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THE EFFECT OF PARTICLE-ASSOCIATED VIRUSES ON DISINFECTION PROCESSES IN WATER TREATMENT

BY

JESSICA L. TOKSON

THESIS

Submitted to the University of New Hampshire

in Partial Fulfillment of

the Requirements for the Degree of

Master of Science

in

Civil Engineering

December, 2009

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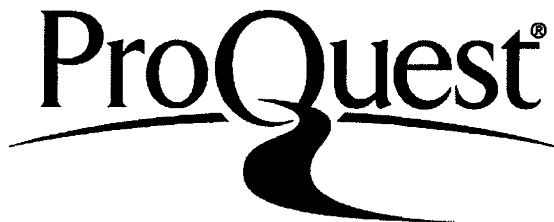

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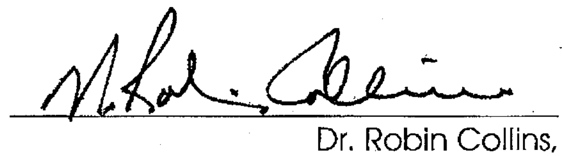

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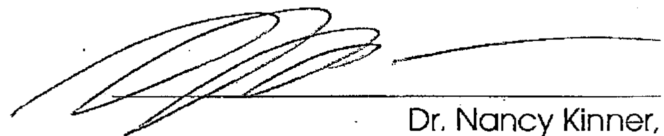

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ABSTRACT

THE EFFECT OF PARTICLE-ASSOCIATED VIRUSES ON DISINFECTION PROCESSES IN WATER TREATMENT

By

Jessica L. Tokson

University of New Hampshire, December, 2009

This research focused on what accelerates or hinders virus-particle association, how the addition of turbidity affects both chlorine and ultraviolet light disinfection, and if sonication can disrupt virus-particle associations exposing viruses to disinfection methods.

A decrease in pH, from 7-4, was found to accelerate association by 2-logs. Calcium increased association by 2.74-logs, and total organic carbon decreased association by 0.36-logs.

An addition of 5 NTU, the maximum turbidity level allowed by USEPA for unfiltered drinking water supplies, affected both chlorine and UV light disinfection showing differences in the rate of kill per dose. The association of viruses and particles can hinder inactivation. These effects must be considered when disinfecting unfiltered drinking water sources because the rate of inactivation is reduced at higher turbidities.

Chapter I

Introduction and Objectives

A growing demand for fresh water and a depletion of fresh water sources has led to increased water reuse and reclamation. This increase in the reuse of water resources has led to a need for more precise testing methods and more suitable pathogen indicators for drinking water treatment. An increase in testing precision has revealed that viruses can be encased or attached to particles and thus are shielded from disinfection, and remain harmful. Particle-associated viruses can remain infective after disinfection, as well as, regrow within distribution systems.

Many organisms of interest in water treatment occur in both a disperse state (not bound to other objects) and a particle-associated state (bound to other objects such as dissolved organic matter, cellular debris, or other bacteria). When talking about disinfection, the fundamental difference between disperse organisms and particle-associated organisms is the intensity or amount of a disinfectant reaching the organism. An organism embedded in a particle will receive a reduced intensity of a disinfectant compared to a disperse organism. This reduction can be due to either shielding from other particles or increased demand or absorbance (depending on the disinfectant) of the water

causing less disinfectant to be available to the organism (Emerick et al., 2000). This phenomenon is illustrated in a typical dose-response curve (Figure 1). A typical dose-response curve has a lag section caused by the waters demand for the disinfectant, a log-linear inactivation section, and a tailing section caused by the shielding of particles (Tchobanoglous, et al., 2003).

This research was performed to quantify the effect of water parameters (pH, TOC and calcium concentration) on particle-virus association, evaluate the impact of turbidity on the inactivation of MS2 bacteriophage by chlorine and ultraviolet light disinfection, and gauge the effectiveness of sonication to break apart virus-particle associations and increase the disinfection efficiency.

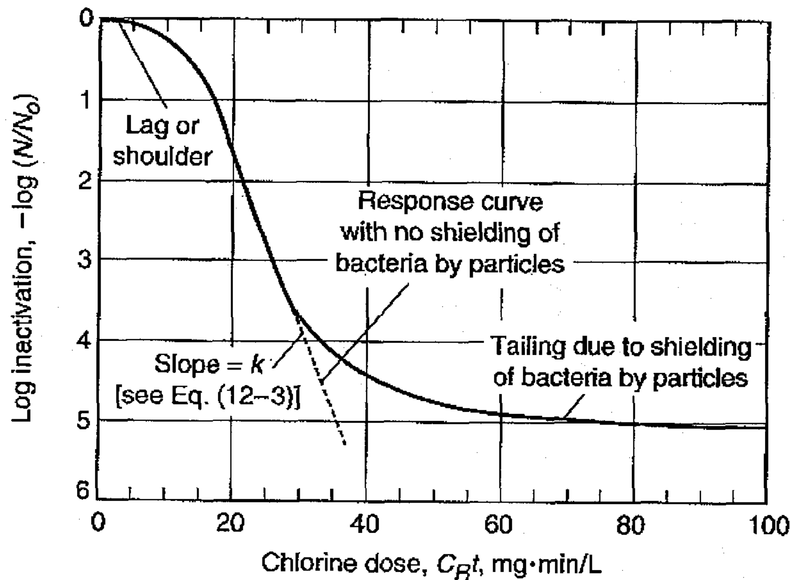


Figure 1: Typical Dose-Response Curve (Tchobanoglous et al., 2003)

Chapter II

Literature Review

Water Reuse

There is a growing demand for water to meet domestic, commercial, industrial, and agricultural needs. Adding to this dilemma is a depletion of fresh water sources. These problems lead us to look for renewable sources of water, the main focus being water reclamation and reuse. Reclaimed effluent generated by domestic wastewater treatment facilities can be used for urban, industrial, agricultural, recreational or environmental applications; as well as groundwater recharge and the augmentation of potable supplies.

Urban use can include irrigation of public parks and golf courses, fire protection, and toilet flushing. Industrial use includes cooling water in cooling tower systems, spray ponds, and industrial process water. Recreational or environmental reuse includes wetland enhancement, stream augmentation, and landscape impoundments (USEPA, 2004). Each use has set standards which differ by State. Table 1 and 2 show the reclaimed water quality and treatment requirements for both unrestricted

and restricted urban reuse. Unrestricted urban reuse is the use of reclaimed water where public exposure is likely, making a high degree of treatment necessary. With restricted urban reuse, public exposure to the reclaimed water is controlled meaning the treatment requirements may not be as strict. The water quality and treatment requirements for agricultural reuse, recreational reuse, environmental reuse, industrial reuse, groundwater recharge, and indirect nonpotable reuse can be found in Appendix C.

Effluent from wastewater treatment plants has been shown to have adverse effects when tested in vitro and in vivo assays. Effluent coming from a chlorinated treatment train was found to have genotoxicity, acute invertebrate toxicity, and adverse effects on medaka embryos development (Cao et al., 2008). Even in grey water (defined as all flows exiting an urban building, excluding toilet water (Winward et al., 2008)) indicator bacteria have been consistently detected, demonstrating the potential for a range of enteric bacteria, protozoa, and viruses to persist in grey water, showing a need for disinfection in grey water reuse (Winward et al., 2008). It is good to note, to date there have been no incidences of illness linked to grey water reuse (Winward et al., 2008a).

	Arizona	California	Florida	Hawaii	Nevada	Texas	Washington
Treatment	Secondary treatment, filtration, and disinfection	Oxidized, coagulated, filtered, and disinfected	Secondary treatment, filtration, and high level disinfection	Oxidized, filtered, and disinfected	Secondary treatment and disinfection	NS (1)	Oxidized, coagulated, filtered, and disinfected
BOD5	NS*	NS	20 mg/l CBOD5	NS	30 mg/l	5 mg/l	30 mg/l
TSS	NS	NS	5.0 mg/l	NS	NS	NS	30 mg/l
Turbidity	2 NTU (Avg) 5 NTU (max)	2 NTU (Avg) 5 NTU (max)	NS	2 NTU (Max)	NS	3 NTU	2 NTU (Avg) 5 NTU (max)
Coliform	Fecal	Total	Fecal	Fecal	Fecal	Fecal	Total
	None detectable (Avg)	2.2/100 ml (Avg)	75% of samples below detection	2.2/100 ml (Avg)	2.2/100 ml (Avg)	20/100 ml (Avg)	2.2/100 ml (Avg)
	23/100 ml (Max)	23/100 ml (Max in 30 days)	25/100 ml (Max)	23/100 ml (Max in 30 days)	23/100 ml (Max)	75/100 ml (Max)	23/100 ml (Max)

*NS - Not specified by state regulations

Table 1: Unrestricted Urban Reuse
Source: Adapted From USEPA, 2004

	Arizona	California	Florida	Hawaii	Nevada	Texas	Washington
Treatment	Secondary treatment and disinfection	Secondary – 23, oxidized, and disinfected	Treatment, filtration, and high-level	Oxidized and disinfected	Secondary treatment and disinfection	NS (1)	Oxidized and disinfected
BOD5	NS	NS	20 mg/l CBOD5	NS	30 mg/l	20 mg/l	30 mg/l
TSS	NS	NS	5 mg/l	NS	NS	NS	30 mg/l
Turbidity	NS	NS	NS	2 NTU (Max)	NS	3 NTU	2 NTU (Avg) 5 NTU (max)
Coliform	Fecal	Total	Fecal	Fecal	Fecal	Fecal	Total
	200/100 ml (Avg)	23/100 ml (Avg)	75% of samples below detection	23/100 ml (Avg)	23/100 ml (Avg)	200/100 ml (Avg)	23/100 ml (Avg)
	800/100 ml (Max)	240/100 ml (Max in 30 days)	25/100 ml (Max)	200/100 ml (Max)	240/100 ml (Max)	800/100 ml (Max)	240/100 ml (Max)
*NS - Not specified by state regulations							

Table 2: Restricted Urban Reuse Water Quality and Treatment Requirements
Source: Adapted from USEPA, 2004

The need for obtaining additional water resources through wastewater reuse has led to the necessity of more precise and sophisticated biological control tools for wastewater recycling. These tools include more suitable pathogen indicators as well as more specific parameters depending on wastewater origins and intended use (Salgot et al., 2006). Wastewater reclamation holds promise as a water resource to decrease demands on finite water sources as well as reducing some pollution of environmentally sensitive regions. With the correct assessment and management of risks, water reuse can be a coveted resource.

Regulations

The Safe Drinking Water Act (SDWA) was enacted in 1974 and amended in 1986, 1988, and 1996. This Act asked the United States Environmental Protection Agency (USEPA) to publish a maximum contaminant level goal (MCLG) and publicize a national primary drinking water regulation (NPDWR) for contaminants that the Administrator determines may have an adverse effect on the health of persons (USEPA, 2001). This Act has led to the establishment of the Interim Enhanced Surface Water Treatment Rule (IESWTR) in 1998 and The Stage 1 Disinfectants and Disinfection Byproduct Rule (Stage 1 DBPR) in 1998. The IESWTR applies to public water systems (PWS) serving at least 10,000 people which use surface water or ground water under direct influence

from surface water (GWUDI). The IESWTR requires: (1) MCLG of zero for Cryptosporidium; (2) 2-log Cryptosporidium removal requirements for systems that filter; (3) increased combined filter effluent (CFE) turbidity performance standards of 1.0 NTU as a maximum and 0.3 NTU or less at the 95th percentile monthly for systems using conventional treatment or direct filtration; (4) requirements for individual filter turbidity monitoring; (5) disinfection profiling and benchmarking provisions; (6) inclusion of Cryptosporidium in the definition of GWUDI and in the watershed control requirements for unfiltered PWSs; (7) requirements for covers on new finished water storage; (8) sanitary surveys for all surface water systems regardless of size (USEPA, 2001).

Cryptosporidium is a widespread contaminant in surface water used as drinking water supplies. Transmission occurs through consumption of water or food contaminated with feces or by direct or indirect contact with infected persons or animals. Ingestion of Cryptosporidium can cause cryptosporidiosis, a gastrointestinal illness. It is a serious concern because Cryptosporidium oocysts are very resistant to standard disinfectants like chlorine (USEPA, 1999a; USEPA, 2006).

The Stage 1 DBPR was set-up to work in unison with the IESWTR to strengthen protection against microbial contaminants, especially Cryptosporidium, while also protecting against the potential health risks from disinfection byproducts. The Stage 1 DBPR established maximum

residual disinfectant goals (MRCLGs) and maximum residual disinfectant levels (MRDLs) for chlorine, chloramines, and chlorine dioxide. It also set maximum contaminant level goals (MCLGs) and maximum contaminant levels (MCLs) for total trihalomethanes (THMs), haloacetic acid (HAAs), chlorite, and bromate. The Rule also requires water systems which use surface water or GWUDI and use conventional filtration treatment are required to remove specific percentages or organic materials, measure as total organic carbon (TOC). TOC may react with disinfectants to form DBPs (USEPA, 2001a).

Disinfectant Byproducts are formed when organic and inorganic materials combine with chlorine and certain other chemical disinfectants. According to the USEPA, more than 260 million people in the U.S. are exposed to disinfected water and DBPs. Trihalomethanes (THMs) and Haloacetic acids (HAAs) are widely occurring classes of DBPs formed during disinfection by chlorine and chloramines. The four THMs (TTHM) and five HAA (HAA5) measured and regulated in Stage 2 DBPR act as indicators for DBP occurrence (USEPA, 2006a). The USEPA has concluded that these is a potential association of chlorinated water with bladder cancer and suggests an association for colon cancers. There is also a potential health concern that DBPs cause adverse developmental or reproductive health effects (USEPA, 2006a).

In January 2002, the Long Term 1 Enhanced Surface Water Treatment Rule extended the requirements of the IESWTR to small PWSs which serve fewer than 10,000 people (USEPA, 2002). In January 2006 the Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) and the Stage 2 Disinfectants and Disinfection Byproducts Rule (Stage 2 DBPR) were enforced. The LT2ESWTR has three main requirements. The first has to do with source water monitoring. PWSs using surface water or GWUDI must monitor their surface water for a two or one year period, depending on the size of the PWS, to determine an average Cryptosporidium level. These monitoring results determine the extent of additional treatment for Cryptosporidium that will be required under this rule. The rule also requires a second round of monitoring to begin 6.5 years after the initial monitoring ends to determine if there have been any changes in the source water characteristics which would require additional treatment (USEPA, 2006). The second requirement of the LT2ESWTR establishes risk-targeted treatment technique requirements. Filtered PWSs will be classified into four "bins" based on their monitoring results. PWSs classified in bins 2, 3, or 4 must achieve an additional treatment of 1.0 to 2.5 log treatment for Cryptosporidium. Unfiltered PWSs must provide at least 2 log inactivation of Cryptosporidium (USEPA, 2006). The third condition requires PWSs with existing uncovered finished water storage facilities to either cover their

storage facilities or treat the storage discharge to achieve removal of 4-log virus, 3-log Giardia, and 2-log Cryptosporidium (USEPA, 2006).

The Stage 2 DBPR has four main provisions. The first requirement is designed to identify higher risk systems by initial distribution system evaluation (IDSE). The purpose of the IDSE is to identify sites that represent each system's highest DBP levels. Compliance will monitored at these sites, and only systems with elevated levels of TTHMs and HAA5 will need to makes changes to bring their system into compliance (USEPA, 2006a). The second requirement bases compliance on a locational running average annual (LRAA) calculation where the annual average is taken at each sampling location along the distribution system. These averages will be used to determine compliance, along the whole length of the distribution system, with the MCLs of 0.080 mg/L and 0.060 mg/L for TTHM and HAA5, respectively. The third provision requires systems exceeding evaluation levels to evaluate system operational practices and identify opportunities to reduce DBP concentrations in the distribution system. This provides systems with a proactive approach to remain in compliance. The fourth provision has to do with uniform regulation of consecutive systems. It ensures consecutive systems deliver drinking water that meets applicable DBP standards, thereby providing equitable public health protection (USEPA, 2006a). A summary of the highlights from the Surface Water Treatment Rule (SWTR), Interim Enhanced Surface Water Treatment

Rule (IESWTR), Long Term 1 Enhanced Surface Water Treatment Rule (LT1ESWTR), Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR), and the Disinfection Byproducts Rules (DBPRs) can be found in Table 3.

In October 2006 the final Groundwater Rule (GWR) was promulgated by the EPA to reduce the risk of exposure to fecal contamination that may be present in public water systems (PWSs) that use groundwater sources. The rule applies to PWSs which use groundwater as well as any system that mixes surface and groundwater if the groundwater is added directly to the distribution system without treatment equivalent to surface water treatment (USEPA, 2009).

Table 3: Summary of USEPA Regulations (USEPA, 2006b)

Surface Water Treatment Rules - Minimum Requirements				
Regulation	**Giardia**	**Viruses**	**Cryptosporidium**	
SWTR	3-log removal and/or inactivation	4-log removal and/or inactivation	Not addressed	
IESWTR & LT1ESWTR	No change from SWTR		2-log removal	
LT2ESWTR	No change from SWTR		0- to 2.5-log additional treatment for filtered systems	
			2- or 3-log inactivation for unfiltered systems	
DBP Rules- MCLs Based on Running Annual Averages (RAAs) or Locational RAAs (LRAAs)				
Regulation	**Total Trihalomethanes (TTHM) (ug/L)3**	**Five Haloacetic Acids (HAA5) (ug/L)3**	**Bromate (ug/L)3**	**Chlorite (ug/L)3**
Stage 1 DBPR	80 as RAA	60 as RAA	10	1000
Stage 2 DBPR	80 as LRAA	60 as LRAA	No change from Stage 1	

The GWR relies on four major components. The first is periodic sanitary surveys of groundwater systems that require the evaluation of eight critical elements (source protection, treatment, distribution system, finished water storage, pumps, monitoring and reporting, water system management and operations, and operator compliance with state requirements) and the identification of significant deficiencies.

The second component is source water must be monitored to test for the presence of E. coli, enterococci, or coliphage in the sample. The

first of two monitoring provisions is triggering monitoring. Systems that do not already provide treatment that achieves at least a 4-log inactivation of viruses and have a total coliform – positive routine sample under the Total Coliform Rule sampling in the distribution systems use triggering monitoring. Assessment monitoring is used as a complement to triggered monitoring. Assessment monitoring gives a State the option to require systems, at any time, to conduct source water assessment monitoring to help identify high risk systems.

The third component involves corrective actions required for any systems with a significant deficiency or source water fecal contamination. The deficient system must implement one or more of four corrective action options. The four options are: correct all significant deficiencies, eliminate the source of contamination, provide an alternative source of water, or provide treatment to achieve 4-log inactivation of viruses. The fourth component of the GWR states that compliance monitoring must be carried out to ensure that treatment technologies reliably achieve a 4-log inactivation of viruses (USEPA, 2009).

To develop the treatment technique requirements, the EPA evaluated existing state requirements and the measures available to systems to address fecal contamination. To provide 4-log treatment of viruses, corrective action technologies include chemical disinfection technologies and membrane filtration technologies.

Chemical disinfection is a demonstrated technology that can achieve 4-log inactivation of viruses. At a temperature of 15 degrees Celsius and a pH of 6-9, a 4-log inactivation of HAV (Hepatitis A Virus) can be achieved for free chlorine with a CT of 4 mg-min/L, for chlorine dioxide with a CT of 16.7 mg-min/L, and for ozone with a CT of 0.6 mg-min/L (USEPA, 2006c). Membrane filtration technologies can achieve 4-log or greater removal of viruses, as long as the absolute MWCO (molecular weight cut-off) of the membrane is smaller than the diameter of viruses. As an example, a reverse osmosis (RO) membrane can achieve greater than 4-log removal of viruses with a diameter larger than 0.5 nm when the absolute MWCO of the membrane is less than 0.5 nm. The absolute MWCOs must be determined for the specific membranes to meet these conditions (USEPA, 2006c). The final GWR does not include specific performance, monitoring, or design requirements related to the use of UV technology to achieve the required 4-log inactivation of viruses. Some viruses, adenovirus in particular, are very resistant to UV light and may require a higher dose than those contemplated in the GWR proposal. Data shows that a dose of 186 mJ/cm^2 is required to achieve 4-log inactivation of adenovirus. This suggests that HAV may not be an appropriate indicator of the virus inactivation performance of UV reactors (USEPA, 2006c).

It is believed that full-scale UV reactor testing is needed to ensure disinfection performance, but no available challenge microorganism is known to demonstrate a 4-log inactivation of adenovirus in a full scale UV reactor. To meet the GWR requirements, UV technology may be used in a series configuration or in combination with other inactivation or removal technologies to provide complete 4-log treatment of viruses. It may become feasible for systems to demonstrate 4-log inactivation of viruses with a single UV light reactor. The GWR allows states to approve and set compliance monitoring and performance parameters for any alternative treatment, including UV light or UV light in combination that will ensure that systems continuously meet the 4-log virus treatment requirements (USEPA, 2006c).

Viruses

A virus is entirely composed of a double or single strand of genetic information (DNA or RNA) surrounded by a protein coat called a capsid. Viruses are typically 0.01 to 0.1 um in size and are very species specific with respect to infection, typically attacking only one type of host (USEPA, 1999a). Viruses are fundamentally different from all other microorganisms in that they are incapable of replicating or adapting to environmental conditions outside of a living host. When not in contact with a host cell the virus remains in a dormant state, during which there are no biological

activities occurring within the virus (Carter, 2005). The virus's capsid coat is composed of protein polypeptides which contain amino acids such as glutamic acid, aspartic acid, and tyrosine. These amino acids contain weakly acidic and basic groups which give the virus a net charge upon ionization (Templeton, 2008). These net charges vary with changing pH because each ionizing polypeptide group has a different dissociation constant. At a certain pH, the isoelectric point (pI), the virion exists at a zero net charge. Above this point the virus will be negatively charged and below it will be positively charged (Templeton, 2008). As an example the bacteriophage MS2 has a pI of 3.9 (Templeton, 2006), showing that in most natural waters MS2, and most viruses, maintain a negative charge.

The life cycle of a virus is simple and can be illustrated using the bacteriophage T4. A bacteriophage is a virus which infects bacteria; T4 selectively infects the bacteria Escherichia coli (commonly E. coli) which is a gram negative bacterium that is commonly found in the lower intestine of warm-blooded animals. T4 exists as an inactive virus until it comes in contact with the surface of an E. coli cell. It uses sensors to recognize binding sites on the surface of the host cell, and this brings the bacteriophage out of its dormant state. The bacteriophage then binds to the surface of the E. coli, punctures the cell with its injection tube, and injects its genetic information into the host cell. The genetic information subverts the host cell's normal operations and sets the cells biosynthetic

machinery to work creating replicas of the virus. The newly created viruses escape from the cell and are inactive until they come in contact with a new host cell.

Viruses in the environment can maintain a dormant state for long time periods, but with the passage of extended periods of time outside of a host cell and especially with exposure to inactivating agents such as sunlight, temperature fluctuations, and pH changes, viruses begin to lose their infectivity. To enhance their survival in the open environment, viruses can protect themselves from environmental factors which can cause their loss of infectivity. This is accomplished by viruses interacting with surfaces of particles such as fecal matter, colloidal clays, soils, and biological and chemical floc particles. These materials can shield the viruses from the harmful environmental factors maintaining their infectivity.

Particles

According to the USEPA, turbidity is "a principal physical characteristic of water and is an expression of the optical property that causes light to be scattered and adsorbed by particles and molecules rather than transmitted in straight lines through a water sample." Some things which interfere with the clarity of water and add to the turbidity include clay, silt, organic and inorganic matter, plankton, and other microorganisms (LeChevallier, 1981; USEPA, 1999). Some sources of these

particles include runoff, waste discharges, or organic particles from the decay of plants, leaves, etc. (USEPA, 1999). The typical unit of turbidity measurement is the nephelometric turbidity unit (NTU). Turbidity is not only aesthetically unpleasing in drinking water, but also represents a health concern. It can provide shelter for pathogens and lead to regrowth of pathogens within the distribution systems even after disinfection. Studies have shown a relationship between turbidity removal and pathogen removal. Neefe et al. (1947) showed that water which was experimentally contaminated with feces containing infectious hepatitis virus and then treated by coagulation, settling, and filtering caused a 40% decrease in disease incidence as compared to untreated samples. When the treated sample was chlorinated the disease incidence went to zero. The untreated sample even when chlorinated still induced disease in volunteers. This study shows the relationship between turbidity removal and pathogen removal.

The particles which make up turbidity in raw water can be categorized into inorganic material, organic material, and biota (USEPA, 1999). Biotic particles include bacteria, viruses, algae, and protozoan (USEPA, 1999). Inorganic particles in water originate from the weathering of minerals and consist of iron oxides, salts, silts, and clays, as a few examples (USEPA, 1999; Templeton, 2008). They vary in diameter from several nanometers to several microns (USEPA, 1999). Organic particles

are compounds which have a carbon molecule as the base of their chemical structure. Both natural and synthetic organics can be found within surface waters. The main sources of organic matter consist of the decomposition of natural organic matter, such as plant matter, in the environment, human activities, such as industrial waste or agricultural runoff, and reactions occurring during water treatment (USEPA, 1999).

Both clays and organic matter can strongly adsorb ions, polar and non-polar molecules, and biological agents. Bitton et al. (1972) showed Klebsiella aerogenes was protected from UV light by both montmorillonite clay and humic acid. Clay minerals are found to display a high adsorptive capacity toward viruses because they have large amounts of surface area and a large ion-exchange capacity (Bitton, 1979).

Colloids are particles of the size 10 to 0.001 microns. They remain suspended in solution and do not settle out, nor can they be effectively removed by filtration alone. Coagulation is needed to form larger particles to then be removed (USEPA, 1999; Templeton, 2008). Colloidal removal is greatly impacted by electrokinetics, which is also one inducer of association among colloidal particles. Most colloidal particles in raw water are hydrophobic particles which are dependent on their electrical charge to remain in suspension. Each colloid carries a similar electric charge, in water most colloidal particles are negatively charged, which maintains a mutual electrostatic repulsion between neighboring particles

keeping them in suspension (USEPA, 1999). The environment surrounding a charged colloid is explained by the double layer model. A colloidal particle, which is negatively charged initially, attracts a layer of positively charged ions. This layer is called a Stern layer and is firmly attached to the colloid. Other positive ions are attracted to the negatively charged colloid while they are also being repelled by the Stern layer as well as by other positive ions that have also been pulled in by the negative colloid. This atmosphere of constant attractive and repulsive forces results is what is called a diffuse layer of charged ions surrounding the colloid and its Stern layer. Together the Stern layer and the Diffuse layer make up the double layer (USEPA, 1999).

