecular biology techniques.

Previous studies show that *G. lamblia* cysts are less susceptible to UV irradiation than rotavirus (Sobsey, 1989). *Cryptosporidium parvum* oocysts appear to be more resistant to UV irradiation over viruses (Campbell et al., 1982). The dose used to obtain a two log reduction in oocysts has been previously used to obtain greater than four log reductions in titer of enteric viruses. However, enteric viruses were not disinfected with the same type of UV reactor in this study.

Inactivation by UV light depends on the dosage and the ability of the irradiation to be absorbed by the hydrogen bonds in the nucleic acid (Havelaar, 1993). In environmental waters with high turbidity and color, such as wastewater, UV light may be less effective as a disinfectant (Havelaar, 1987). Color and solids act to absorb irradiation which lowers the effective dose absorbed by the nucleic acid.

UV is not a chemical disinfectant. It does not leave a residual dose in water (Chang et al., 1985). This is viewed as a drawback to using UV as a disinfectant. Any organisms surviving the initial dose survive in the effluent. UV disinfection strategies also suffer from



regrowth of organisms downstream from the UV reactor (Qualls and Johnson, 1983). Regrowth is defined as the propagation of injured organisms after UV irradiation. These organisms adhere to and form colonies on pipes downstream from the UV reactor (LeChevallier et al., 1987). Later, they slough off the piping and are carried in the permeate water potentially causing illness to consumers.

Design and maintenance problems with present day UV disinfection strategies exist. Typically, radiometers that measure dosage on a planar scale are used and incorrectly determine dosage in cylindrical pipes. The dose read from a radiometer is typically higher than the actual dose applied to the water because of the dispersion of UV irradiation by particulates in the water. Incorrect formulas are used to determine applied dosage as well (Qualls and Johnson, 1983). Pilot and bench scale data is needed to adequately determine the effective microcidal doses needed in various types of waters. This data also supplies better understanding of the construction of a more effective treatment plant design.

UV disinfection does have a number of benefits that make it a worthy alternative disinfection strategy to those presently used in groundwater treatment facilities. UV light is a potent disinfectant of viruses and bacteria (Battigelli et al., 1993). UV does not require personal trained in the handling and transport of hazardous materials. UV light does not combine or alter naturally occurring organics or other compounds to form harmful by-products (Sobsey et al., 1991). In the future, treatment facilities not meeting the requirements of the DBP/P may be required to use UV light to replace or act in conjunction with their existing chlorine disinfection strategy.

Previously, it was shown that UV irradiation is a less costly disinfection procedure over chlorine, chlorine dioxide, and ozone for smaller treatment facilities (<0.05 million gallons per day (mgd)) (Wolfe, 1990). In European countries, UV light is extensively used as a disinfectant in drinking water facilities (Wiendermann, 1993). In these treatment plants, properly maintained prefiltration removes particulate matter lowering the turbidity which increases the effectiveness of UV dosage. Smaller wastewater treatment facilities have used UV irradiation in conjunction with post-filtration and found UV to be cost effective when compared to chlorine disinfection (Wiendermann, 1993).

### **UV Disinfection Experimentation**

The following work uses various groundwaters to determine the effect of color, turbidity, elemental composition, overall UV absorption, and pH on UV inactivation of viruses. The RNA viruses, poliovirus (PV), rotavirus (RV), HAV, and MS2 used in the assessment of UV's disinfection capability. RV is the only ds RNA virus used in this study. Inactivation is said to follow a second order kinetic rate because of the possibility for strand or base replacement and the reduced amount of UV absorbance by the ds genome (Patrick and Rahn, 1976). RV is the only virus in these studies possessing a double concentric capsid and additional core protein layer. These three layers of protein are capable of absorbing UV irradiation and shielding the nucleic acid from its effects. The genome is the largest, about 15,000 nucleotides, which requires more absorption to take place in order to inactivate the virus (Battigelli et al., 1993).

MS2 has been proven to be less sensitive to UV irradiation in filtered water. The

only speculation that possibly explains this effect is that MS2 has a relatively high degree of tertiary structure in its genome and a low uracil content. It has also recently been postulated that the capsid density or composition allows for substantial UV absorption (Wiendermann, 1993). As previously stated, this virus is inexpensive, simple, and rapid to enumerate. Its nonpathogenicity makes the virus suitable for pilot and full scale experimentation. It would appear to be the perfect surrogate for UV inactivation criteria. However, there is also speculation that the increased dosage needed to inactivate MS2 over RV is a function of the assay used for their enumeration and not the MS2's lower susceptibility to UV disinfection.

#### **Cell Culture-RT PCR Assay**

Currently, cell culture is the accepted method of choice for detecting enteroviruses in environmental water samples (Reynolds et al., 1996). Monolayers of a continuous cell lines are inoculated with concentrated water samples and the presence of cytopathic effects (CPE) is used as the criteria for a positive result. Some cytopathic effects include decreased adherence of the cell monolayer, increased refractility of the cell monolayer to the bottom of the culture flask, and other morphological changes in cell appearance. While this assay benefits from its ability to detect infectious viruses, it is time-consuming. Positive identification of viruses requires the extraction of the virus after CPE determination and subsequent inoculations onto new monolayers. The assay is also relatively expensive and does not identify the virus or viruses present in the sample (Hurst et al, 1989). No one cell line can be used for the identification of all enteric viruses

(Schmidt et al., 1978) and not all viruses exhibit cytopathic effects on continuous cells.

An alternative assay used for the detection of RNA enteric viruses is reverse transcriptase polymerase chain reaction (RT PCR). RT PCR is less laborious and less expensive than cell culture and is a very sensitive assay for the detection of viral RNA. As little as 0.005 pfu of rotavirus have been detected in environmental isolates using RT PCR (Grinde, 1996).

However, RT PCR is not without its disadvantages as a detection assay for the presence of viruses in environmental water samples. RT PCR is capable of distinguishing the type of viral RNA present in an environmental sample. RT PCR is incapable of determining if the RNA detected is from infectious viruses. In other words, RT PCR will detect viral RNA that has been liberated from a virus inactivated by disinfection or environmental stresses.

RT PCR is also limited by small reaction volumes (Ma et al., 1994). Typically, water sample concentrates are 20 to 30 ml (Ma et al., 1994). A ten to 20  $\mu$ l aliquot of this sample concentrate is used in RT PCR to detect viral RNA. A false negative may result from RT PCR because the aliquot may not be sufficient to represent the entire concentration of viruses in the original water sample concentrate.

Fulvic and humic acids, heavy metals, and some proteins found in environmental waters may also inhibit the enzymes used in RT PCR and make the assay less sensitive increasing the chance for a false negative result. Purification assays that are done prior to RT PCR are capable of removing these substances but can decrease the concentration of virus in the sample (Ma et al., 1995).

The combined cell culture-RT PCR assay involves a single inoculation of a continuous cell line, replication of the virus, and subsequent detection of viral RNA by RT PCR. The cell culture step serves to increase the concentration of viruses in the sample increasing the chance for viral detection by RT PCR (Reynolds et al., 1996). The cell culture step dilutes compounds which may inhibit the enzymes utilized in RT PCR resulting in increased detection of viruses.

The cell culture-RT PCR assay is also a more rapid approach to detecting viruses in environmental samples. Traditional cell culture assays detect viruses by the presence of CPE in cells. Once this occurs, the virus is extracted and this sample is inoculated onto a new monolayer. Positive identification by the presence of CPE is again confirmed on this cell line. In the cell culture-RT PCR step, the second inoculation is removed and replaced by RT PCR. While the second inoculation in the traditional cell culture assay may take up to 14 days, the RT PCR step in the cell culture-RT PCR assay will take four to five hours. Previously, the cell culture-RT PCR assay detected enteric viruses in three-fold less time than a traditional cell culture detection assay (Reynolds et al., 1996). The cell culture-RT PCR assay also allows for the positive identification of viruses that do not produce typical CPE such as rotavirus and hepatitis A virus (Reynolds et al., 1996). Here, this assay is used in place of a standard plaque assay for the determination of log reduction of rotavirus Wa strain due to UV irradiation. The assay has greater reproducibility and is more sensitive than the plaque assay methodology in detecting rotavirus.

## METHODOLOGY

### Ultraviolet Light Irradiation of Viruses

#### **MS2 Propagation**

MS2 was propagated using the host: *Escherichia coli* C3000. The host was grown to mid-exponential phase in 1L of tryptic soy broth (Sigma) supplemented with 0.005 M CaCl<sub>2</sub> (Sigma, St. Louis, MO). The bacterial suspension was placed in a coffin shaker at 200 rpm. A total of  $1 \times 10^{13}$  pfu of MS2 was added to the host and the suspension was shaken slowly for 150 min at 37 °C. The lysates were decanted into 250 ml centrifuge bottles (Nalgene, Rochester, NY) and chloroform treated. The suspensions were centrifuged at 10,000 x g for 10 min at 4 °C. The supernate was recovered and then stored at 4 °C until use. Initial virus titers ranged from  $2.43 \times 10^{11}$  pfu/ml to  $3.75 \times 10^{11}$  pfu/ml.

#### **MS2 Quantification**

The double agar overlay plaque assay (Debartolomeis et al., 1991) was used to enumerate MS2. Virus was diluted from  $10^0$  to  $10^{-8}$  in 1 X PBS. Approximately 0.2 ml of *E. coli* C3000 and 0.1 ml of virus dilution were added to 5.0 ml of soft 2 X tryptic soy overlay (Difco, Detroit, MI) and poured onto 100 x 15 mm<sup>2</sup> plastic petri dishes containing

tryptic soy agar (Difco). Positive controls received 0.1 ml of virus of known concentration. The negative control received 0.1 ml of 1 X PBS. Upon solidification of the overlay, plates were inverted and placed in a 37 °C incubator. All plaques were counted in 12 to 16 hours.

### **Poliovirus propagation**

Buffalo Green Monkey (BGM) kidney cells (ATCC, Rockville, MD) were utilized to propagate poliovirus type I (LSc). The cells were grown in closed 75 cm<sup>2</sup> cell culture flasks (Corning, Corning, NY). Eagle's Minimal Essential Media (MEM) (Sigma) supplemented with 8% fetal bovine serum (Gemini BioProducts, Calabasas, CA) was used to grow the cells to confluency. The cells were washed twice with 1 X PBS (Sigma) and then 1 ml of poliovirus stock ( $1 \times 10^8$  pfu/ml) was added to the cell monolayer. The multiplicity of infection (MOI) was at a ratio of 1:100. The cells were incubated at 37 °C for 80 minutes. The cells were rocked every 15 min in order to ensure complete hydration of the cell monolayer. MEM without serum was added to the flasks and they were further incubated at 37 °C for 24 to 48 hours. When cells exhibited cytopathic effect (CPE) the flasks were freeze-thawed twice in order to liberate any cellular bound viruses. The lysate was decanted into sterile 30 ml Oakridge tubes (Nalgene, Rochester, NY) and centrifuged at 10,000 rpm, for 10 min at 4 °C to pellet the cellular debris. The supernate was aliquoted into 1.5 ml microfuge tubes and stored at -80 °C until use.

### **Poliovirus quantification**

Poliovirus stocks were quantified using 25 cm<sup>2</sup> flasks (Corning, Corning, NY) containing confluent monolayers of BGM cells (BioWhittaker, Walkersville, MD). The stock was diluted ten-fold in 1 X PBS from 10<sup>0</sup> to 10<sup>-8</sup>. Duplicate flasks were used for each dilution. Each flask was rinsed with 1X PBS and then inoculated with 0.1 ml of each dilution. The flasks were incubated at 37 °C for 80 min. The negative control flask received 0.1 ml of 1 X PBS. Two positive control flasks were inoculated with 0.1 ml of poliovirus of known titer. Flasks were rocked every 15 min during the incubation period.

A 2 X agar overlay was used as the solid support matrix for subsequent quantification. The overlay was composed of 2% flake agar (Difco) and 2X M199 (Sigma) and was brought to pH 6.9 with 1N HCl. At the end of the incubation period 5 ml of agar overlay was pipetted onto the interior side of all of the flasks except the second positive control flask. The overlay was then distributed onto the cell monolayers. The overlay was allowed to harden for 20 min in the absence of light at room temperature. All flasks containing overlay were inverted and incubated at 37 °C. The second positive control flask received 5 ml of MEM (Sigma) and was incubated at 37 °C until CPE was observed. Plaques were counted after 48 to 72 hours.

### **Hepatitis A virus propagation**

Fetal rhesus kidney (FRhK-4) cells (ATCC) were used to propagate hepatitis A virus (HAV) HM-175. The cells were grown in 75 cm<sup>2</sup> closed cell culture flasks (Corning). The cells were grown to confluency using MEM (Sigma) supplemented with



12% fetal bovine serum (Gemini Bioproducts). The cells were washed three times with 1 X PBS and then inoculated with 1 ml of hepatitis A virus stock ( $1 \times 10^6$  pfu/ml). The flask was incubated at 37 °C for 120 min. The flask was rocked every 15 min in order to ensure complete hydration of the monolayer. Upon completion of the incubation period, 10 ml of MEM (Sigma) supplemented with 2% fetal bovine serum (Gemini BioProducts) was added. The flasks were incubated until every cell showed CPE. The flask was freeze-thawed three times in order to liberate any cellular bound viruses. The lysate was decanted into 30 ml Oakridge tubes (Nalgene) and centrifuged at 10,000 rpm for 10 min at 4 °C. The supernate was then allotted into 1.5 ml microfuge tubes and stored at -80 °C until use.

#### **Hepatitis A virus quantification**

Hepatitis A virus stocks were quantified using 25 cm<sup>2</sup> (Corning) containing confluent monolayers of FRhK-4 cells. The cells were grown to confluency using MEM (Sigma) supplemented with 12% fetal bovine serum (Gemini BioProducts). The stock was serially diluted in 1 X PBS from  $10^0$  to  $10^{-7}$ . Each dilution was assayed in duplicate. Flasks were rinsed three times with 1 X PBS and then inoculated with 0.1 ml of each dilution. The negative control flask received 0.1 ml of 1 X PBS and two positive control flasks were inoculated with 0.1 ml of hepatitis A virus stock of known titer. Each flask was incubated at 37 °C for 120 min, rocking every 15 min.

After the incubation period was completed, 5 ml of agar overlay was pipetted onto the interior side of all flasks except the second positive control flask. The agar was then distributed onto the cell monolayers. The overlay consisted of 2% flake agar (Difco)

and 2 X M199 supplemented with 2% fetal bovine serum and 100  $\mu$ M diethylaminoethyl (DEAE) dextran (Sigma). The overlay was brought to pH 6.9 with 1 N HCl. Overlay was allowed to harden in the absence of light at room temperature for 20 min. Flasks containing overlay were inverted and incubated at 37 °C. The second positive control flask received 5 ml of MEM supplemented with 2% fetal bovine serum and was incubated at 37 °C until CPE was observed. Plaques were counted after four to seven days.

### **Rotavirus propagation**

MA-104B cells (BioWhittaker, Walkersville, MD) were used to propagate rotavirus Wa (ATCC strain VR-2018). The cells were grown in 75 cm<sup>2</sup> closed cell culture flasks (Corning). Eagle's MEM (Sigma) supplemented with 8% fetal bovine serum (Gemini BioProducts) was used to grow the cells to confluency. A 1.0 ml aliquot of virus was incubated at 37 °C for 10 min with trypsin 1:250 (ICN, Costa Mesa, CA) at final concentration of 10  $\mu$ g/ml. The cells were rinsed three times with 1 X PBS and inoculated with 0.5 ml of rotavirus Wa stock ( $1 \times 10^6$  pfu/ml). The flasks were incubated at 37 °C for 90 min. The flasks were rocked every 15 min. Upon completion of the incubation period, 5 ml of MEM supplemented with 0.5  $\mu$ g/ml of trypsin 1:250 (ICN) was added to the flask. Flasks were incubated until every cell showed CPE. The flasks were freeze-thawed three times to liberate any cellular bound viruses. The lysate was decanted into 30 ml Oakridge tubes (Nalgene) and centrifuged at 10,000 rpm for 10 min at 4 °C. The supernate was allotted into 1.5 ml microcentrifuge tubes and stored at -80 °C until use.

### **Rotavirus quantification (Cell culture-RT PCR method)**

Rotavirus samples were quantified using 25 cm<sup>2</sup> flasks that contained confluent monolayers of MA-104B cells. Cells were grown using MEM supplemented with 8% fetal bovine serum. Samples were serially diluted in MEM from 10<sup>0</sup> to 10<sup>-5</sup>. Each dilution was incubated at 37 °C for 10 min with trypsin 1:250 at a final concentration of 10 µg/ml. Cell culture flasks were rinsed three times with 5 ml of MEM and 0.1 ml of each dilution was added to the cell monolayers. Duplicate flasks were used for all dilutions.

The negative control received 0.1 ml of 1 X PBS. The positive control received 0.1 ml of rotavirus whose titer equaled that of the highest dilution in the experimental series. Another control received 0.1 ml of suspended rotavirus ds RNA. Proteinase K (Sigma), at a concentration of 400 µg/ml, was added to a titer of rotavirus equal to that of the lowest dilution in the experimental series. The virus was then incubated at 56 °C for 60 min in a water bath to destroy the capsid and release the ds RNA. The suspension was heated to 99 °C for 5 min to destroy the proteinase K. The samples were then placed on ice for 5 min. The solution was then centrifuged at 12,000 rpm for 10 min at 4 °C to pellet the viral and cellular protein. The supernate was then added to the control flask.

The flasks were incubated for 90 min, rocking every 15 min. After incubation was completed 5 ml of MEM containing 0.5 µg/ml of 1:250 trypsin was added to each flask. Flasks were incubated until CPE was observed and then the flask was frozen at -80 °C. The control flask containing suspended ds RNA was frozen concomitantly with the first experimental flask. After ten days any flasks not showing CPE were discarded. All previously frozen flasks including the positive and negative controls were freeze-thawed

three times to liberate any cellular bound viruses. Each lysate was decanted into a 30 ml Oakridge tube and centrifuged at 10,000 rpm for 10 min at 4 °C. All supernates were stored at -80 °C until use.