Virus-Particle Association

It has been previously concluded that viruses and bacteria are regularly associated with organic and inorganic particles in water and when associated are protected from both chemical and physical disinfectants (LeChevallier et al., 1981; Schaub and Sagik, 1975; Bitton, 1972; Cantwell, 2006; Li, D. et al., 2008; Cantwell and Hofmann, 2008; Stagg et al., 1977; Berman et al., 1988; Templeton et al., 2005; Templeton et al., 2006). Viruses can become enmeshed in particles which are <10 um in diameter due to the small size of viruses. These sized particles can pass through well operated filters and other treatment processes. These

associated viruses, once thought to be inactivated, are now accepted to be as infective, if not more infective, as free virions (Bitton, 1979; Neefe et al., 1947). Schaub and Sagik found EMC virus adsorbed to montmorillonite clay were actually more infective than viruses in filtered water where no clay was present (Schaub and Sagik, 1975).

The association of colloidal particles with viruses is complex and highly dependent on the particle, the virus, and the environmental conditions, such as pH and dissolved constituents. Particles and viruses both have an overall negative charge in water. How these two negatively charged particles can become associated has to do, at a basic level, with the double layer model introduced in the previous section. When two colloidal particles which are correspondingly charged approach each other their diffuse layers interact. Their primary negative charges repel and this keeps the particles from associating, but this repulsion can possibly be counteracted by the attractive van der Waals forces. If the colloids can come amply close to overcome the electrostatic repulsion, van der Waals forces will dominate and the particles will be associated. Brownian movement is the random movement of colloids caused by collisions with water molecules. This Brownian movement can bring the particles close enough together for van der Waals forces to govern (USEPA, 1999; Templeton, 2005; Bitton, 1979; Lipson and Stotzy, 1983) Viruses in water act as hydrophobic

colloidal particles, and so hydrophobic interactions play a role in particle-association. Water molecules will group hydrophobic particles together causing more association between hydrophobic particles (viruses and particles). This arrangement is thermodynamically favored because it allows for less reordering of water molecules and so a maximization of hydrogen bonding among water molecules in the salvation layer surrounding the particles. This hydrophobic bonding is a consequence of the thermodynamically unfavored interaction of hydrophobic substances with water molecules and is not due to interactions between the hydrophobic particles themselves (Wait and Sobsey, 1983).

Dissolved constitutes, pH, and organic matter in water have all been found to affect virus-particle association. The pH of the water affects the thickness of the repulsive double-layer on both the virus and particle. At a higher pH, viruses and particles will behave as anions and will have strong repulsive forces. As the pH decreases, it allows for lower repulsive forces due to a thinning of the electron double layer. Schulze-Makuch et al. (2003) found at pH 6.1 there was a significant decrease in MS2 breakthrough in a model aquifer due to increased attachment to particles. Schaub and Sagik (1975) found that a pH increase from 5.5 to 9.5 caused a 2.5% decrease in virus adsorption. They also found that a decrease in pH to 3.5 caused a larger decrease in virus adsorption, decreasing it down 6.5%. This shows that if the pH decreases below or

close to the pI values of both the particle and the virus, association is hindered.

An increase in ionic strength causes an increase in virus adsorption (Lance and Gerba, 1984; Moore et al., 1982; Schaub and Sagik, 1975; Lipson and Stotzky, 1983). This increase is due to a decrease in the thickness of the layers of charged ions around the viruses and particles. This decrease in the double-layer thickness would allow the colloidal particles and virus to come close enough for van der Waals forces to take effect. Schaub and Sagik (1975) found an addition of 10^{-3} M $CaCl_2$ caused 98.4% of the virus to become attached to montmorillonite clay; in deionized water containing the same concentration of clay only 32% of the viruses were found to be attached. Reducing the divalent cation concentration to 10^{-4} M of $CaCl_2$ decreases the adsorbed percentage of viruses to 55% (Schaub and Sagik, 1975). Divalent cations increase virus adsorption, but multivalent cations, such as Al^{+3} and $PO4^{-3}$, have been found to have no influence on virus adsorption (Taylor et al., 1981).

A confounding factor is the location and orientation of the adsorbed viruses on negatively charged particles. Tailed phages, such as bacteriophage T4, have different charges for their heads as compared to their tails. The tail fibers are positively charged, in most tailed phages, so they will attach to the negatively charged sites on the clay molecule. This can leave the viruses head vulnerable to inactivating agents (Templeton

et al., 2006; Templeton et al., 2008). Different viruses will attach to different sites on particle surfaces. As an example, Coliphage T1 attaches to montmorillonite clay on the planar surface of the clay molecule. Coliphage T7, on the other hand, attaches to montmorillonite on the edge of the clay mineral (Templeton et al., 2006). These differing association sites can affect the shielding effect of the clay molecule.

Soluble organic compounds, such as microbial enzymes or heavy metals, have been found to hinder the virus-particle association (Mitchell and Jannasch, 1969). The organic compounds compete with the viruses for specific adsorption sites on the particle surfaces. Proteins and other soluble organics block negatively charged sites on particles which are important sites for the adsorption of viruses (Lipson and Stotzky, 1983; Lipson and Stotzky, 1984; Schaub and Sagik, 1975; Templeton et al, 2008; Wait and Sobsey, 1985). Lipson and Stotzky (1984) found the protein complexes Chymotrypisin and ovalbumin reduced the adsorption of reovirus to kaolinite and montmorillonite. Comparing the titers of experimental (clay-protein complexes) and control (clay only) virus suspensions it was seen that chymotrypsin reduced the adsorption of reovirus to kaolinite and montmorillonite by 26% and 66% respectively. Ovalbumin reduced the reovirus adsorption to kaolinite and montmorillonite by 39% and 45% respectively (Lipson and Stotzky, 1984). Schaub and Sagik (1975) found the addition of serum protein greatly

reduced virus adsorption. A mixture of deionized water, clay, and 10^{-3} M $CaCl_2$ allowed for 99.74% virus adsorption. The addition of 0.6 mg/ml of serum protein to this mixture decreased the virus adsorption to 16% (Schaub and Sagik, 1975).

Soluble organics can also disturb already associated viruses and particles. A typical virus elution method includes adding a protein-rich elutent such as tryptose broth or beef extract. The protein-rich elutent can disrupt the electrostatic bonds between virus and particle (Templeton, 2008). The smaller protein molecules insert themselves between the larger virus and their adsorption site on the particles. This reduces the strength of the bond and can cause the viruses to become resuspended (Wait and Sobsey, 1983). Overall, particle association depends on a number of factors; these factors include the type of virus, the type of particle, the concentration of the virus and the particles, and the physiochemical properties of the water.

Disinfection

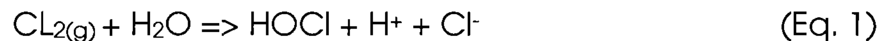
According to the USEPA, the three primary mechanisms of pathogen inactivation by disinfection are: "(1) destroy or impair cellular structural organization by attacking major cell constituents, (2) interfere with energy-yielding metabolism through enzyme substrates in combination with prosynthetic groups of enzymes thus rendering them

non-functional, and (3) interfere with biosynthesis and growth by preventing synthesis of normal proteins, nucleic acids, coenzymes, or the cell wall" (USEPA, 1999a). The primary factor controlling disinfection efficiency is the ability of the disinfectant to permeate into the cell and interfere with the cell activity (USEPA, 1999a; Templeton et al., 2008; Hoff et al., 1986). Maximum disinfection efficiency is achieved when the disinfectant agent has unhindered access to the target organism. This access is disturbed by particulate matter, either by creating a chemical disinfectant demand or by physically shielding the organism from the disinfectant (Steward and Olson, 1996). This shielding causes the "tailing" portion of the inactivation curves for both chlorine and ultraviolet light (USEPA, 1999a; Tchobanoglous, et al., 2003).

Chlorine Disinfection

The majority of all surface water and groundwater systems in the United States use chlorine for disinfection. It has a reputation for successful implication in improving water treatment operations. It also is effective in inactivating a large array of commonly found pathogens in water, and leaves a measurable and controllable residual for disinfection (USEPA, 1999a). Chlorine can react with organic and inorganic compounds to produce undesirable disinfection byproducts (DBPs) and can be hazardous to handle (USEPA, 1999a).

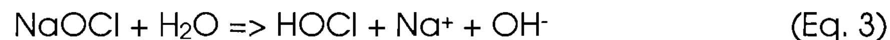
Chlorine used for disinfection is usually distributed in one of three forms. Chlorine gas when added to water quickly hydrolyses to form hypochlorous acid (HOCl) (Equation 1). Hypochlorous acid is a weak acid which will dissociate and become hydrogen and a hypochlorite ion (Equation 2) (USEPA, 1999a; Tchobanoglous, et al., 2003).



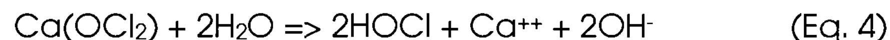
$$CL_{2(g)} + H_2O \Rightarrow HOCl + H^+ + Cl^- \quad \text{(Eq. 1)}$$

$$HOCl \Leftrightarrow H^+ + OCl^- \quad \text{(Eq. 2)}$$

Between 6.5 and 8.5 pH values both HOCl and OCl^- species are present. The total quality of HOCl and OCl^- present in water is the "free available chlorine." The allocation of these two species is important since HOCl has a 40 to 80 times stronger germicidal effect than OCl^- (Tchobanoglous, et al., 2003). Sodium hypochlorite contains about 12.5% available chlorine. The reaction of sodium hypochlorite in water is shown in Equation 3. Calcium hypochlorite contains about 65% available chlorine. The reaction of calcium hypochlorite in water is shown in Equation 4 (USEPA, 1999a).



$$NaOCl + H_2O \Rightarrow HOCl + Na^+ + OH^- \quad \text{(Eq. 3)}$$



$$Ca(OCl_2) + 2H_2O \Rightarrow 2HOCl + Ca^{++} + 2OH^- \quad \text{(Eq. 4)}$$

One of the most important factors for finding the germicidal efficiency of any disinfectant is the CT factor. The CT factor is a product of the Chick-Watson Law, which finds the disinfectant contact time. The

main Chick-Watson equation is seen in Equation 5, where N_t is the number of organisms at time t, N_o is the number of organisms when t equals 0, k' is the die-off constant, C is the concentration of disinfectant, n is the coefficient of dilution, and t is time (Tchobanoglous, et al., 2003). CT is defined as the concentration of the residual disinfectant, C, in mg/L, multiplied by the time, T (in minutes), that the residual disinfectant is in contact with the water. Because direct monitoring of individual organisms in the field is difficult, CT is used as a standard for disinfection design and performance and is based on the disinfectant, required log reduction, target organism, pH, and water temperature. Under the surface water treatment rule (SWTR), the USEPA developed CT values for Giardia and viruses (USEPA, 1999a; Tchobanoglous, et al., 2003; USEPA, 1999).

$$Ln\ (N_t/N_o) = -\ k'C^n t \qquad \text{(Eq. 5)}$$

Chlorine causes damage to the cell wall membrane, promotes leakage through the cell membrane, can produce lower levels of DNA synthesis, and can disrupt cell transport (EPA, 1999a). Environmental factors which influence chlorine inactivation efficiency include water temperature, pH, contact time, mixing, turbidity, interfering substances, and the concentration of available chlorine. Temperature and pH have the most significant effect on the germicidal effectiveness of chlorine. The distribution of chlorine species ($HOCl$ and OCl^-) is decided by pH, $HOCl$ being the stronger germicidal. $HOCl$ dominated at a lower pH, so

chlorination is more efficient at a low pH. It has been shown that higher temperatures increase chlorination efficiency (USEPA, 1999a; Tchobanoglous, et al., 2003). Chlorine is highly effective at bacteria and virus inactivation, but has limited success with protozoa, such as Giardia, and little impact on Cryptosporidium oocysts (USEPA, 1999a).

One major issue with chlorine disinfection is the formation of disinfection byproducts (DBPs). Trihalomethanes (THMs) and other DBPs are formed as a result of a series of complex reactions between free chlorine and a group of organic acids known as humic acids (Tchobanoglous, et al., 2003). Toxicology experiments have shown several DBPs are carcinogenic in laboratory animals, while other DBPs have shown adverse reproduction of development effects in laboratory animals. The Stage 1 and 2 Disinfectants and Disinfection Byproducts Rules have set maximum contaminant levels for harmful DBPs (USEPA, 2001a; USEPA, 2005). The formation of DBPs is related to total organic carbon (TOC) at the point of disinfection. They can be controlled by removing the DBP precursors (TOC, NOM), modifying the chlorination strategy, changing disinfectants, or removing the DBPs themselves (USEPA, 1999a).

Particle Effect on Chlorine Disinfection

Particles can hinder chlorine disinfection in two ways: either causing a higher chlorine demand or by shielding the organism from penetration

of the chlorine (Tchobanoglous, et al., 2003). LeChevallier et al. (1981) found that coliforms in high turbidity water (13 NTU) were reduced to only 20% of the initial count; where in low turbidity water (1.5 NTU) coliforms were undetectable. The chlorine dose ranged between 0.5-2.5 mg/L for 1 hr (LeChevallier, 1981). Turbid water samples were examined in an attempt to determine what factors could have led to increased survival. Scanning electron photomicrographs showed that bacteria were embedded in turbidity particles, appeared to be coated in amorphous material, or both. They also found that the chlorine demand of the water was positively correlated with both turbidity and total organic carbon (TOC). This positive correlation showed turbidity and TOC potentially impacts the maintenance of a free chlorine residual (LeChevallier, 1981).

Stagg et al (1977) found an increase in the time required to activate 99% of the starting virus titer (t_{99}) when comparing freely suspended virus and clay attached virus. At 0.5 mg/L HOCl dose the t_{99} doubles from 10 seconds for the freely suspended virus to 20 seconds for the clay attached virus. Berman et al. (1988) showed for water with particles larger than 7 um a 99% inactivation a CT of 2.7 was needed, while water with particles smaller than 7 um had a 99% inactivation at a CT of 0.9. These examples show how particles hinder chlorine disinfection efficiency.

Dietrich et al. (2003) found chlorine is capable of penetrating into particles through macroporous and microporous networks of pathways. The degree to which chlorine penetrates particles is influenced by a variable initial chlorine concentration (mg/L) at a fixed CT dose (mg-min/L). For a fixed CT dose of 270 mg-min/L and an initial chlorine concentration of 27 mg/L, particles up to 145 um showed inactivation of coliform bacteria. It is important to make the connection with increasing chlorine concentrations that may be needed to overcome particle-association shielding and disinfection byproducts in water caused by humic matter reacting with chlorine. Even though according to Dietrich, chlorine application could be tailored to penetrate particles of know size by adjusting chlorine dose and initial concentration, care should be taken to see what consequences could be brought about with ever increasing chlorine doses.

Ultraviolet Disinfection

Ultraviolet (UV) radiation energy waves are the range of the electromagnetic spectrum from 100 to 400 nm long. The UV range is divided into Vacuum UV (100-200 nm), Short wave or UV-C (200-280 nm), Middle wave or UV-B (280-315 nm), and Long wave or UV-A (315-400 nm) (Tchobanoglous, et al., 2003). The optimum range for germicidal effects is between 245 and 285 nm. UV radiation is produced when lamps

containing mercury vapor are charged by striking an electrical arc. The energy generated by the excitation of the mercury vapor results in the emission of UV light (Tchobanoglous, et al., 2003). UV disinfection uses three kinds of lamps: Low-pressure lamps which can be either low-intensity which uses mercury-argon lamps to generate in the UV-C regions wavelengths or high-pressure lamps which replaces mercury with a mercury-indium amalgam to provide greater stability and lamp life. Medium-pressure lamps emit energy at wavelengths from 180 to 1370 nm. Only about 7% to 15% of the output is near the optimal 254 nm wavelength, but they generate 50 to 100 times the total UV-C output of conventional low-pressure lamps. Finally there are lamps that emit at other wavelengths in a high intensity "pulsed" manner but these are not in widespread commercial use (Tchobanoglous, et al., 2003; USEPA, 1999a).

Ultraviolet radiation is a physical disinfectant. It penetrates the cell wall and is absorbed by the nucleic acids (DNA or RNA). The DNA or RNA is necessary for reproduction, so damage to these substances basically sterilize the organism. Damage usually results from the formation of double bonds (dimerization) in pyrimidine molecules. The three primary types of pyrimidine molecules are thymine found in DNA, uracil found in RNA, and cystosine found in both DNA and RNA. Once the pyrimidine molecules are bonded together by the double bond, replication of the nucleic acid becomes difficult because of the distortion of the DNA

helical structure (USEPA, 1999a). Many microorganisms have enzyme systems that repair damage caused by UV light. This repair does not prevent inactivation although it can increase the UV dose needed to achieve a given degree of inactivation. Repair mechanisms are classified as photorepair or dark repair.

During photorepair, enzymes energized by exposure to light between 310 and 490 nm (near and in the visible light range) break the covalent bonds that form the pyrimidine dimers. This repair requires reactivating light and repairs only pyrimidine dimers. Photorepair can be prevented by keeping the UV disinfected water in the dark for at least two hours before exposure to room light or sunlight (USEPA, 2006b).

Dark repair is defined as any process that does not require the presence of light. One form of dark repair, excision repair, is an enzyme-mediated process in which the damaged section of DNA is removed and regenerated using the existing complementary strand of DNA. The extent of dark repair varies with the microorganism. Because with bacteria and protozoa dark repair enzymes immediately start to act following UV light exposure, reported dose-response data (found in Table 4) are assumed to account for dark repair.

UV disinfection is sufficient for inactivating bacteria and viruses at relatively low doses, 2 to 6 mW s/cm^2 is enough for 1-log inactivation in many cases. Adenovirus however requires a significantly higher dose for

inactivation than most other pathogens of concern due to its ability to biochemically repair damage. Protozoan cysts and oocysts can be inactivated by UV light, although a somewhat higher dose is needed (USEPA, 1999a). Table 4 shows UV dose requirements for log inactivation of Cryptosporidium, Giardia, and viruses.

The effectiveness of UV disinfection is based on the UV dose. UV dose (D) is defined as UV intensity (I) (mW/cm^2) multiplied by exposure time (t) (sec).

$$D = I \times T \qquad \text{(Eq. 6)}$$

This dose term is akin to the chlorine disinfection dose term CT (Tchobanoglous, et al., 2003). UV dose is the integral of UV intensity during the exposure time. The total UV intensity at a point in space is the sum of the intensity of UV light from all directions. In a completely mixed batch system, the UV dose received by the microorganisms is equal to the volume-averaged UV intensity within the system (USEPA, 2006b).

Table 4: UV Dose Requirements (mJ/cm^2) (USEPA, 2006b)

Target Pathogens	Log Inactivation							
	0.5	**1**	**1.5**	**2**	**2.5**	**3**	**3.5**	**4**
Cryptosporidium	1.6	2.5	3.9	5.8	8.5	12	15	22
Giardia	1.5	2.1	3	5.2	7.7	11	15	22
Viruses	39	58	79	100	121	143	163	186

As UV light emits from its source, it interacts with constituents in the water through adsorption, reflection, refraction, and scattering. UV

absorbance or UV transmittance (UVT) is the parameter that incorporates the effect of adsorption and scattering on water quality. Absorption is the transformation of light to other forms of energy as it passes through a substance. The components of a UV reactor and the water passing through a reactor all absorb UV light to varying degrees depending on their material composition. When UV light is absorbed by these different components, it is no longer available to disinfect microorganisms (USEPA, 2006b).

The phenomena of refraction, reflection, and scattering change the direction of UV light, but it is still available to disinfect microorganisms. Refraction occurs in UV reactors when light passes through the interfaces from the UV lamp into an air gap, from the air gap into the lamp sleeve, and from the lamp sleeve into the water. The angle that UV light strikes target pathogens is changed as the UV light passes through the interfaces.

Reflection is the change in direction of light when it is deflected by a surface. Reflection may take place at interfaces which do not transmit UV light as well as interfaces which do. The material of the surface dictates the type and intensity of the reflection. Scattering is the change in direction of light caused by interaction with a particle. Particles can cause scattering in all directions, including back-scattering (USEPA, 2006b).

UV absorbance measures the decrease in the amount of light as it passes through a water sample over a specified distance. In UV disinfection, A^{254} (UV absorbance at 254 nm) is used to measure the amount of UV light passing through the water and reaching the target organisms. UV transmittance (UVT) is also widely used to describe the behavior of UV light. UVT is the percentage of light passing through material over a specified distance, and can be calculated using Beer's Law: as shown in Equation 7.

$$\%UVT = 100*(I/Io) \qquad \text{(Eq. 7)}$$

Where I is the intensity of light transmitted through the sample (mW/cm2), Io is the intensity of light incident on the sample (mW/cm2), and UVT is the UV transmittance at a specified wavelength (e.g., 254 nm) and pathlength (e.g. 1 cm). UVT can also be calculated by relating it to UV absorbance (A):

$$\%UVT = 100 * 10^{-A} \qquad \text{(Eq. 8)}$$

UVT is typically reported at 254 nm because UV manufacturers and PWSs widely use A^{254} (USEPA, 2006b; Tchobanoglous, et al., 2003).

UV reactor performance is affected by UVT, particle content, upstream water treatment processes, constituents that foul reactor components, and algae. UVT has a strong effect on the UV dose delivery. As UVT decreases, the intensity throughout the reactor decreases reducing the dose the reactor delivers. Typical UV absorbers include

soluble and particulate forms of humic and fulvic acids, other aromatic organics, metals, and anions.

Unit processes and chemical addition upstream of UV reactors can significantly affect UV performance. Water treatment processes upstream of the UV reactors can maximize UVT, thereby optimizing the design and costs of the UV reactor. The processes of coagulation, flocculation, and sedimentation remove soluble and particulate matter increasing UVT. The addition of oxidants (such as chlorine and ozone) degrades natural organic matter, reducing soluble material and precipitating metals which can increase UVT (USEPA, 2006b).

UVT is decreased by some chemicals used in water treatment, such as ferric iron, permanganate, and ozone, because they will absorb UV light. If a significant concentration of ozone is present in the water passing through a UV reactor, the UVT will be reduced. On the other hand, the addition of ozone (an oxidant) prior to UV disinfection can increase the overall inactivation by degrading NOM in the water (USEPA, 2006b).

Algal growth or occurrence can reduce UVT and interfere with the UV disinfection process. Algal growth may occur upstream or downstream of UV reactors. Visible light emitted from the lamps is transmitted through the water beyond their germicidal wavelengths. This growth depends on nutrient concentration in the water, hydraulics of the

systems, and the amount of light emitted beyond the reactor (USEPA, 2006b).

UV disinfection has been found to have no effect on many water parameters such as pH, dissolved organic carbon content, or UVT up to doses of 200 mJ/cm^2 (USEPA, 2006b). There can be byproducts produced from UV disinfection through photochemical reactions or reactions occurring with products of photochemical reactions. Photochemical reactions occur when UV light is absorbed into a chemical species and the resulting excited state reacts to form a new species. Mainly, the research has focused on the potential formation of halogenated DBPs after subsequent chlorination, the transformation of organic material to more degradable components, and the potential formation of other DBPs (USEPA, 2006b).

At UV doses less than 400 mJ/cm2, no significant affect on formation of THMs (trihalomethanes) or HAAs (haloacetic acids) has been found upon subsequent chlorination. Liu et al (2006) found a UV dose of 60 mJ/cm2 with subsequent chlorination cause some significant increases in concentrations of chloroform, DCAA (dichloroacetic acid), TCAA (trichloroacetic acid), and CNCl (cyanogens chloride), DBPs not regulated by the EPA, compared to cases using only chlorination. Non-regulated DBPs have also been found to form as a result of UV light doses greater than 400 mJ/cm^2 being applied to wastewater and raw drinking

water sources. At doses typical for UV disinfection in drinking water (<140 mJ/cm^2), however, no significant increase in DBPs was observed (USEPA, 2006b).

Particle Effect on UV Disinfection

Particles affect UV disinfection by decreasing the UV transmittance of the water affecting dose delivery or they can shield microorganisms from UV light. The shielding effect of clumping or particle-association can cause a tailing or flattening of the dose-response curve at high inactivation levels (Passantino et al., 2004; Wen-jun and Yong-ji, 2006; USEPA, 2006b).

Bitton et al. (1972) found bacterial survival during UV disinfection is directly correlated with the specific adsorption of the clay minerals. Halloysite, a clay with negligible absorbance had about 25% survival of K. aerogenes after 1 min of UV irradiation. K-montmorillonite, a clay with a high specific absorption had 75% survival of K. aerogenes after 1 minute of UV irradiation; showing the absorbance of the clay or particles is an important factor to be considered. Templeton et al. (2005) found a concentration of 150 mg/L of humic acid protected phage T4 and MS2 from UV inactivation, even after the absorbance due to the humic acid was accounted for in the UV dose calculation. This shows that humic acid can shield organisms from UV light, and that absorbance of UV light by

the particles is not the only effect of the particles. Cantwell and Hofmann (2008) found three natural water with relatively low turbidities (0.84, 0.85, and 2.9 NTU) exhibited significantly tailing (due to particle shielding) in their dose-response curves at a UV dose of 10 mJ/cm2. They found particles as small as 11 um were able to shield coliform bacteria even up to a UV dose of 40 mJ/cm2. Important to note, viruses are much smaller (0.01 to 0.1 um) than coliform bacteria and so could logically be shielded by much smaller particles.

In contrast, Batch et al (2004) found at low turbidities (<0.3 NTU), there was no significant decrease in inactivation of MS2 with increasing turbidities for both LP and MP lamps. For unfiltered waters, source water with turbidity up to 10 NTU had no effect on the ability of UV light to inactivate seeded MS2 virus (Passantino et al., 2004).

Elution

There are methods used to break apart or elude viruses which are attached to particles. Desorption of viruses may occur when the physiochemical properties of the water change, or physical forces are used. These physiochemical changes include lowering the ionic strength of water. This is illustrated with soil columns washed out with rain water. The rain water dilutes the soil column, lowers the ionic strength, and so there is a washout of viruses, thought to be attached to soil particles, into

the groundwater (Lance and Gerba, 1984). The addition of proteins or other soluble organics, such as beef extract, can lead to the elution of viruses from particles as was talked about in the virus association section of this paper (Rao et al., 1986; Wait and Sobsey, 1983; Lipson and Stotzky, 1984). Raising the pH of the water also helps to resuspended viruses because it increases the negative repulsive forces between the virus and the particle (Templeton et al., 2008; Wait and Sobsey, 1983). Physical shearing forces can be used to shear particle surfaces and break apart virus-particle association. These physical methods, often used in conjuncture with physiochemical methods, include vigorous mixing, sonication, and centrifugation (Wellings et al., 1975; Rao et al., 1986).

Wellings et al. (1975) treated wastewater with a 3% beef extract solution, raised the pH to 9.4 - 9.6 and sonicated or mixed the solution. Both the sonication and mixing methods showed about 60% efficiency of virus elution. Wait and Sobsey (1983) tested the ability of different elutents (beef extract, disodium EDTA, and citrate) to recover enteric viruses from estuarine sediments. A 3% concentration of beef extract eluted the viruses more efficiently than any of the other elutents. These experiments were also run at different pH levels to see if any effect was seen from raising the pH. At a pH of 5.5 the beef extract had 5% efficiency; compared to a pH of 10.5 where the beef extract had 22% efficiency. These results showed a higher pH does increase elution. These studies

show the typical method of elution which involves an addition of a protein elutant followed by a physical treatment.

Chapter III

Materials and Methods

Experimental Design

The experimental design of this research consists of three main phases. Phase 1 studied the effect of water parameters (TOC, calcium concentration, and pH) on particle-virus association to find optimal association conditions. Phase 2 (non-pellet method) studied the change in the dose-response of MS-2 virus for chlorine and UV disinfection when 5 NTU turbidity was present. These experiments were done in a batch experimental setup. The pH was buffered to 7 and the samples were directly measured to try to mimic more natural conditions. Phase 2 concluded with the chlorine dose-response curves showing an effect from the increased turbidity, but the UV dose-response curves showed no significant effect. The small particle-shielding effect for UV disinfection in Phase 2 led to a procedure which combined the findings and methods from phase 1 and the conditions from phase 2 to try to ensure particle-virus association. Phase 3 (pellet method) used a pH of 4.5-5, found in phase 1 to accelerate virus-particle association. The samples were

centrifuged after mixing for 24 hours. The supernatant and pellet were separated and used as the "free" and "associated" virus samples respectively. This more controlled procedure in phase 3 ensured virus-particle association where the previous procedure could not, and UV disinfection dose-response curves showed to be affected by the elevated turbidity.

Microbial Preparation

Tryptic Soy Broth

Sterile tryptic soy broth (DF0370, Fisher Scientific) was prepared according to manufacturer's specifications. 500mL of the broth was prepared and 2.5 mL of 1% $MgCl_2$ per 500 ml of broth was added before autoclaving at 121 degrees Celsius, 15 psi for 15-20 minutes. After the mixture cooled, 5 mL of a Strep/Amp antibiotic solution was added.

The Strep/Amp antibiotic solution was prepared by adding of 0.15 grams of each Streptomycin Sulfate Salt (S6501-50G, Sigma-Aldrich Inc.) and Ampicillian Sodium Salt (A9518-5G, Sigma-Aldrich Inc.) to 100 mL of DI water. The mixture was filter sterilized (syringe and 0.22 um syringe filter) under a hood into 50 mL sterile conical tubes. The antibiotic mixture was stored at 4° C in the dark for up to a month.

Escherichia coli F_{AMP}

Escherichia coli F_{AMP} (ATCC 15597) was grown in 50 mL portions of tryptic soy broth supplemented with 0.25 mL of 1% Magnesium Chloride (BP214-500, Fisher Scientific) solution and 0.5 mL of the Strep/Amp antibiotic solution. 5 mL of E. coli F_{AMP} stock culture was added to the broth and stirred continuously on a stir plate to aerate the culture for 18-24 hours at 37° C.

On the day the culture was to be used, a 5 mL of previously grown culture was added to 50 mL of fresh tryptic soy broth supplemented with the 1% magnesium chloride solution and Strep/Amp antibiotic solution as described above. The culture was grown for 3.5 hours with continuous stirring at 37° C.

All microbial cultures were stored at 4° C for short-term storage and at -80° C for long-term storage. For long-term storage, the cultures were first mixed with 10% glycerol to prevent bacterial cell damage.

MS2 Bacteriophage Propagation

MS2 Bacteriophage (ATCC 15597-B1) propagation was performed by first preparing 50 mL of sterile tryptic soy broth containing 0.25 mL of 1% magnesium chloride and 0.5 mL of the Strep/Amp antibiotic solution with 5 mL of E. coli F_{AMP}. The culture was grown for 3.5 hours at 37° C with

continuous stirring. After 3.5 hours, 0.1 mL of the MS2 stock culture was added to the actively growing E. coli F_{AMP}. The culture was incubated at 37° C for 16-18 hours without stirring. After overnight incubation, the culture was poured into sterile, Nalgene ® centrifuge bottles and centrifuged in a Beckman Coulter J2-HS centrifuge at 9000 revolutions per minute for 15 minutes. The supernatant was collected, and concentration of the phage was enumerated using the double agar overlay method. The MS2 was stored in a sterile plastic container at 4° C. The MS2 titer will decrease about a log every 8-12 months.

MS2 Enumeration

A standard double agar overlay assay was used for enumeration. To prepare the overlay, 15 grams of tryptic soy broth, 5 grams of yeast extract, 7.5 grams of bacto agar, and 0.075 grams of calcium chloride (C79-500, Fisher Scientific) were added to 500 mL of DI water. This mixture was stirred and brought to a boil. The overlay mixture was allocated at 5 mL each into sterile 20 mL test tubes, capped and autoclaved at 121° C for 15 minutes, removed, and placed in a 52° C water bath.

The bacteriophage culture was serially diluted to a suspected endpoint titer. The dilutions were begun by adding 100 µL of the undiluted stock to 900 µL of RO water. The process was then repeated serially to the desired dilution. To enumerate the phage by the double agar overlay method, 100 µL of the appropriate bacteriophage dilution was added

into 5 mL of overlay containing 200 µL of actively growing E. coli F_{AMP}. This was repeated in triplicate for each dilution. The overlay mixture was then poured into a previously poured tryptic soy agar petri dish. The plates were allowed to dry on the bench for about 15 minutes and were then are inverted and placed at 37° C for 16-18 hours. The plates were observed for plaques the next day. Plaques were visualized and counted for each dilution with a light box. Each plaque was assumed to represent one phage and was referred as a plaque forming unit (PFU). To calculate a geometric mean titer (pfu/mL); only plates containing between 30-300 plaques were used.

Clay-Virus Mixture

Montmorillonite Clay Preparation

Montmorillonite Clay (Wards Natural Science Cat. # 46E0435) was prepared by weighing out 100 grams of the clay and adding it to a 1000 mL flask with a stir bar. 1L of DI water was added and the mixture was stirred on a stir plate. Once mixed sufficiently, approximately 250 mL of the mixture was poured into each of 4 autoclaved, 500 mL Nalgene centrifuge bottles. The centrifuge bottles were placed into a Branson 5510 ultrasonic mixer (Branson Ultrasonics Corporation, Danbury, CT) overnight. After mixing overnight the bottles were centrifuged at 9000 revolutions per minute for 30 minutes. The supernatant was discarded and replaced with

fresh DI water. This overnight mixing and centrifuging was repeated two more times to ensure the clay was cleaned. After the third centrifuge, the pellet had separated into a top jelly-like layer and a bottom sandy mix layer. The jelly-like layer was scraped from each bottle and placed into a 2000 mL glass flask. One liter of DI water was added and mixed to resuspend the clay. Then 2.0 moles (M) of sodium chloride (S671-500, Fisher Scientific) were added and mixed until dissolved. This mixture was poured into 4 new 500 mL Nalgene centrifuge bottles, mixed for 2 hours in the ultrasonic mixer, and centrifuged at 9000 rpm for 30 minutes. The supernatant was then discarded and replaced with new DI water. This 2 hour mixing and centrifugation was repeated two more times. After the third centrifuge, pour off the supernatant into a 1000 mL flask to allow total mixing. This supernatant is then tested for chloride by titrating with 0.041 N $AgNO_3$.

The clay pellets were washed with DI water for 2 hours, centrifuged, and tested for chloride until the supernatant is free of any chloride residual. Once the chloride residual was gone, the clay pellets were resuspended for 2 hour in the ultrasonic mixer and poured into one 1L Nalgene bottle and stored at 4° C for up to 2 weeks.

Clay, Groundwater, MS2 mixture

Groundwater was collected from a well in front of Gregg Hall on the University of New Hampshire campus in Durham, NH. The temperature,

pH, and TOC for the groundwater were taken at the time of collection. The clay solution was prepared as discussed above. The MS2 stock was prepared as previously mentioned, and a plaque count of 10^9 was achieved.

Six flasks were labeled 1 NTU, 200 NTU, 400 NTU, 600 NTU, 800 NTU, and 1000 NTU. Each bottle was filled with 250 mL of groundwater and adjusted to a pH of 7 using a phosphate buffer. The clay solution was added to each flask to attain the correct turbidity, as they were labeled. This was confirmed using a LaMotte 2020 turbiditmeter (LaMotte, Chestertown, MD). After the desired turbidities were reached, some solution was emptied until each flask again contained 250 mL. For the MS2 addition, the theory was to have a 10^6 MS2 pfu/mL. Using the equation $C_1V_1 = C_2V_2$, the correct amount of virus was added to each flask to attain the correct concentration.

Phosphate Buffer Solution

0.001M of sodium bicarbonate (S233-500, Fisher Scientific) was added into CDFW (chlorine demand free water) (0.084 g/L). This mixture was then autoclaved at 121 degrees C for 15 minutes. After it cooled to 25 degrees C, final adjustments were made to pH 7 by using filtered and dried standard CO_2.

Chlorine Demand Experiments

Mixtures of groundwater and MS2 (10^6 PFU/mL) and groundwater, MS2 (10^6 PFU/mL) and clay (5 NTU) were prepared and buffered to pH 7 using a phosphate buffer. Six 500 mL flasks and stir bars were prepared chlorine demand free and autoclaved. Three were filled with 250 mL each of the no clay solution (groundwater and MS2) and three were filled with 250 mL each of the clay solution (groundwater, MS2, and enough clay to have a turbidity of 5 NTU), and placed on stir plates at a high enough rpm to create a vortex. A 100 mg/L Sodium Hypochlorite (NaOCl) (SS290-1, Fisher Scientific) solution was made using CDFW at the time of the experiment in brown glass. This is used to dose either 2 mg/L, 1.5 mg/L, or 1 mg/L into the 250 mL of the clay and no clay solutions. Samples were taken at 1, 5, 10, and 30 minute intervals, and the free Cl_2 concentration in mg/L were found using the DPD Colorimetric Method (4500-Cl, Standard Methods) with a Hach DR/2000 direct reading spectrophotometer.

Chlorine Particle Shielding Experiments

One liter mixtures of 500 mL of groundwater (GW) and 500 mL of the phosphate buffer solution (buffer) were prepared and a pH of 7 was finalized by bubbling filtered dried standard CO_2 through the buffer solution. This GW-buffer mixture was split into two beakers, one containing

500 mL and one containing 250 mL of the mixture. The 500 mL beaker was spiked with enough MS2 to obtain a 10^6 MS2 concentration; this was the no clay sample. The 250 mL sample had 250 mL of the clay mixture added to it and enough MS2 to obtain a 10^9 MS2 concentration, this was the clay mixture. This ensured a high particle count was allowed to come in contact with the virus to allow for attachment to occur. Both the clay and no clay mixtures were allowed to mix for 20-24 hours. After mixing, 250 mL of the no clay mixture was poured into a 500 mL beaker with a stir bar. Another 500 mL beaker was filled with 250 mL of the GW-buffer mixture and was adjusted to a pH of 7 using the standard CO_2. Enough of the clay mixture was added to this to obtain 5 NTU turbidity and a 10^6 MS2 concentration; this was perfected through trial and error before the experiment took place. The idea here was to allow attachment to occur in the 24 hour mixing period and then transfer just 5 NTU of that virus-particle mixture to see if that amount of turbidity would affect the disinfection efficiencies.

Dilution tubes were prepared, for each sample to be taken at time zero (before chlorine dosing), 1, 5, 10, and 30 minutes, with 500 µl of 10 mg/L sodium thiosulfate to dechlorinate samples. Samples of 500 µl were taken at time zero, 1, 5, 10, and 30 minutes, and placed in the dilution tubes containing 500 µl of 10 mg/L sodium thiosulfate solution. The 250 mL clay and no clay samples were dosed with the approximate amount of

chlorine stock. 100 mg/L chlorine stock was made up at the time of each experiment. 500 µl samples were taken at the approximate times and placed into the dilution tubes with the sodium thiosulfate. These samples were then diluted out to 10^{10} PFU and plated in duplicate using the double agar overlay method. The plates were allowed to dry on the bench for about 15 minutes and then were inverted and placed at 37° C for 16-18 hours. The plates were observed for plaques the next day. Plaques were visualized and counted for each dilution with a light box. Each plaque was assumed to represent one phage and was referred to as a plaque forming unit (PFU).

Chlorine Sonication Experiments

The same procedure was followed as for the particle shielding experiment except the amounts were doubled for the no clay and the clay mixtures. This gives 4 flasks, 2 flasks each with 250 mL of the no clay mixture (10^6 MS2) and 2 flasks each with 250 mL of the clay mixture (5 NTU, 10^6 MS2). One 250 mL flask for each the clay and no clay mixtures were placed into autoclaved Nalgene bottles and sonicated for 10 minutes and then placed back into their flasks and dosed with the appropriate chlorine dosage. Samples were taken after the correct contact times, diluted, and plated as stated above. The Branson 5510 ultrasonic mixer

was warmed up for at least 15-20 minutes before the samples were placed inside.

Collimated Beam Studies

The Collimated Beam was chosen for these experiments due to its high level of condition control and its accuracy and repeatability (Bolton and Linden, 2003). Collimated beam studies were run using a low-pressure high output Infilco Degremont collimated beam unit (Infilco Degremont, Richmond, VA) equipped with a low-pressure mercury UV lamp. The water samples were prepared as explained in the chlorine particle shielding procedure. The 254-nanometer absorbance of the waters was found using a U-2000 Hitachi Spectrophotometer (Hitachi Instruments Inc.). The irradiance of the lamp at 254 nm was read by a recently calibrated research radiometer, IL-1700 (International Light, Inc. Newburyport, MA). The fluence or UV dose (lamp intensity x time) was calculated according to a spread sheet created by James Bolton of Bolton Photosciences, Inc. (IUVA 2002). Bolton's spreadsheet corrects for the spectrum of light passing through the 254 nm filter, the radiometer sensitivity, the Petri factor, the UV absorbance and the depth of the sample. The Petri factor was needed because the irradiance varies over the surface of the surface area of the sample. This factor was found by scanning the radiometer detector in increments over the area of the petri dish at the height that the water surface of the sample will be placed

(Bolton and Linden, 2003). The depth of the 15 mL of water in the dishes was 0.65 cm. The depth and the distance from the lamp to the surface of the samples were entered previously into the Bolton spreadsheet. The samples were stirred continuously during irradiation for the specific times found using the Bolton spreadsheet to achieve the desired UV doses. The desired UV doses which were used are 20, 40, 60, 80, and 100 mJ/cm^2.

Two methods were used for the ultraviolet light experiments. These methods showed a progression in the procedures. The non-pellet method was first used to mimic real water conditions. This method was found to not be sufficient for ensuring virus-particle association. The pellet method which was originally used for testing the effect of water parameters on virus-particle association was modified to be used for collimated beam testing because this method could ensure virus-particle association.

Ultraviolet Light Particle-Shielding and Sonication (Non-Pellet Method)

The same clay and no clay mixtures were prepared as for the chlorine particle shielding and sonication procedures. Dilution tubes were prepared for samples at doses of 20, 40, 60, 80, and 100 mJ/cm2. Small petri dishes were labeled in duplicate for the different doses for both clay and no clay samples for the particle shielding experiments and for both sonicated and nonsonicated for the sonication experiments. All Petri dishes were prepared with micro stir bars. 15 mL samples of the different

mixtures were placed in the appropriate petri dishes. These plates were then exposed to the UV light in the collimated beam for the appropriate time found using the Bolton Spreadsheet (as explained in the procedure above). All samples were diluted 10-fold with dilution tubes and plated in duplicate and counted as explained in the chlorine particle shielding procedure.

Pellet Method for Water Parameter Testing

This method was used to see what affected particle association, as well as, to try and achieve greater particle-virus association. Enough clay was added to a known titer of MS2 to attain a turbidity of 1,000 NTU. This high turbidity was used to allow for the virus to come in contact with a large amount of particles. Clay and virus were mixed for 24 hours and then allowed to settle. The mixture was centrifuged to remove the solids into a pellet. An MS2 assay was performed on the centrifugate to get a final unassociated MS2 titer (pfu/mL).

pH

The same procedure (pellet method) was used. The pH was varied during the 24 hour mixing stage from 3-9.

TOC

The total organic carbon was varied from 2-9 mg/L during the 24 hour mixing period with the addition of Swanee River Fulvic Acid.

Calcium

The calcium concentration was varied from 10-70 mg/L as calcium during the 24 hour mixing period by the addition of calcium carbonate ($CaCO_3$).

Collimated Beam

The same pellet procedure was used as explained above. The most optimal pH, TOC, and calcium concentrations were used to optimize particle-virus association. Once the centrifugate was removed after the 24 hour mixing period and centrifugation, the pellet was resuspended by rapid mixing for 60 minutes. The appropriate UV dose was applied to both the centrifugate and the resuspended pellet sample. The samples were then plated and final MS2 titers for both the disperse (free) and the particle-associated (resuspended pellet) virus were counted.

Pellet Method for Ultraviolet Light Testing

Water Preparation

A water sample was prepared the day before UV exposure. Enough montmorillonite clay stock solution was added to RO lab water to attain a turbidity of 5 NTU. The turbidity was lowered to 5 NTU (max

turbidity allowed by the EPA for unfiltered waters) to test if this turbidity affected disinfection (USEPA, 2006b). MS2 virus was then added to attain a 1×10^7 pfu/ml concentration. Although natural waters do not have such a high concentration of viruses, this exaggeration was used so that there would be virus growth and quantifiable data generated for statistical analysis. The pH of the water sample was controlled in order to maximize the association of particles of montmorillonite clay with the MS2. 4.5 was found to be the ideal pH for MS2 association. Using a dilute (1:100) HCl (hydrochloric acid) solution the pH of the water sample was maintained between 4.5 and 5. This mixture was then allowed to mix for 24 hours. After 24 hours of mixing, the mixture was centrifuged for 15 minutes at 9,000 rpm and 4 degrees Celsius. A pellet formed in the centrifuge bottle. This pellet was assumed to contain the particle-associated virus. The supernatant was collected and used as unassociated virus because it contained viruses that remained free floating in the water. The pellet was resuspended for 3 hours by slow mixing in groundwater (collected from well in front of Gregg Hall at the University of New Hampshire in Durham, NH every 2 weeks) buffered to pH 7. This was intended to mimic natural conditions of water supplies.

Collimated Beam Testing

Times were calculated for the samples using the Bolton Spreadsheet as explained in the Collimated Beam Studies section above. Both the

samples were transferred to 12 petri dishes, 6 for particle associated (pellet) virus and 6 for free virus. The covers were removed and each sample was exposed to the UV light for the specified amount of time while being continuously stirred using a micro stir bar. Once the sample had been exposed for the appropriate time, it was sonicated for 10 minutes using a Branson 5510 ultrasonic mixer which was warmed up for at least 15-20 minutes before the samples were placed inside. This step was added to break up the particle association that had occurred in order to get an accurate count on viable MS2 in the sample after UV irradiation. Without this step some particle-associated viruses may have remained inside the clay particles and caused the results to misrepresent the actual conditions. After sonication the samples were diluted ten-fold. Each dilution was plated with duplicates using the overlay technique; the plates were incubated overnight (about 18 hours) before reading. MS2 was visible as circular areas on the plate where the *E. coli* lawn was broken up. Each circle was assumed to be one plaque forming unit (pfu), and plate counts between 30 and 300 pfu were recorded.

Statistical Analysis

An analysis of variance (ANOVA) is a technique for analyzing the way in which the mean of a variable is affected by different factors. The factors being compared in the experiments (Table 13) are clay (C) in the sample and sonication (S). The idea is to find the chance of incorrectly

concluding that there is a difference between two groups (rejecting the null hypothesis), as an example incorrectly concluding that experiment 1 (1.1 mg/L chlorine dose) shows a difference in the dose-response of the samples with clay (C) and those without clay (NC).

The p-value shows the probability of making this incorrect conclusion. When the p-value is small, it can be concluded that there is a significant difference between two groups and it is acceptable to reject the null hypothesis. A p-value which is less than 0.05 is commonly used as a cut-off, meaning 5% is the significant level above which the null hypothesis can not be rejected and no significant difference is seen between the factors.

Chapter IV

Results and Discussion

Water Properties Effect on MS2-Particle Association

pH

The pH trial showed the optimum pH for MS-2-particle association ~ 4 to 5 (Table 5). At a pH of 4 and 5 the unassociated MS2 count is 2-logs lower than at other pH values, showing more of the virus was removed along with the solids during centrifuging. At a pH of 4, the MS2 concentration was 8.5×10^2 PFU/mL, and at a pH of 5 the MS2 concentration was 1.2×10^3 PFU/mL. At a pH of 3 or 6, the amount of unassociated MS2 increases to 9.9×10^4 and 9×10^4 PFU/mL respectively. For these pH trials, no total organic carbon was added, the ionic strength was fixed using NaCl, and the calcium concentration was added to set the desired level of 20 mg/L based on the level in typical soft waters.

When pH is increased both particles and viruses behave as anions and repel each other (Schulze-Makuch et al, 2003). A decrease in pH allows for a reduction of these repulsive forces allowing the particle and virus to come close enough for van der Waals forces to take effect. The

data shows that between a pH of 5 to 6, the electron double-layer increases in size enough that association is hindered. A decrease in pH below 4 also shows a decrease in association. This coincides with the pH of the isoelectric point (pI) for the clay and virus. MS2 has a pI of 3.9 (Templeton, 2006) and montmorillonite clay has a pI of 2.5 (Cai et al., 2007). The decrease in association from pH 4 to pH 3 is due to the MS2 undergoing charge reversal as it falls below its pI and the clay approaching its pI, so the electrostatic forces between the two colloids are weakened. A similar pattern was shown by Schijven and Hassanizadeh (2000) where the attachment of MS2 decreased with increasing pH, but hit a plateau at a pH of 5.5-6, remaining constant for higher pHs. They found most attachment to occur between 3.5 and 5.5, similar to the results shown in this research. It is important to note that although the optimum pH for association was ~ 4 to 5; the chlorine and non-pellet UV light disinfection experiments as well as the water parameter experiments were performed at a pH of 7 to mimic more typical real-life applications. The pellet UV light experiments used the optimum pH (between 4-5) for association.

Table 5: Effect of pH on MS2 Association (n=1)

Target pH	MS2 Initial (pfu/mL)	MS2 Final (pfu/mL)	log Change in MS2	Actual pH
3	1.20E+06	9.90E+04	1.08	3.22
4	1.05E+06	8.50E+02	3.09	3.95
5	1.85E+06	1.20E+03	3.19	5.01
6	1.00E+06	9.00E+04	1.05	5.98
7	1.15E+06	6.50E+04	1.25	7.00
8	9.80E+05	8.90E+04	1.04	7.89
9	1.35E+06	1.05E+05	1.11	8.79

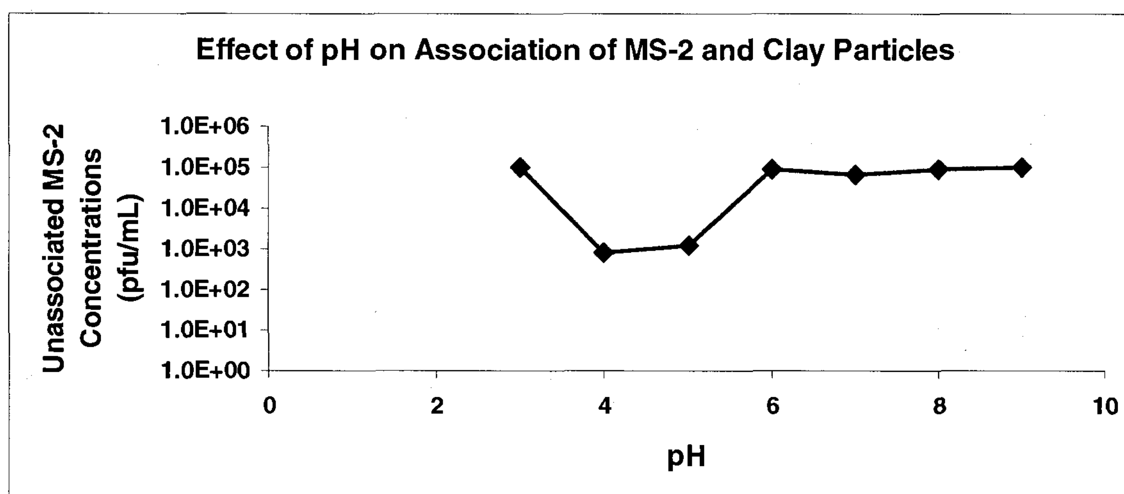


Figure 2: Effect of pH on Association of MS2 and Clay Particles (Ca^{2+} concentration is 20 mg/L, no addition of total organic carbon)

Total Organic Carbon

The unassociated MS2 concentration did not show high variability when the total organic carbon concentration was varied from 1.97 mg/L to 8.9 mg/L (Table 6). The highest unassociated MS2 concentration (2.42 x10^5 pfu/mL) was read at a TOC concentration of 4.97 mg/L, and the lowest unassociated MS2 concentration (7.5 x 10^4 pfu/mL) was read at a

TOC concentration of 7.56 mg/L, there is not a definite trend (Figure 3). For all TOC concentrations, the difference in unassociated MS2 concentration was maintained around a 1-Log difference (Table 6 and Figure 3).

The pH was kept constant at 7 for these experiments, the ionic strength was fixed using NaCl, and the calcium concentration was fixed at 20 mg/L. There was a 1.25 log difference for a pH of 7 showing about a 1-log increase in particle-associated MS2 (Table 5). For a TOC concentration of 3.58 mg/L, the log difference was 0.89 (Table 6). This shows about a 0.36-log decrease in MS2 association due to the increased TOC concentration. This decrease is typical to what was found in the literature. Sobsey and Hickey (1985) showed with the addition of 3 mg/L of fulvic acid as TOC at a pH of 7.5, there was a 40% decrease in poliovirus adsorption. Organic matter has been shown to hinder virus-particle association due to competition with viruses for the same binding sites (Gerba, 1984). In these experiments TOC was added using Swanee River Fulvic Acid. Stagg et al. (1977) found that the presence of 3 mg/L of TOC reduced adsorption of MS2 virus to bentonite clay from 35-95%.