Reverse transcription polymerase chain reaction (RT PCR) was utilized to detect viral ds RNA in the cell culture supernates. The positive identification of virus RNA in a cell culture supernate would demonstrate that infectious virus was originally present in a specific dilution. In future experimentation, log reductions in titer were calculated using the formula:  $\log N_T/N_0$ , where  $N_0$  represents the original titer and  $N_t$  represents the highest dilution to yield a positive RT PCR result.

An adaptation of the RT PCR procedure cited by Grinde, et al, 1995 was performed for rotavirus RNA detection. Before reverse transcription (RT), 10 µl of each supernate and 1 µM downstream primer (5'-GATCCTGTTGGCCATCC-3') were heated at 99 °C for 8 min in the presence of 2 mM ethylenediaminetetraacetic acid (EDTA) (Sigma), pH 8.0, and 4 mM dithiothreitol (DTT) (Sigma) in a 0.2 ml thin walled microfuge tube. The reaction mixture was placed on ice for 5 min following the ds RNA denaturation.

In the same microfuge tube, 2.5 µl of 10 X PCR buffer (Perkin Elmer Cetus, Norwalk, CT), 8 mM MgCl<sub>2</sub> (Perkin Elmer), 0.42 mM dNTPs (Perkin Elmer), 0.5 U RNasin (Perkin Elmer), and 1 µl of MuLV reverse transcriptase (Perkin Elmer) was added and heated to 42 °C for 30 min in a Perkin Elmer 2400 Thermal Cycler. Following reverse transcription, the reaction mixture was heated to 99 °C for 5 min and then placed on ice.

To each tube, 2.5  $\mu$ l of 10X PCR buffer, 2  $\mu$ M upstream primer (5'-GTATGGTATTGAATATACCA-3') 1  $\mu$ M downstream primer, and 0.02% gelatin (Sigma), was added together for a reaction volume of 59.5  $\mu$ l. The tubes were heated to 99 °C for 4 min and 0.5 ml of AmpliTaq DNA polymerase (Perkin Elmer) was added to each reaction mixture at 72 °C. The reaction proceeded for 36 cycles. Denaturation occurred at 95 °C for 15 s, annealing occurred at 55 °C for 30 s, and extension occurred at 72 °C for 15 s. A final extension step occurred at 72 °C for 7 minutes and the reaction mixture was store at 4 °C until its use. A 1.0% low EEO agarose (Sigma) gel was cast and electrophoresis in 1X tris borate EDTA (TBE) was performed to visualize 203 bp PCR products.

### **Column Purification**

Sephadex G-200 (Sigma) and Chelex 100 (Bio-Rad, Hercules, Ca) were made for purification of environmental water samples prior to the cell culture-RT PCR assay. Slurries of both, Sephadex and Chelex, were made with molecular biology grade water (Sigma). Glass wool (Fisher Scientific, Springfield, NJ) was placed in at the bottom of a 1.0 cc syringe. A 200  $\mu$ l aliquot of the Chelex 100 was added onto the wool. The columns were centrifuged at 1500 rpm in a Sorvall swinging bucket rotor (VWR, Bridgeport, NJ). A slurry of Sephadex was added to the column and the centrifuge step was repeated. A 200  $\mu$ l aliquot of virus sample was pipetted onto the Sephadex G-200 slurry. The column was centrifuged at 1500 rpm. Spin column supernates were then analyzed by RT PCR.

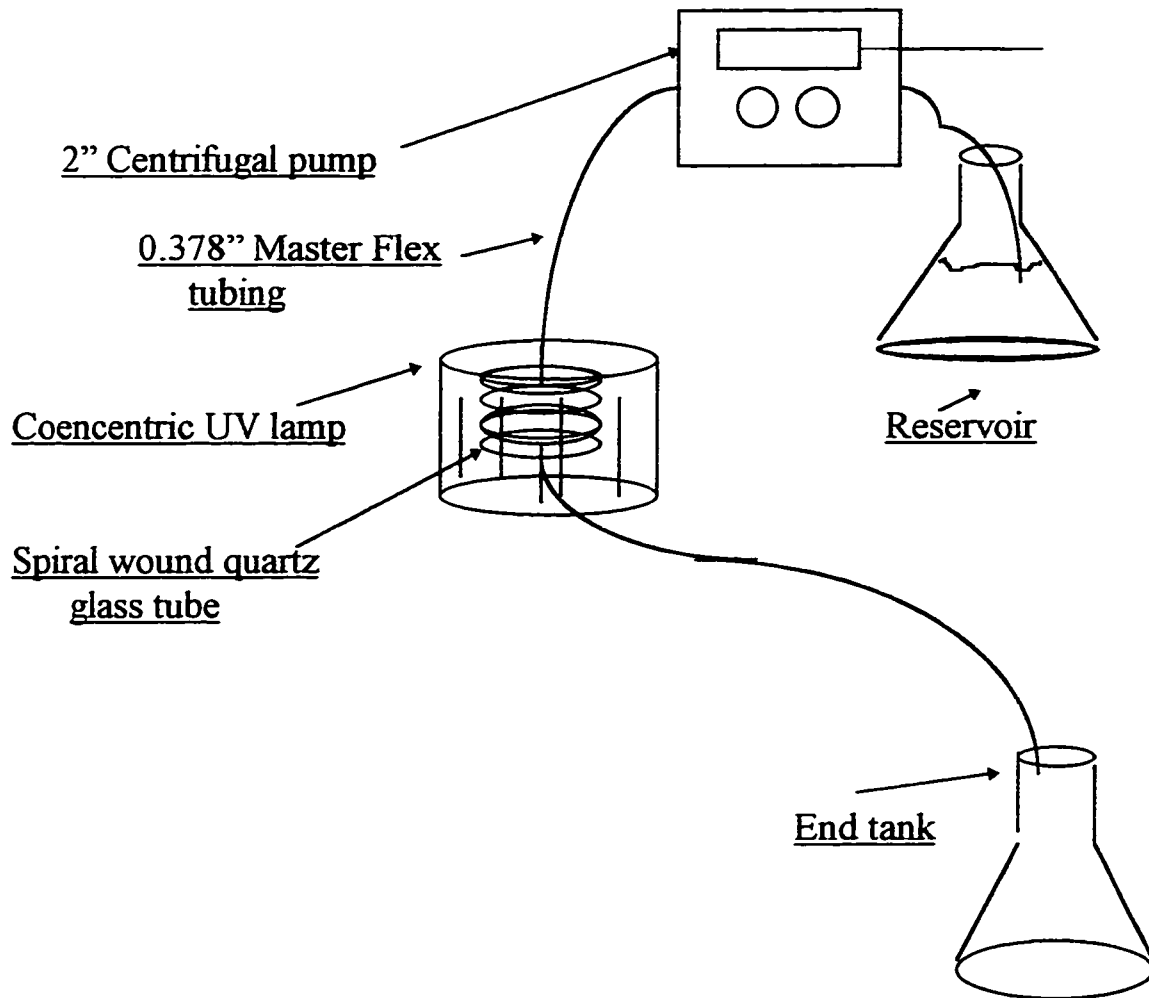
### **Dynamic ultraviolet light disinfection (spiral quartz coil)**

The dynamic bench scale apparatus, see Figure 10, was employed to determine the effectiveness of UV irradiation on the inactivation of MS2 in MilliQ water. The initial reservoir contained approximately four liters of MilliQ water and MS2 at a titer ranging from  $3.12 \times 10^8$  pfu/ml to  $1.34 \times 10^7$  pfu/ml. A two inch peristaltic pump (Cole Parmer, Vernon Hills, IL) was used to pump the virus suspension from the initial reservoir to the spiral coil tube reactor via a 172.72 cm section of 0.375 inch Masterflex (Cole Parmer) tubing. The reactor tube was suspended within a circular UV light apparatus containing eight cylindrical short wave (234.3 nm) UV lights. The lights were spaced evenly on the interior perimeter of the disinfection apparatus. A UV meter (Fisher Scientific, Pittsburgh, PA) was placed on the UV reactor to record UV intensity. Another 172.72 cm section of 0.375 inch Masterflex tubing delivered the virus to the end reservoir where final titer samples were obtained.

Prior to each sampling event, the UV lights were activated for 30 minutes to equilibrate the reactor. Initial virus titer, for each flow rate used, was calculated using 50 ml samples taken at the end of the outflow tubing prior to UV light activation. After the activation of the UV lights, respective flow rates were allowed to equilibrate for a 10 minute period. Final titer was calculated from 50 ml samples taken after the equilibration period. Log reduction in virus titer, for each flow rate, was determined using the formula:  $\log N_o / \log N_t$ , where  $N_o$  represents the initial virus titer and  $N_t$  represents the final virus titer.

Figure 10

Dynamic UV Bench Scale Apparatus



The three flow rates, 60 ml/min, 80 ml/min, and 120 ml/min were initially used to determine a range of virus inactivation that contained a four log reduction in MS2 titer. These flow rates produce IT values of 203.7 mWs/cm<sup>2</sup>, 130.0 mWs/cm<sup>2</sup>, and 67.5 mWs/cm<sup>2</sup> respectively. UV dose was related to flow rate using the formula  $\log(\text{flow rate}) = -\log(\text{dose}) + 9.25$ . The formula was derived from UV actinometry experimentation using MilliQ water at 22.5 °C.

Initially, virus adherence to the bench scale system components or inactivation due to experimental manipulation were performed in triplicate at all three flow rate. The UV lights were not activated in these experiments. Eight separate UV inactivation experiments were performed at the 80 ml/min flow rate to determine log reduction of MS2 due to UV dose. Three series of UV irradiation experiments using a flow rate progression of 60 ml/min, 80 ml/min, and 120 ml/min were performed to determine virus inactivation at varying flow rates. Experiments similar to these were then performed where flow rate progression was randomized to ensure that dose was proportional to log reduction in virus titer. Finally, UV irradiation trials with enhanced flow rates of 180 ml/min (96.7 mWs/cm<sup>2</sup>), 240 ml/min (42 mWs/cm<sup>2</sup>), 360 ml/min (24.5 mWs/cm<sup>2</sup>), and 1080 ml/min (15.3 mWs/cm<sup>2</sup>) were performed to obtain a more accurate relationship between UV dose and log reduction of MS2. Flow rate progression was randomized in these trials.



### **Dynamic ultraviolet light disinfection (straight tube)**

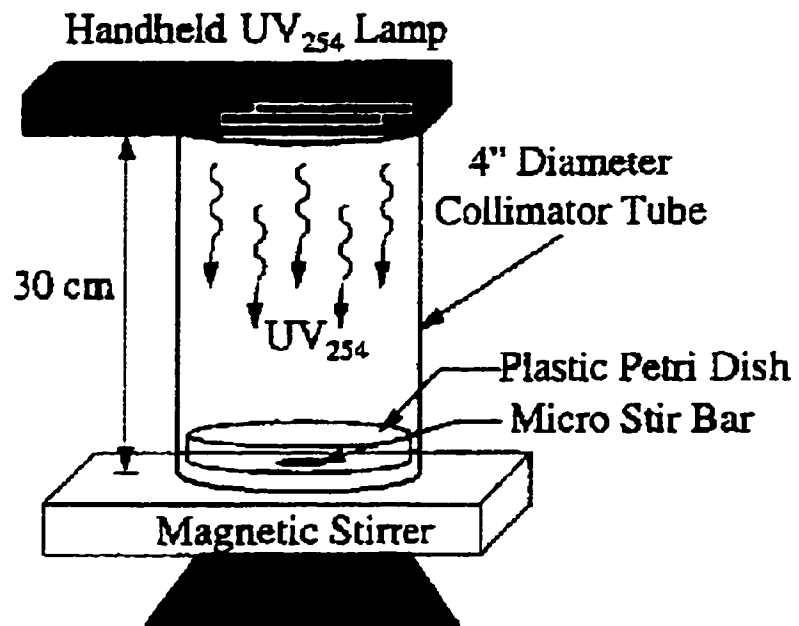
The spiral tube reactor was replaced by a 30 cm straight quartz reactor tube. Experimentation with this tube was performed similar to the coil reactor tube. Flow rates of 25 ml/min. (41.35 mWs/cm<sup>2</sup>), 40 ml/min. (66.16 mWs/cm<sup>2</sup>), 70 ml/min. (115.78 mWs/cm<sup>2</sup>), 140 ml/min. (231.56 mWs/cm<sup>2</sup>), and 220 ml/min. (363.88 mWs/cm<sup>2</sup>) were used to obtain a range of inactivation which included a four log reduction in MS2 titer. Three separate trials were performed where the flow rate progression for the straight tube experiments was randomized.

### **Static ultraviolet light disinfection**

All static disinfection experimentation was performed using the apparatus shown in Figure 11. The handheld 6-Watt short wave UV lamp (Fisher Scientific, Springfield, NJ) was activated at least 30 minutes prior to each experiment to equilibrate the instrument output. The intensity was measured with a UV radiometer (Fisher Scientific). The 30 inch collimated tube was used to focus the UV beam to the petri dish. The 100 x 15 mm petri dish (VWR Scientific, Bridgeport, NJ) contained virus suspended in 20 ml of water and a 1.0 cm micro stir bar. All suspensions were continuously stirred using the magnetic stir plate throughout the duration of the experiments. This size stir bar was used due to its negligible interference to the UV intensity received by the suspension. MilliQ water was used first for each of the viruses in order to determine a baseline range of inactivation without the interference of naturally occurring particulates.

Figure 11

**Static UV disinfection apparatus**



The titer of the viral suspensions ranged from  $10^7$  pfu/ml to  $10^5$  pfu/ml. Before UV exposure, the suspension's UV absorbance was measured with a UV spectrophotometer.

The average UV intensity,  $I_{ave}$  in the suspension is determined using the formula:

$$I_{ave} = I_0 0.96(1 - e^{-aL}) / -aL$$

$a = 254$  nm absorbance per cm of virus suspension

$L =$  solution depth irradiated by the collimated beam

$I_0 =$  measured intensity ( $mW/cm^2$ )

$I_{ave} =$  intensity average ( $mW/cm^2$ )

The lamp intensity ranged from 0.35 to 0.37  $mW/cm^2$ . Dose was varied as a function of time. The suspensions were irradiated to achieve a range of log reductions in viral titer including a four log reduction in titer. Log reduction was calculated using the formula:  $\log N_T/N_0$  where  $N_T$  represents the titer of virus at a particular dose and  $N_0$  represents the initial titer of virus. Virus log reduction was plotted against UV dose and other environmental parameters such as iron concentration, pH, water turbidity, and UV absorbance of an environmental water for MS2 and poliovirus trials. Linear regression analysis, multiple linear regression analysis, and One Way Analysis of Variance (ANOVA) were calculated using SigmaStat<sup>R</sup> and SigmaPlot<sup>R</sup> (Jandel Corp., San Rafael, CA).

### **UV Irradiation of MS2 in MilliQ Water**

An MS2 titer of  $3.2 \times 10^8$  pfu/ml was diluted in 18.0 ml of MilliQ water to yield an experimental titer of  $3.2 \times 10^7$  pfu/ml. The virus suspension was irradiated at doses of 13.5  $mWs/cm^2$ , 30  $mWs/cm^2$ , 47  $mWs/cm^2$ , 65.2  $mWs/cm^2$ , an 80  $mWs/cm^2$  and 0.1 ml

samples were taken for each dose. Dose was varied as a function of time. The samples were serially diluted in 1 X PBS and assayed via the double agar overlay.

An experiment was performed to determine the effect of iron, manganese, and nitrate on log reduction of virus due to UV irradiation. Final concentrations of 3.0 mg/L, 0.1 mg/L, and 0.1 mg/L of FeCl<sub>3</sub>, MnCl<sub>2</sub>, and NaNO<sub>3</sub>, respectively, in 18.0 ml of MilliQ water, were used as the diluent for MS2 to yield an experimental titer of  $3.2 \times 10^7$  pfu/ml. The virus suspension was UV irradiated at the above doses and 0.1 ml samples were taken at each dose to determine final log reduction. A double agar overlay assay was performed to determine the titer of poliovirus after each dose. Log reductions in titer were calculated using the formula:  $\log N_t/N_0$ .

#### **UV Inactivation of MS2 in Groundwater**

The effects of varying groundwater quality matrices- iron, total organic carbon (TOC), UV absorbance, specific absorbance, turbidity, and pH- on MS2 inactivation via UV irradiation were analyzed using ten different groundwaters. All groundwaters were seeded with MS2 to yield a final titer of  $3.2 \times 10^7$  pfu/ml and irradiated at a series of doses which included 13 mWs/cm<sup>2</sup>, 30 mWs/cm<sup>2</sup>, 65.2 mWs/cm<sup>2</sup>, 80 mWs/cm<sup>2</sup>, and 99 mWs/cm<sup>2</sup>. All experiments were performed in duplicate. One ml samples were taken for each dose and a double agar overlay was performed for each sample.

### **UV Irradiation of Poliovirus in MilliQ Water**

Poliovirus was diluted in MilliQ water at a final concentration of  $1 \times 10^5$  pfu/ml and irradiated using the static UV system similar to the experimentation involving MS2 bacteriophage. The virus suspension was irradiated at  $21.7 \text{ mWs/cm}^2$  and  $29 \text{ mWs/cm}^2$  and viral titers after each dose was determined using the plaque assay mentioned previously. Log reduction due to UV dose was determined using the formula:  $\log N_t/N_0$ , shown previously.

### **UV Inactivation of Poliovirus in Groundwaters**

Seven of the ten groundwaters previously used in the MS2 experimentation were employed to determine the effect of groundwater constituents on UV inactivation of poliovirus. Poliovirus was diluted to a final concentration of  $1.0 \times 10^5$  pfu/ml and irradiated at  $21.7 \text{ mWs/cm}^2$  and  $29.0 \text{ mWs/cm}^2$ . One ml samples were taken for each dose and titer determination was performed using the plaque assay method.