Gerba and Lance (1978) made argued that the effect of dissolved organic matter may not always be apparent. They found that an increase in TOC from 10 mg/L to 70 mg/L had no effect on the adsorption of poliovirus 1, due to an excess of adsorption sites on the soil media.

Likewise, Powelson et al. (1993) found that when comparing secondary and tertiary wastewater effluent, there was no difference in the adsorption of MS2. The Bacteriophage PRD1 had 3 times less adsorption in the secondary effluent than the tertiary effluent.

Table 6: Effect of Total Organic Carbon on MS2 Association (n=1)

MS2 Initial (pfu/mL)	MS2 Final (pfu/mL)	log Change in MS2	Actual pH	Measured TOC (mg/L)
2.10E+06	2.00E+05	1.02	6.95	1.97
1.03E+06	1.13E+05	0.96	6.89	2.66
1.16E+06	1.49E+05	0.89	6.86	3.58
2.22E+06	2.42E+05	0.96	7.05	4.97
1.25E+06	9.70E+04	1.11	6.98	6.03
9.40E+05	7.50E+04	1.10	7.02	7.56
1.76E+06	1.75E+05	1.00	6.99	8.90

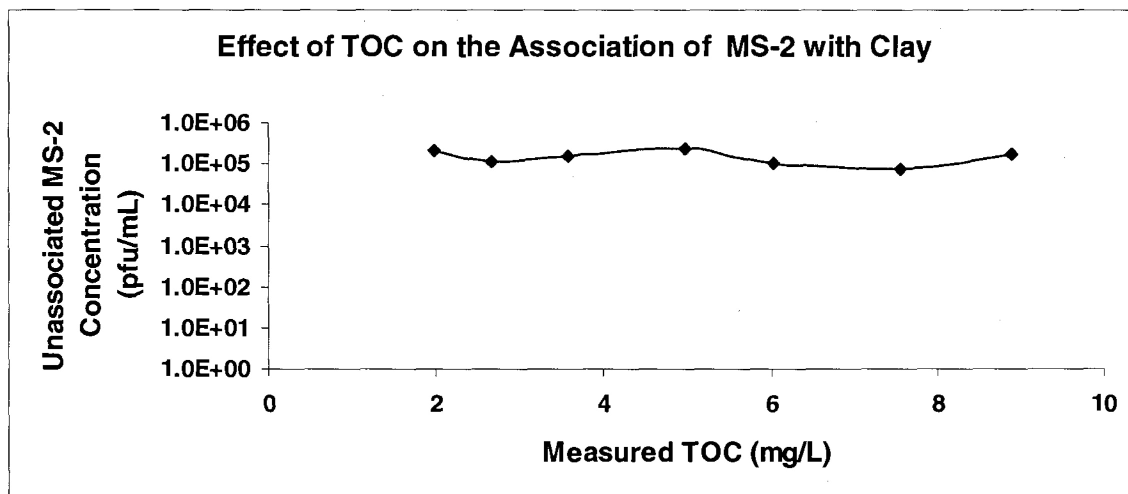


Figure 3: Effect of TOC on the Association of MS2 with Clay (Ca^{2+} concentration of 20 mg/L, pH buffered to 7)

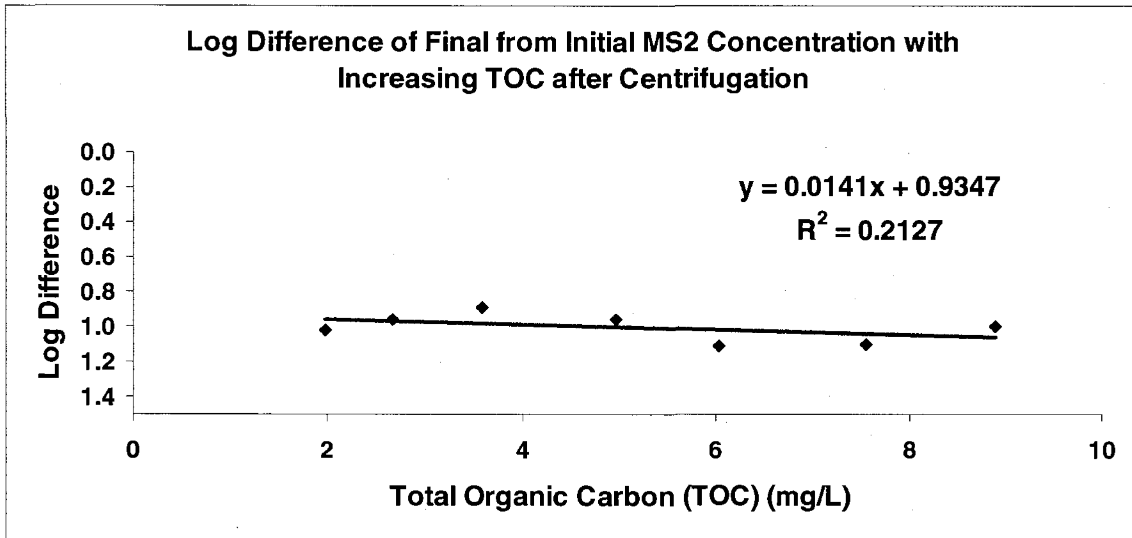


Figure 4: Log Difference of Final from Initial MS2 Concentration with Increasing TOC (Ca^{2+} concentration of 20 mg/L, pH buffered to 7)

Calcium Concentration

An increase in calcium caused a decrease in the unassociated MS2 concentration. More MS2 was becoming associated with the clay particles and so being removed in the centrifuge process with increasing calcium concentrations. A measured calcium concentration of 10.1 mg/L gave an unassociated MS2 concentration of 1.45×10^5 pfu/mL. When the calcium concentration was increased to 50.5 mg/L there was a decrease in the unassociated MS2 concentration to 1.8×10^3 pfu/mL, and at 71.9 mg/L of calcium the unassociated MS2 concentration decreased to 1.25×10^2 pfu/mL (Table 7 and Figure 5). Figure 6 shows the log differences in the unassociated MS2 concentration with increasing calcium concentrations. At 10.1 mg/L of calcium, there was only a log difference

of 0.82, where at 71.9 mg/L of calcium there was almost a 4-log difference showing most of the original MS2 was contained in the pellet after centrifuging.

The pH was kept at 7, the ionic strength was fixed using NaCl, and there was no TOC added for this experiment. The findings in Table 5 show the log difference for a pH of 7 to be 1.25, showing a 1.25-log increase in particle-associated MS2. In Table 7, for the middle calcium concentration of 42.1 mg/L as calcium the log difference was 1.35-logs. This showed a 0.1-log increase in MS2 association due to the calcium concentration. At the highest calcium concentration, 71.9 mg/L as calcium, the log difference increased to 3.99-logs. This showed a 2.74-log increase in MS2 association due to the increase in calcium concentration.

An increase in the calcium concentration probably leads to an increase in virus adsorption due to the increase in Ca^{2+} ions (Jenkins and Snoeyink, 1980). Multivalent ions have been shown to link virus and particles of like charge forming salt bridges between them (Schijven and Hassanizadeh, 2000). Bales et al. (1991) found a 10 fold increase in MS2 attachment in the presence of 10 mg/L Ca^{2+} than in control samples. This is consistent with the data found in Table 7, after 10-20 mg/L Ca^{2+} addition there was about a 10-fold increase in attachment.

Controlling Factor for Association

For MS2 under the conditions studied in this research, pH and ionic concentrations have a much higher effect on adsorption than the TOC content. When comparing the TOC data and the pH data, at a pH of 6-7, the pH at which the TOC and calcium experiments were conducted, the log difference in the unassociated MS2 concentration is about 1-log. The log difference is maintained at about 1-log until about a 42 mg/L calcium concentration is reached (Table 7). This is the point where the ionic concentration begins to influence the adsorption in conjunction with pH effects. Therefore, pH was the controlling factor for MS2 adsorption under these conditions. Calcium can act as a coagulant due to the fact that complexation of calcium with colloids can reduce the negative charge allowing the colloids to clump together and settle (Tchobanoglous, et al., 2003). If the negative charge and so the diffuse layer of the viruses or particles was reduced due to complexation with calcium, it would encourage association. The increase in calcium concentration caused the highest increase in virus-particle association with a 2.74-log increase. A decrease in pH from 7-4 caused a 2-log increase, and an increase in total organic carbon content caused a decrease in association by 0.38-logs.

MS2	MS2	log	Actual	Measured	Measured Calcium	Calculated Calcium Hardness
Initial	Final	Change	pH	TOC (mg/L)	(mg/L as Calcium)	(mg/L as CaCO3)
(pfu/mL)	(pfu/mL)	in MS2				
9.50E+05	1.45E+05	0.82	6.95	1.97	10.1	25.22
9.80E+05	8.80E+04	1.05	6.89		20.4	50.94
1.11E+06	1.25E+05	0.95	6.86		30.0	74.91
1.01E+06	4.50E+04	1.35	7.05	2.21	42.1	105.12
1.00E+06	1.80E+03	2.74	6.98		50.5	126.10
9.50E+05	6.90E+02	3.14	7.02		59.8	149.32
1.23E+06	1.25E+02	3.99	6.99	2.18	71.9	179.53

Table 7: Effect of Calcium on MS2 Association (n=1)

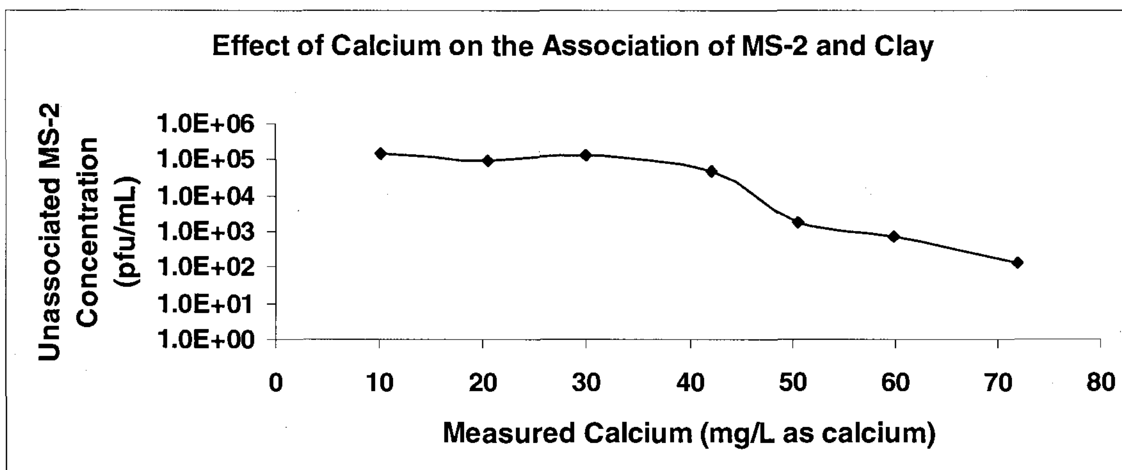


Figure 5: Effect of Calcium on the Association of MS2 and Clay (pH buffered to 7, no addition of total organic carbon)

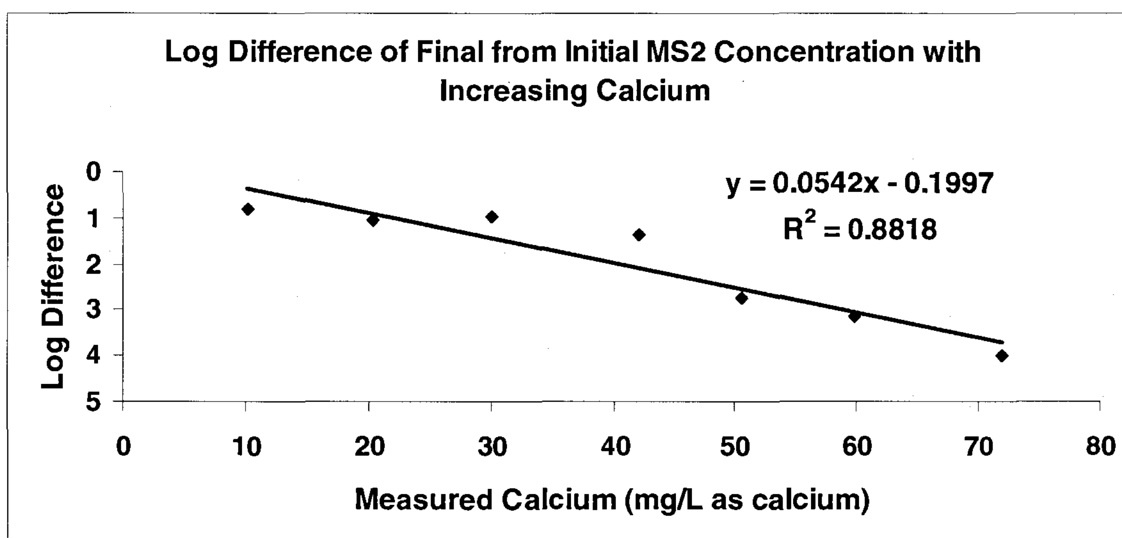


Figure 6: Log Difference of Final from Initial MS2 Concentrations with Increasing Calcium Concentrations (pH buffered to 7, no addition of total organic carbon)

Chlorine Demand

The chlorine demand results for the groundwater and phosphate buffer mixture showed a 0.4 mg/L chlorine concentration decrease over a 30 minute period for the 1.1 mg/L chlorine dosage (Table 8 and Figure 7). For the same water, a 2 mg/L chlorine dose showed a 0.86 mg/L chlorine concentration decrease over 30 minutes (Table 9 and Figure 7). For both dosages, the largest decrease in chlorine concentration occurred in the first minute, 0.23 mg/L for the 1.1 mg/L chlorine dose and 0.43 mg/L for 2 mg/L dose after a 1 minute period.

When enough MS2 virus was added to the groundwater and buffer mixture to obtain a 10^6 pfu/mL concentration (calculation can be found in Appendix A), the chlorine demand was increased (Tables 10 and 11). For the 1.1 mg/L chlorine dose, the total decrease in chlorine concentration increased to 0.76 over a period of 30 minutes. For the 2 mg/L chlorine dose, the total decrease in chlorine concentration increased to 1.35 mg/L over a 30 minute period. This was an increase in demand of 0.36 mg/L for the 1.1 mg/L chlorine dose and 0.49 mg/L for the 2 mg/L dose from the mixture without the virus addition. The largest decrease still occurred in the first minute (Figure 8). A decrease of 0.68 mg/L occurred for the 1.1 mg/L chlorine dose after a 1 minute period, and a decrease if 1.23 mg/L occurred for 2 mg/L chlorine dose after a 1 minute period.

Enough clay was added to the groundwater, buffer, and MS2 mixture to obtain a turbidity reading of 5 NTU. This amount of turbidity complicated the method which was being used to test the chlorine concentration, and so the experiment could not be carried out. The 5 NTU turbidity caused a misreading of the chlorine residual by the visual DPD colorimetric method.

Chlorine demand is the difference between the amount of chlorine applied to the sample and the amount of free, combined, or total chlorine residual remaining at the end of the contact time (Tchobanoglous et al., 2003). Living cells, turbidity, and TOC can all react with chlorine and reduce its concentration. Figure 7 shows a lower chlorine demand for the groundwater (GW) and buffer mixture as there was a low turbidity ~ 1 NTU. When the virus was added (Figure 8), the demand increased as more of the chlorine was used in the inactivation of the virus. In addition, tryptic soy broth in which the E.coli and the MS2 virus are grown in is organic matter. Virto et al. (2005) saw an increase in resistance to chlorination for Y. enterocolitica and E. coli in the presence of tryptic soy broth as compared to distilled water. This resistance resulted from the heightened chlorine demand due to the organic matter; this was illustrated in a prolonged shoulder or lag section of the dose-response curve (Virto et al., 2005). A similar increase should also have been seen for an increase in turbidity of 5 NTU. LeChevallier et al. (1981) found a positive

correlation between chlorine demand and turbidity. Because of the inability in this research to find the chlorine demand of the 5 NTU water samples using the DPD colorimetric method, the doses used for the chlorine particle-association experiments were found using trial and error. For future research, a method which allows for correct chlorine demand and residual readings should be used. There was much work with the DPD Colorimetric Method (4500-Cl G, Standard Methods) as well as the DPD Ferrous Titration Method (4500-Cl F, Standard Methods) to find a correct chlorine demand for the 5 NTU water. The biggest problem with these methods is that they are colorimetric. The turbidity added interfered with the reagents so an accurate colorimetric assessment of chlorine could not be made.

Table 8: Cl_2 Demand for GW and Buffer (1.1 mg/L Cl_2 Dose)

Parameters	**1st Trial**		**2nd Trial**	
1 NTU	**Time**	**Residual Chlorine**	**Time**	**Residual Chlorine**
7.2 pH	(mins)	(mg/L)	(mins)	(mg/L)
62 degrees F	0	1.1	0	1.1
	1	0.87	1	0.87
	5	0.82	5	0.82
	10	0.8	10	0.8
	30	0.7	30	0.7

Table 9: Cl_2 Demand for GW and Buffer (2 mg/L Cl_2 Dose)

Parameters	1st Trial		2nd Trial	
1 NTU	Time	Residual Chlorine	Time	Residual Chlorine
7.2 pH	(mins)	(mg/L)	(mins)	(mg/L)
62 degrees F	0	2	0	2
	1	1.57	1	1.57
	5	1.49	5	1.49
	10	1.34	10	1.34
	30	1.14	30	1.14

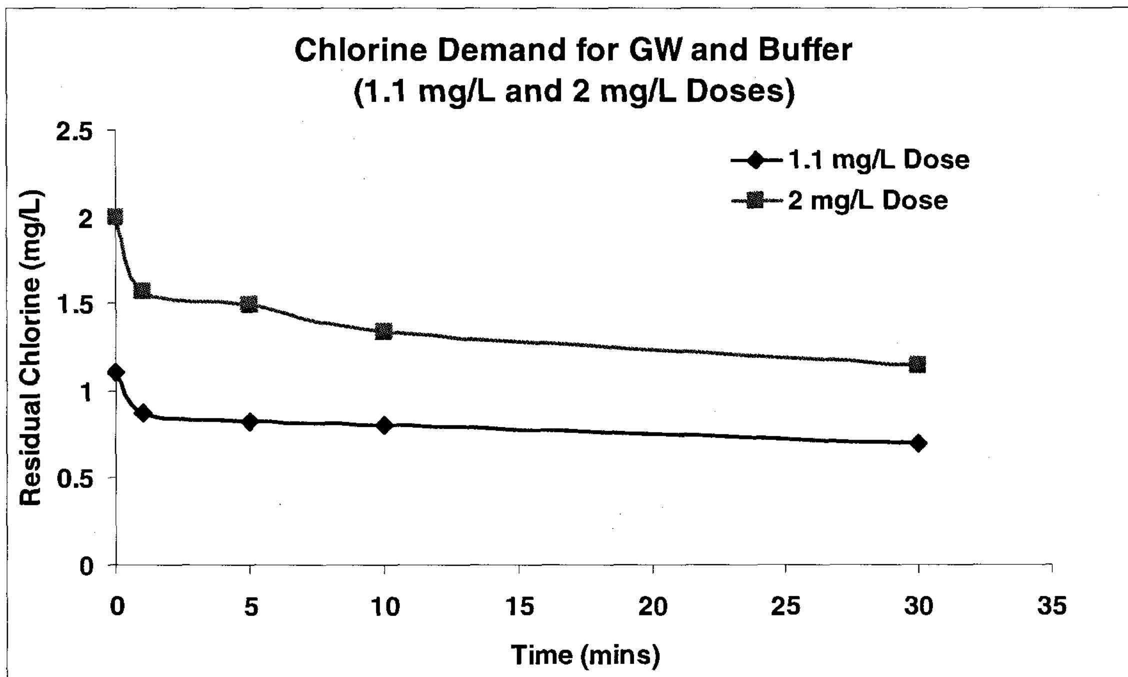


Figure 7: Cl_2 Demand of GW and Buffer (1.1 mg/L and 2 mg/L Cl_2 Dose)

Table 10: Cl_2 Demand for GW, Buffer and MS2 (1.1 mg/L Cl_2 Dose)

Parameters	1st Trial		2nd Trial	
1 NTU	Time	Residual Chlorine	Time	Residual Chlorine
6.3 pH	(mins)	(mg/L)	(mins)	(mg/L)
62 degrees F	0	1.1	0	1.1
10^6 PFU	1	0.42	1	0.42
	5	0.4	5	0.4
	10	0.39	10	0.39
	30	0.34	30	0.34

Table 11: Cl_2 Demand for GW, Buffer, and MS2 (2 mg/L Cl_2 Dose)

Parameters	1st Trial		2nd Trial	
1 NTU	Time	Residual Chlorine	Time	Residual Chlorine
6.3 pH	(mins)	(mg/L)	(mins)	(mg/L)
62 degrees F	0	2	0	2
10^6 PFU	1	0.77	1	0.77
	5	0.75	5	0.75
	10	0.71	10	0.71
	30	0.65	30	0.65

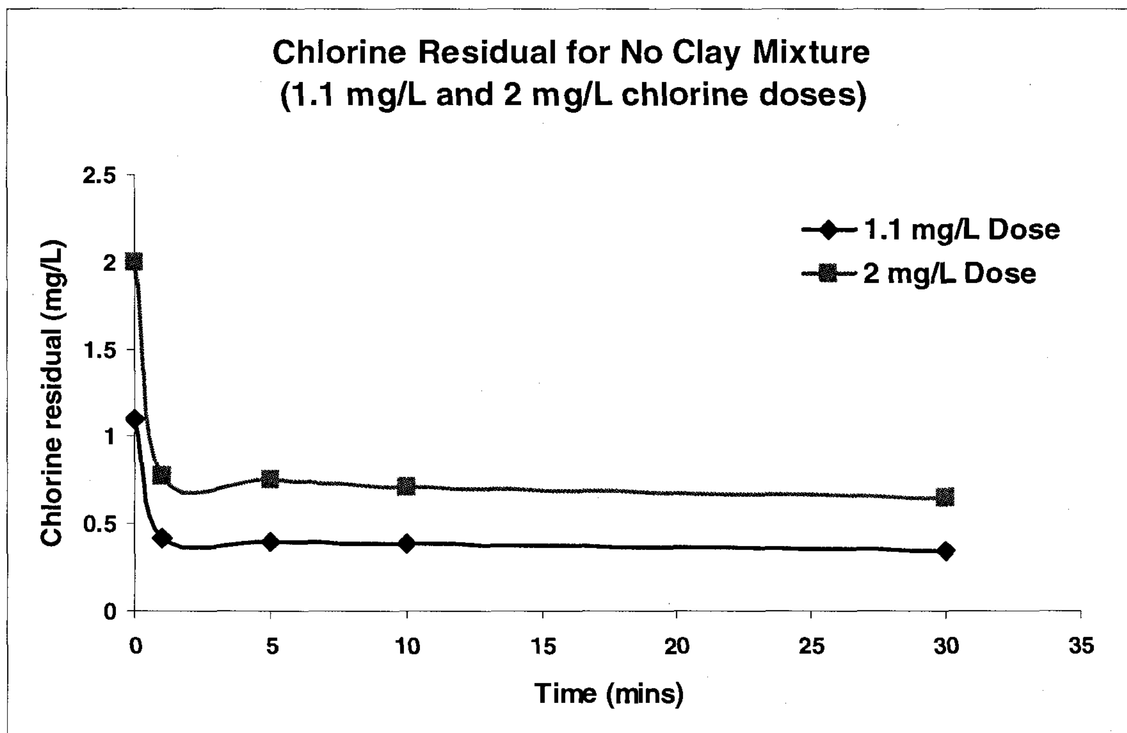


Figure 8: Cl_2 Demand for GW, Buffer, and MS2
(1.1 mg/L and 2 mg/L Cl_2 Dose)

Particle-Association Effect on MS2 Inactivation

Overview

The main objectives of these experiments were to compare the dose-response of the disinfectants chlorine and ultraviolet light as a function of:

- the addition of clay in the 24 hour mixing period allowing for MS2-particle association, and;
- the application of sonic sound waves before the use of the disinfectant to destroy the MS2-particle associations allowing the disinfectant to come in contact with more of the virus.

Mixtures were made using a buffered groundwater which was spiked to a 10^6 pfu/mL MS2 titer as was discussed in the Clay-Virus Mixture section in Chapter II: Materials and Methods. To this initial mixture, either clay (C) or no clay (NC) was added and the mixtures were sonicated (S) or not sonicated (NS). This shorthand notation will be used throughout the tables and figures and text for this thesis.

Statistical Data

Table 13 shows the p-values, found using Analysis of Variance as discussed in the Materials and Methods section. It is helpful to compare the p-values from all the experimental runs. In Table 13 only one set of data, Cl_2 1.1 mg/L data, had a significant difference between the C

sample and the NC sample with a low p-value which is. Although many of the p-values are high, it is important to note that the chlorine data showed a higher significant difference with overall lower p-values compared to the UV p-values. The UV data were more conservative because the UVT (UV transmittance) of the water was taken into account, but the chlorine demand of the water was not. The slopes of the trend lines for the pellet method UV light experiments were also significantly different (p=0.05).

Table 12: Water Parameters for Chlorine and UV Experiments

Water Parameters					
		pH	NTU	TOC (mg/L)	%UVT
Cl_2 1.1 mg/L dose	C	7.03	5.7	0.83	-
	NC	7.01	1.3		-
Cl_2 1.5 mg/L dose	C	6.49	5.4	0.69	-
	NC	6.33	1.6		-
Cl_2 2 mg/L dose	C	7.2	5.1	0.64	-
	NC	6.9	1.54		-
Cl_2 Sonication	S	7.03	5.7	0.83	-
	NS	7.03	5.5		-
UV Exp. 1	C	7.02	5.1	0.77	84.33
	NC	6.89	1.2		91.83
UV Exp. 2 / UV sonication	C	7.3	5.4	0.77	83.36
	NC	9.92	1.1		91.20

Table 13: Statistical Data

Clay or No Clay	
	p - values
Cl_2 1.1 mg/L dose	0.081
1.1 mg/L 1 min excluded	0.031
Cl_2 1.5 mg/L dose	<0.0001
Cl_2 2 mg/L dose	0.191
UV Exp. 1	0.874
UV Exp. 2	0.396
UV Pellet Method	0.208
Sonication or no Sonication	
Cl_2 Sonication	0.295
UV Sonication	0.741

Chlorine

Table 12 shows the water parameters for the chlorine and ultraviolet light experiments. Table 13 shows the statistical ANOVA data for the chlorine experiments. Figure 9 shows the chlorine dose-response at a dose of 1.1 mg/L. After a 30 minute contact time, the NC sample had a 3.3-log inactivation while the C had a 1.3-log inactivation. This was an increase of 2-log inactivation for the NC sample compared to the C sample. Table 13 shows a p-value of 0.081 for this data (1.1 mg/L of chlorine). This is a bit over the 0.05 cut-off p-value, but when the 1 minute data point is removed the p-value decreases to 0.031. After a 1 minute contact time there is no difference between the C and NC samples, but after that 1 minute contact time the difference is apparent.