### **UV Inactivation of Hepatitis A Virus in MilliQ Water and Groundwater**

All hepatitis A virus experimentation was conducted in the same manner as the poliovirus experimentation except for UV dose and the number of groundwaters examined. UV doses of  $3.0 \text{ mWs/cm}^2$  and  $6.0 \text{ mWs/cm}^2$  were used to irradiate the virus suspensions. Two of the 10 groundwaters were examined to determine their effect on hepatitis A virus inactivation.

### **UV Inactivation of Rotavirus in MilliQ Water**

Rotavirus Wa strain was diluted in MilliQ water to yield a final viral titer ranging from  $1.1 \times 10^5$  pfu/ml to  $1.1 \times 10^7$  pfu/ml. Virus suspensions were irradiated in the static system in order to determine a range of UV doses which yielded one to five log reductions in final titer. Doses of 65.2 mWs/cm<sup>2</sup>, 77.0 mWs/cm<sup>2</sup>, 86.3 mWs/cm<sup>2</sup>, 97.0 mWs/cm<sup>2</sup>, and 110.0 mWs/cm<sup>2</sup> were used for this purpose. Virus titer was determined using the cell culture-RT PCR method described previously. Log reductions in titer were determined using the formula:  $\log N_t/N_0$ , where  $N_t$  is the titer at a particular UV dose and  $N_0$  is the initial titer of virus stock.

### **UV Inactivation of Rotavirus in Groundwaters**

Two of the 10 groundwaters were examined for their effect on rotavirus inactivation. UV irradiation of the virus suspensions was similar to the MilliQ water experimentation. Virus suspensions were irradiated at 77 mWs/cm<sup>2</sup> and 97 mWs/cm<sup>2</sup> in order to determine the effect of the groundwater on log inactivation of rotavirus. Log reductions in titer were calculated similar to the methodology used in the MilliQ water experimentation.

## RESULTS

### Inactivation of Viruses by Ultraviolet Irradiation

Previously, UV irradiation has been used to inactivate viruses such as MS2, poliovirus, hepatitis A virus, and rotavirus in static experiments. These UV disinfection studies were performed in order to determine virus sensitivity to UV irradiation in both dynamic and static systems. The ultimate goal was to establish UV doses that can be used by water treatment plants to disinfect drinking waters in compliance with EPA mandated regulations. However, most of the earlier studies do not determine the effects of various environmental water matrices on UV disinfection capability.

Both static and dynamic bench scale UV disinfection systems were used here to determine virus sensitivity to UV irradiation. In the static studies, both laboratory grade and groundwaters were used to determine water matrices effects on virus inactivation. The Wa strain of rotavirus was used instead of the simian strain, SA-11, because it is a human pathogen. Other experimentation has shown that Wa has greater survivability in groundwaters and surface waters at different pHs and temperatures.

#### **Nonspecific adsorption to dynamic system**

The nonspecific adsorption of MS2 to components of the bench scale system was determined at three flow rates: 60 ml/min, 80 ml/min, and 120 ml/min. No log reduction

in virus titer occurred at 60 ml/min Average log reductions of 0.008 and 0.19 occurred at 80 ml/min and 120 ml/min respectively. It was determined that each reduction in titer was within the standard error of titer determination ( $\pm 0.5$  log) of the double agar overlay assay (APHA, 1989).

### **Log reduction of MS2 in dynamic system using spiral quartz reactor**

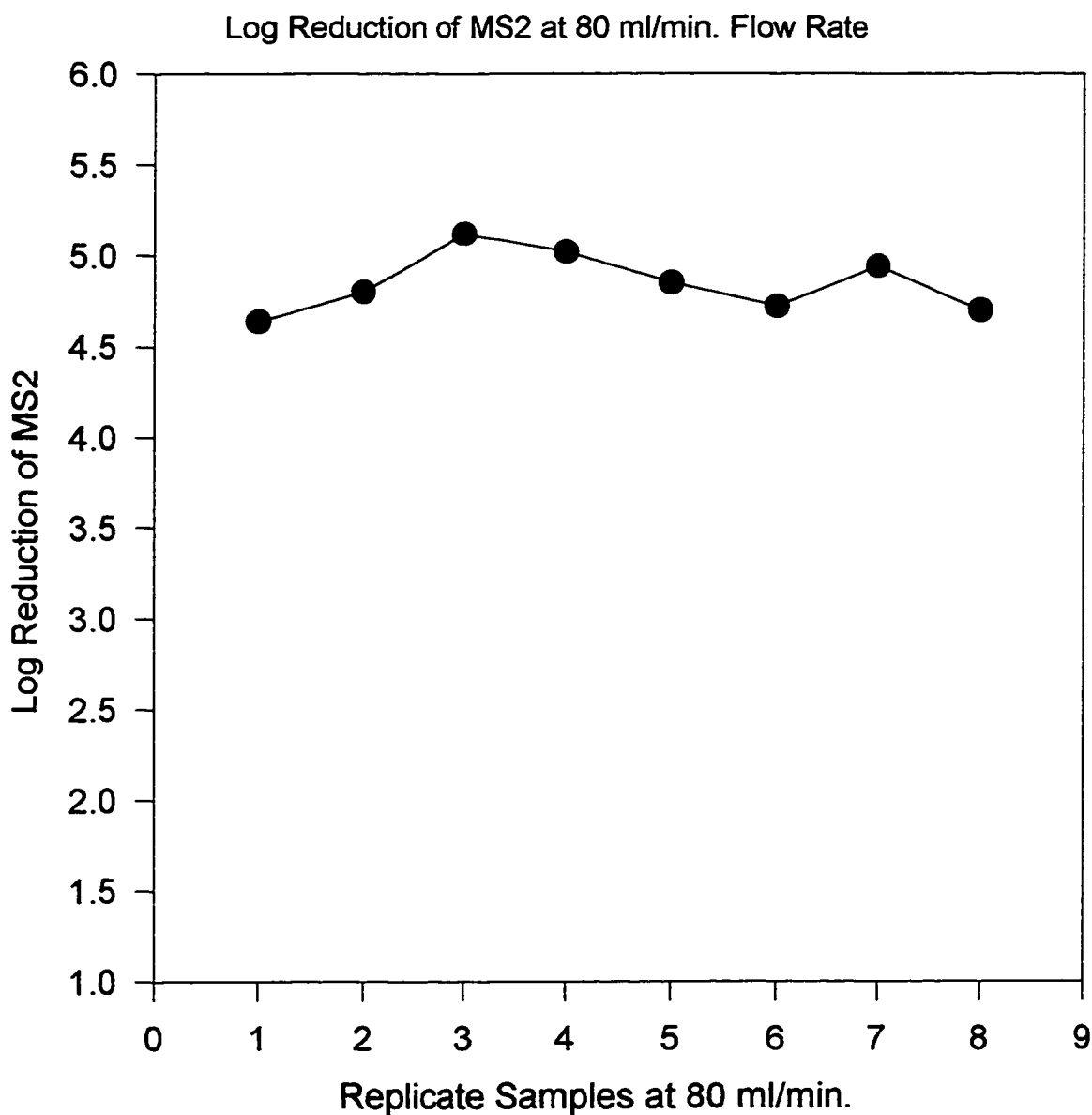
Eight replicate trials were performed to ascertain the log reduction of MS2 at the 80 ml/min. flow rate in the dynamic system. A total of  $1.0 \times 10^{11}$  pfu of MS2 was added to four liters of RO water to produce initial virus titers of  $1 \times 10^7$  pfu/ml. The system was turned on, flow rate was adjusted, and the UV reactor was activated. The 80 ml/min. flow rate represented an IT value of  $130 \text{ mWs/cm}^2$ . The range of log reduction at this flow rate was 4.64 and 5.12, mean reduction of 4.858 with a standard deviation of 0.1596 (Figure 12).

### **Log reduction of MS2 at varying flow rates**

Dynamic UV inactivation trials were done using three flow rates which yield three different UV doses. Flow rate order for each trial was as follows: 60 ml/min ( $203 \text{ mWs/cm}^2$ ), 80 ml/min ( $130 \text{ mWs/cm}^2$ ), and 120 ml/min ( $67 \text{ mWs/cm}^2$ ). Inactivation of MS2 at the 60 ml/min, 80 ml/min, and 120 ml/min flow rates is shown in Figure 13. Figure 13 elucidates a trend of increasing log reduction with decreasing UV dose since dose decreases with increased flow rate. A similar trend was noted in other trials, where complete inactivation of MS2 occurred at the 80 ml/min and 120 ml/min flow rates.

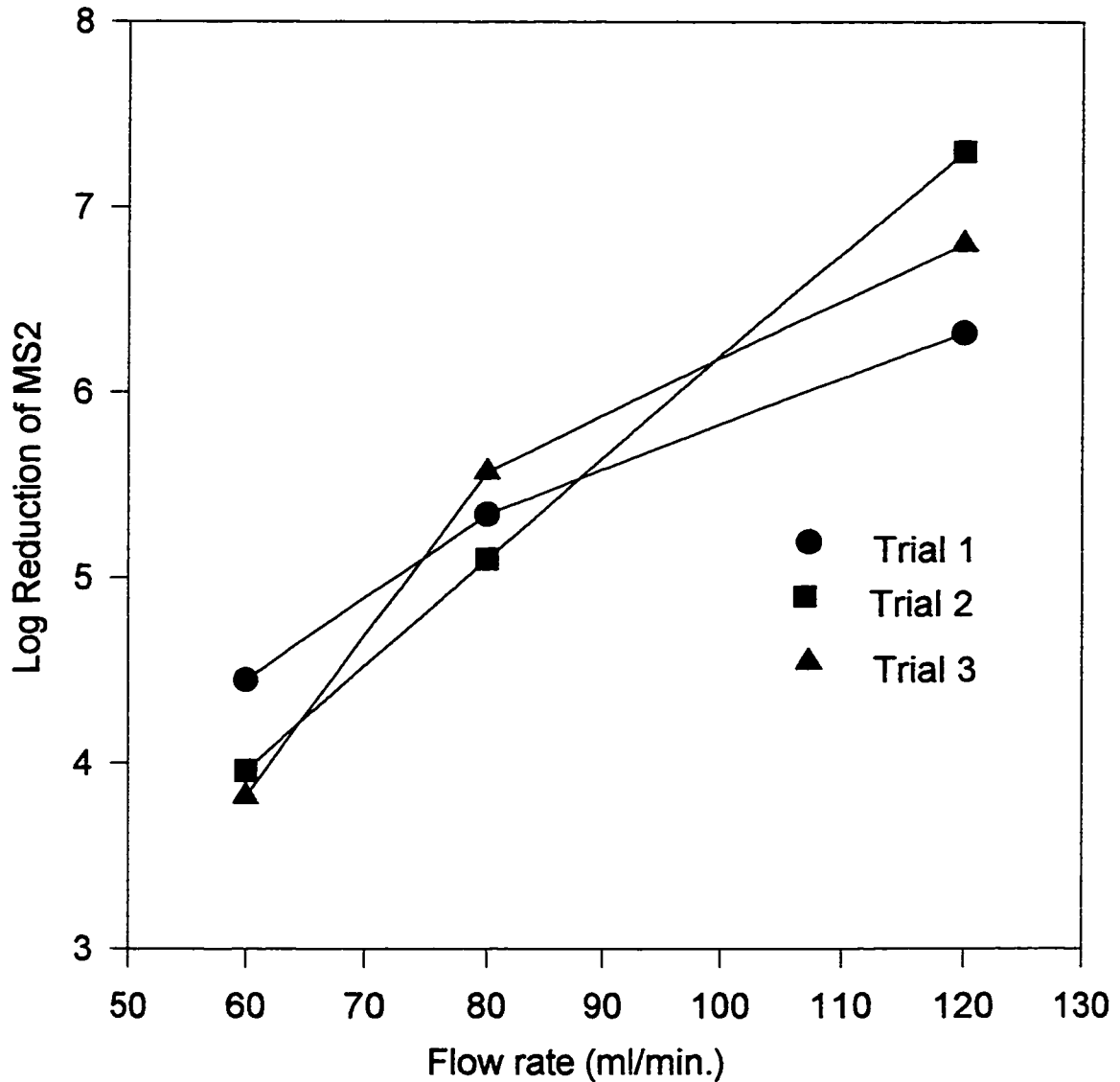


Figure 12



Replicate Samples at 80 ml/min.  
Log reductions in MS2 titer in eight replicate trials. The spiral quartz tube reactor was used in the dynamic system and a final UV dose of 130 mWs/cm<sup>2</sup> was achieved

Figure 13  
Log Reduction of MS2 At Varying Flow  
Rates Using the Spiral Quartz Tube Reactor



Flow rate order for each of the three trials was as follows:  
60 ml/min (203.0 mWs/cm<sup>2</sup>), 80 ml/min (130.0 mWs/cm<sup>2</sup>), and  
120 ml/min (67.0 mWs/cm<sup>2</sup>).

The average log reductions in titer for the trials shown in Figure 14 at 60 ml/min, 80 ml/min, 120 ml/min were 4.08, 5.34, and 6.81 respectively. From these data, increasing log reduction is inversely proportional to flow rate. The broad confidence bars indicate that a range of flow rates or IT values would produce one particular log reduction.

### **Log reduction of MS2 using a randomized flow rate succession**

UV inactivation trials were performed similarly to previous trials except that flow rate succession was randomized. Table 8 shows the data generated from three separate trials. Log reduction of MS2 increased over time independent of UV dose. The three UV doses used were not capable of producing different log reductions in virus titer. All three UV doses yielded a similar log reduction in virus titer when at the same position in flow rate succession.

### **Log reduction of MS2 at enhanced flow rates**

To determine the relationship between MS2 inactivation and UV dose, dynamic trials were performed using a broader range of flow rates. Flow rates of 120 ml/min (96.7 mWs/cm<sup>2</sup>), 240 ml/min (42 mWs/cm<sup>2</sup>), 360 ml/min (24.3mWs/cm<sup>2</sup>), and 1080 ml/min (15.3 mWs/cm<sup>2</sup>) were used in random succession producing log reductions in MS2 titer of >7.09 (complete inactivation), 4.73, 4.49, and 2.22 respectively (Figure 15). The broader range of flow rates allows for a range of virus inactivation that includes a 4 log reduction in MS2 titer. The formula generated from the linear regression indicates that a 581.5