The rate of inactivation was different for the clay and no clay samples shown in Figure 9a. The slope of the C linear fit trendline was 2.7% while the NC line has about a 7.7% slope. The slopes are significantly different at the 95% confidence level. The increase in slope for the NC trendline shows a higher rate of inactivation per dose. The clay line has a flatter slope and so a lower rate of inactivation per dose. The flattening of the clay line shows the influence from particles. In Figure 11b, a polynomial trendline was fit to the data. Both the C and NC lines appear to have a flattening at the longer contact times, but the NC has a much steeper log-inactivation at the shorter contact times. The difference in the rate of inactivation per dose or the flattening of the slope of the clay trendline shows an effect of particle shielding. A typical dose-response curve shows a flattening at higher doses due to particle shielding, and the effect is seen at the 1.1 mg/L chlorine dose.

Figure 10 shows the chlorine dose response at a dose of 1.5 mg/L. Table 12 shows the water parameters for Figure 10. After 30 minutes of contact time, the NC sample had a 3.09-log inactivation while the C sample had a 1.7-log inactivation. This was an increase of 1.4-log inactivation for the NC sample compared to the C sample. The p-value (Table 13) for this data was < 0.0001. This low p-value shows indicates that the null hypothesis can be rejected, i.e. clay and no clay samples show a significant difference. Therefore, inactivation occurs after 1 minute of

contact time and then it levels off; most of the chlorine was taken up after the 1 minute (Figures 9 and 10).

The C and NC linear trend lines (Figure 10) slopes are 0.08% and 0.5% respectively, significantly different at the 95% confidence level. After 1 minute, all of the virus which could be inactivated was, and the remaining virus was shielded by particles. The flat slopes indicate a particle-shielding effect.

Figure 11 shows the chlorine dose-response at 2 mg/L. Table 12 shows the water parameters for this run. After 30 minutes of contact time, the NC and C samples both had about a 2.8-log inactivation. The NC sample obtained over a 2-log inactivation after only 5 minutes of contact time. The C sample had a log inactivation below 1.5-logs until 30 minutes. This shows a 2 mg/L dose and 30 minute contact time overcame the particle-virus shielding effect on the chlorine inactivation (Dietrich et al., 2003; LeChevallier et al, 1981).

The data shown in Figure 11 is different from those in Figures 9 and 10 in that the C linear trendline is steeper, with a slope of 6.6% compared to the NC trendline with a slope of 3.3%. At the higher chlorine dose, any particle effects were overcome allowing the clay sample, which had a higher beginning virus concentration to have a higher rate of inactivation per dose.

Figure 12 shows the chlorine dose-response curve at a 1.1 mg/L dose, with 5 NTU turbidity for S and NS samples. The 1 minute to 10 minute contact times did not show much variation in inactivation, they were all under a 1-log inactivation for the S and NS samples. At the 30 minute contact time, the NS sample has a 1.3-log inactivation, while the S sample has a 2.8-log inactivation, ~ a 1-log difference in the inactivation in the S and NS samples. This data suggested there may have been some particle shielding occurred after the 30 minute contact time, as indicated by difference in the inactivation for the S and NS samples.

In Figure 12, the effects of sonication were apparent: the NS and S samples had a slope of 2.65% and 7.93% respectively. This shows a definite increase in the rate of inactivation per dose with sonication of the sample, the slopes are significantly different at the 95% confidence level.

In order to break the virus and particle association, typically a physiochemical method, such as the addition of a protein, is used in conjunction with a physical force such as sonication (Wellings et al., 1975; Templeton et al., 2008). The reason no physiochemical method was applied along with the sonication in my research was to test if a chemical addition was necessary. Even with a physiochemical addition and a physical shearing force, the elution is not consistent. Wellings et al. (1975) found with an addition of 3% beef extract solution and the application of sonic forces for 15 minutes viruses were eluted 60% of the time. There was

elution of viruses seen in these samples shown with the increase in the rate of inactivation or the slope increase (Figure 12). The flattening out of the trendline at the higher doses which is seen in the NS sample was eliminated in the S sample.

Overall, there were particle-virus shielding effects between the C and NC samples for chlorine disinfection. These differences were seen through the varying rate of inactivation per dose between the C and NC samples. The typical flattening of the dose-response curve due to particle shielding was found in the samples which had 5 NTU of clay added. Unfortunately, the chlorine residual could not be found for the C samples due to turbidity complication with the method. A chlorine residual method that is not affect by increased turbidity must be developed. Another way to help ensure greater particle association would be to change the water parameters, such as pH. This was not done in these experiments because more real life situations were being simulated. It was shown that higher chlorine doses may be used to overcome particle shielding effects, but this is a tradeoff because increasing chlorine doses can bring about complications with DBP formation.

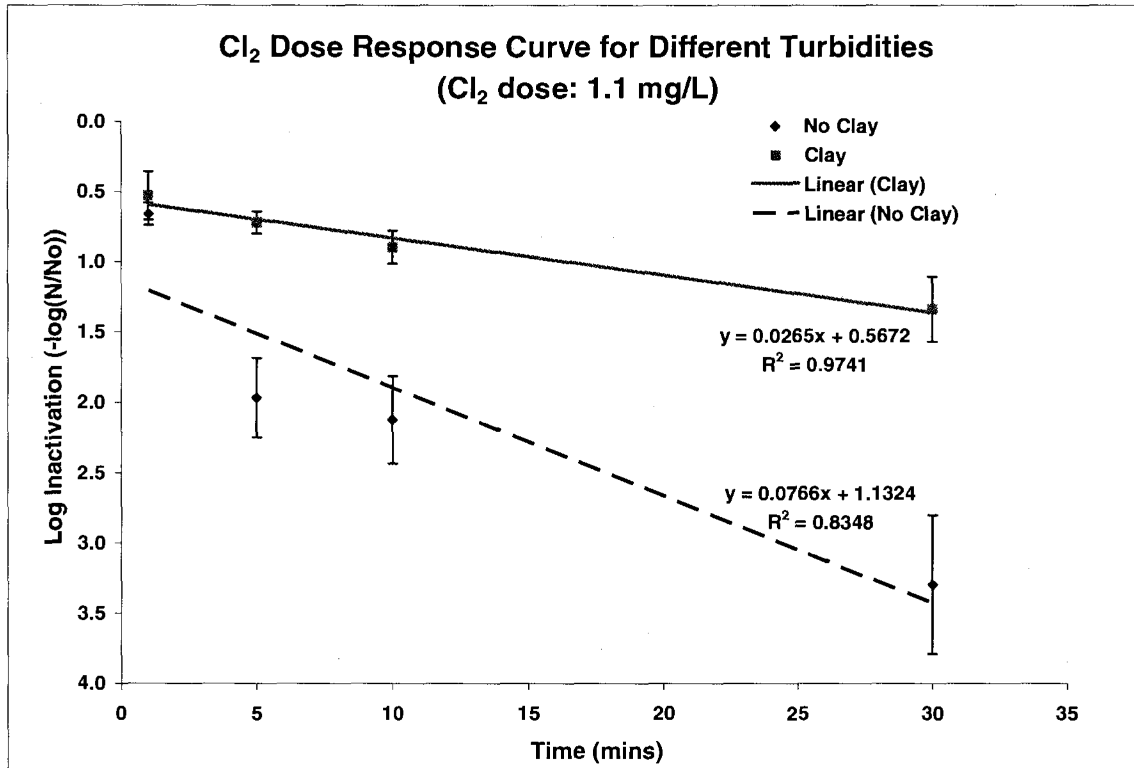


Figure 9: Comparing Cl_2 Dose Response Curves with Different Turbidities (1.1 mg/L Dose)

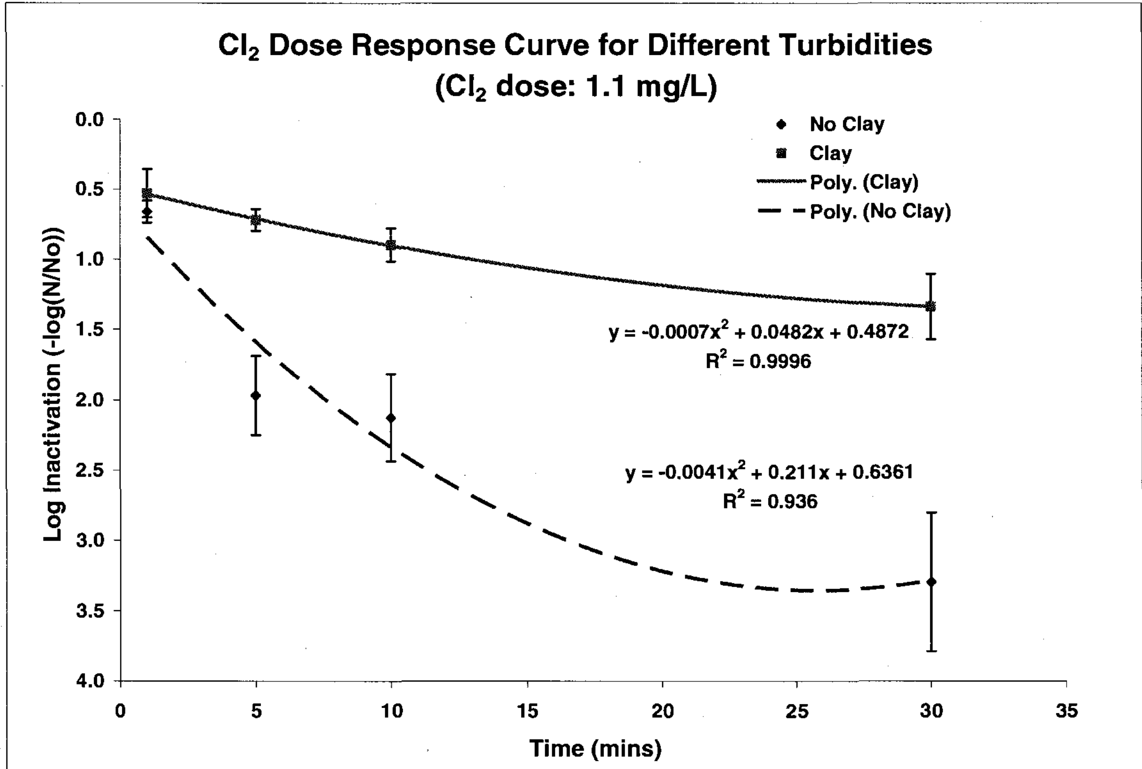


Figure 9a: Comparing Cl_2 Dose Response Curves with Different Turbidities (1.1 mg/L Dose)

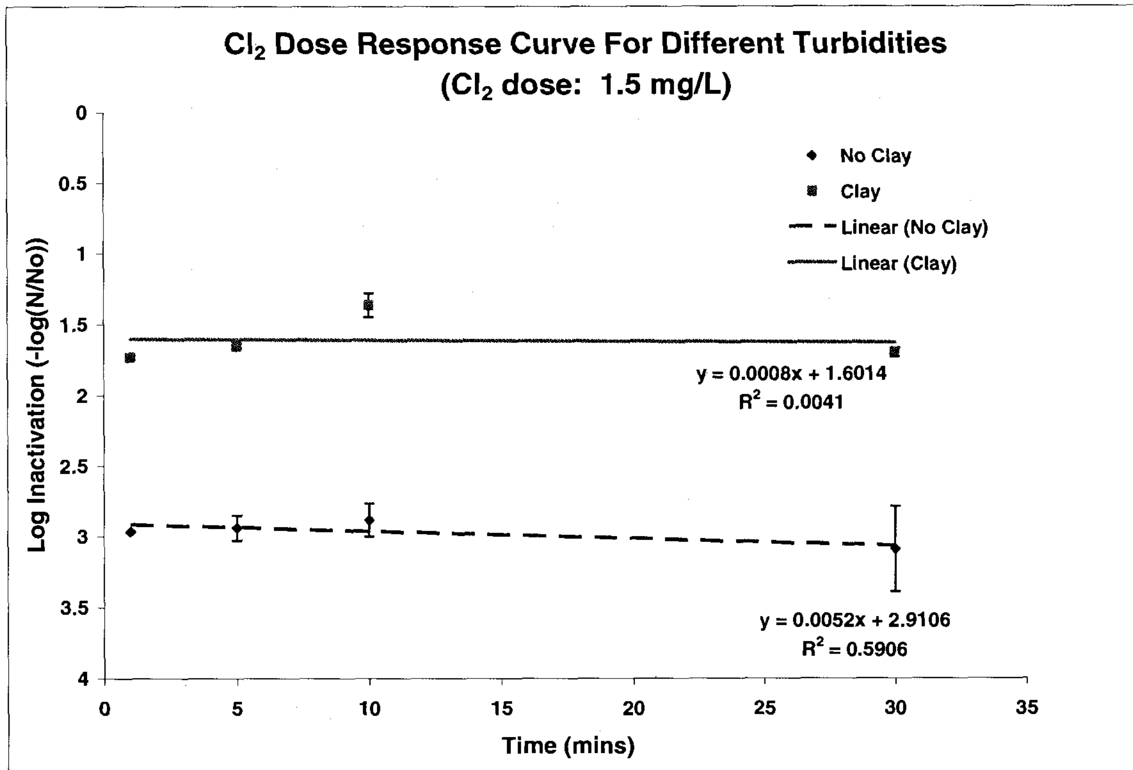


Figure 10: Comparing Cl_2 Dose Response Curves with Different Turbidities (1.5 mg/L Cl_2 Dose)

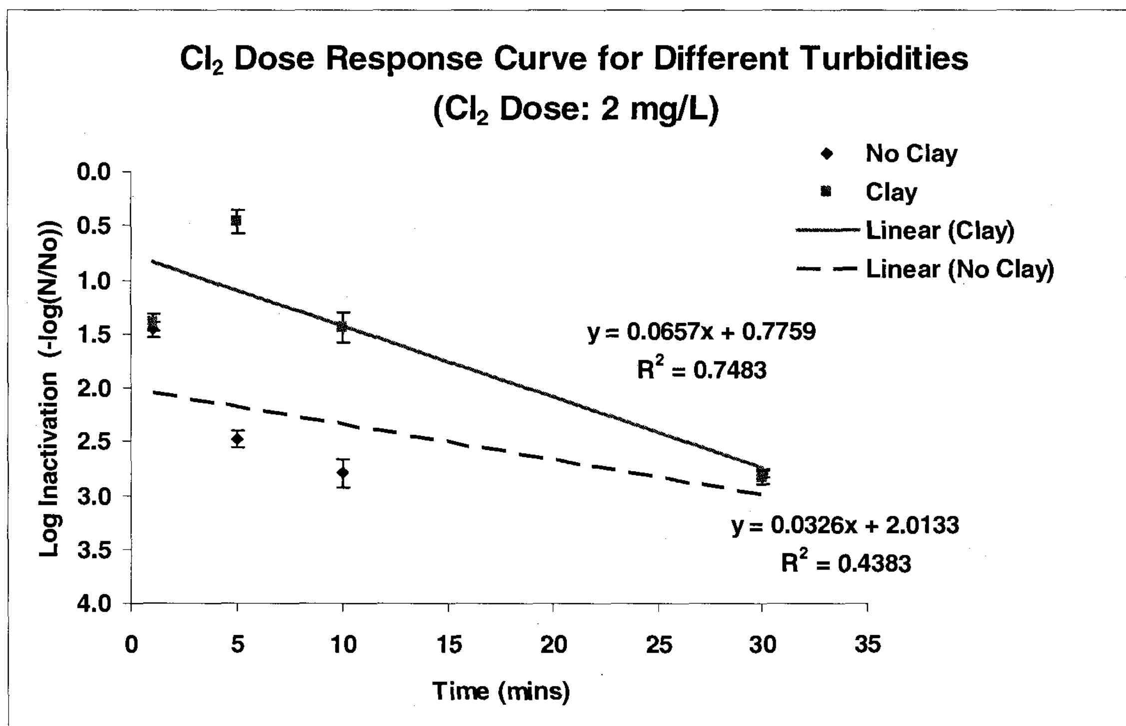


Figure 11: Comparing Cl_2 Dose Response Curves with Different Turbidities (2 mg/L Cl_2 Dose)

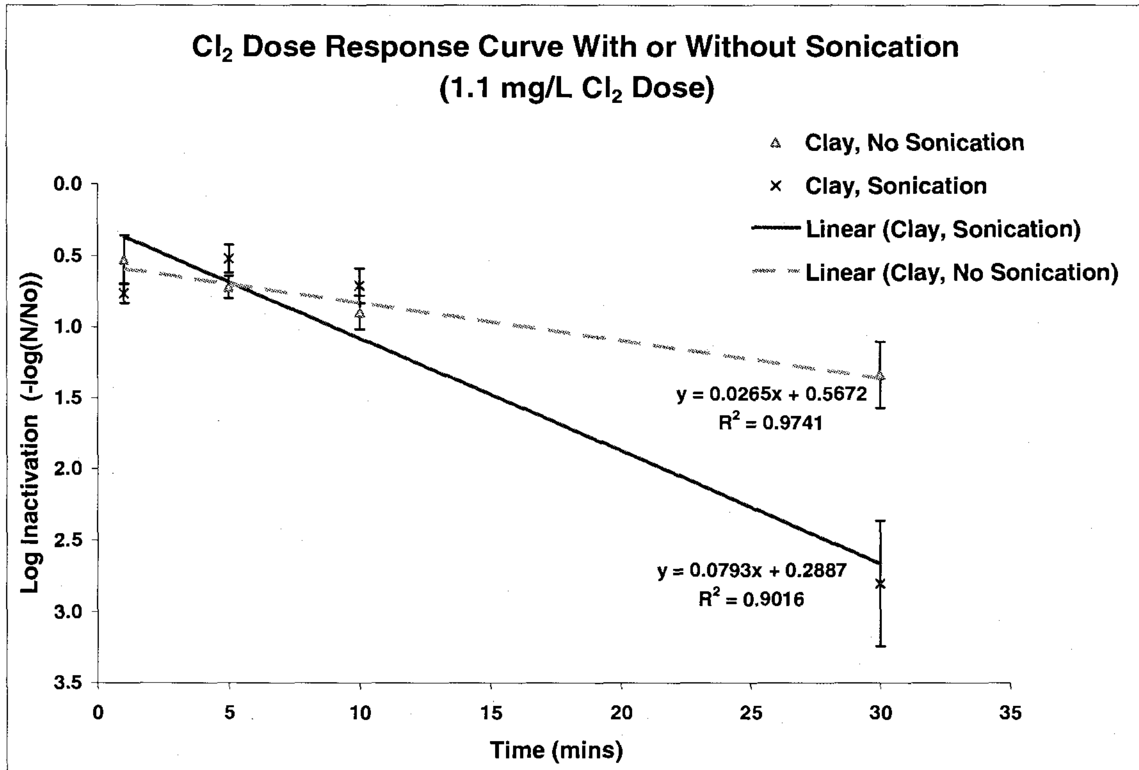


Figure 12: Comparing Cl_2 Dose Response Curves with or without Sonication (1.1 mg/L Cl_2 Dose)

Ultraviolet Light

Non-Pellet Method

Figure 13 shows the UV dose response curve for the C and NC samples.. The C and NC samples had similar inactivation; there was never even a 0.5-log difference in the inactivation. The p-value, found in Table 13, was 0.874. This was high and showed little variability between the C and NC samples. The slopes of the linear regression (rate of inactivation per dose) lines were not significantly different at the 95% confidence level.

This low variability between the C and NC samples was most likely due to the fact that the UVT (UV transmittance) of the water was accounted for in the dose delivered and there were no demonstrated virus-particle associated particulates. There may have been scattering of the UV light from the particles not bound to viruses instead of particle shielding. The UV light could have been scattered and so it remains germicidal when it comes in contact with the viruses.

The slopes of the C and NC trendlines in Figure 13 were similar, 3.87% and 3.56% respectively. This show similar inactivation rates per dose for both samples. There is no flattening of the lines showing no particle effect.

Figure 14 shows another UV dose response for the C and NC samples. For these samples, there was some difference between the C and NC samples. At a 20 mJ/cm2 dose the C sample had a 1.23-log inactivation while the NC sample had a 1.4-log inactivation. There is less than a 1-log difference between the C and NC samples. The p-value for this data was 0.396; not as high as the previous data, but still too high to be able to say there is a significant difference between the C and NC data. The C trendline has a slope of 2.43% and the NC trendline has a slope of 2.79%. There is no flattening seen due to particle-shielding.

Figure 15 shows the log inactivation of C and NC samples using the pellet method with a high turbidity (1000 NTU) allowed during the mixing period for higher particle to virus ratios which was thought to increase

virus-particle association. The Pellet Method was discussed at the end of Chapter II: Materials and Methods. This method shows more of a difference in the inactivation between the C and NC due to the increased effort to ensure particle virus association. At a 60 mJ/cm2 dose, the C sample had a 1.54-log inactivation, while the NC sample had a 3.04-log inactivation. This shows some shielding of the virus from the clay particles as the UVT was taken into account. The pellet method p-value (0.208) was not low enough to show a significant difference between the C and NC samples, but it was lower than the other UV experiments. This showed that although there was some particle shielding found in these data, the fact that the UVT was corrected kept the variance low.

The pellet method shows a difference in the inactivation rates per dose for the C and NC samples. The clay trendline has a slope of 2.97%, and flattened at higher UV doses. The slope of the no clay trendline is higher at 5.11% and shows no flattening at higher UV doses. This shows an effect from particle shielding.

The reason for the disagreement between this method and the nonpellet method used for the other experiments has to do with the fact that the pellet method accomplished particle-virus association. Table 14 shows a MS2 assay using the pellet method where the pH was lowered to 4.5 during the overnight mixing period; this pH was optimal for association (Chapter III). The experiment showed equal MS2 titer of 10^8 in both the

centrifugate and the pellet (the beginning titer of the MS2 was 10^8). There was definite virus-particle attachment, something which the nonpellet method did not accomplish effectively. The pellet method was used in later UV experiments and the turbidity was lowered to 5 NTU, the maximum turbidity level allowed by USEPA for unfiltered drinking water supplies (USEPA, 2006b).

Figure 16 shows a UV dose response of C samples which were S or NS. The water parameters are found in Table 12. There is not a large difference seen between the S or NS samples. At 20 mJ/cm^2, the S sample had a 1.22-log, inactivation while the NS sample had a 1.23-log inactivation. The UV sonication data showed no difference in the inactivation rate between the NS and the S samples. The slope of the S trend line was 2.16% and the slope of the NS trendline was 2.43%. The slopes were not significantly different at the 95% confidence level, found using a student t-test. The difference between this sonication data and the chlorine sonication data was most likely due to the fact that UV disinfection was able to overcome the particle effects which were brought about in the nonpellet method, as none of the UV experiments done with that methods showed any difference in the Inactivation or the rate of inactivation between the C and the NC samples. With the pellet method, particles and viruses were associated to cause a difference in the inactivation and the rate of inactivation with the UV disinfection.

The pellet method allowed for particle-virus association, so some shielding effect was seen. This result is consistent with the literature review that viruses can be shielded from UV light (Templeton et al., 2006).