Figure 14

Log Reduction of MS2 vs. Flow Rate (ml/min)  
Average of Three Successive Trials

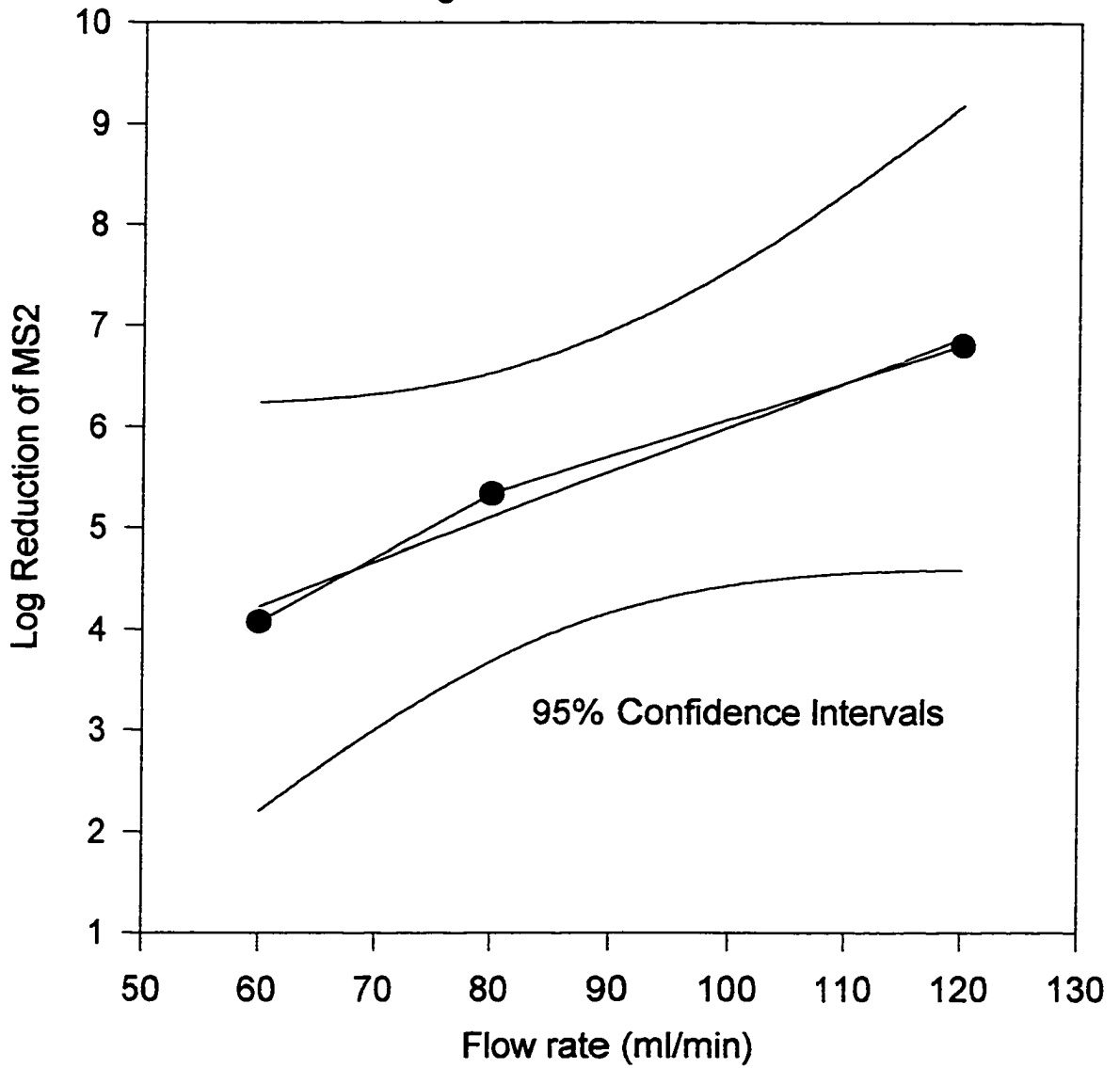
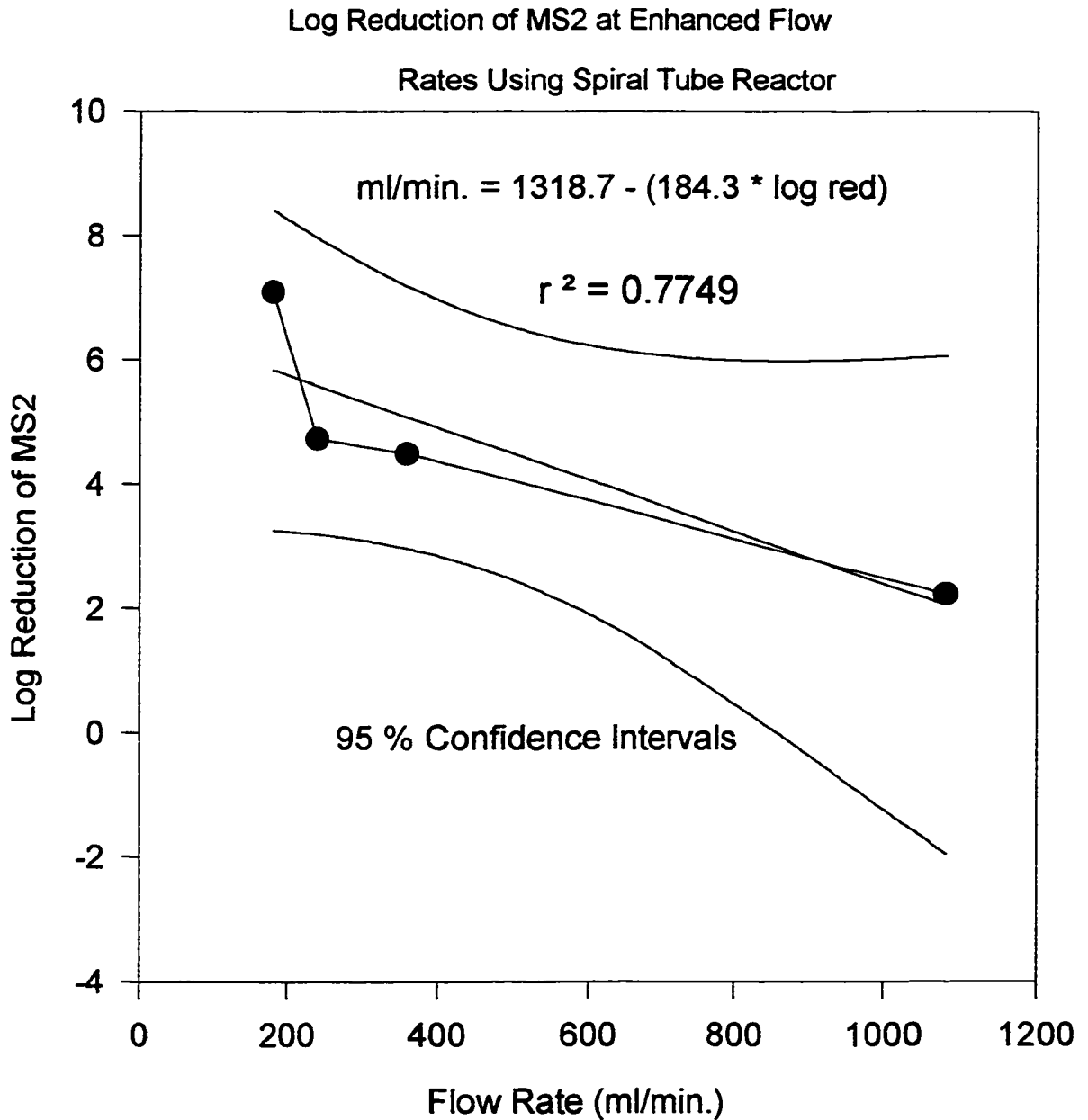


Table 8

Log reduction in separate trial using a randomized flow rate succession  
randomized flow rate succession: Series 1: 80 ml/min, 120 ml/min, 60 ml/min  
Series 2: 120 ml/min, 80 ml/min, 60 ml/min  
Series 3: 80 ml/min, 120 ml/min, 60 ml/min

Series 1	80 ml/min	120 ml/min	60 ml/min
	4.92	5.49	7.47
Series 2	120 ml/min	80 ml/min	60 ml/min
	6.77	6.77	7.17
Series 3	80 ml/min	120 ml/min	60 ml/min
	7.47	8.07	8.07

Figure 15



Flow rates of 120.0 ml/min. (96.7 mWs/cm<sup>2</sup>), 240.0 ml/min. (42.0 mWs/cm<sup>2</sup>), 360 ml/min. (24.5 mWs/cm<sup>2</sup>), and 1080 ml/min. (15.3 mWs/cm<sup>2</sup>) were used in random succession.

ml/min flow rate is needed to achieve a four log reduction in titer. The  $r^2 = 0.7749$ ,  $p = 0.1197$ , signifies that a direct linear relationship between flow rate and log reduction may not exist. The 95% confidence intervals show that a broad range of doses may be used to achieve the same log reduction. Large confidence intervals are due to the inherent variability found in the MS2 quantification assay.

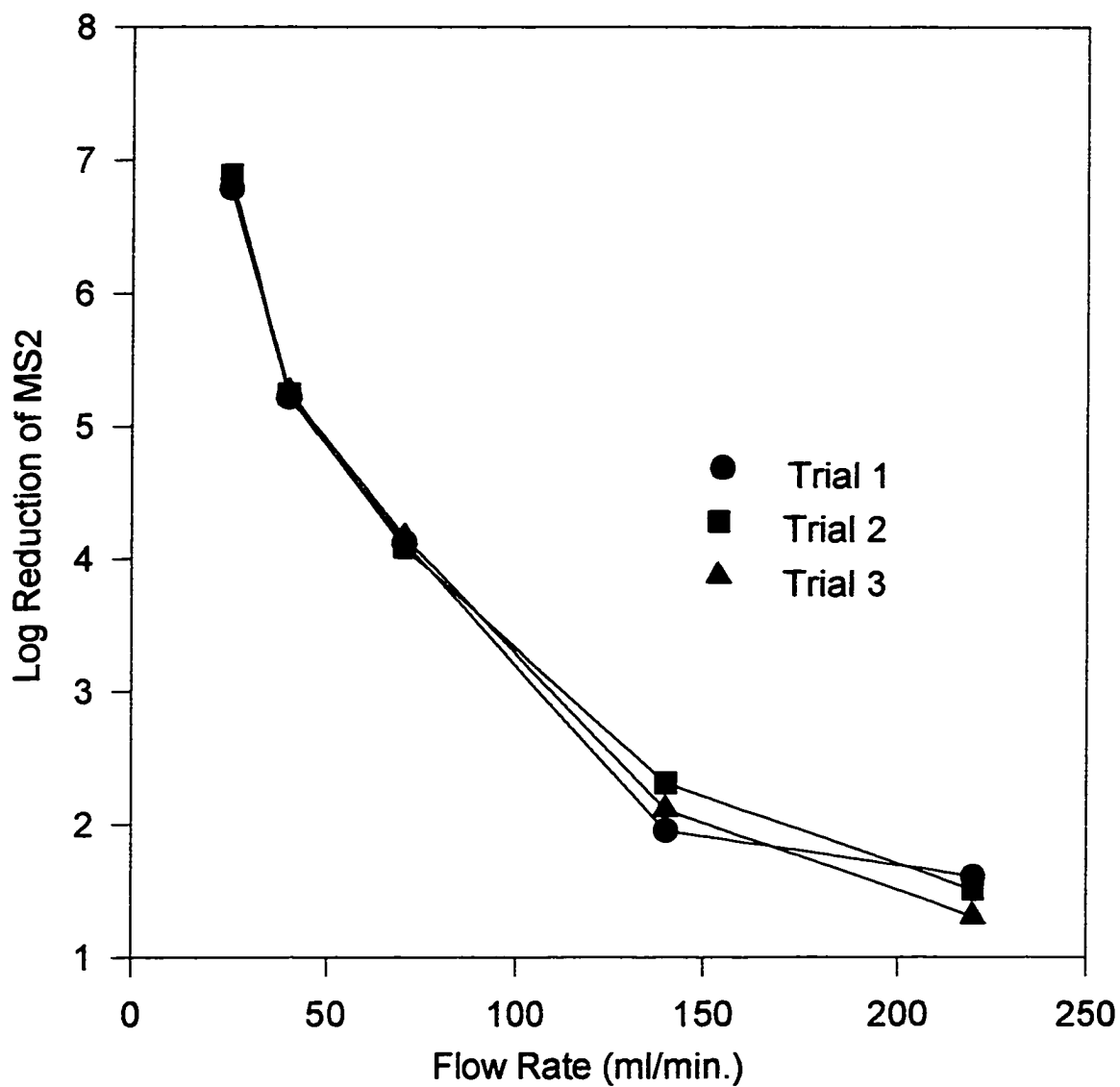
### **Inactivation of MS2 in dynamic system using the straight quartz reactor tube**

The 30 cm straight quartz reactor tube was used for the following dynamic bench scale UV disinfection experiments. The straight tube was used to decrease the flow rates needed to generate UV doses that would achieve four log reductions in virus titer. The decrease in flow rate decreases the volume of water and titer of viruses needed in trials. MS2 can be propagated in large volumes at titers of  $1 \times 10^{10}$  pfu/ml to  $1 \times 10^{12}$  pfu/ml. Human enteric viruses are propagated in smaller volumes at lower titers,  $1 \times 10^8$  pfu/ml to  $1 \times 10^4$  pfu/ml. The volume of water required for trials using enhanced flow rates would dilute human virus stocks so that the initial virus titers would be below  $1 \times 10^3$  pfu/ml. The generation of 4 log reduction data would not be possible.

Three replicate trials were performed using flow rates of 25 ml/min (41.4 mWs/cm<sup>2</sup>), 40 ml/min (66.2 mWs/cm<sup>2</sup>), 70 ml/min (115.8 mWs/cm<sup>2</sup>), 140 ml/min (231.6 mWs/cm<sup>2</sup>), and 220 ml/min (363.9 mWs/cm<sup>2</sup>) in a random succession (Figure 16). The three trials demonstrate that log reduction decreases as the flow rate increases. Figure 16 shows good reproducibility between trials where flow rate order was randomized. The

Figure 16

Log Reduction of MS2 Using Straight Quartz Reactor Tube





expected trend of increasing UV dose yielding increasing log reduction of virus titer is shown (Figure 17). A linear relationship between flow rate and log inactivation is evidenced by a  $r^2 = 0.8840$ ,  $p = 0.02$ . The mean plus standard deviation indicates a four log reduction would occur at 96.2 ml/min.

### **Static UV disinfection experiments using MS2**

It was impractical to produce the titers of human viruses required to perform the previous dynamic UV disinfection trials. Static trials were performed using the protocol stated previously. Trials demonstrating reductions in titer due to UV dose were performed in MilliQ water. Baseline data on the effects of UV dose on particular viruses was established from this experimentation. A final titer of  $3.2 \times 10^7$  pfu/ml of MS2 was irradiated in MilliQ water at UV doses of 13.5 mWs/cm<sup>2</sup>, 30 mWs/cm<sup>2</sup>, 47 mWs/cm<sup>2</sup>, 65.2 mWs/cm<sup>2</sup>, and 80 mWs/cm<sup>2</sup>. These values were used to determine a range of inactivation which would include a four log reduction in titer. The log reductions in MS2 titer were 1.01, 1.66, 2.71, 3.42, 4.14 respectively (Figure 18). The  $r^2 = 0.9949$ ,  $p = 0.0002$ , indicates that a direct linear relationship between log inactivation of MS2 and UV dose exists. The mean plus standard deviation indicates that a 4 log reduction in titer occurs at 77.18 mWs/cm<sup>2</sup>.

Figure 17  
Average Log Reduction of MS2 Using  
Straight Reactor Tube

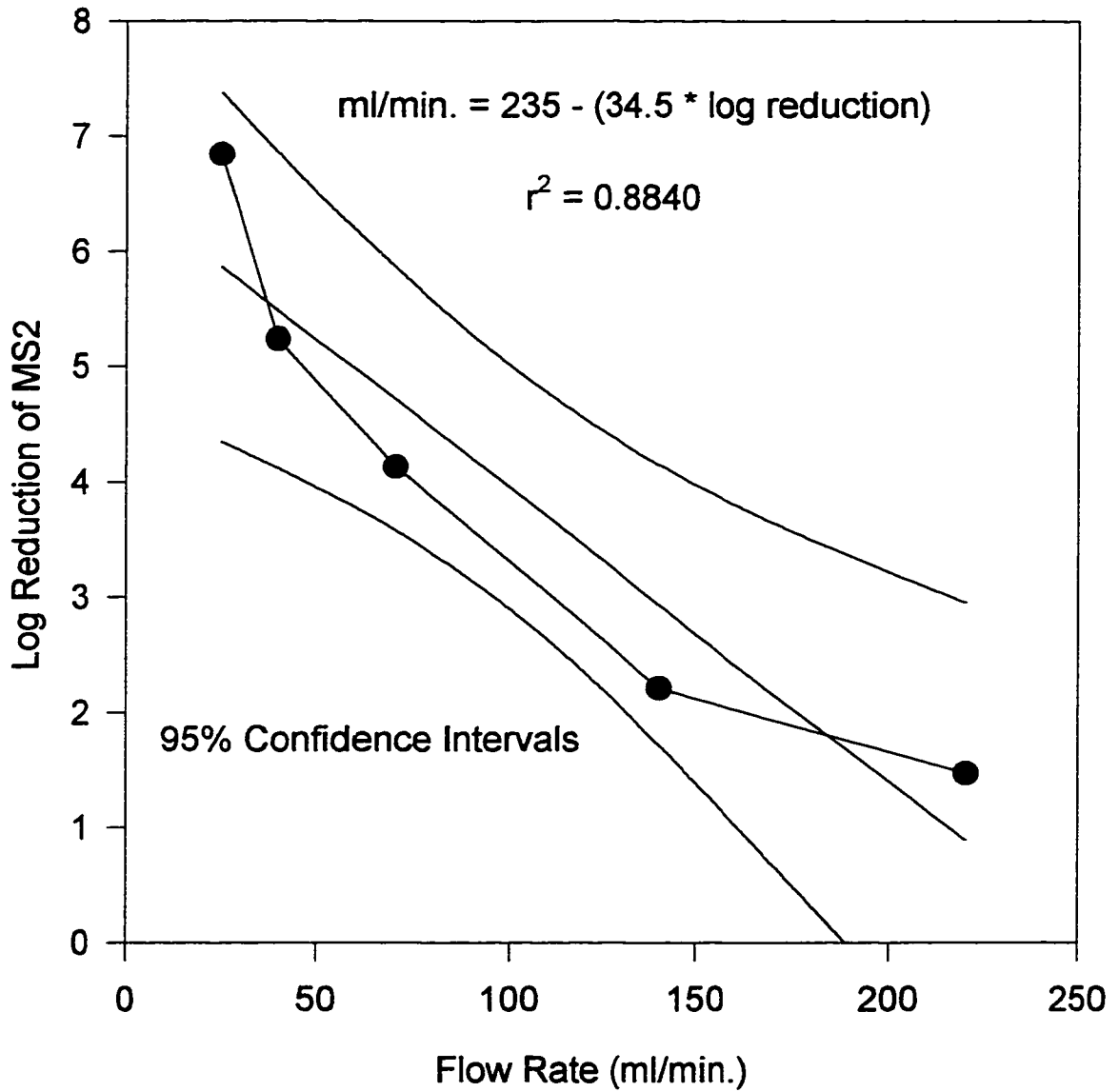
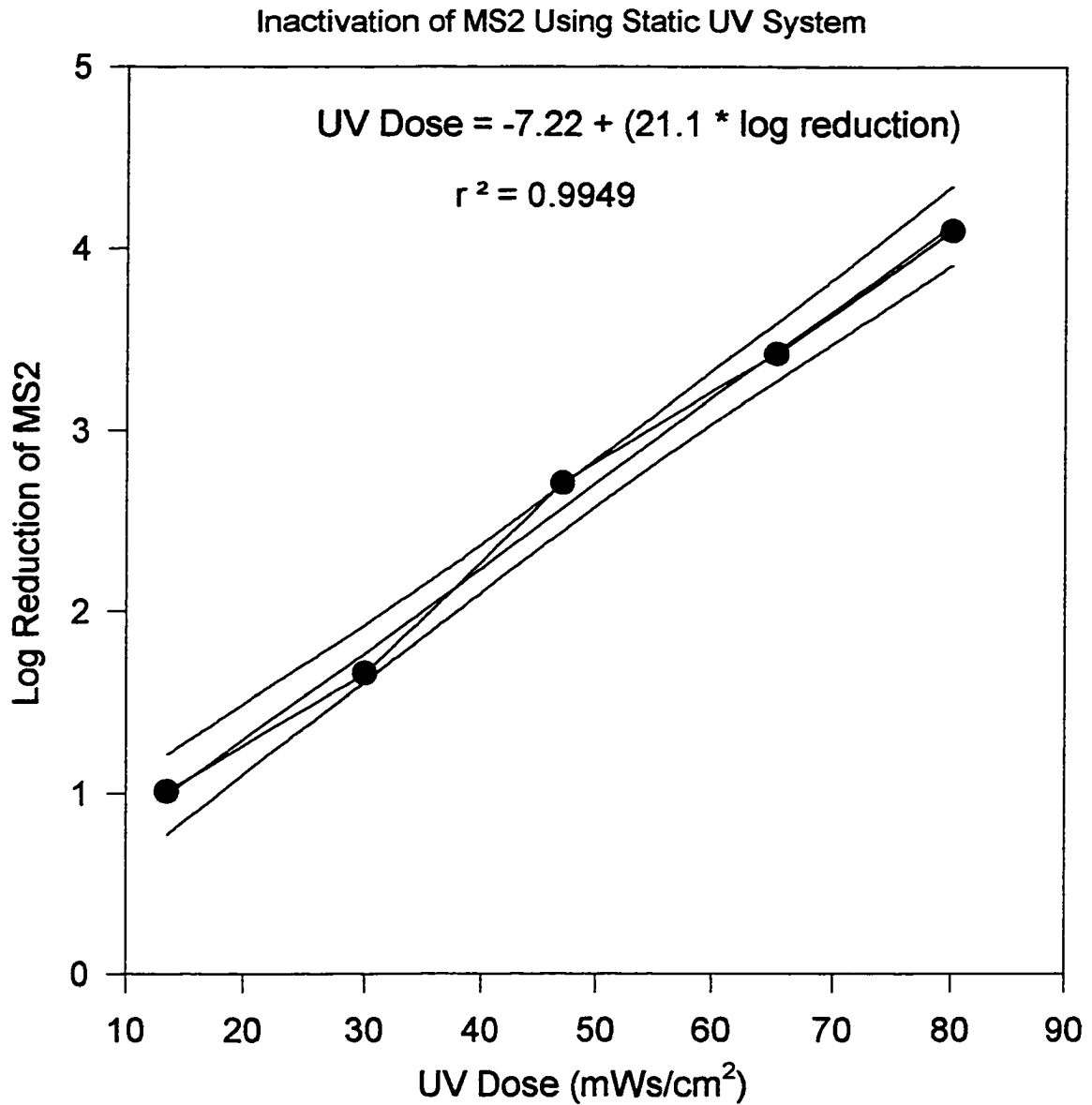


Figure 18



UV doses of 13.5 mWs/cm<sup>2</sup>, 30.0 mWs/cm<sup>2</sup>, 47.0 mWs/cm<sup>2</sup>, 65.2 mWs/cm<sup>2</sup>, 80.0 mWs/cm<sup>2</sup> producing log reductions of 1.01, 1.66, 2.71, 3.42, 4.14 respectively.