Table 14: MS2 Titer using Pellet Method showing MS2 Concentration in Free and Pellet Form (PFU/mL)

	Free		Pellet	
10^1	TNTC	TNTC	TNTC	TNTC
10^2	TNTC	TNTC	TNTC	TNTC
10^3	TNTC	TNTC	TNTC	TNTC
10^4	TNTC	TNTC	TNTC	TNTC
10^5	TNTC	TNTC	TNTC	TNTC
10^6	201	164	TNTC	TNTC
10^7	32	57	77	83
10^8	17	31	29	10
10^9	23	23	26	11
10^10	23	22	17	25

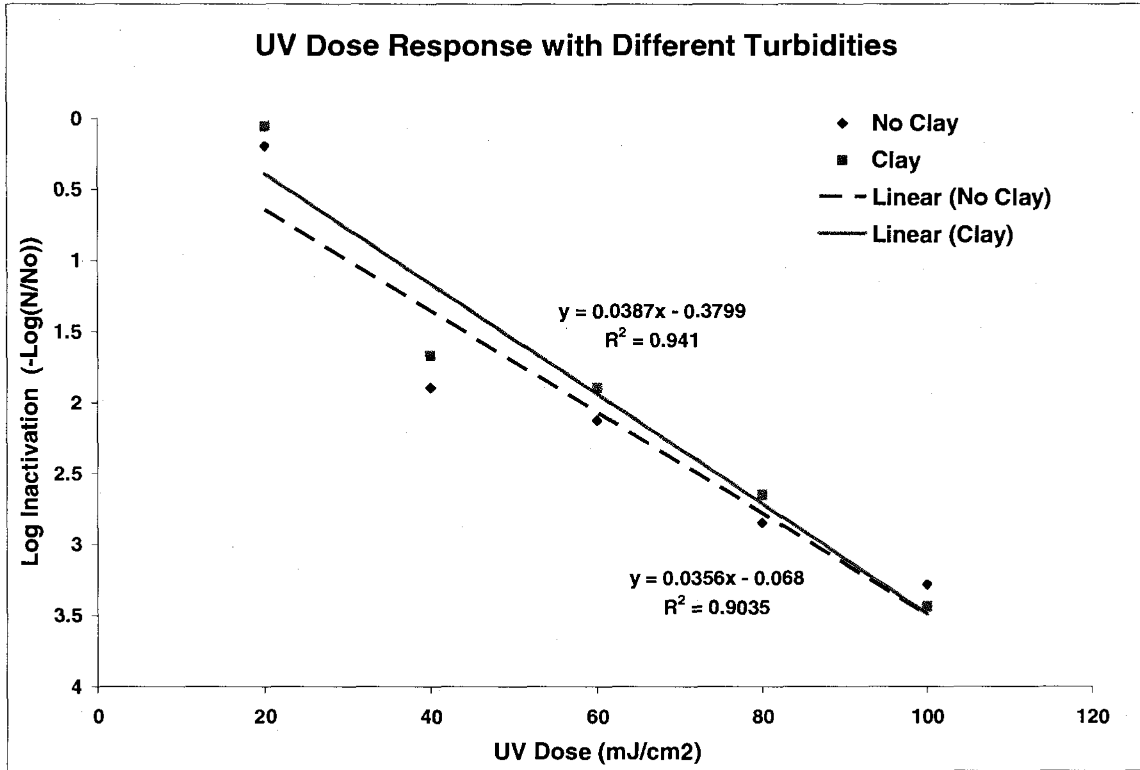


Figure 13: Comparing UV Dose Response with Different Turbidities (UV Exp. 1)

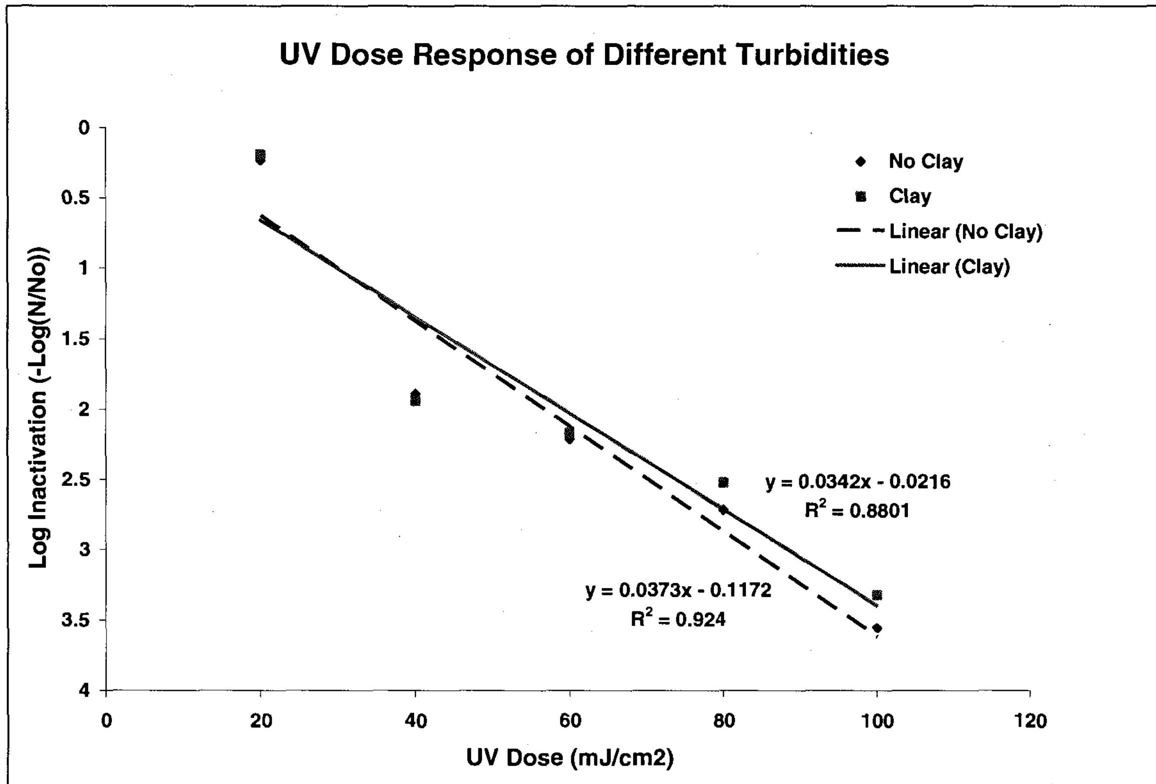


Figure 14: Comparing UV Dose Response with Different Turbidities (UV Exp. 2)

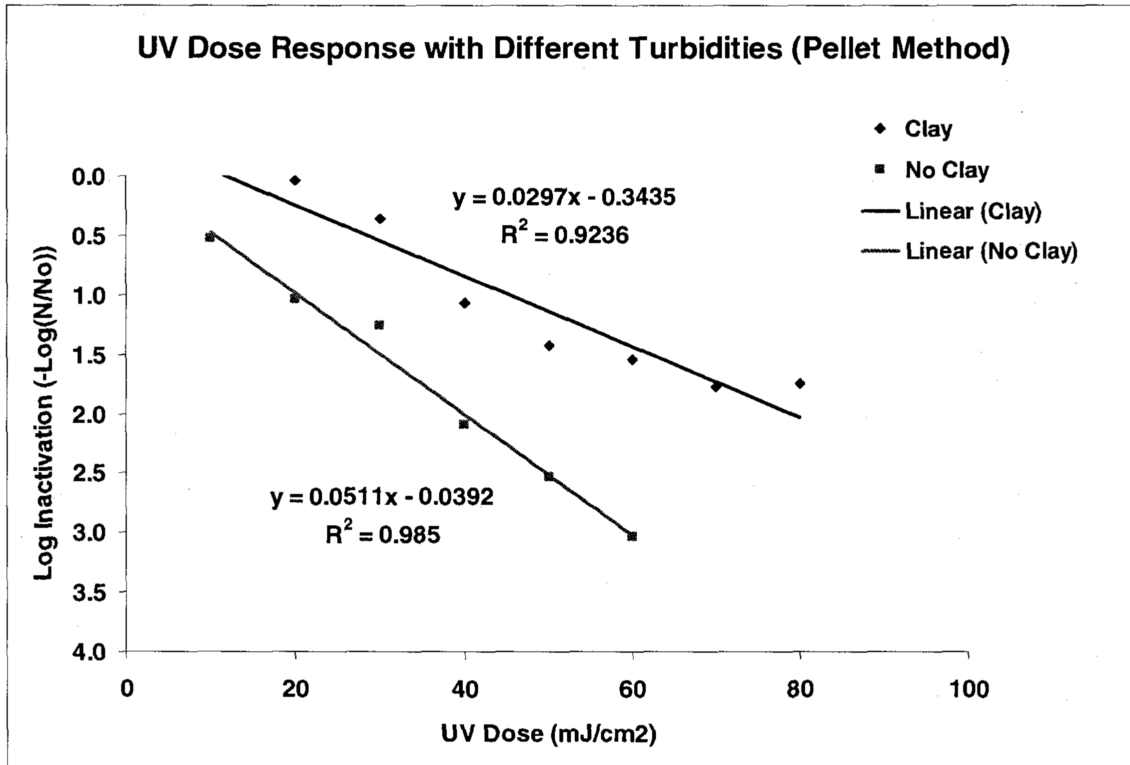


Figure 15: Comparing UV Dose Response with Different Turbidities (Pellet Method, 1,000 NTU)

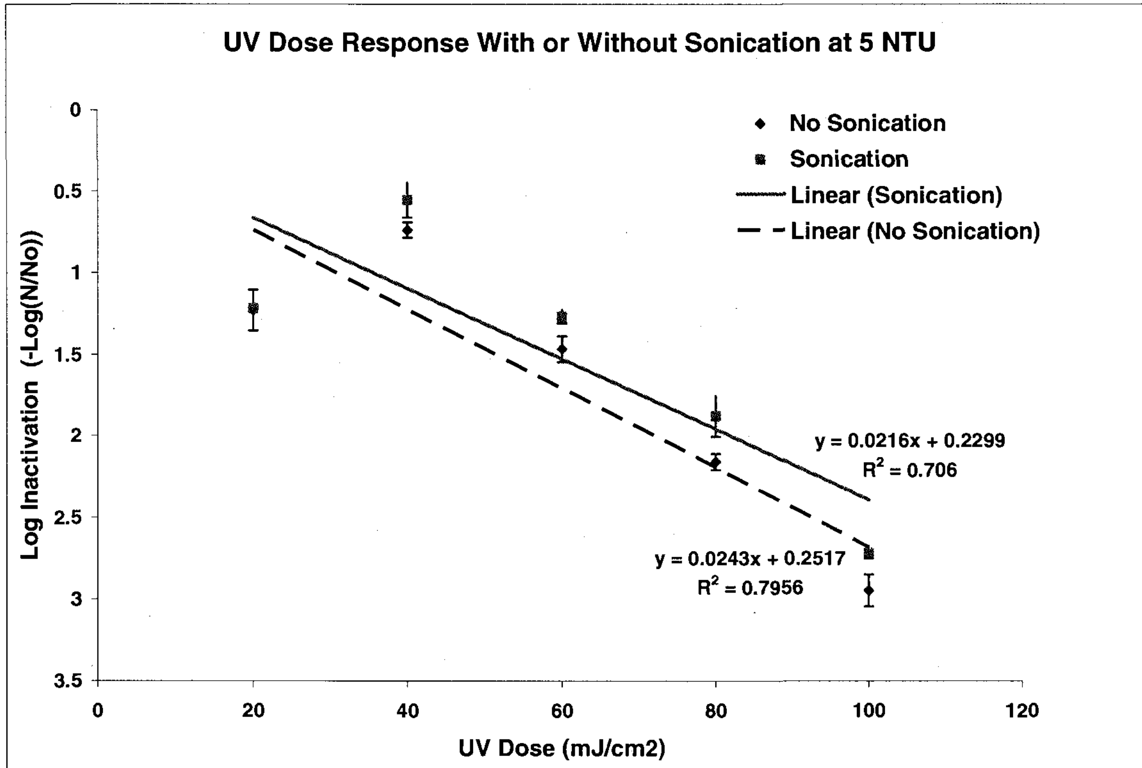


Figure 16: Comparing UV Dose Response With or Without Sonication

Pellet Method (5 NTU)

Because no shielding effect was seen using the non-pellet method for UV light and the pellet method at a turbidity of 1,000 NTU showed some particle shielding effects, these methods were combined to see if particle-virus association could be ensured using the pellet method (i.e. would a low turbidity of 5 NTU show an effect on UV light disinfection?) The unassociated (free) virus and the associated (pellet) virus dose-response curves were generated and compared to see if a turbidity of 5 NTU showed a significant effect on the log-inactivation of MS2 virus. Figure 17 shows the unassociated virus dose-response curve. The

published MS2 UV inactivation guidelines for UV testing with MS2 bacteriophage for quality assurance purposes are shown as the dark lines for the purpose of comparison. These guidelines were published by the National Water Research Institute (NWRI) in collaboration with the American Water Works Association Research Foundation (AWWARF) (NWRI/AWWARF, 2000). Comparing the findings for the unassociated MS2 dose-response curve to the NWRI/AWWARF, the unassociated data fell between the typical MS2 dose-response for low-pressure ultraviolet light. For the unassociated virus, there was about a 1-log reduction for each dose increase from 20 to 60 mJ/cm^2. The inactivation rate changed to about 0.5-log inactivation per dose increase for 80-100 mJ/cm^2.

Figure 18 showed the associated virus dose-response curve. The NWRI/AWWARF MS2 guidelines were plotted on this graph as well, for the means of comparison. There was a distinct difference in this dose-response curve from the typical MS2 dose-response for LP ultraviolet light and from the dose-response in Figure 17 for the unassociated virus. For the 20 to 40 mJ/cm^2 doses, there was about a 1-log inactivation for each dose increase, but after 40 mJ/cm^2 the dose-response curve showed a definite flattening in the log inactivation for the 60 to 100 mJ/cm^2 doses. This flattening in the dose-response showed a disturbance in the inactivation due to shielding of the viruses by the clay particles.

To clearly show the differentiation between the associated and unassociated MS2 dose-response curves, a linear regression was fit to the data. Figure 19 showed the linear regression lines as well as the corresponding slopes. The slopes showed the rate of inactivation per dose. The unassociated linear regression had a slope of 3.51%, while the associated linear regression has a slope of 1.57%. The slopes were significantly different at the 95% confidence level. The unassociated dose-response showed a significantly higher inactivation per dose rate than the associated dose-response curve.

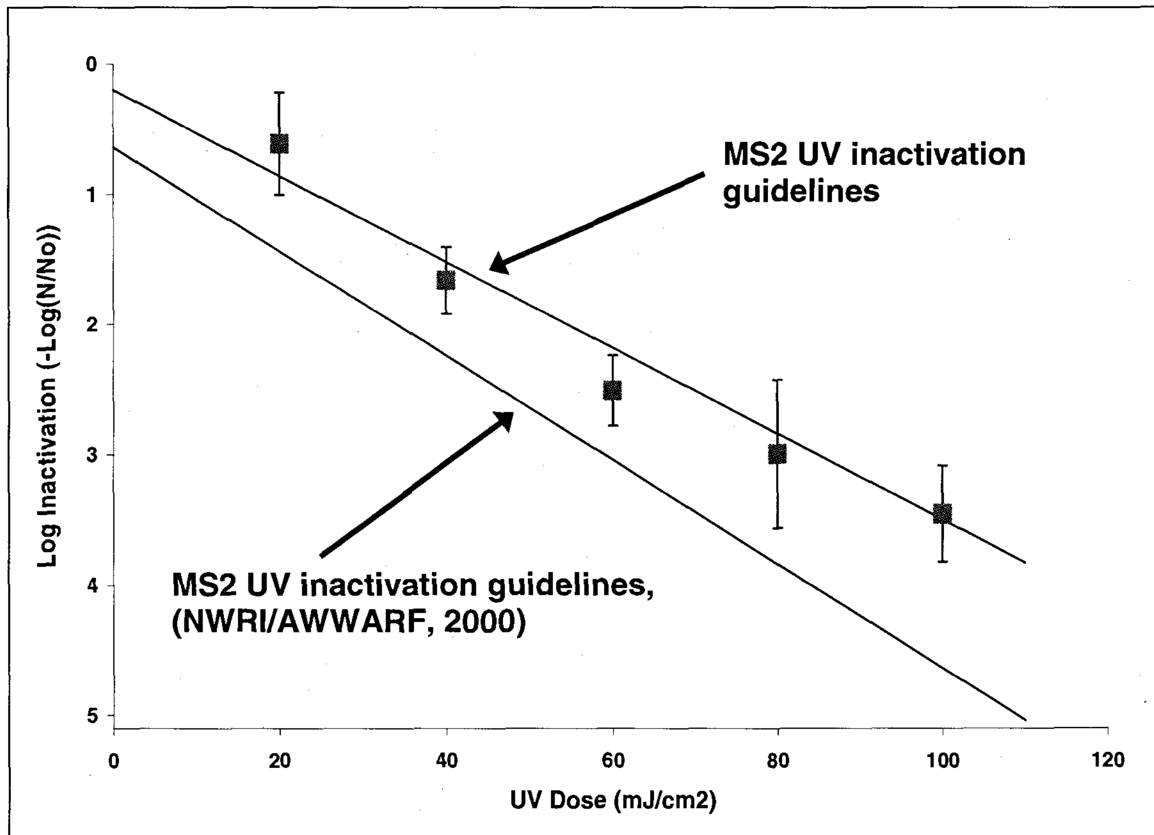


Figure 17: UV Dose Response for Unassociated (free) MS2 (Pellet Method, 5 NTU)

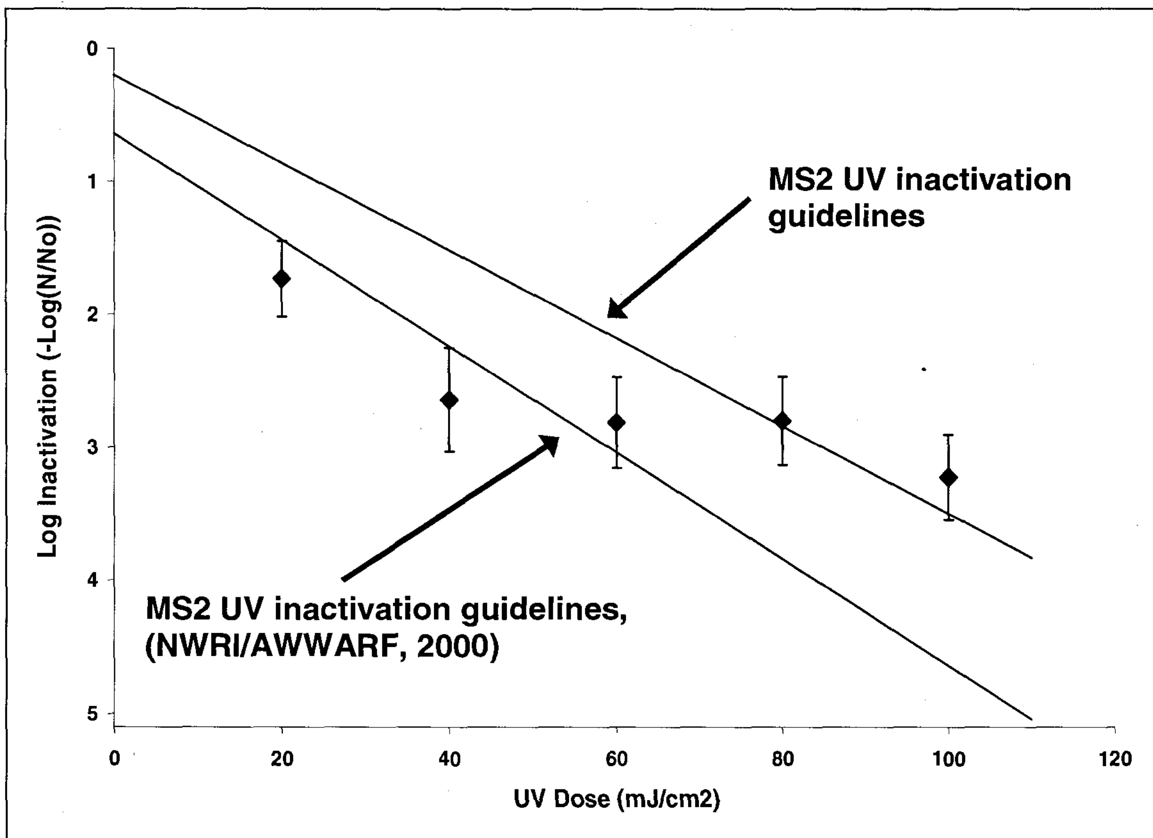


Figure 18: UV Dose Response for Associated (pellet) MS2 (Pellet Method, 5 NTU)

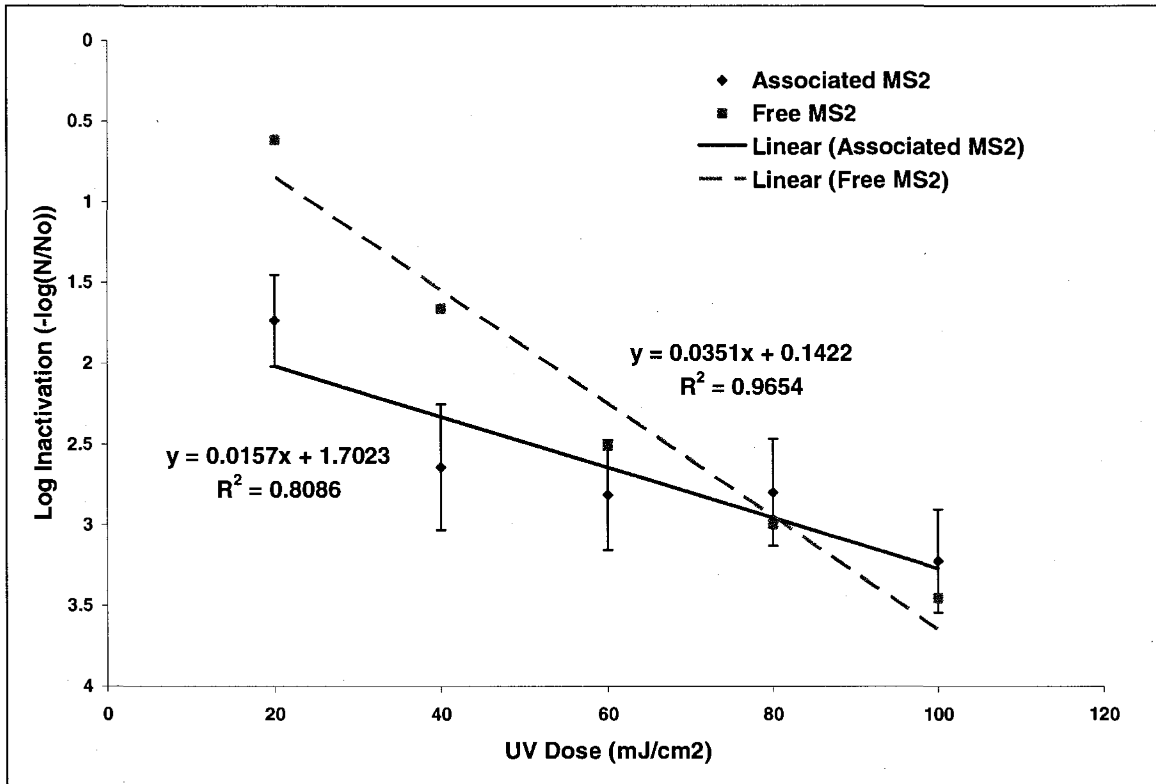


Figure 19: Comparison of Linear Regressions of Dose-Response for Unassociated and Associated MS2 (Pellet Method, 5 NTU)

Chapter V

Conclusions and Recommendations

Conclusions

Virus-particle association is hindered by the repealing negative charges on both the particle and the virus. Manipulation of pH was found to be the most efficient method for facilitating association. A decrease in pH from 7 to 4 increased association by 2-log. The addition of calcium can accelerate association by as much as 2.74-log. Total organic carbon was found to hinder association by 0.36-log. Lowering the pH to an optimum association level was an important step, in past research as well as in the earlier steps of this research, when steps were not taken to encourage association, there was not a significant difference in dose-response due to the same increase in turbidity of 5 NTU for the ultraviolet light data (Passantino, 2004).

The effect of the addition of 5 NTU turbidity for the non-pellet experiments was more noticeable in the chlorine experiments than the ultraviolet light disinfection. For a chlorine dose of 1.1 mg/L, the rate of inactivation per dose was decreased in the clay sample (a flattening of the slope of the trend line). It was at this lower chlorine dose where the

particle-association effect was best seen. The chlorine demand in the presence of turbidity was not addressed because the colorimetric methods used did not work when 5 NTU turbidity was present. Because of the inability to measure residual chlorine, the chlorine dosing could not be adjusted correctly as a function of turbidity. Higher chlorine doses may be used to overcome particle shielding effects, but increasing chlorine doses can bring about complications with DBPs and so may not be a good option for waters where particle shielding may be occurring.

UV dosing was adjusted for the different UVT in the waters with higher turbidities. Ultraviolet disinfection, using the non-pellet method, showed little difference between the clay and no clay samples for both the overall inactivation as well as the rate of inactivation per dose the samples were not significantly different at the 95% confidence level. The pellet method using a high turbidity (1000 NTU) did show a difference in the rate of inactivation per dose through a flattening of the slope of the clay trend line. The pellet method at 5 NTU turbidity showed a distinct difference in the unassociated MS2 and associated MS2 dose-response curves. The associated MS2 dose-response curve showed a flattening at higher doses, a characteristic of particle-shielding. The flattening seen in this research would prevent the 4-log inactivation needed to meet drinking water standards (USEPA, 2006b). A significant difference was seen between the rates of inactivation per dose for the two conditions.

The unassociated MS2 had a higher rate of inactivation per dose when compared to the associated MS2.

UV disinfection had the ability to overcome the particle shielding effects due to any association that was achieved using the nonpellet method, but when association was ensured using the pellet method there was a reduction in inactivation at the 5 NTU turbidity.

Sonication of the chlorine samples resulted in an increased reduction after the 30 minute contact time showing some particle-association was occurring. When the samples were sonicated, there was an increase in the rate of inactivation per dose. Sonication of the UV samples using the non-pellet method showed little difference. Sonication was able to break apart the virus-particle association, but more work is needed to quantify the efficiency of this method of elution.

This research concluded that the 5 NTU max turbidity allowed for unfiltered Public Water Systems can reduce the rate of inactivation per dose for chlorine and low-pressure UV disinfection systems. This reiterates the necessity for validation studies at individual treatment plants and/or the implementation of systems which ensure turbidity reduction and 4-log inactivation of viruses.

Recommendations for Future Research

Recommendations for future research include using a method which takes into account turbidity to find accurate chlorine demand readings of samples. This will allow for correct dosing of the chlorine samples, as well as confirmation responses are due to association effects.

Future research examining the effects of particles on UV disinfection may include studies using medium-pressure ultraviolet lamps. These lamps emit at lower wavelengths and so may have a stronger effect on particle-associated organisms. Other studies may use different organisms, such as Cryptosporidium, to see the effect of particles on their inactivation. Other particles could be tested, including other types of clay (Kaolinite), humic acids, or cellular debris.

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APPENDIX A

Chlorine Studies

Chlorine Demand Trials

Calculation of dose: $C_1V_1 = C_2V_2$

Calculation of 100 mg/L chlorine stock solution: 50,000 mg/L = original stock solution

$(50{,}000 \text{ mg/L})(V_1) = (100 \text{ mg/L})(1000\text{mL})$
$V_1 = 2$ mL

Trial #1: Groundwater and Buffer Chlorine Demand

Calculation of dose: $C_1V_1 = C_2V_2$

1.1 mg/L Chlorine Dose Calculation: $(100 \text{ mg/L})(V_1) = (1.1 \text{ mg/L})(250 \text{ mL})$
$V_1 = 2.75$ mL

2 mg/L Chlorine Dose Calculation: $(100 \text{ mg/L})(V1) = (2 \text{ mg/L})(250 \text{ mL})$
$V_1 = 5$ mL

Chlorine Dose: 1.1 mg/L				
Parameters	**1st Trial**		**2nd Trial**	
1 NTU	**Time**	**Residual Chlorine**	**Time**	**Residual Chlorine**
7.2 pH	(mins)	(mg/L)	(mins)	(mg/L)
62 degrees F	0	1.1	0	1.1
	1	0.87	1	0.87
	5	0.82	5	0.82
	10	0.8	10	0.8
	30	0.7	30	0.7

Chlorine Dose: 2 mg/L				
Parameters	**1st Trial**		**2nd Trial**	
1 NTU	**Time**	**Residual Chlorine**	**Time**	**Residual Chlorine**
7.2 pH	(mins)	(mg/L)	(mins)	(mg/L)
62 degrees F	0	2	0	2
	1	1.57	1	1.57
	5	1.49	5	1.49
	10	1.34	10	1.34
	30	1.14	30	1.14

Trial #2: Groundwater, Buffer, and Virus Chlorine Demand

Calculation of dose: $C_1V_1 = C_2V_2$

1.1 mg/L Chlorine Dose Calculation: (100 mg/L)(V_1) = (1.1 mg/L)(250 mL)
V_1 = 2.75 mL

2 mg/L Chlorine Dose Calculation: (100 mg/L)(V1) = (2 mg/L)(250 mL)
V_1 = 5 mL

Original MS-2 titer = 10^9

Calculation of MS-2 10^6 titer: (10^9)(V_1) = (10^6)(250mL)
V_1 = 0.25 mL

Chlorine Dose: 1.1 mg/L				
Parameters	**1st Trial**		**2nd Trial**	
1 NTU	**Time**	**Residual Chlorine**	**Time**	**Residual Chlorine**
6.3 pH	(mins)	(mg/L)	(mins)	(mg/L)
62 degrees F	0	1.1	0	1.1
10^6 PFU	1	0.42	1	0.42
	5	0.4	5	0.4
	10	0.39	10	0.39
	30	0.34	30	0.34
Chlorine Dose: 2 mg/L				
Parameters	**1st Trial**		**2nd Trial**	
1 NTU	**Time**	**Residual Chlorine**	**Time**	**Residual Chlorine**
6.3 pH	(mins)	(mg/L)	(mins)	(mg/L)
62 degrees F	0	2	0	2
10^6 PFU	1	0.77	1	0.77
	5	0.75	5	0.75
	10	0.71	10	0.71
	30	0.65	30	0.65

Studies of Effect of Clay and Sonication on MS-2 Inactivation by Chlorine

Trial #1: Clay and No Clay Study

MS-2 Assay 7.12.08		
	1	2
10^0	TNTC	TNTC
10^1	TNTC	TNTC
10^2	TNTC	TNTC
10^3	TNTC	TNTC
10^4	TNTC	TNTC
10^5	TNTC	TNTC
10^6	TNTC	TNTC
10^7	TNTC	TNTC
10^8	161	161
10^9	114	100
10^10	80	70

Water Parameters	
Cl_2 Dose: 1.5 mg/L	
GW TOC: 0.69 mg/L	
Clay Mixture	**No Clay Mixture**
pH: 6.39	pH: 6.05
NTU: 5.2	NTU: 1.44

Clay Plaque Assay Results					
Time (mins)	**Dilution**	**Plaques**	**pfu/mL**	**Log**	**Log Reduction**
0	10^3	221/134			
0	10^4	47/58	1.78×10^5	5.25	N/A
0	10^5	6/10			
1	-	NG*	-	-	-
5	-	NG	-	-	-
10	-	NG	-	-	-
30	-	NG	-	-	-
* NG: no growth					

No Clay Plaque Assay Results					
Time (mins)	Dilution	Plaques	pfu/mL	Log	Log Reduction
0	10^3	146/142	1.44×10^5	5.158	0
0	10^4	71/83			
0	10^5	7/2			
1	10^3	45/56	5.05×10^4	4.703	0.457
1	10^4	5/3			
5	10^3	60/46	5.3×10^4	4.724	0.437
5	10^4	2/4			
10	10^3	32/37	3.45×10^4	4.538	0.622
10	10^4	2/4			
30	10^3	33/41	3.7×10^4	4.568	0.593
30	10^4	1/5			

Trial #2: Clay and No Clay Study

Water Parameters	
Cl_2 Dose: 1.5 mg/L	
GW TOC: 0.69 mg/L	
Clay Mixture	**No Clay Mixture**
pH: 6.49	pH: 6.33
NTU: 5.4	NTU: 1.6

No Clay Plaque Assay Results					
Time (mins)	**Dilution**	**Plaques**	**pfu/mL**	**Log**	**Log Reduction**
0	10^3	123/118			
0	10^4	16/22	1.205×10^5		
0	10^5	6/9		5.081	0
1	10^1	13/13	1.3×10^2	2.114	2.967
5	10^1	12/16	1.4×10^2	2.146	2.935
10	10^1	19/13	1.6×10^2	2.204	2.877
30	10^1	19/6	1.25×10^2	2.097	2.984

Clay Plaque Assay Results					
Time (mins)	**Dilution**	**Plaques**	**pfu/mL**	**Log**	**Log Reduction**
0	10^4	182/205			
0	10^5	17/23	1.94×10^6		
0	10^6	6/4		6.288	0
1	10^3	35/37			
1	10^4	8/10			
1	10^5	1/3	3.6×10^4	4.556	1.732
5	10^3	42/44			
5	10^4	16/10			
5	10^5	0/1	4.3×10^4	4.633	1.655
10	10^3	73/96	8.45×10^4		
10	10^4	2/3		4.927	1.361
30	10^3	37/41			
30	10^4	1/8	3.9×10^4	4.591	1.697

Analysis of Variance Trial 2

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Batch	1	3.7019205	3.70192	203.9270	<.0001*
Error	6	0.1089190	0.01815		
C. Total	7	3.8108395			

Means for Oneway Anova of Log Reductions Trial 2

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
Clay	4	1.61000	0.06737	1.4452	1.7748
No Clay	4	2.97050	0.06737	2.8057	3.1353

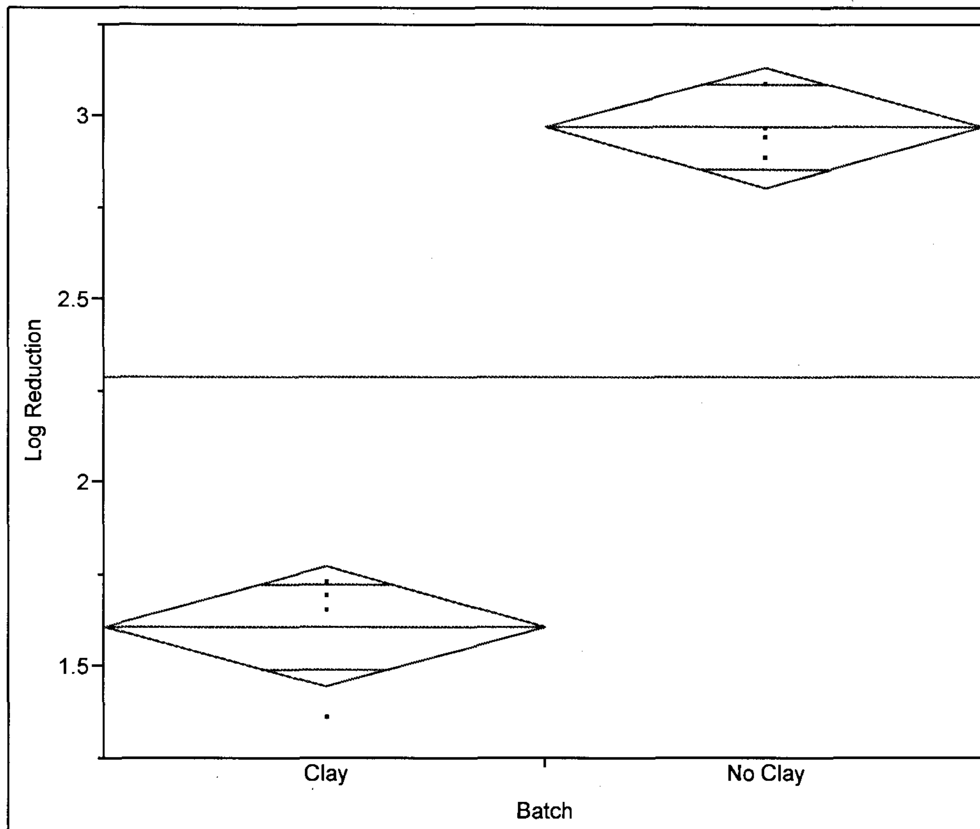

Figure A1: Diamond Plots for Trial 2

Trial #3: Clay, No Clay, Sonication, and No Sonication Study

MS-2 Assay 1.9.09		
	1	2
10^0	TNTC	TNTC
10^1	TNTC	TNTC
10^2	TNTC	TNTC
10^3	TNTC	TNTC
10^4	TNTC	TNTC
10^5	TNTC	TNTC
10^6	TNTC	TNTC
10^7	TNTC	TNTC
10^8	230	234
10^9	20	26
10^10	4	5

Water Parameters	
Cl_2 Dose: 2 mg/L	
TOC: 0.64 mg/L	
Clay Mixture	**No Clay Mixture**
pH: 7.2	pH: 6.9
NTU: 5.1	NTU: 1.54

Clay, No Sonicate Plaque Assay Results					
Time (mins)	**Dilution**	**Plaques**	**pfu/mL**	**Log**	**Log Reduction**
0	10^3	298/307			
0	10^4	182/205	1.94×10^6		
0	10^5	17/23		6.288	0
1	10^3	89/70	7.95×10^4		
1	10^4	10/7		4.9	1.388
5	10^3	158/96	6.75×10^5		
5	10^4	59/76		5.829	0.459
10	10^3	56/87	7.15×10^4		
10	10^4	16/30		4.854	1.434
30	10^2	30/27	2.85×10^3		
30	10^3	7/0		3.455	2.833

Clay, Sonicate Plaque Assay Results					
Time (mins)	**Dilution**	**Plaques**	**pfu/mL**	**Log**	**Log Reduction**
0	10^3	204/275			
0	10^4	108/136	1.22×10^6		
0	10^5	14/8		6.086	N/A
1	-	NG	-	-	-
5	-	NG	-	-	-
10	-	NG	-	-	-
30	-	NG	-	-	-

No Clay, No Sonicate Plaque Assay Results					
Time (mins)	**Dilution**	**Plaques**	**pfu/mL**	**Log**	**Log Reduction**
0	10^2	319/304			
0	10^3	97/62			
0	10^4	12/6	7.95×10^4	4.9	0
1	10^2	24/31	2.75×10^3	3.439	1.461
5	10^1	22/31	2.65×10^2	2.423	2.477
10	10^1	16/10	1.3×10^2	2.114	2.786
30	10^1	14/11	1.25×10^2	2.097	2.803

No Clay, Sonicate Plaque Assay Results					
Time (mins)	**Dilution**	**Plaques**	**pfu/mL**	**Log**	**Log Reduction**
0	10^3	107/86			
0	10^4	31/21			
0	10^5	5/2	9.56×10^4	4.985	N/A
1	-	NG	-	-	-
5	-	NG	-	-	-
10	-	NG	-	-	-
30	-	NG	-	-	-

Analysis of Variance of Log Reduction Trial 3

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Batch	1	1.4706125	1.47061	2.1626	0.1918
Error	6	4.0800750	0.68001		
C. Total	7	5.5506875			

Means for Oneway Anova of Log Reduction Trial 3

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
Clay	4	1.53000	0.41231	0.5211	2.5389
No Clay	4	2.38750	0.41231	1.3786	3.3964

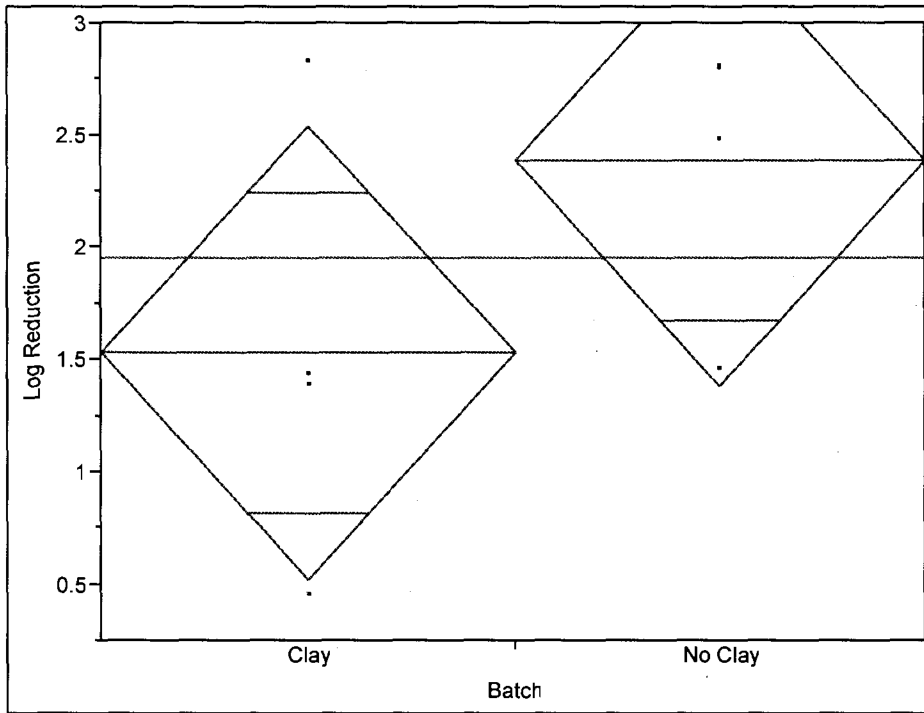

Figure A2: Diamond Plot for Trial 3

Trial #4: Clay, No Clay, Sonication, and No Sonication Study

Water Parameters	
Cl_2 Dose: 1.1 mg/L	
TOC: 0.61 mg/L	
Clay Mixture	**No Clay Mixture**
pH: 7.01	pH: 6.89
NTU: 5.1	NTU: 1.2

Clay, No Sonicate Plaque Assay Results					
Time (mins)	**Dilution**	**Plaques**	**pfu/mL**	**Log**	**Log Reduction**
0	10^3	40/35	3.75×10^4	4.574	0
0	10^4	6/2			
1	10^3	25/24	2.45×10^4	4.389	0.185
1	10^4	3/4			
5	10^3	27/23	2.5×10^4	4.398	0.176
5	10^4	2/3			
10	10^3	22/18	2.0×10^4	4.301	0.273
10	10^4	4/5			
30	10^3	45/50	4.75×10^4	4.677	N/A
30	10^4	3/7			

Clay, Sonicate Plaque Assay Results					
Time (mins)	**Dilution**	**Plaques**	**pfu/mL**	**Log**	**Log Reduction**
0	10^3	56/68	6.2×10^4	4.792	0
0	10^4	7/5			
1	10^3	12/22	1.7×10^4	4.23	0.562
1	10^4	2/2			
5	10^3	16/17	1.65×10^4	4.217	0.575
5	10^4	3/2			
10	10^3	20/23	2.15×10^4	4.332	0.46
10	10^4	5/4			
30	10^2	9/3	6.0×10^2	2.778	2.014

No Clay, No Sonicate Plaque Assay Results					
Time (mins)	Dilution	Plaques	pfu/mL	Log	Log Reduction
0	10^3	154/105			
0	10^4	11/12			
0	10^5	2/1	1.29×10^5	5.111	0
1	10^2	11/18	1.45×10^3	3.161	1.95
5	10^2	7/3	5.0×10^2	2.699	2.412
10	10^2	5/6	5.5×10^2	2.74	2.371
30	-	NG	NG	-	-

No Clay, Sonicate Plaque Assay Results					
Time (mins)	Dilution	Plaques	pfu/mL	Log	Log Reduction
0	10^3	147/93			
0	10^4	13/12			
0	10^5	1/1	1.20×10^5	5.079	0
1	10^2	13/14	1.35×10^3	3.13	1.949
5	10^2	5/6	5.5×10^2	2.74	2.339
10	10^2	7/4	5.5×10^2	2.74	2.339
30	10^2	8/9	8.5×10^2	2.929	2.15

Analysis of Variance of Log Reduction

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Batch	1	2.9282000	2.92820	4.4253	0.0801
Error	6	3.9701500	0.66169		
C. Total	7	6.8983500			

Means for Oneway Anova of Log Reduction

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
Clay	4	0.81250	0.40672	-0.183	1.8077
No Clay	4	2.02250	0.40672	1.027	3.0177

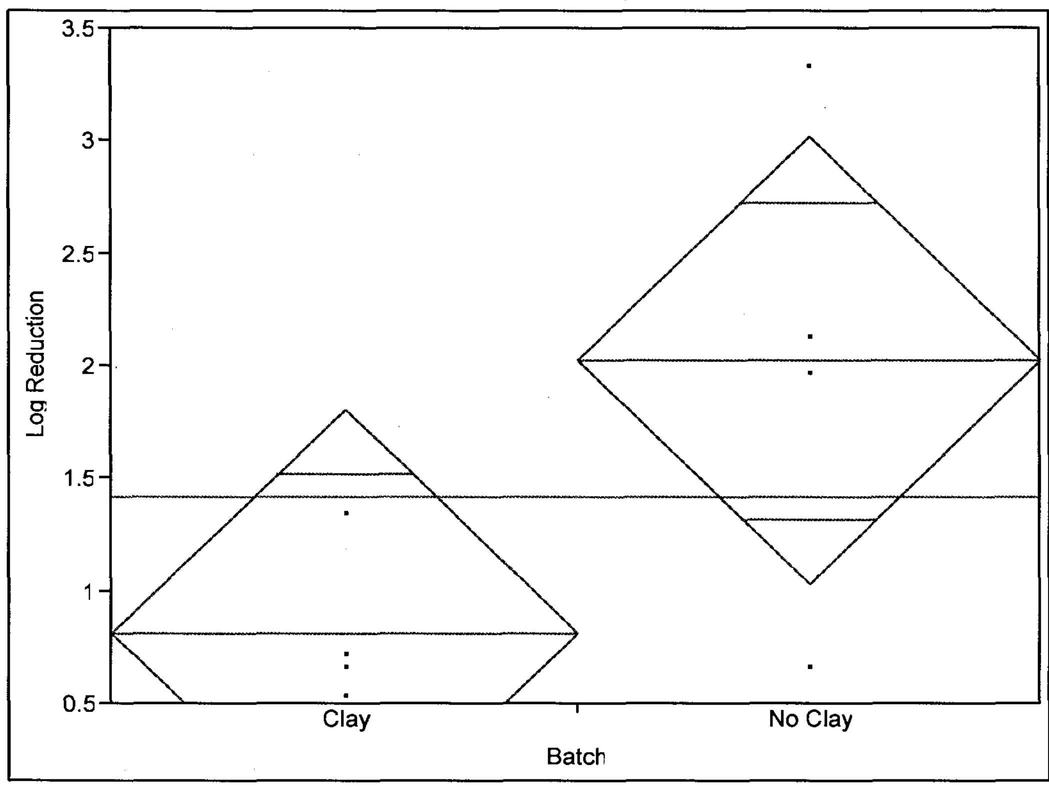

Figure A3: Diamond Plot for Trial 4

Trial #5: Clay, No Clay, Sonication, and No Sonication Study

MS-2 Assay 3.2.09		
	1	2
10^0	TNTC	TNTC
10^1	TNTC	TNTC
10^2	TNTC	TNTC
10^3	TNTC	TNTC
10^4	TNTC	TNTC
10^5	TNTC	TNTC
10^6	TNTC	TNTC
10^7	TNTC	TNTC
10^8	46	29
10^9	3	4
10^10	-	-

Water Parameters	
Cl_2 Dose: 1.1 mg/L	
TOC: 0.83 mg/L	
Clay Mixture	**No Clay Mixture**
pH: 7.03	pH: 7.01
NTU: 5.7	NTU: 1.3

Clay, No Sonicate Plaque Assay Results					
Time (mins)	**Dilution**	**Plaques**	**pfu/mL**	**Log**	**Log Reduction**
0	10^3	140/147			
0	10^4	20/17			
0	10^5	6/1	1.44×10^5	5.158	0
1	10^3	32/36			
1	10^4	16/14			
1	10^5	2/1	3.4×10^4	4.531	0.627
5	10^3	31/24			
5	10^4	10/7	2.75×10^4	4.439	0.719
10	10^3	22/15			
10	10^4	4/7	1.85×10^4	4.267	0.891
30	10^2	45/96			
30	10^3	9/3	7.05×10^3	3.848	1.31

Clay, Sonicate Plaque Assay Results					
Time (mins)	**Dilution**	**Plaques**	**pfu/mL**	**Log**	**Log Reduction**
0	10^3	153/159			
0	10^4	19/24			
0	10^5	2/9	1.56×10^5	5.193	0
1	10^3	24/30			
1	10^4	12/7	2.7×10^4	4.431	0.762
5	10^3	40/55			
5	10^4	22/17			
5	10^5	4/5	4.75×10^4	4.677	0.516
10	10^3	37/25			
10	10^4	8/3	3.1×10^4	4.491	0.702
30	10^2	12/5	8.5×10^2	2.929	2.264

No Clay, No Sonicate Plaque Assay Results					
Time (mins)	**Dilution**	**Plaques**	**pfu/mL**	**Log**	**Log Reduction**
0	10^3	107/70			
0	10^4	17/12			
0	10^5	2/4	8.85×10^4	4.947	0
1	10^3	22/17	1.95×10^4	4.29	0.657
5	10^2	15/6	1.05×10^3	3.021	1.926
10	10^2	11/4	7.5×10^2	2.875	2.072
30	10^1	2/10	6×10^1	1.778	3.169

No Clay, No Sonicate Plaque Assay Results					
Time (mins)	**Dilution**	**Plaques**	**pfu/mL**	**Log**	**Log Reduction**
0	10^3	56/89			
0	10^4	16/12			
0	10^5	4/8	7.25×10^4	4.86	0
1	10^3	20/17	1.85×10^4	4.267	0.593
5	10^2	14/10	1.2×10^3	3.079	1.721
10	10^2	7/5	6×10^2	2.778	2.082
30	10^2	7/2	4.5×10^2	2.653	2.207

Analysis of Variance of Log Reductions Trial 4

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Batch	1	0.8071281	0.807128	1.2544	0.2952
Error	8	5.1476508	0.643456		
C. Total	9	5.9547789			

Means for Oneway Anova of Log Reductions Trial 4

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
NS	5	0.88900	0.35874	0.06175	1.7162
S	5	1.45720	0.35874	0.62995	2.2844

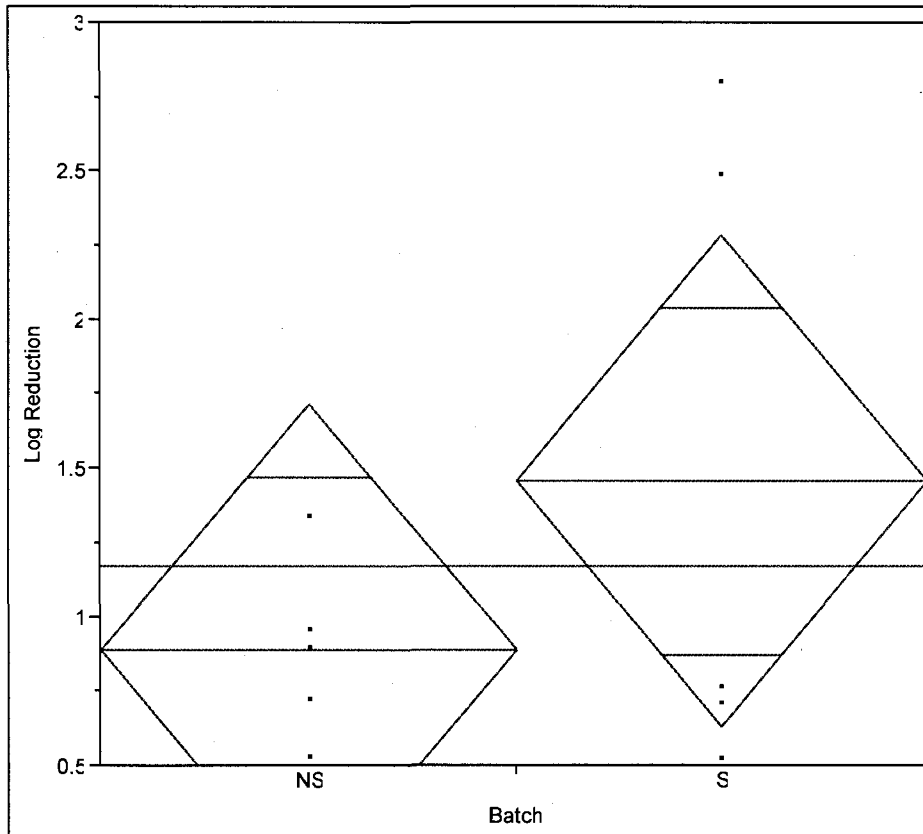

Figure A4: Diamond Plot for Trial 5

APPENDIX B

Ultraviolet Light Studies

Studies of Effect of Clay and Sonication on MS-2 Inactivation by Ultraviolet Light (Non-pellet Method)

Trial #1: Clay, No Clay Study

A^{254} Clay sample = 0.075
A^{254} No Clay sample = 0.036

Bolton Speadsheet Results	
Clay sample	
Dose (mJ/cm^2)	Time (mins)
20	2.37
40	4.75
60	7.12
80	9.5
100	11.87
No Clay Sample	
Dose (mJ/cm^2)	Time (mins)
20	2.45
40	4.88
60	7.33
80	9.78
100	12.23

Clay Plaque Assay Results					
Dose (mJ/cm^2)	**Dilution**	**Plaques**	**pfu/mL**	**Log**	**Log Reduction**
0	10^3	254/283			
0	10^4	57/71			
0	10^5	16/21	2.69 x 10^5	5.43	0
20	10^3	134/218			
20	10^4	67/92			
20	10^5	10/6	1.76 x 10^5	5.245	0.185
40	10^2	38/31	3.45 x 10^3	3.538	1.892
60	10^1	178/225			
60	10^2	27/19	2.02 x 10^3	3.305	2.125
80	10^1	39/37	3.8 x 10^2	2.579	2.851
100	10^1	18/11	1.45 x 10^2	2.161	3.269

No Clay Plaque Assay Results					
Dose (mJ/cm^2)	Dilution	Plaques	pfu/mL	Log	Log Reduction
0	10^3	189/237			
0	10^4	38/42			
0	10^5	27/11	2.115 x 10^5	5.325	0
20	10^3	163/230			
20	10^4	45/61			
20	10^5	27/23	1.97 x 10^5	5.294	0.031
40	10^2	68/30	4.9 x 10^3	3.69	1.635
60	10^1	302/239			
60	10^2	13/5	2.71 x 10^3	3.433	1.892
80	10^1	53/42	4.75 x 10^2	2.677	2.648
100	10^1	12/5	8.5 x 10^1	1.929	3.396

Analysis of Variance of Log Reductions Trial 1

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Batch	1	0.039690	0.03969	0.0265	0.8747
Error	8	11.968320	1.49604		
C. Total	9	12.008010			

Means for Oneway Anova of Log Reductions Trial 1

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
Clay	5	1.94400	0.54700	0.68262	3.2054
No Clay	5	2.07000	0.54700	0.80862	3.3314