## **The effects of water matrices on UV inactivation of MS2**

The effects of water matrices such as iron, manganese, and nitrate on UV inactivation were examined using MS2. Final concentrations of 3.0 mg/L, 0.1 mg/L, and 0.1 mg/L of FeCl<sub>3</sub>, MnCl<sub>2</sub>, and NaNO<sub>3</sub> were added independently to MilliQ water containing an initial titer of  $1.0 \times 10^7$  pfu/ml of MS2. The solutions were irradiated using the same IT values mentioned in the previous experimentation. Log reductions in titer followed the same trend (Figure 19). A one way ANOVA indicated that the mean standard deviation of the log reductions of MS2 at each IT value did not vary significantly.

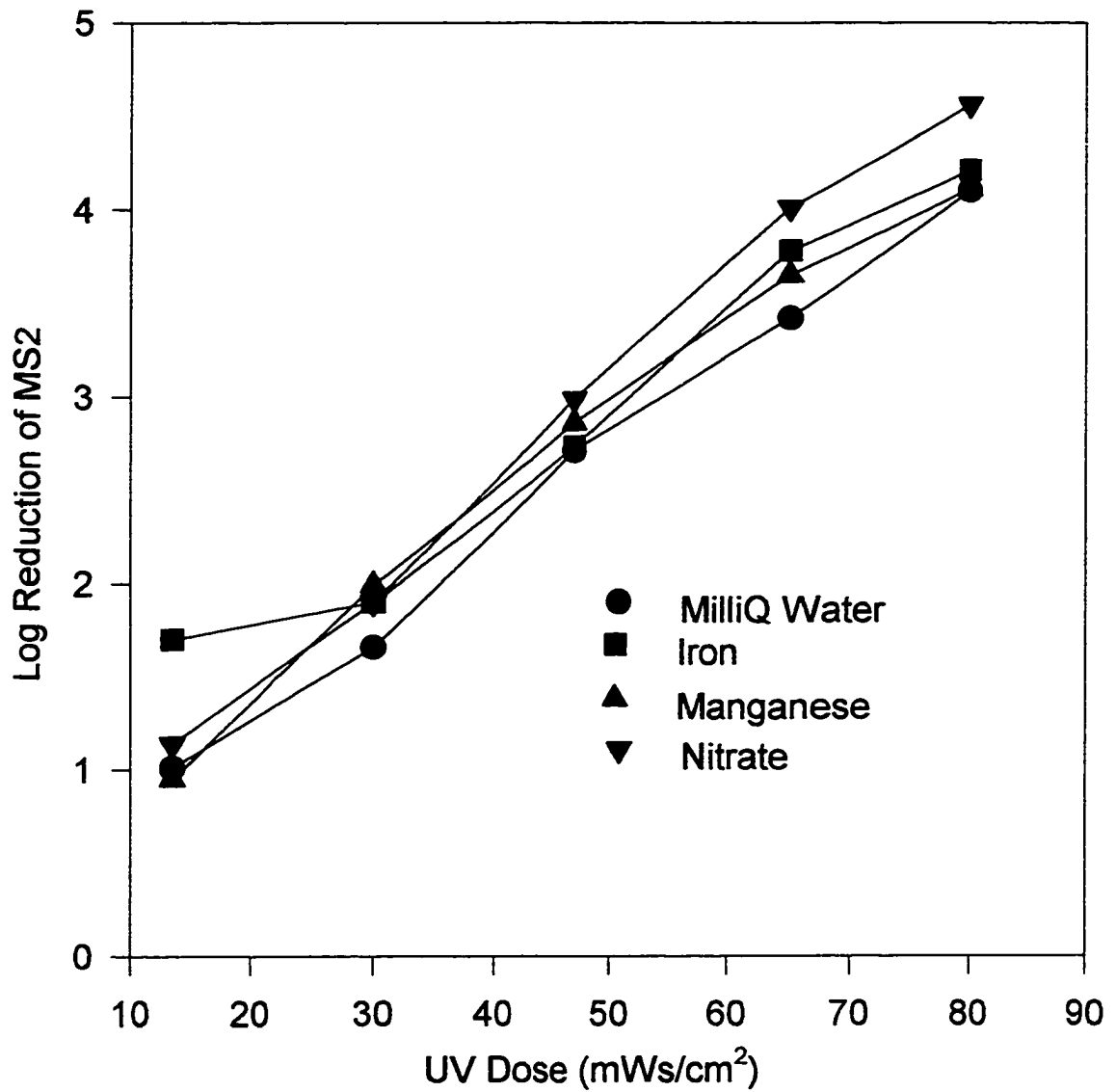
It was speculated that the human enteric viruses would follow a similar trend in the environmental waters used later in the study. The MS2 trials were performed to gain an initial understanding of each compound's effects on virus inactivation separately. The groundwaters used later would possess a combination of water matrices and the total effect of these constituents on virus inactivation would be analyzed.

## **UV irradiation of MS2 in groundwaters**

UV irradiation trials were performed similarly to previous trials with the exception that different groundwaters were used in place of MilliQ water. Figure 20 demonstrates the UV doses required to achieve one, two, three, four, and five log reductions in MS2 titer in all ten waters assayed. A four log inactivation window has been created to show the range of UV doses required for the four log reductions in MS2 titer. The doses spanned in the window range from 64.0 mWs/cm<sup>2</sup> to 93.0 mWs/cm<sup>2</sup>. The UV doses required for log inactivation of MS2 are represented by a dashed line. Positive and

Figure 19

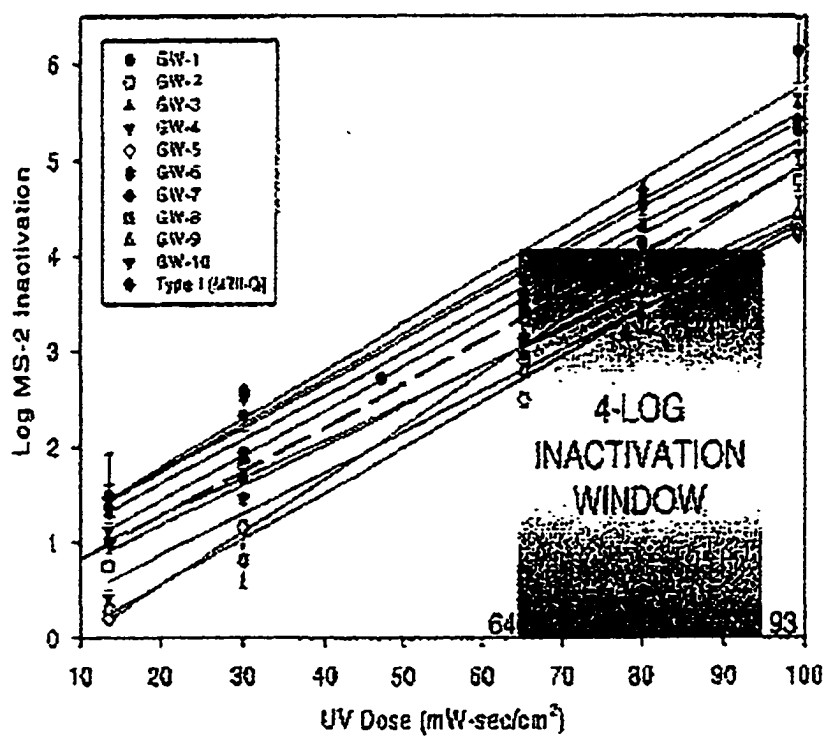
UV Inactivation of MS2 with Different Water Parameters



Static UV experiment where MS2 is diluted in MilliQ water is 3.0 mg/L FeCl<sub>3</sub>, 0.1 mg/L MnCl<sub>2</sub>, or 0.1 mg/L NaNO<sub>3</sub>

Figure 20

Log Reduction of MS2 in Various Waters



negative interferences by the different groundwater types are displayed by the existence of lines on either side of the MilliQ water line.

### **The effects of groundwater matrices on MS2 inactivation**

Figure 21 shows the linear regression calculated from a comparison of total organic carbon (TOC) levels and UV doses required for four log reductions in MS2 titer. Outlying data points are represented as triangles in the figure. These outlying points were not used in the regression analysis. Little correlation between TOC and inactivation exists,  $r^2 = 0.274$ .

Figure 22 shows that no correlation between turbidity (NTU) and log reduction due to UV dose exists,  $r^2 = 0.053$ . Turbidity values ranged from 0.5 mg/L to 1.5 mg/L for all groundwaters tested.

Specific UV absorbance and log reduction due to UV dose showed only a loose correlation,  $r^2 = 0.480$ , for waters with higher absorbances (represented by circles) (Figure 23). The lower absorbance waters (represented by triangles) had only minimal absorbance and no correlation to UV dose could be concluded using these waters.

Iron was the only water parameter that produced a direct correlation,  $r^2 = 0.922$ , with the UV dose required for 4 log reduction in MS2 titer, see Figure 24. Eight of the 10 groundwaters used in this experimentation had iron levels of  $>0.05$  mg/L. These groundwaters were used to determine correlation of iron concentration to inactivation of MS2. Groundwater 9 had an iron concentration of 15.7 mg/L and was considered to be an

Figure 21

UV doses for 4 log reduction of MS2  
versus groundwater TOC

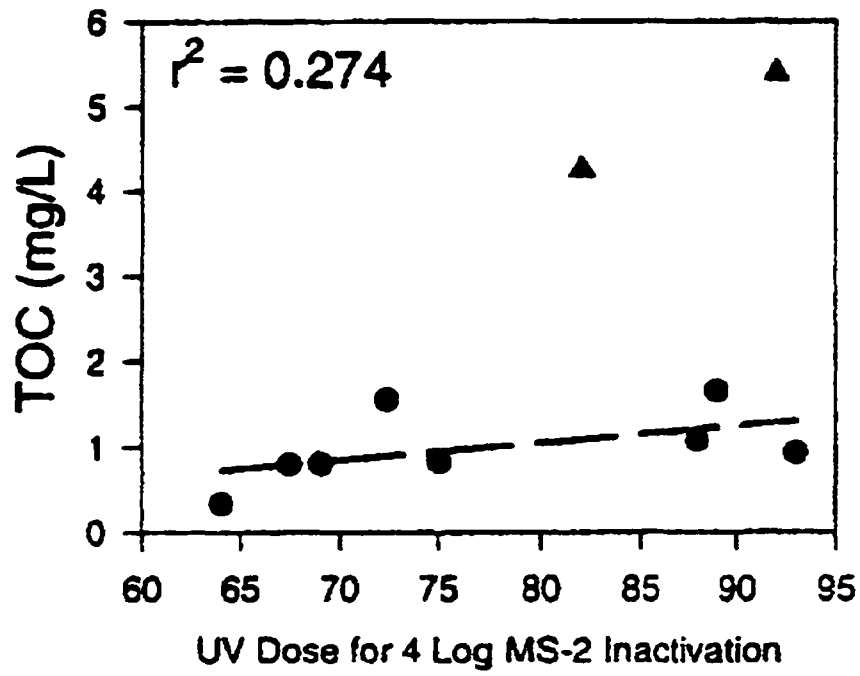




Figure 22

UV doses for a 4 log reduction of MS2  
versus groundwater turbidity

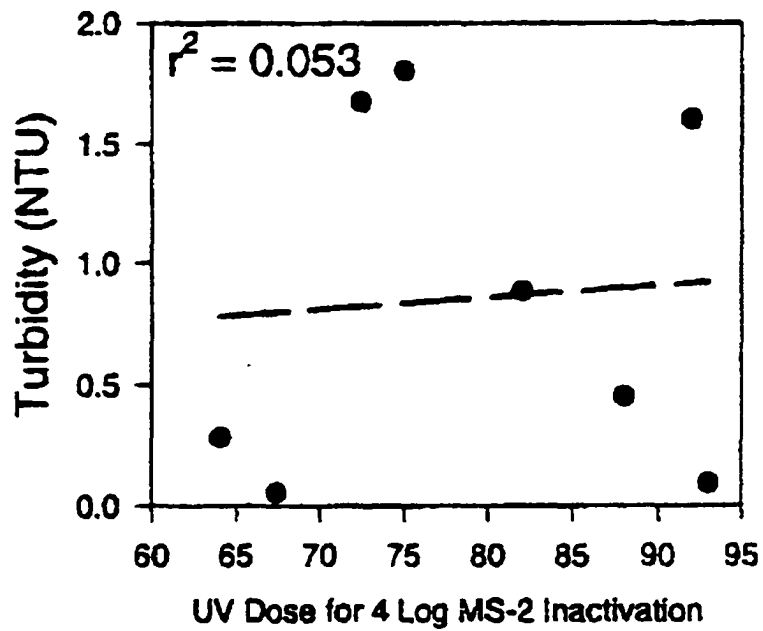


Figure 23

UV doses for 4 log reduction of MS2  
versus groundwater specific UV absorbance

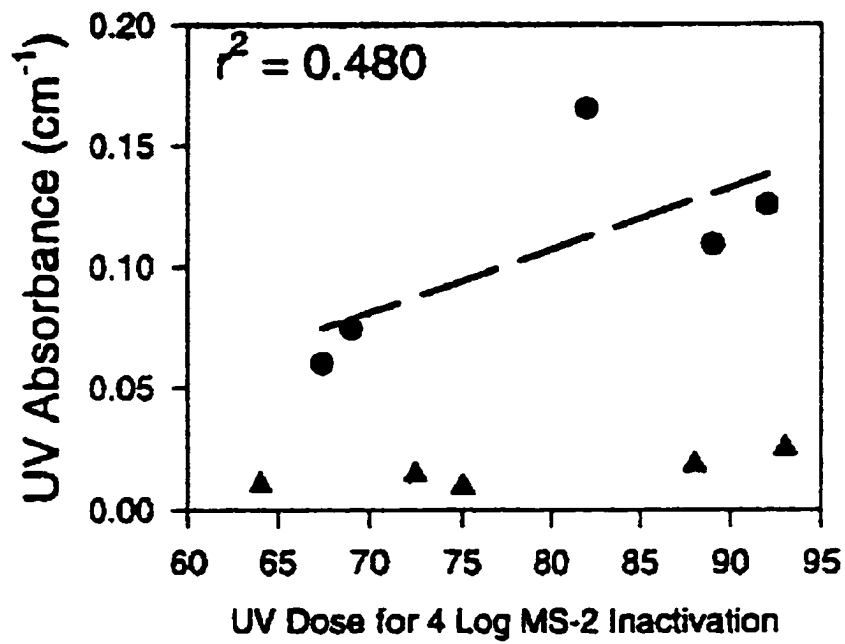
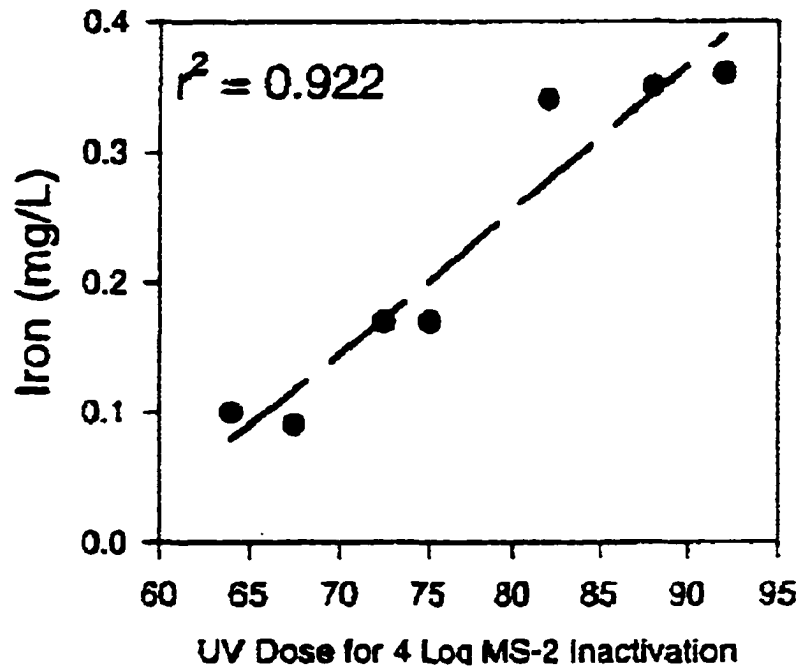


Figure 24

UV doses for 4 log reduction of MS2  
versus groundwater iron concentration



outlying data point. It was not used in the determination of correlation by linear regression.

Figure 25 shows that no correlation between UV dose and pH exists when a first order linear regression (dashed line) is performed,  $r^2 = 0.008$ . A second order linear regression (curved line),  $r^2 = 0.625$ , elucidates a loose correlation between pH and dose required for 4 log reduction in MS2 titer.

### **Inactivation of poliovirus by UV irradiation**

Eight different waters were tested to determine poliovirus LSc-1 susceptibility to UV irradiation. UV doses of 21.7 mWs/cm<sup>2</sup> and 29.0 mWs/cm<sup>2</sup> were used to determine the range of UV doses required to achieve a 4 log inactivation of poliovirus LSc-1, see Figure 26. The range of UV doses required for the 4 log reduction spanned from 23.0 mWs/cm<sup>2</sup> to 29.0 mWs/cm<sup>2</sup>. The dose response curve for MilliQ water is represented by a dashed line. Negative interference by groundwater matrices is evidenced for the groundwaters examined.

### **Inactivation of hepatitis A virus by UV irradiation**

Similar static trials were performed to determine the susceptibility of HAV to UV inactivation. Both MilliQ water and groundwaters were used in these trials. HAV was the most susceptible to UV irradiation in MilliQ water and the two groundwaters tested (Figure 27). Four log reductions in virus titer would be achieved at 6 mWs/cm<sup>2</sup> and 15 mWs/cm<sup>2</sup> for GW1 and GW2 respectively, as determined by linear regression.

Figure 25

UV doses for 4 log reduction of MS2  
versus groundwater pH

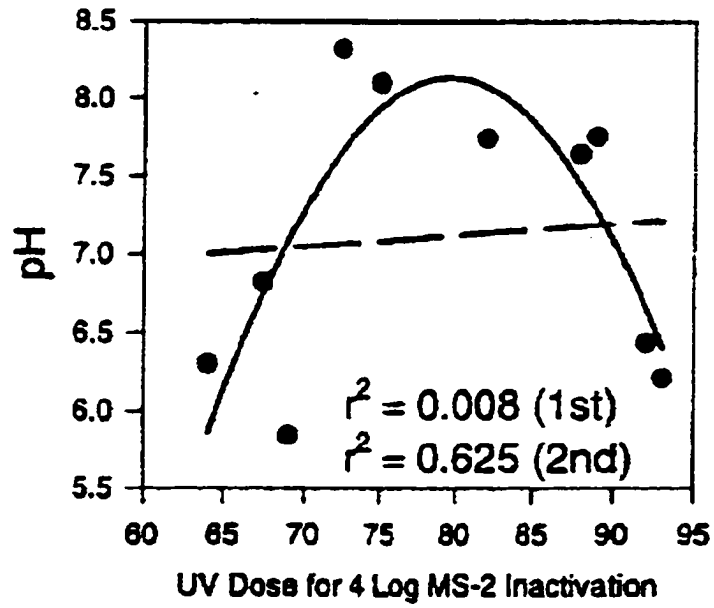


Figure 26

Log Reductions of Poliovirus in Various Waters

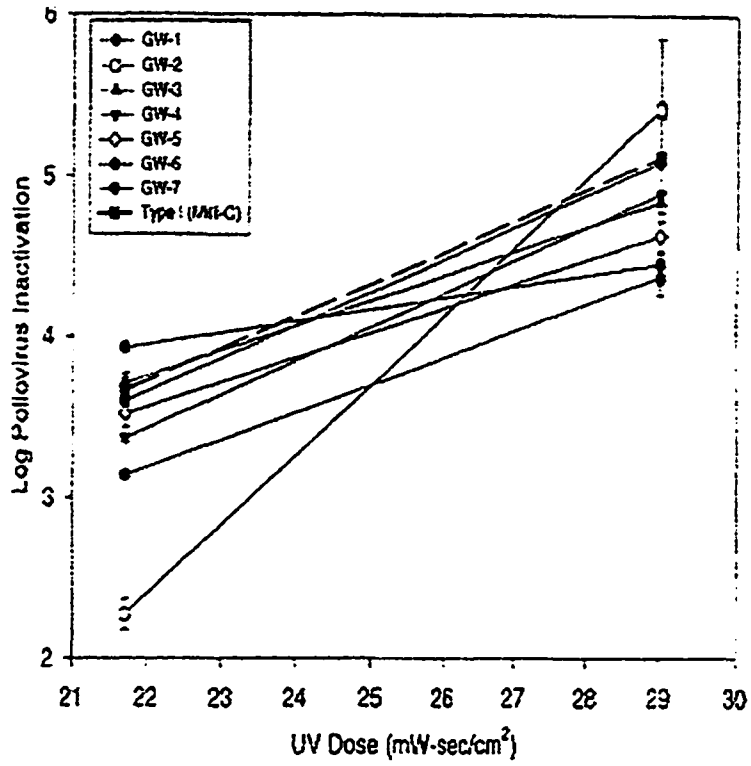
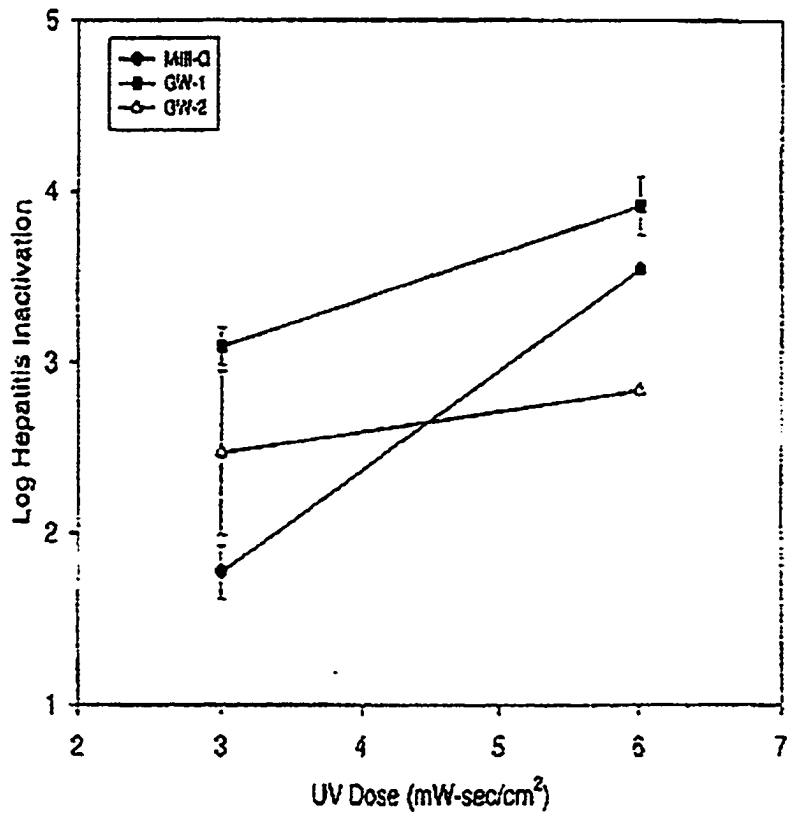


Figure 27

Log Reductions of Hepatitis A Virus in Various Waters



### **Inactivation of rotavirus by UV irradiation**

Similar static trials were performed to determine the susceptibility of rotavirus to UV inactivation. Both MilliQ water and groundwaters were used in these trials. Rotavirus was the least susceptible to UV irradiation in MilliQ water of all the viruses examined. Figure 28 shows the dose response curve for rotavirus in MilliQ water. A 4 log reduction in titer occurred at 97 mWs/cm<sup>2</sup>.

A 1.0 % agarose gel showing the 203 bp products from RT PCR is shown in Figure 29. The rotavirus stock was serially diluted seven times in MEM L-15 media,, using 1:10 dilutions of the virus stock. Lane 1 was intentionally left empty. Lanes 2, 3, 4, 5, 6 show products which represent titers of  $1.0 \times 10^2$  IAU (integrated cell culture-RT PCR assay units),  $1.0 \times 10^3$  IAU,  $1.0 \times 10^4$  IAU,  $1 \times 10^5$  IAU,  $1.08 \times 10^6$  IAU, respectively. Lane 7 represented the last dilution which failed to yield product. Lane 8 is a negative control. Lane 9 is the positive control, undiluted rotavirus stock. Lane 10 is a 100 bp molecular weight marker set.

In each water, 4 log reductions in titer were achieved in duplicate at 77 mWs/cm<sup>2</sup>. At a UV dose of 97 mWs/cm<sup>2</sup>, a 4 log reduction in titer was achieved in GW1 and a greater than 5 log reduction occurred in GW2 (Table 9).

### **Column purification prior to RT PCR**

Sephadex G100/Chelex 100 spin columns were used to remove inhibitory substances to RT PCR. A 200- $\mu$ l aliquot of cell culture supernatant was added to the spin columns and centrifuged at 1500 rpm. The supernate from this procedure was then



Figure 28

Log reductions of rotavirus Wa due to UV dose

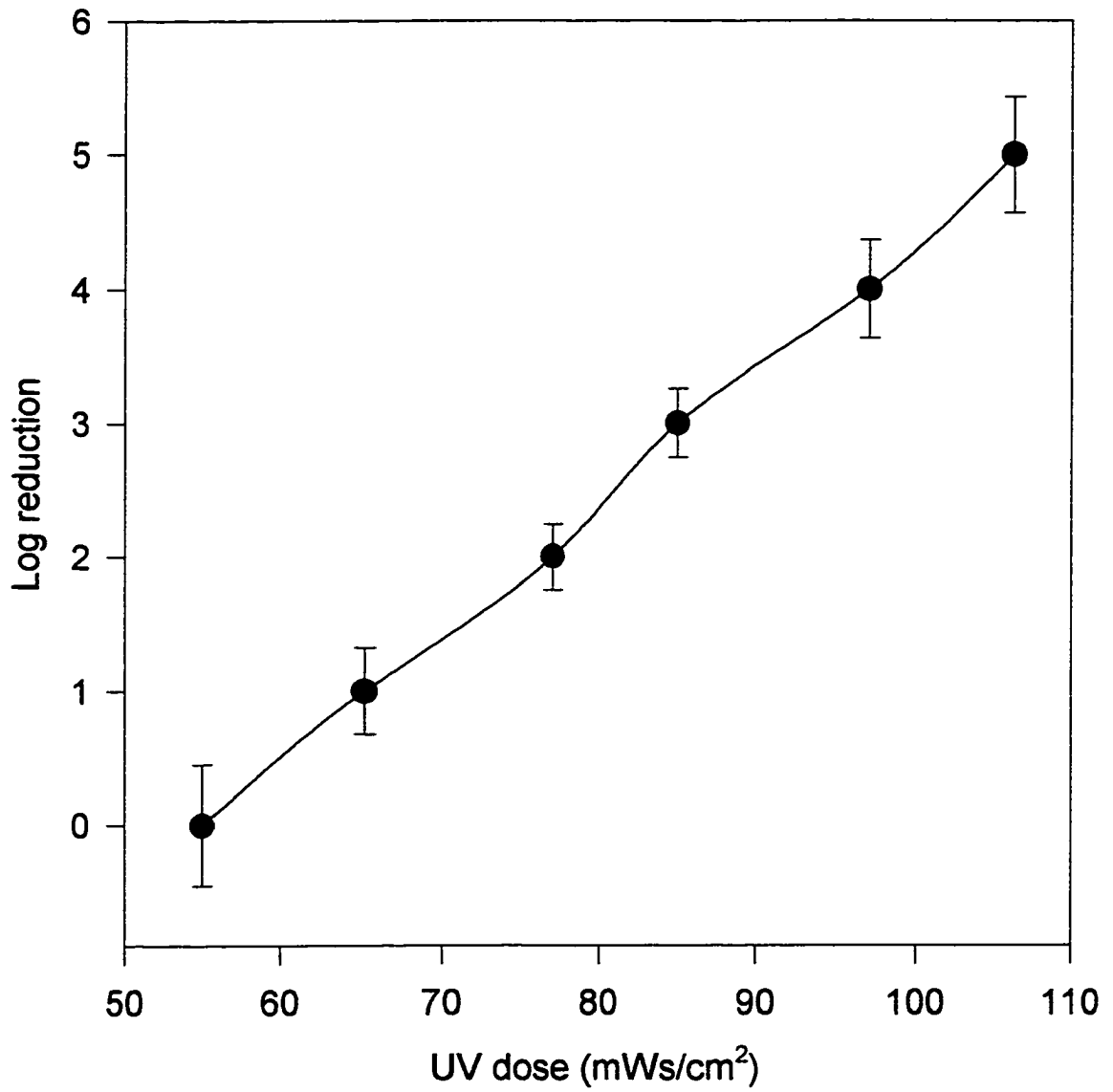
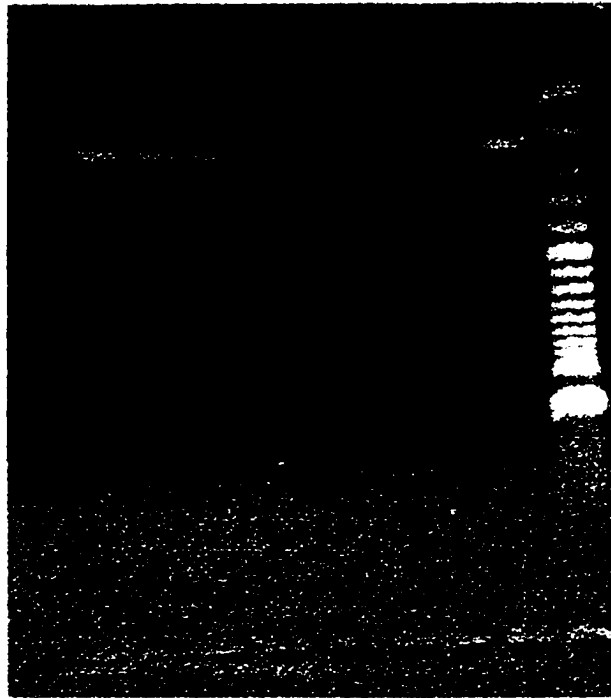


Figure 29

1.5 % Agarose gel showing 203 ds DNA products  
from cell culture-RT PCR assay



Lanes 2 through 7 represent ten-fold dilutions of rotavirus stock. A final titer of  $1.0 \times 10^6$  IAU is represented by the end-point product in Lane 6. Lane 7 is the negative control. Lane 9 is the positive control. Lane 10 is the 100 bp ds DNA ladder

Table 9

Log reduction of rotavirus in GW1 and GW2 determined by cell culture-RT PCR assay with and without Sephadex G-200/Chelex 100 column purification.

Groundwater Type	Log Reduction of RV at 77mWs/cm <sup>2</sup>	Log Reduction of RV at 77mWs/cm <sup>2</sup> with purification	Log Reduction of RV at 97mWs/cm <sup>2</sup>	Log Reduction of RV at 97mWs/cm <sup>2</sup> with purification
GW1	4	3	4	4
GW2	4	3	>5	4

assayed by RT PCR and finally gel electrophoresis. With column purification prior to RT PCR, a 3 log reduction in titer occurred at 77 mWs/cm<sup>2</sup> for GW1 and GW2. At 97 mWs/cm<sup>2</sup>, a 4 log reduction in titer occurred for both GW1 and GW2 (Table 9). The decrease in log reduction in the procedure which included column purification is due to the increase in detection sensitivity after the removal of inhibitory compounds prior to RT PCR. When the column purification step was added to the protocol, the RT PCR assay detected a one log higher concentration of virus in UV treated groundwater samples. Therefore, log reductions in titer were subsequently decreased by one log due to the increase in final titer of RV.

## DISCUSSION

### Introduction to UV Irradiation of Viruses

Over 150,000 public water systems depend on groundwater wells for their drinking water supply. Cities, small towns, and hospitals are supplied with groundwater, which is often untreated, as drinking water. The GWDR will state that a  $10^{-4}$  microbial risk must be attained by a drinking water facility to minimize outbreaks of enteric illness from viruses and other pathogens. For many water utilities, this translates into a disinfection practice that is capable of achieving a four log (99.99%) inactivation of viruses. In addition, the D/DBP states that water utilities meet a Maximum Contaminant Level (MCL) on disinfection by-products. These by-products are formed from the interaction of chlorine and naturally occurring organic matter. Compounds such as trihaloacetic acids, haloacetic acids, and trihalomethanes are toxic to plant and animal life and are also suspected carcinogens. Some facilities may need to increase their doses of chlorine to meet the regulations stated in the GWDR. However, this serves to increase the DBP output of the facility which may exceed the MCL allowed by the D/DBP rule. Other disinfection methodologies are needed to use in conjunction with chlorine disinfection.

The GWDR will state that UV irradiation is an alternative disinfection method that may be used to meet pathogen removal criteria. UV disinfection is used in European drinking water facilities (Chang et al., 1985). UV is also used in the US in wastewater

treatment plants (Chang et al., 1985). UV irradiation does not produce by-products (Sobsey et al., 1994).