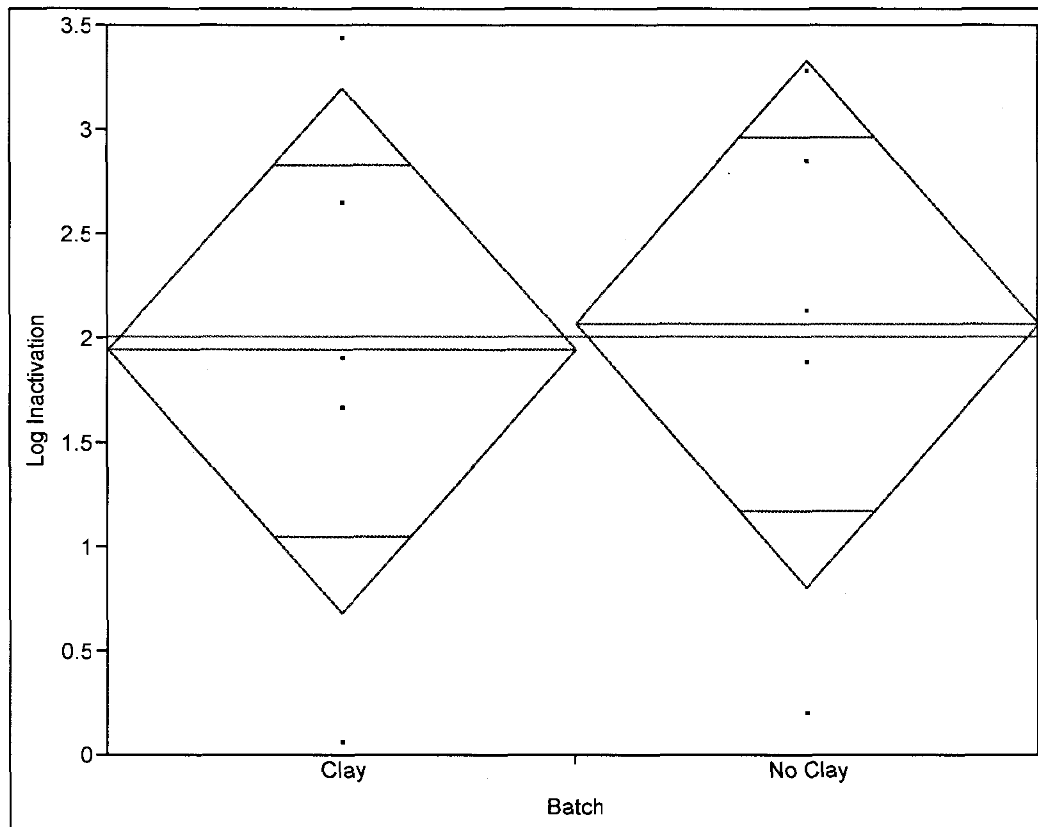

Figure B1: Diamond Plot UV Trial 1

Trial #2: Clay, No Clay Study

A254 Clay sample = 0.074
A254 No Clay sample = 0.037

Bolton Speadsheet Results	
Clay sample	
Dose (mJ/cm^2)	Time (mins)
20	2.62
40	5.25
60	7.86
80	10.48
100	13.1
No Clay Sample	
Dose (mJ/cm^2)	Time (mins)
20	2.55
40	5.1
60	7.65
80	10.2
100	12.75

Clay Plaque Assay Results					
Dose (mJ/cm^2)	**Dilution**	**Plaques**	**pfu/mL**	**Log**	**Log Reduction**
0	10^3	327/346			
0	10^4	84/64	7.4 x		
0	10^5	19/8	10^5	5.869	0
20	10^3	202/230			
20	10^4	75/69	2.16 x		
20	10^5	11/14	10^5	5.334	0.535
40	10^2	41/36	3.85 x 10^3	3.585	2.284
60	10^1	228/224	2.26 x		
60	10^2	18/16	10^3	3.354	2.515
80	10^1	114/89	1.02 x 10^3	3.009	2.86
100	10^1	16/16	1.6 x 10^2	2.204	3.665

No Clay Plaque Assay Results					
Dose (mJ/cm^2)	Dilution	Plaques	pfu/mL	Log	Log Reduction
0	10^3	276/291			
0	10^4	62/73			
0	10^5	10/12	2.84 x 10^5	5.453	0
20	10^3	210/137			
20	10^4	89/62			
20	10^5	4/9	1.69 x 10^5	5.228	0.225
40	10^2	40/33	3.65 x 10^3	3.562	1.891
60	10^1	117/256			
60	10^2	13/8	1.87 x 10^3	3.272	2.181
80	10^1	48/61	5.45 x 10^2	2.736	2.717
100	10^1	7/9	8.0 x 10^1	1.903	3.55

Analysis of Variance of Log Reductions Trial 2

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Batch	1	0.6400900	0.640090	0.8058	0.3956
Error	8	6.3547200	0.794340		
C. Total	9	6.9948100			

Means for Oneway Anova of Log Reductions Trial 2

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
Clay	5	1.71000	0.39858	0.7909	2.6291
No Clay	5	2.21600	0.39858	1.2969	3.1351

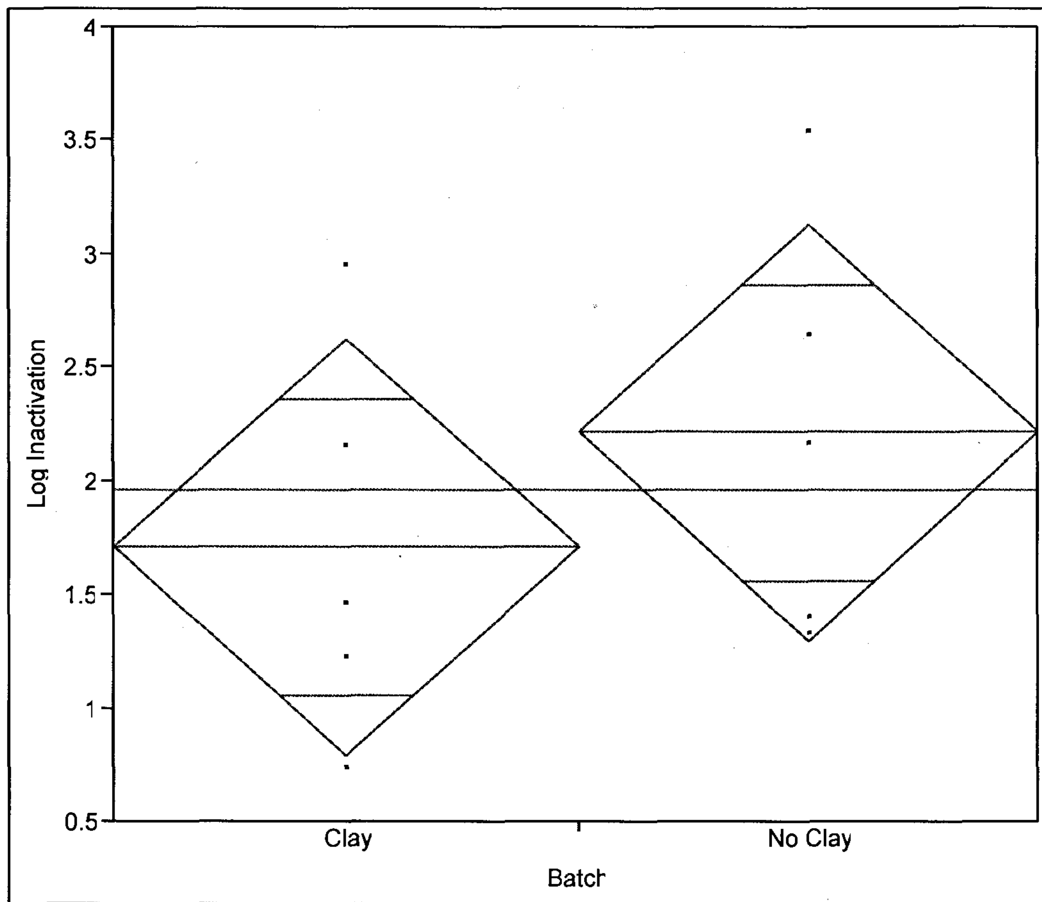

Figure B2: Diamond Plot for UV Trial 2

Trial #3: Clay, No Clay, Sonicate, No Sonicate Study

A^{254} Clay sample = 0.079
A^{254} No Clay sample = 0.04

Bolton Speadsheet Results	
Clay sample	
Dose (mJ/cm^2)	Time (mins)
20	2.63
40	5.27
60	5.9
80	10.52
100	13.15
No Clay Sample	
Dose (mJ/cm^2)	Time (mins)
20	2.55
40	5.12
60	7.67
80	10.22
100	12.77

No Clay, Sonicate Plaque Assay Results					
Dose (mJ/cm^2)	**Dilution**	**Plaques**	**pfu/mL**	**Log**	**Log Reduction**
0	10^3	316/294			
0	10^4	41/53			
0	10^5	11/12	4.7 x 10^5	5.672	0
20	10^3	38/37	3.75 x 10^4	4.574	1.098
40	10^2	127/158	1.43 x 10^4	4.155	1.517
60	10^1	193/207			
60	10^2	24/27	2.00 x 10^3	3.301	2.371
80	10^1	69/79			
80	10^2	2/2	7.4 x 10^2	2.869	2.803
100	10^1	8/6	7.0 x 10^1	1.845	3.827

No Clay, No Sonicate Plaque Assay Results					
Dose (mJ/cm²)	Dilution	Plaques	pfu/mL	Log	Log Reduction
0	10^3	271/285			
0	10^4	42/56			
0	10^5	11/7	2.78 x 10^5	5.444	0
20	10^3	10/12	1.1 x 10^4	4.041	1.403
40	10^2	123/136	1.295 x 10^4	4.112	1.332
60	10^1	178/194			
60	10^2	21/24	1.86 x 10^3	3.269	2.175
80	10^1	63/64			
80	10^2	2/5	6.35 x 10^2	2.803	2.641
100	10^1	9/7	8.0 x 10^1	1.903	3.541

Clay, Sonicate Plaque Assay Results					
Dose (mJ/cm²)	Dilution	Plaques	pfu/mL	Log	Log Reduction
0	10^3	54/45			
0	10^4	23/14			
0	10^5	2/4	4.95 x 10^4	4.695	0
20	10^3	3/1	3.0 x 10^3	3.477	1.218
40	10^2	146/116	1.31 x 10^4	4.117	0.578
60	10^1	322/286			
60	10^2	16/17	3.04 x 10^3	3.483	1.212
80	10^1	62/80			
80	10^2	5/4	7.1 x 10^2	2.851	1.844
100	10^1	10/9	9.5 x 10^1	1.978	2.717

Clay, No Sonicate Plaque Assay Results					
Dose (mJ/cm²)	Dilution	Plaques	pfu/mL	Log	Log Reduction
0	10^3	84/82			
0	10^4	25/27			
0	10^5	3/4	8.3 x 10^4	4.92	0
20	10^3	4/6	5.0 x 10^3	3.699	1.221
40	10^2	163/140	1.52 x 10^4	4.182	0.738
60	10^1	250/249			
60	10^2	27/20	2.495 x 10^3	3.397	1.523
80	10^1	53/62			
80	10^2	3/1	5.75 x 10^2	2.76	2.16
100	10^1	11/8	9.5 x 10^1	1.978	2.942

Analysis of Variance of Sonication/No Sonication of Log Reductions Trial 3

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Batch	1	0.0828100	0.082810	0.1176	0.7405
Error	8	5.6316800	0.703960		
C. Total	9	5.7144900			

Means for Oneway Anova of Sonication/No Sonication of Log Reductions Trial 3

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
Clay	5	1.52800	0.37522	0.66274	2.3933
No Clay	5	1.71000	0.37522	0.84474	2.5753

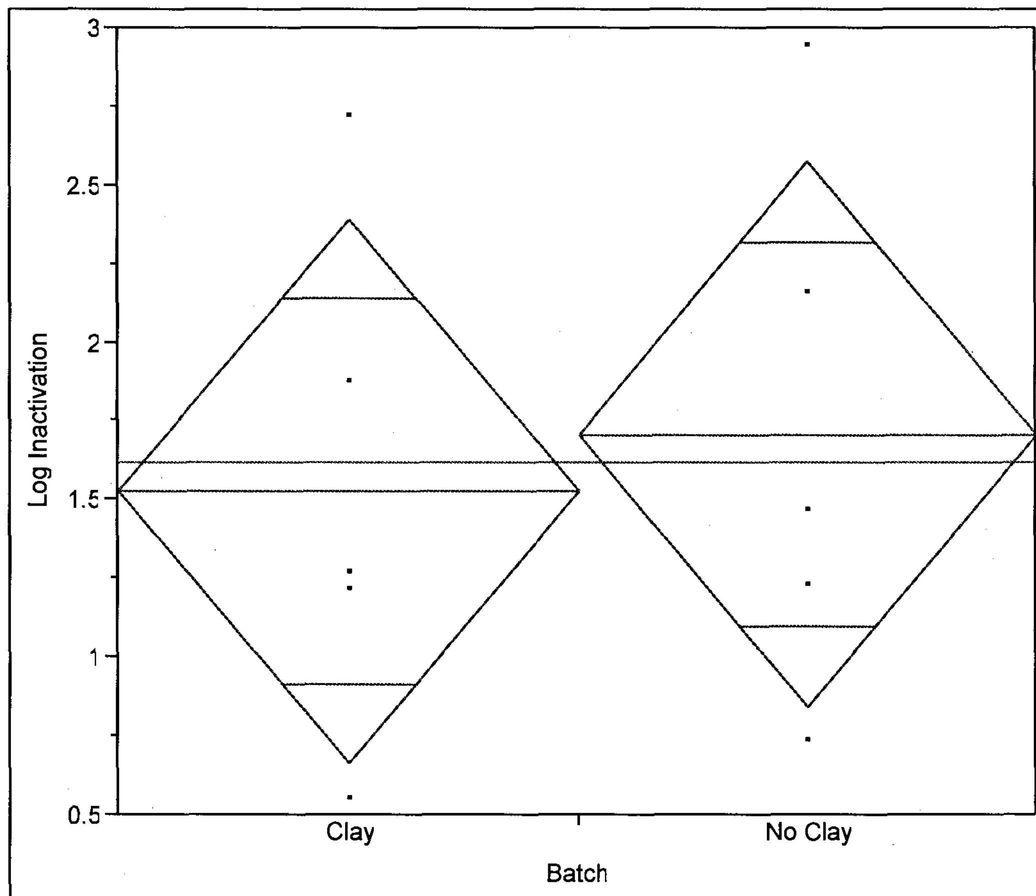

Figure B3: Diamond Plots of UV Trial 3

Studies of Effect of Clay on MS-2 Inactivation by Ultraviolet Light (Pellet Method)

The absorbance's for the free samples are often higher then the pellet sample for these experiments due to the high absorbance characteristics of the tryptic soy broth the virus was grown in.

Trial #1

A^{254} Pellet sample = 1.204
A^{254} Free sample = 1.776

Bolton Speadsheet Results	
Pellet sample	
Dose (mJ/cm^2)	Time
20	5m 26s
40	10 53s
60	16m 19s
80	21m 45s
100	27m 11s
Free Sample	
Dose (mJ/cm^2)	Time
20	7m 14s
40	14m 28s
60	21m 42s
80	28m 56s
100	36m 10s

Collimated Beam Results		
Pellet Virus		
Dose (mJ/cm^2)	(PFU/mL)	Log Reduction
0	1.53E+06	
20	3.10E+04	1.69
40	2.35E+03	2.81
60	9.00E+02	3.23
80	2.65E+03	2.76
100	1.10E+03	3.14
Free Virus		
Dose (mJ/cm^2)	(PFU/mL)	Log Reduction
0	1.74E+06	
20	5.50E+06	-0.50
40	8.05E+05	0.33
60	1.70E+05	1.01
80	2.40E+04	1.86
100	2.40E+03	2.86

Trial #2

A^{254} Pellet sample = 1.204
A^{254} Free sample = 1.776

Bolton Speadsheet Results	
Pellet sample	
Dose (mJ/cm²)	Time
20	6m 46s
40	13m 31s
60	20m 17s
80	27m 2s
100	33m 48s
Free Sample	
Dose (mJ/cm²)	Time
20	6m 23s
40	12m 46s
60	19m 10s
80	25m 33s
100	31m 56s

Collimated Beam Results		
Pellet Virus		
Dose (mJ/cm^2)	(PFU/mL)	Log Reduction
0	5.50E+06	
20	4.40E+04	2.10
40	3.00E+04	2.26
60	1.60E+04	2.54
80	3.00E+03	3.26
100	8.60E+03	2.81
Free Virus		
Dose (mJ/cm^2)	(PFU/mL)	Log Reduction
0	1.80E+07	
20	2.40E+06	0.88
40	6.20E+05	1.46
60	1.10E+05	2.21
80	1.00E+04	3.26
100	5.70E+03	3.50

Trial #3

A^{254} Pellet sample = 1.204
A^{254} Free sample = 1.776

Bolton Speadsheet Results	
Pellet sample	
Dose (mJ/cm2)	Time
20	7m 56s
40	15m 53s
60	23m 49s
80	31m 45s
100	39m 41s
Free Sample	
Dose (mJ/cm2)	Time
20	7m 31s
40	15m 2s
60	22m33s
80	30m 4s
100	37m 35s

Collimated Beam Results		
Free Virus		
Dose (mJ/cm^2)	(PFU/mL)	Log Reduction
0	3.40E+08	
20	3.10E+08	0.04
40	1.96E+06	2.24
60	9.50E+04	3.55
80	1.43E+05	3.38
100	5.60E+04	3.78
Pellet Virus		
Dose (mJ/cm^2)	(PFU/mL)	Log Reduction
0	4.40E+06	
20	1.80E+05	1.39
40	1.79E+04	2.39
60	1.30E+04	2.53
80	1.50E+04	2.47
100	1.65E+03	3.43

Trial #4

A^{254} Pellet sample = 1.204
A^{254} Free sample = 1.776

Bolton Speadsheet Results	
Pellet sample	
Dose (mJ/cm^2)	Time
20	7m 19s
40	14m 39s
60	21m 58s
80	29m 18s
100	36m 37s
Free Sample	
Dose (mJ/cm^2)	Time
20	6m 34s
40	13m 9s
60	19m 43s
80	26m 17s
100	32m 51s

Collimated Beam Results		
Pellet Virus		
Dose (mJ/cm^2)	(PFU/mL)	Log Reduction
0	9.50E+06	
20	1.65E+05	1.76
40	7.40E+03	3.11
60	1.04E+04	2.96
80	1.87E+04	2.71
100	2.90E+03	3.52

Free Virus		
Dose (mJ/cm^2)	(PFU/mL)	Log Reduction
0	3.10E+08	
20	3.70E+07	0.92
40	7.40E+05	2.62
60	1.65E+05	3.27
80	1.00E+05	3.49
100	6.50E+04	3.68

APPENDIX C

Water Reuse Requirements for Water Quality and Treatment

Water Quality and Treatment Tables adapted from USEPA, 2004

Agricultural Reuse - Non-Food Crops

	Arizona	California	Florida	Hawaii	Nevada	Texas	Washington
Treatment	Secondary treatment and disinfection	Secondary -23, Oxidized, and disinfected	Secondary treatment, basic disinfection	Oxidized, filtered, and disinfected	Secondary treatment and disinfection	NS*	Oxidized and disinfected
BOD6	NS	NS	20 mg/l CBOD5	NS	30 mg/l	5 mg/l	30 mg/l
TSS	NS	NS	20 mg/l	NS	NS	NS	30 mg/l
Turbidity	NS	NS	NS	2 NTU (Max)	NS	3 NTU	2 NTU (Avg)
							5 NTU (Max)
Coliform	Fecal	Total	Fecal	Fecal	Fecal	Fecal	Total
	200/100 ml (Avg)	23/100 ml (Avg)	200/100 ml (Avg)	2.2/100 ml (Avg)	200/100 ml (Avg)	20/100 ml (Avg)	23/100 ml (Avg)
	800/100 ml (Max)	240/100 ml (Max in 30 days)	800/100 ml (Max)	23/100 ml (Max)	400/100 ml (Max)	75/100 ml (Max)	240/100 ml (Max)

*NS - Not specified by state regulations

Agricultural Reuse - Food Crops

	Arizona	California	Florida	Hawaii	Nevada	Texas	Washington
Treatment	Secondary treatment, filtration, and disinfection	Oxidized, coagulated, filtered, and disinfected	Secondary treatment, filtration, and high-level disinfection	Oxidized, filtered, and disinfected	Secondary treatment and disinfection	NS*	Oxidized, coagulated, filtered, and disinfected
BOD6	NS	NS	20 mg/l CBOD5	NS	30 mg/l	5 mg/l	30 mg/l
TSS	NS	NS	5 mg/l	NS	NS	NS	30 mg/l
Turbidity	2 NTU (Avg)	2 NTU (Avg)	NS	2 NTU (Max)	NS	3 NTU	2 NTU (Avg)
	5 NTU (Max)						5 NTU (Max)
Coliform	Fecal	Total	Fecal	Fecal	Fecal	Fecal	Total
	None detectable (Avg)	2.2/100 ml (Avg)	75% of samples below detection	2.2/100 ml (Avg)	200/100 ml (Avg)	20/100 ml (Avg)	2.2/100 ml (Avg)
	23/100 ml (Max)	23/100 ml (Max in 30 days)	25/100 ml (Max)	23/100 ml (Max in 30 days)	400/100 ml (Max)	75/100 ml (Max)	23/100 ml (Max)

*NS - Not specified by state regulations

Unrestricted Recreational Reuse							
	Arizona	**California**	**Florida**	**Hawaii**	**Nevada**	**Texas**	**Washington**
Treatment	NR**	Oxidized, coagulated, clarified, filtered, and disinfected	NR	NR	Secondary treatment and disinfection	NS*	Oxidized, coagulated, filtered, and disinfected
BOD6	NR	NS	NR	NR	30 mg/l	5 mg/l	30 mg/l
TSS	NR	NS	NR	NR	NS	NS	30 mg/l
Turbidity	NR	2 NTU (Avg)	NR	NR	NS	3 NTU	2 NTU (Avg)
		5 NTU (Max)					5 NTU (Max)
Coliform		Total			Fecal	Fecal	Fecal
	NR	2.2/100 ml (Avg)	NR	NR	2.2/100 ml (Avg)	20/100 ml (Avg)	2.2/100 ml (Avg)
		23/100 ml (Max in 30 days)			23/100 ml (Max)	75/100 ml (Max)	23/100 ml (Max)

*NS - Not specified by state regulations

**NR - Not regulated by the state

Restricted Recreational Reuse							
	Arizona	**California**	**Florida**	**Hawaii**	**Nevada**	**Texas**	**Washington**
Treatment	Secondary treatment, filtration, and disinfection	Secondary-23, oxidized, and disinfected	NR**	Oxidized, filtered, and disinfected	Secondary treatment and disinfection	NS	Oxidized and disinfected
BOD6	NS*	NS	NR	NS	30 mg/l	20 mg/l	30 mg/l
TSS	NS	NS	NR	NS	NS	NS	30 mg/l
Turbidity	2 NTU (Avg)	NS	NR	2 NTU (Max)	NS	NS	2 NTU (Avg)
	5 NTU (Max)						5 NTU (Max)
Coliform	Fecal	Total	NR	Fecal	Fecal	Fecal	Total
	detec table (Avg)	2.2/100 ml (Av g)		2.2/100 ml (Av g)	200/100 ml (Av g)	200/100 ml (Av g)	2.2/100 ml (Av g)
	23/100 ml (Max)	23/100 ml (Max in 30 days)		23/100 ml (Max)	23/100 ml (Max)	800/100 ml (Max)	23/100 ml (Max)

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**NR - Not regulated by the state

Environmental Reuse - Wetlands							
	Arizona	**California**	**Florida**	**Hawaii**	**Nevada**	**Texas**	**Washington**
Treatment	NR**	NR	Advanced treatment	NR	NR	NR	Oxidized, coagulated, and disinfected
BOD6	NR	NR	5 mg/l CBOD5	NR	NR	NR	20 mg/l
TSS	NR	NR	5 mg/l	NR	NR	NR	20 mg/l
Coliform							Fecal
	NR	NR	NS*	NR	NR	NR	2.2/100 ml (Avg)
							23/100 m l (Max)
Total Ammonia	NR	NR	2 mg/l	NR	NR	NR	Not to exceed chronic standards for freshwater
Total Phosphorus	NR	NR	1 mg/l	NR	NR	NR	1 mg/l

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**NR - Not regulated by the state

Industrial Reuse***							
	Arizona	California	Florida	Hawaii	Nevada	Texas	Washington
Treatment	NR**	Oxidized and disinfected	Secondary treatment and basic disinfection	Oxidized and disinfected	NR	NS	Oxidized and disinfected
BOD6	NR	NS*	20 mg/l	NS	NR	20 mg/l	NS
TSS	NR	NS	20 mg/l	NS	NR	---	NS
Turbidity	NR	NS	NS	NS	NR	3 NTU	NS
Coliform	NR	Total	Fecal	Fecal	NR	Fecal	Total
		23/100 ml (Avg)	200/100 ml (Avg)	23/100 ml (Avg)		200/100 ml (Avg)	23/100 ml (Avg)
		240/100 ml (Max in 30 days)	800/100 ml (Max)	200/100 ml (Max)		800/100 ml (Avg)	240/100 ml (Avg)
*NS - Not specified by state regulations							
**NR - Not regulated by the state							
***All state requirements are minimum values. Additional treatment may be required depending on expected public exposure.							

Groundwater Recharge***							
	Arizona	California	Florida	Hawaii	Nevada	Texas	Washington
Treatment	NR	Determined on a Case-by-Case Basis	Secondary treatment and basic disinfection	Determined on a Case-by-Case Basis	NR	NR	Oxidized, coagulated, filtered, and disinfected
BOD6	NR		NS		NR	NR	5 mg/L
TSS	NR		10 mg/L		NR	NR	5 mg/L
Turbidity	NR		NS		NR	NR	2 NTU (Avg)
							5 NTU (Max)
Coliform	NR		NS		NR	NR	Total
							2.2/100 ml (Avg)
							23/100 ml (Max)
Total Nitrogen	NR		12 mg/L		NR	NR	NS

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**NR - Not regulated by the state

***All state requirements are for groundwater recharge via rapid-rate application systems.

Indirect Nonpotable Reuse							
	Arizona	**California**	**Florida**	**Hawaii**	**Nevada**	**Texas**	**Washington**
Treatment	NR	Determined on a Case-by-Case Basis	Adavnced treatment, filtration, and high-level disinfection	Determined on a Case-by-Case Basis	NR	NR	Oxidized, coagulated, filtered, reverse-osmosis treated, and disinfected
BOD6	NR		20 mg/L		NR	NR	5 mg/L
TSS	NR		5.0 mg/L		NR	NR	5 mg/L
Turbidity	NR		NS*		NR	NR	0.1 NTU (Avg)
							0.5 NTU (Max)
Coliform	NR		Total		NR	NR	Total
			All samples less than detection				1/100 ml (Avg)
							5/100 ml (Max)
Total Nitrogen	NR		10 mg/L		NR	NR	10 mg/L
TOC	NR		3 mg/L (Avg)		NR	NR	1.0 mg/L
			5mg/L (Max)				
Primary and Secondary Standards	NR		Compliance with most Primary and Secondary		NR	NR	Compliance with most Primary and Secondary

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