UV disinfection benefits from its potent virucidal capability as shown by our laboratory and others (Battigelli et al., 1992), (Wiendernmann et al., 1993), (Meng and Gerba, 1996). UV disinfection does not require the handling and transport of hazardous materials such as sodium hypochlorite used in chlorine disinfection. It also may be a more cost-effective procedure for groundwater disinfection.

UV disinfection may be a less expensive disinfection methodology than chlorine disinfection in small treatment systems (<0.05 million gallons per day(mgd)). Operation and maintenance (O&M) costs for small utilities, based on eight percent interest annually for 20 years, are less than those for chlorine, chlorine dioxide, and ozone (Wolfe, 1990). For larger treatment facilities, 0.5 mgd to 1.0 mgd, O&M costs may surpass those of chlorine but not of chlorine dioxide and ozone (Wolfe, 1990).

In order to meet existing and upcoming disinfection regulations, some treatment facilities have decided to build larger chlorine contact basins. The construction of atmospheric contact basins is a proposed scheme for water treatment plants that are incapable of meeting the regulations in the GWDR. Contact basins have the benefit of employing longer contact time between chlorine and pathogens for increased reductions in titer. In a study performed by the University of New Hampshire Engineering Research Group (UNH-ERG), UV disinfection was a less expensive alternative to chlorine disinfection in treatment plants building new contact basins. The implementation of UV

light as an alternative disinfectant is necessitated by its cost effectiveness, powerful virucidal capability, and inability to create harmful by-products.

UV irradiation does not leave a residual amount of disinfectant to destroy pathogens surviving treatment. This is sometimes viewed as a disadvantage of the treatment. Biofouling is another problem found in UV disinfection treatment plants. Biofouling is caused by oligotrophic bacteria adhering to pipes in treatment plants. This layer of bacteria absorbs UV light and decreases its disinfection potential (Qualls and Johnson, 1983). Filtration prior to UV irradiation is used to decrease the bacterial population in waters and decrease the potential for biofouling.

### **Bench scale system**

The purpose of bench scale apparatus was to yield data that provides a probable representation of a pilot scale system. The data from this facility in turn allows for the design of a full scale water treatment facility. Here, a spiral quartz reactor tube was employed in order to best imitate the hydrodynamic effects experienced when larger volumes of water enter and are processed by a larger system. Water passing through the spiral tube form eddies and whirlpools which occur in treatment facilities. The reactor was also used to provide a path length for UV light that was representative of the path lengths found in water treatment facilities. Path length is the distance between the pathogens in the water and the UV irradiation source. The flow rates used with the original bench scale system were chosen in order to produce a range of log reductions in virus titer that would include a four log reduction.

Based on data from other laboratories (Battigelli et al., 1993), MS2 was used in these studies because it is the most resistant RNA virus to UV irradiation. The advantages to using this virus in bench scale and other applications are its nonpathogenicity to humans, the ability to propagate large volumes of the virus at high titers for relatively little expense, and the assays used for its enumeration require little time and technical ability. These advantages permit the use of MS2 in full scale drinking water facilities. Data generated represents disinfection in full scale water treatment plants.

### Dynamic UV Irradiation Studies

#### **Spiral reactor tube design**

The 80 ml/min flow rate produced an IT of 130.0 mWs/cm<sup>2</sup> which yielded a mean log reduction in virus titer of 4.833 in eight consecutive experiments with a standard deviation of 0.1596. The 60 ml/min (203.7 mWs/cm<sup>2</sup>) and 120 ml/min (67.3 mWs/cm<sup>2</sup>) flow rates were selected to provide a range of log reductions in virus titer surrounding a four log reduction. Repeatedly, dynamic flow experimentation revealed a trend of increasing log reduction in virus titer with increasing flow rate (decreasing UV dose). The linear regression provides a direct relationship between increasing log inactivation due to decreasing UV dose. This is intuitively an incorrect assumption as evidenced by static UV irradiation experimentation (Abbaszadegan and Gerba, 1992). Another shortcoming of these trials is their high degree of experimental variability. Broad confidence intervals surround the regression relating UV dose and log reduction using the spiral reactor, see



Figure 14. This indicates that a wide range of flow rates or IT values would produce a particular log reduction in titer of MS2.

It appeared that the set of flow rates utilized was not broad enough to produce distinct log reductions due to flow rate or IT value. Any UV dose in this range would produce the same log reductions in titer. The hypothesis was confirmed by experiments where the 60 ml/min., 80 ml/min., and 120 ml/min. flow rate order was altered. Increasing log reduction occurred over time independent of UV dosage. All three UV doses yielded a similar log reduction in virus titer when at the same position in flow rate succession. It was concluded that this range of IT values did not produce an accurate representation of log inactivation due to UV dose.

The same reactor tube was used in later experiments where enhanced flow rates of 180 ml/min, 240 ml/min, 360 ml/min, and 1080 ml/min produced IT values of 96.7 mWs/cm<sup>2</sup>, 42 mWs/cm<sup>2</sup>, 24.5 mWs/cm<sup>2</sup>, and 15.3 mWs/cm<sup>2</sup>, respectively. The flow rate order was performed in random succession. The mean plus standard deviation from a linear regression indicates that a 4 log reduction in virus titer would typically occur at a flow rate of 581.5 ml/min. The  $r^2$  value of 0.7749,  $p = 0.1197$ , indicates that a direct linear relationship between UV dose and log reduction may be assumed. Log reduction in virus titer did decrease as a function of increasing UV dose. However, the broad 95% confidence intervals indicate that a high degree of experimental variability is present in these trials. Large confidence intervals show that a particular log reduction could be achieved using a range of UV doses.

At enhanced flow rates, the physical stability of the dynamic system is compromised making it difficult to obtain reliable samples. The system leaks from various components creating a serious health risk to the researcher when human pathogens are used. The higher flow rates needed to conduct these experiments are also impractical for experimentation with human viruses. Titers achieved from propagation of human viruses are much lower than those obtained for MS2. The volume of MilliQ water would dilute the stock concentration of human viruses so that the initial experimental virus titer would be below  $1 \times 10^3$  pfu/ml. With this initial titer, it would not be possible to calculate a 4 log reduction due to UV dose. To correct for this inability, a 30 cm straight quartz reactor tube was substituted for the spiral quartz reactor tube.

### **Straight tube reactor design**

The flow rates used in the experimentation were 25 ml/min, 40 ml/min, 70 ml/min, 140 ml/min, and 220 ml/min, which yielded UV doses of 41.40 mWs/cm<sup>2</sup>, 66.20 mWs/cm<sup>2</sup>, 115.78 mWs/cm<sup>2</sup>, 231.46 mWs/cm<sup>2</sup>, and 363.90 mWs/cm<sup>2</sup>, respectively. Figure 16 illustrates good reproducibility between experiments where flow rate order was randomized. A linear relationship,  $r^2 = 0.8840$ ,  $p = 0.02$ , between flow rate and log inactivation of virus is shown in Figure 17. The smaller confidence intervals illustrate that a singular log reduction is produced by a range of doses. This range of doses for a particular log reduction is smaller than those shown with the spiral tube design. The experimental variability is decreased using the straight tube reactor. However, the goal of

determining a single UV dose that can be used to achieve a 4 log reduction of all viruses is still improbable.

Another shortcoming of these trials is the flow rate required to achieve a 4 log reduction of viruses in this system. The mean flow rate for a 4 log reduction is 96.2 ml/min., calculated from the mean plus standard deviation. This flow rate is more than 5 fold less than the flow rate required for a four log reduction in titer in the spiral tube reactor system. However, the volume of MilliQ water needed to perform these trials would dilute the stock concentration of human viruses so that the initial experimental virus make initial titers below  $1 \times 10^3$  pfu/ml. Therefore, it would not be possible to calculate a 4 log reduction due to UV dose.

A static UV irradiation system was designed to produce UV dose data that would yield 4 log reductions in virus titer for MS2 as well as the human pathogens. This design suffers from its inability to provide inactivation data for viruses under hydrodynamic stresses more related to those found in a full scale water treatment facility. However, the information from this experimentation should convey the comparative susceptibility of human pathogenic viruses to MS2. If MS2 is not the most resistant virus to UV irradiation than comparative susceptibility data may still be used by treatment facilities. This data can be used to determine the UV dose requirements for inactivating different types of viruses found in groundwaters. Human enteric viruses cannot be used for this purpose due to the expense of their propagation and their pathogenicity to humans. As stated previously, the titers achieved from propagation are too low for practical use in full scale treatment plants.

## Static UV Irradiation of Viruses

### **MS2 inactivation in MilliQ water**

The static UV system design produced data which yielded stronger relationships between log reduction of viruses and UV dose. A range of log reductions in MS2 titer in MilliQ water due to UV dose is shown in Figure 18. A linear relationship,  $r^2$  value of 0.9949,  $p=0.0002$ , exists between log reduction and UV dose. The tight confidence intervals indicated that little experimental variability existed in the treatment design. The mean plus standard deviation formula illustrated that a 4 log reduction in MS2 would occur at a UV dose of 77.18 mWs/cm<sup>2</sup>. Similar UV inactivation experiments were performed using MilliQ water containing concentrations of iron, manganese, and nitrate, see Figure 19. It was hypothesized that these groundwater constituents would decrease UV's virucidal capability. A one-way ANOVA performed on the data generated from this experiment indicated that this did not occur. No significant difference in log reduction due to UV dose occurred in waters containing these added constituents.

The components of enumeration assays for particular viruses may vary substantially. MS2 uses bacteria for its host while poliovirus uses a continuous mammalian cell line. The agars used for the solid support matrix in these assays are chemically different as well. These components are affected differently by other substances.

Continuous cell lines are more fastidious than bacteria. They are more likely to be affected by the concentrations of divalent cations found in the groundwaters used in these

studies. Viral adherence to mammalian cell receptors may be diminished because of the presence of cations. This factor decreases the amount of viruses in a sample that could infect the cell monolayer. The concentration of viruses in a sample would appear to be less than the actual titer of viruses in that sample. A lower final titer would give the appearance that greater log reductions of human viruses were achieved in these waters. This would denote that the human virus being studied is more sensitive to UV irradiation than it actually is.

### **MS2 inactivation in groundwaters**

Figure 20 illustrates the log reductions in titer of MS2 due to UV irradiation in MilliQ water and different groundwaters with varying water quality matrices: iron content, pH, total organic carbon (TOC), specific UV absorbance, and turbidity. The four log inactivation window shows that 4 log reductions in titer occur between 64.0 mWs/cm<sup>2</sup> and 93.0 mWs/cm<sup>2</sup>. The best fit line for the MilliQ water trials is centered between the various groundwater lines elucidating both negative and positive interferences from the groundwaters' constituents. The data suggest that different groundwaters may require different UV doses to achieve a 4 log reduction in virus titer.

UV doses yielding 4 log reductions in MS2 titers in the various groundwaters were correlated with concentrations of constituents found in these groundwaters, see Figure 21 through Figure 25. No significant correlation was determined for TOC and turbidity. It was suspected that a correlation would exist due to the ability of dissolved carbon compounds to absorb UV energy. The failure to find a correlation between these

matrices and log reduction of virus is attributed to the narrow range, 0.5 mg/L to 1.5 mg/L, of TOC examined. Surface waters and wastewaters would probably provide direct correlations due to their increased TOC and turbidity levels. However, in surface water and wastewater disinfection, pretreatment in the form of filtration or settling would reduce the water TOC levels to concentrations such as those found in these studies. The data presented here provide a realistic understanding of TOC's effects on UV irradiation of viruses in waters that have TOC levels that would be acceptable for groundwaters.

No correlation was found to exist between UV dose used to obtain a four log reduction in virus titer and UV absorbance of the waters tested. UV absorbance represents the quantity of UV energy absorbed from a groundwater. Increased absorbances reflect a groundwater's ability to diminish the amount of UV irradiation available for disinfection. Five of the waters examined possessed minimal UV absorbances. The five groundwaters possessing appreciable absorbances presented a small positive correlation to UV dose required for four log reduction in titer. It is speculated that a larger range of absorbances, similar to those found in surface waters, would have produced a significant relationship. However, pretreatment of surface waters may reduce absorbance to levels illustrated in this study. Thus, data would provide an accurate depiction of the inability of UV absorbance to effect surface water and groundwater disinfection.

Linear regression analyses showed that iron was the only constituent that correlated with the dose required for inactivation of MS2 by UV irradiation. Increased iron content decreased UV irradiation's ability to inactivate MS2. It was hypothesized that the positive charge of the iron cations had an aggregative effect on MS2 virus.

Aggregates of viruses shield inner particles from UV irradiation requiring a larger UV dose for inactivation. Iron particulates have also been proven to scatter UV light in waters. These scattering effects reduce the disinfection capability of UV light (Qualls and Johnson, 1983). TOC and turbidity are measurements of particulates found in groundwaters. There was no decrease in UV's ability to inactivate MS2 due to TOC or turbidity. Therefore, iron's effect on UV irradiation is most likely due to aggregation effects on viruses added to these waters.

First order regression analysis showed no relationship between pH and UV dose. A second order regression illustrated a loose correlation to UV dose. The second order pH dependence of MS2 to other disinfection methodologies has been shown previously by Malley et al., 1996. A multiple linear regression including all constituents measured in this experimentation yielded no significant correlation. Lack of correlation between water matrices and virus reduction may be due in part to the idea that a heterogeneous population of viruses is produced in a single propagation. Viruses with differing physical characteristics may be more or less susceptible to the effects of water matrices. The use of a homogeneous population of viruses may decrease experimental variability and increase the probability of obtaining correlations between UV dose required for virus inactivation and constituents found in the groundwaters tested.

### **Poliovirus inactivation in MilliQ water and groundwaters**

Poliovirus data, see Figure 26, elucidates a window of 4 log inactivation ranging from 23.0 mWs/cm<sup>2</sup> to 29.0 mWs/cm<sup>2</sup>. The range of UV doses is smaller than the MS2 range and indicates that MS2 is 3.5 times less susceptible to UV inactivation. In these experiments, MS2 appears to be a good surrogate for the human enteric virus because of its decreased susceptibility to UV irradiation. A treatment facility achieving a four log reduction in MS2 titer would probably achieve a four log reduction in poliovirus titer. However, part of the decreased susceptibility to UV irradiation could be due to the difference in assays used to perform quantification of the virus. Poliovirus assays may be more susceptible to negative effects produced from constituents naturally occurring in the groundwaters. These negative effects elucidate themselves as greater log reductions in virus titers due to UV dose. Viruses will have a decreased ability to infect the cells used in these assays. Therefore, titer estimations will be lower than the actual titer of viruses present in the sample. When log reduction calculations are performed, higher log reductions would be calculated due to the incorrect titer determination. The need for a standard assay that is used for all virus titer determination in these types of studies is apparent.

### **Hepatitis A virus inactivation in MilliQ water and groundwaters**

Hepatitis A virus proved to be the virus most susceptible to UV inactivation in MilliQ water, see Figure 27. HAV was irradiated to achieve a 4 log reduction in two groundwaters. Groundwater 1 (GW1) was chosen because of its relatively large specific



UV absorbance and turbidity. Turbidity and UV absorbance are two important physical factors which negatively affect UV irradiation disinfection capability. GW1 possessed the largest UV absorbance of the groundwaters. With the exception of one of the waters used, GW1 was the most turbid of all the waters. High turbidity and specific UV absorbance measurements are indicative of waters possessing large concentrations of particulates. These particulates scatter UV light are expected to negatively affect UV's germicidal capability.

Groundwater 2 (GW2) was chosen because of its large iron content. With the exception of one of the waters, GW2 possessed the highest iron concentration of the waters. In environmental waters, divalent and trivalent iron cations lead to the formation of viral aggregates (Bitton, 1980). With the formation of these aggregates, fewer viruses are exposed to UV irradiation. A higher dose of UV light is then needed to disinfect a population of viruses. The iron particles will also scatter UV light, decreasing its disinfection capability.

A four log reduction in HAV titer was achieved using a UV dose of 6.0 mWs/cm<sup>2</sup> in GW1 and 15.0 mWs/cm<sup>2</sup> in GW2. The MilliQ water experiment elucidates that MS2 is about 12 times more resistant to UV inactivation than HAV. A dose that achieves a 4 log reduction in MS2 titer may inactivate the equivalent titer of HAV in various groundwaters. As before, the difference in UV dose requirements for 4 log reductions in virus titer may be due to the differences in quantification assays.

## **Rotavirus inactivation in MilliQ water and groundwaters**

Theoretically, rotavirus has a number of morphological features that would positively affect its resistance to UV irradiation. Rotavirus possesses a double layer icosahedral capsid with an inner protein core (Kapikian and Chanock, 1985). The three layers of protein shield the viral genome from UV irradiation effects. The rotavirus genome is double-stranded (ds) RNA which is highly ordered due to its hydrogen bonding within the helices of the nucleic acid (Kapikian and Chanock, 1985). The increased stability of the genome confers a decreased absorbance of UV irradiation (Meng and Gerba, 1996). The hydration of adjacent uracils, creating uracil-uracil dimers in RNA, occurs less readily than thymine-thymine dimer formation in DNA (Abbaszadegan and Gerba, 1996). The larger genome size of rotavirus (<15,000 bp), confers increased resistance to the mutagenic effects of UV irradiation due to the number of hits needed to destroy the genome (Battigelli et al., 1993). The complementary strands of the ds genome provide appreciable redundancy for added resistance to the effects of UV irradiation (Battigelli et al., 1993). Rotavirus inactivation by chemical and physical factors follows a second order kinetic rate which establishes a retardant die-off effect in the decline of viral titer at higher doses (Hill et al., 1971).

In 1987, Pancorbo et al. demonstrated that the Wa strain was less affected by chlorine disinfection in tap water than the SA-11 strain. In this same study, the Wa strain showed longer survival in groundwater over time at 4 °C and 22 °C. It was postulated that the SA-11 strain may not have the same susceptibility to UV irradiation. A four log reduction of the simian rotavirus strain, SA-11, was achieved at a dose of 42.0 mWs/cm<sup>2</sup>

(Battigelli et al., 1993) and 39.4 mWs/cm<sup>2</sup> (Abbaszadegan and Gerba, 1996). However, in MilliQ water, the Wa strain of rotavirus was more than twice as resistant to UV irradiation in the studies using column purification with cell culture-RT PCR assay. These results are due to differences in virus type or detection methodology of the two strains. The SA-11 rotavirus strain shares a similar morphology and some cross reactive antigenic sites with the Wa strain but its overall genetic relatedness, as shown by cDNA sequencing and electrophoretic data, is low (Pancorbo et al., 1987). Among different strains, intergenic and intragenic reassortment allows for considerable drift in dominant antigenic sites, such as the outer capsid VP7 glycoprotein and VP4 protein. These drifts may allow for a significant alteration in the infectious capability of a particular strain of rotavirus (Hoshino et al., 1994).

The human strain of rotavirus was the least susceptible virus to UV disinfection in MilliQ water and groundwaters. Our studies showed that rotavirus is 1.5 times more resistant than MS2 to UV irradiation in MilliQ water. A 4 log reduction in titer occurred at 97.0 mWs/cm<sup>2</sup> in MilliQ water. In groundwaters, a 4 log reduction in rotavirus titer occurred at 97.0 mWs/cm<sup>2</sup>. Four log reductions in MS2 occurred between 64.0 mWs/cm<sup>2</sup> to 93.0 mWs/cm<sup>2</sup> in groundwaters. Rotavirus is 1.0 to 1.5 times more resistant to UV light in groundwaters.

Previously, it was reported that MS2 is a more resistant virus than any of the enteric viruses. MS2 has long been employed as a surrogate virus for poliovirus and other enteroviruses because of its similar morphological characteristics, response to environmental stresses, and susceptibility to disinfectants. It was proposed that MS2 could

act as a surrogate virus in full scale water treatment facility disinfection studies. The data from this study seriously question the efficacy of MS2 as a surrogate virus for UV disinfection since it appears to be more sensitive to UV irradiation than rotavirus. This difference in susceptibility may be due to the difference in quantification assays. The use of a universal quantification assay for all viruses is needed to decrease experimenter variability and to determine the comparative susceptibility of different viruses to UV irradiation.

It is believed that the data generated from this study are different from those of earlier studies due to the strain of rotavirus used and its enumeration assay. The rotavirus Wa strain has been shown to be more resistant to chemical disinfection (Pancorbo et al., 1987). The Wa strain may be less sensitive to UV irradiation effects over the SA-11 strain used in previous studies.

The assay used for SA-11 titer determination is a plaque assay. It is proposed that this assay yields lower titer estimations of the viruses in a sample. Previously, it was explained that lower final titer estimates result in the appearance of greater log reductions of virus due to UV dose. The virus would be perceived as being more susceptible to UV irradiation than it actually is. The cell culture-RT PCR assay provides a more accurate estimation of the titer of rotavirus Wa strain in MilliQ water and in various groundwaters. Therefore, the assay provides a more accurate representation of rotavirus sensitivity to UV irradiation.

### **Cell-culture RT PCR assay**

The plaque assay used for the quantification of the human strain of rotavirus is unreliable. In our laboratory, it lacked reproducibility as well as sensitivity for titer determination. Therefore, the cell culture RT-PCR assay was used to determine initial and final titers of rotavirus in disinfection trials. The integrated cell culture-RT PCR assay provides the detection sensitivity of RT PCR with the ability to discriminate between infectious and non-infectious particles. Assays that employ only cell culture are time-consuming, expensive, and have a high degree of experimenter subjectivity. Cell culture detection and enumeration of a particular enteric virus in groundwater is limited due to the fact that cell lines may exhibit CPE from a number of different viruses. Differentiation of viral CPE and CPE caused by chemical constituents in groundwater is difficult and some viruses such as rotavirus do not always exhibit a typical viral CPE (Reynolds et al., 1996). Cell culture may be less sensitive in detecting virus in environmental samples. Previously, direct cell culture detection of rotavirus was determined to be 10 to 1000 times less sensitive than RT PCR in sewage concentrates (Kopecka et al., 1993).

Many steps in this assay have the advantage of increasing detection sensitivity of infectious rotaviruses. The cell culture step in the assay increases virus detection efficiency by serving to dilute inhibitory compounds and to increase the concentration of virus in samples (Reynolds et al., 1996). Ethylenediaminetetraacetic acid (EDTA), at a concentration of 0.05 M, was used in the denature step prior to RT PCR step in order to chelate naturally occurring iron. Gelatin was used in the PCR step in order to decrease the possibility of non-specific primer annealing yielding false negative results. The use of the

RNA negative control ensured that only infectious rotavirus RNA would be converted to cDNA and later amplified in PCR. Lastly, specific primer sets employed in RT PCR guarantee the detection of one particular type of virus.

The cell culture-RT PCR assay is a more sensitive assay for the quantification of rotavirus in both laboratory grade water and groundwaters. The assay is less sensitive to groundwater matrix effects than traditional enumeration assays. Quantification sensitivity of rotavirus in groundwater is increased by one log with Sephadex/Chelex column purification, see Table 10. This increase in sensitivity is due to the removal of organic and inorganic substances by the columns. These substances are inhibitory to RT PCR and negatively affect the assay in terms of RNA detection or virus titer determination.

#### **Increasing sensitivity of the cell culture-RT PCR assay with column purification**

The detection of viruses in environmental samples by direct RT PCR analysis is inhibited by naturally occurring substances such as humic acids and fulvic acids (Abbaszadegan et al., 1993) and heavy metals (Saiki et al., 1988). Organic constituents may bind divalent magnesium ( $Mg^{2+}$ ) used to enhance the activation of polymerases used in RT PCR (Tsai and Olson, 1992). This decreases the sensitivity of the assay.

Purification of a sample, prior to RT PCR, dilutes or reduces the concentration of certain organic and inorganic inhibitors. Purification of samples with Sephadex G-200 Chelex 100 columns have been used extensively to remove naturally occurring organics and metals (Straub et al., 1994). However, column purification may remove up to 99% of the viruses

found within a particular environmental sample (Ma et al., 1995). In the cell culture-RT PCR assay used for this experimentation, this 2 log reduction in titer was not observed.

Other methods of purification are used to increase RT PCR sensitivity. Viral RNA extraction kits are commercially available to remove inhibitory substances found in urine, blood, and stool samples. Their use in our laboratory suggests that they fail to increase RT PCR detection sensitivity of rotaviruses in environmental waters. Freon (1,1,2-trichlorofluoroethane) purification is capable of removing organic compounds from environmental samples containing viruses and serves to monodisperse viruses (Meng and Gerba, 1996). Freon is a chlorofluorocarbon whose use is limited by EPA mandated regulations and is clearly antithetic to use in environmental research. Freon purification was not found to increase RT PCR detection of rotavirus in our laboratory.

By the cell culture-RT PCR assay alone, a greater than 4 log reductions in titer occurred at 77 mWs/cm<sup>2</sup> in GW1 and GW2. By the same method, 3 log reductions were achieved at this dose in MilliQ water. One possible explanation for the increased sensitivity to UV dose in GW1 and GW2 may be a function of some inhibitory effect on the assay used for enumeration of rotavirus. Using column purification combined with the cell culture-RT PCR assay detection sensitivity was increased. Three log reductions in rotavirus titer at 77 mWs/cm<sup>2</sup> and 4 log reductions in titer at 97 mWs/cm<sup>2</sup> were observed after the implementation of column purification.

## **Future Experimentation**

Immunomagnetic bead capture allows increased sensitivity in viral detection without a decrease in virus concentration. Grinde et al. (1996) were able to detect 0.005 pfu of rotavirus Wa in seeded seawater samples. Studies from our laboratory also suggest increased virus detection in sludge using oligo d(T) probe magnetic beads. This relatively new technology may allow for the more representative determination of rotavirus initial and final titers.

It is apparent that UV disinfection of groundwater to remove pathogens is in its infancy. Very few laboratory data exist on UV disinfection of viruses in groundwater. All of the journal articles reviewed for this paper determined virus inactivation in laboratory grade water. The data presented here are obtained from both MilliQ water and groundwaters obtained from various treatment facilities.

There exists a need for a standard detection assay which does not suffer from decreased sensitivity due to groundwater constituents. There also exists the need to use a human strain of rotavirus instead of the simian strain when determining virus susceptibility to disinfectants. Simian and human strains may not be equivalent in the physical characteristics which confer infectivity or resistance to mutagens.

Data gathered from this study can be used to identify those viruses most resistant to the effects of UV irradiation in laboratory grade water and groundwaters. More research needs to be conducted in order to complete susceptibilities of viruses in groundwaters possessing a wider range of water matrices. Dynamic bench scale UV disinfection systems that simulate hydrodynamic effects found in treatment facilities need



to be constructed. Virus inactivation data generated with the dynamic bench scale system used here provided baseline data which could be useful in designing UV treatment facilities. However, the system lacked the ability to produce data for the human viruses. The static bench scale design provided dose response curves for MS2, poliovirus, HAV, and rotavirus. Data for inactivation in groundwaters were also generated. In the future, treatment facilities forced to comply with the GWDR will have guidelines allowing a more objective understanding of virus inactivation by UV irradiation.

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## APPENDIX A

### PREPARATION OF MEDIA

#### Agar overlay (2X)

30 g of tryptic soy broth powder (Difco)

5 g of yeast extract (Difco)

7.5 g Bacto-agar (Difco)

0.075 g CaCl<sub>2</sub> (Sigma)

*Bring up to 500 ml with MilliQ water. Mix with a magnetic stir plate with heat.*

*Autoclave 15 minutes. Store at 57 °C until use.*

#### Flake agar (2%)

2 g agar flake (Difco, Detroit, MI)

*Bring up to 100 ml with MilliQ water. Autoclave 15 minutes. Leave at 56 °C until use.*

#### Medium 199 (2X)

1.96 g Medium 199 (Sigma)

0.13 g NaHCO<sub>3</sub>

0.95 g hepes

0.06 g L-glutamine

1 ml nystatin (Sigma)

1 ml penicillin/streptomycin (Sigma)

0.5 ml kanamycin (Sigma)

1.2 ml neutral red solution (Sigma)

1 ml 1% MgCl

*Bring up to 100 ml with MilliQ water. Filter sterilize.*

#### Minimal Essential Media (MEM) with 12% Fetal Bovine Serum (Growth Media for FRhK-4 cells)

2.65 g MEM (Sigma)

3.45 g L-15 Medium Leibovitz (Sigma)

5 ml Non-essential amino acids (10 mM each amino acid) (Sigma)

2 g hepes

0.5 g NaHCO<sub>3</sub>

5 ml nystatin (10,000 U/ml) (Sigma)

5 ml penicillin/streptomycin (10,000 U pen. and 10 mg strep./ml) (Sigma)

2 ml kanamycin (Sigma)

60 ml fetal bovine serum (Gemini Bioproducts)

*pH to 6.9. Bring up to 500 ml with MilliQ water. Filter sterilize.*

**Minimal Essential Media with 8% Fetal Bovine Serum  
(Growth media for BGM and MA-104 cells)**

Same as 12% MEM, except add 40 ml FBS instead of 60 ml.

**Tryptic soy agar**

40 g tryptic soy agar powder (Difco)

*Bring up to 1000 ml with MilliQ water. Mix with a magnetic stir plate with heat for 20 minutes. Autoclave for 15 minutes. Leave at 57 °C until use.*

**Tryptic soy broth**

30 g tryptic soy broth powder (Difco, Detroit, MI)

*Bring up to 1000 ml with MilliQ water. Autoclave 15 minutes. Leave at 4 °C or room temperature until use.*

## APPENDIX B

### ENZYMES

#### Proteinase K (Sigma)

*Five ml of RNase/DNase free water was added to 100 mg of proteinase K. The solution was stored at -20 °C until use.*

#### Trypsin 1:250 (ICN)

*10 ml of MEM without FBS was added to 0.01 g of trypsin 1:250. The solution was heated at 56 °C for 10 minutes. Filter sterilize.*

## APPENDIX C

### BUFFERS AND SOLUTIONS

#### Chelex 100

1.0 g Chelex 100

*Bring up to 5.0 ml with UV treated MilliQ water. Vortex until slurry forms at room temperature.*

#### DTT (0.5M)

1.86 g powdered dithiothreitol.

*Bring up to 1.0 ml with UV treated MilliQ water in UV irradiated microfuge tube. Store at -20 °C in 0.1 ml aliquots*

#### EDTA (1M)

73.05 g disodium ethylenediaminetetraacetic acid 2H<sub>2</sub>O

NaCl pellets to adjust to pH 8.0 (approximately 10 g)

*Bring up to 500 ml with UV treated MilliQ water in UV irradiated bottle.*

#### Gelatin (0.012%)

0.240 g porcine pancreas gelatin

*Bring up to 20 ml with UV treated MilliQ in UV irradiated 50 ml conical tube.*

*Dissolve with vortexing at 56 °C*

#### Sephadex G-200

1.0 g Sephadex G-200

*Bring up to 5.0 ml with UV treated MilliQ water. Vortex until slurry forms at room temperature.*

#### Phosphate Buffered Saline (PBS) (1X)

9.785 g of PBS powder (Sigma)

*Bring up to 1000 ml with MilliQ water. Autoclave for 15 minutes. Store at 4 °C or room temperature. Final pH 6.9.*

#### Tris-Borate-EDTA

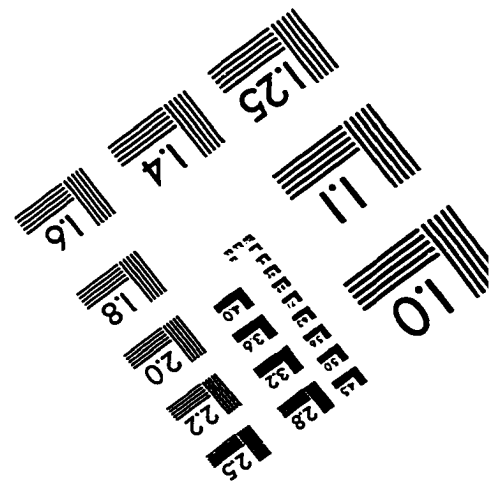
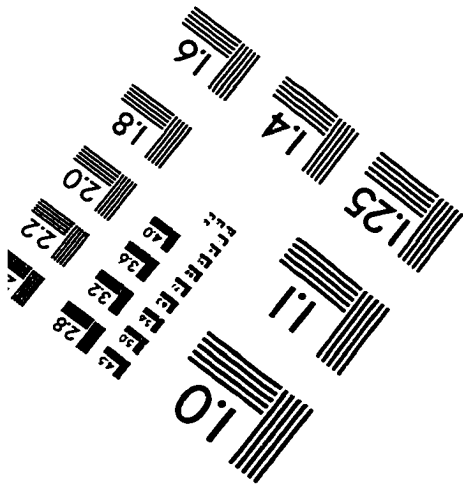
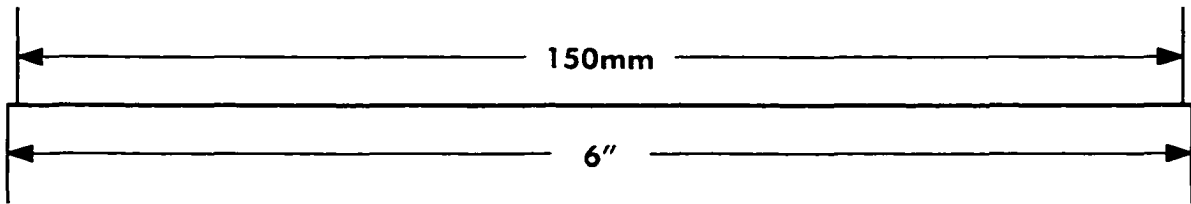
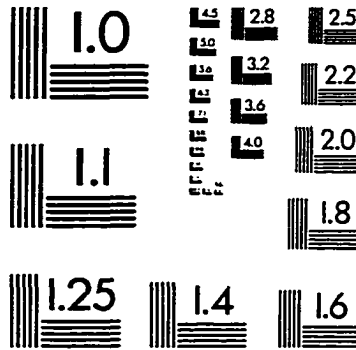
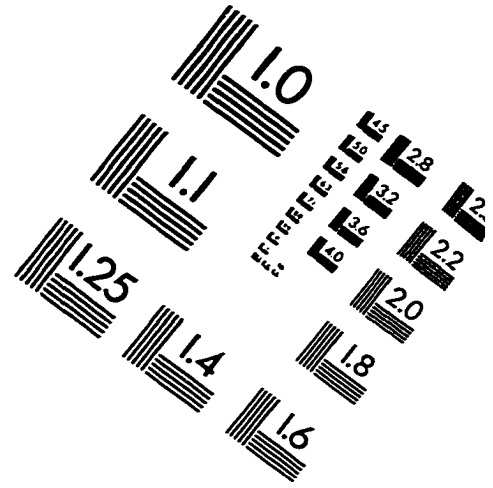
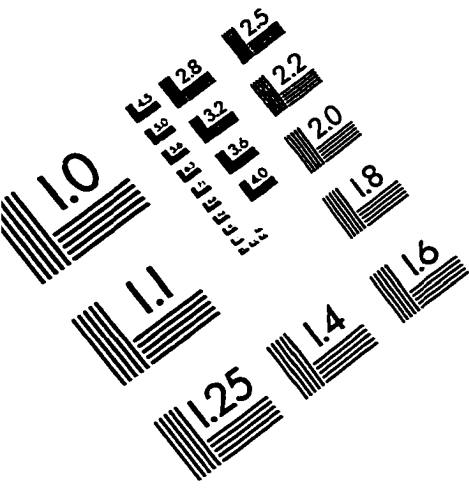
54.0 g tris base

27.5 g boric acid

20 ml 0.5M EDTA

*Bring up to 1000 ml with MilliQ water. Store at room temperature.*

# IMAGE EVALUATION TEST TARGET (QA-3)



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