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Evaluation of an enhanced magneto-chemical process for the removal of pathogens in wastewater

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EVALUATION OF AN ENHANCED MAGNETO-CHEMICAL PROCESS FOR THE REMOVAL OF PATHOGENS IN WASTEWATER

BY

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DISSERTATION

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INFORMATION TO USERS

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DEDICATION

This dissertation is dedicated to Jessica for believing in me even when I didn't believe in myself; I could not have done it without your support, encouragement and motivation. To my parents, Chester and Elisa, who always emphasized the importance of education, hard work, persistence and personal sacrifice. And to my sisters, Dominique and Michele, for providing much needed perspective and humor along the way.

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ABSTRACT

EVALUATION OF AN ENHANCED MAGNETO-CHEMICAL PROCESS FOR THE REMOVAL OF PATHOGENS IN WASTEWATER

by

Christine N. Wright

University of New Hampshire, May, 2008

New wastewater treatment technologies are a necessity as a result of increasingly stringent discharge standards, particularly concerning viruses. The traditional approach to wastewater treatment consists of biological treatment, such as trickling filters, biofilms, and activated sludge. Although these biological processes have been an adequate means of wastewater treatment, they are intrinsically limited by their biological nature. A magneto-chemical process, known as CoMag™, has been developed to improve coagulation and solids separation and expand upon conventional wastewater treatment techniques.

The research objective of this project was to develop and evaluate a bench-scale model of the CoMag™ process for the removal of MS2, poliovirus type 1, rotavirus strain Wa, and adenovirus type 2 from secondary effluent wastewater, at 24°C and 4°C. Additionally, the removal of MS2 was assessed using a 100 gpm CoMag™ pilot plant. The

results indicate that there was a statistically significant difference (when α=0.050) in the removal of MS2, when magnetite is added, versus when magnetite is not added. In addition, results suggest that MS2 was removed more effectively using the bench-scale model then the 100 gpm pilot plant. Employing the bench scale model, the mean Log Reduction Value for MS2, poliovirus type 1, rotavirus strain Wa and adenovirus type 2 was 2.9182, 3.3893, 3.5313, and 3.482 respectively. Moreover, there was no statistically significant difference in the removal of MS2, rotavirus strain Wa, and adenovirus type 2 at 24° and 4°C. There was, however, a statistically significant difference in the removal or poliovirus type 1 at 24° and 4°C.

This research demonstrates that the CoMag™ process has the ability to achieve > 2 log removal of MS2 and >3 log removal of poliovirus type 1, rotavirus strain Wa and adenovirus type 2. Therefore, the CoMag™ process has the potential to aid wastewater and water treatment facilities meet their more stringent water quality permits.

INTRODUCTION

Enteric viruses that can be transmitted from environmental sources to humans are present in excess of 150 serotypes (Fong and Lipp, 2005). Viruses are negatively charged particles and are readily adsorb to particle surfaces. The adsorption of viruses to a surface is affected by the viral capsid proteins and more importantly the viral surface charge. Factors which influence viral adsorption are pH, ionic strength, electrolytes and interfering substances present in the water such as organics and heavy metals (Bitton et al., 1976).

Wastewater disinfection strategies are designed on the basis of bacterial removal and do not necessarily protect the public against viral infection. Viruses are not generally removed as successfully as bacteria by conventional disinfection processes. In water, the reduction of viral particles fundamentally depends on two processes: inactivation and adhesion-aggregation (Gassilloud and Gantzer, 2005). The use of disinfectants to inactivate microorganisms in public water supplies is credited as one of the greatest public health advancements of the 20th century.

Chlorination is the most widely used method of disinfection in the

United States and has been vital in minimizing the occurrence of microbial waterborne disease. However, chlorine can combine with contaminants naturally present in source waters, such as organic matter, to produce harmful disinfection by-products (DBPs). DBPs are toxic to aquatic life, even in small quantities, and are linked with a variety of adverse human health effects ranging from reproductive disorders to cancer (Nieuwenhuijsen et al., 2000). Furthermore, chlorine disinfection, using practical doses and contact time, does not eliminate waterborne protozoan pathogens, such as *Cryptosporidium* and *Giardia* (Sobsey, 1989). Alternative disinfection processes, ozone and ultraviolet light, are somewhat successful at destroying these pathogens. However, the processes do not always efficiently inactivate pathogenic viruses, because inactivation efficiency is dependant upon the viral species and turbidity level (Clancy et al., 2000)(Sano et al., 2004). Moreover, new water treatment technologies are needed because discharge standards concerning DBPs and disinfectants, are becoming increasingly stringent (USEPA, 2006a).

MS2

Coliphages are bacterial viruses that infect and replicate in *Escherichia coli* (*E. coli*). They are ubiquitous inhabitants of the intestinal tract of humans and animals and are encountered wherever fecal contamination occurs (Stetler et al., 1984). One type of coliphage that is commonly assayed for is MS2, a male specific coliphage from the family Leviviridae. MS2 is a coliphage that is often used as a model or surrogate to evaluate the presence of human viruses in water quality assessment, because it resembles enteric viruses more closely then the commonly used bacterial indicators of fecal pollution, such as coliforms and enterococci (Goyal and Gerba, 1983)(Grabow, 1996)(Vaughn and Metcalf, 1975). The MS2 virion is icosahedral in shape and consists of a protein coat containing a linear, single-stranded, RNA genome. MS2 infects male *E. coli* cells via attachment to the F-pilus. After attachment, the phage genome enters the cell where it replicates exponentially, resulting in lysis of the bacterial cell (Cole et al., 2003).

The size, structure, and survival rate of MS2 in the environment is similar to those of enteric viruses (Grabow, 1986). Laboratory experiments with individual coliphages have confirmed that many are more resistant to environmental stresses and survive longer in natural aquatic

environments than enteric viruses (Kott, 1984). Furthermore, these coliphages are at least as resistant, if not more resistant, to commonly-used disinfectants, such as chlorine. In addition, MS2 is removed, from water, at comparable rates as enteric viruses, during treatment processes. Thus, male-specific RNA coliphages are useful surrogates for human enteric viruses in waters.

Poliovirus

Poliovirus is a small, 20 to 30 nm, single-stranded RNA virus belonging to the Picornaviridae family. It consists of non-enveloped particles which are comprised of a protein shell surrounding naked RNA genome. The poliovirus capsid is composed of 60 copies of 4 viral proteins (VP1, VP2, VP3, and VP4), which are arranged in an icosahedral symmetry. The surface of poliovirus has a corrugated topography with a prominent, star-shaped plateau at the 5-fold axis of symmetry, surrounded by a deep depression and a protrusion at the 3-fold axis (Belnap et al., 2000). The poliovirus genome is a single-stranded, positive-sense RNA approximately 7,440 nucleotides in length (Fields and Knipe, 1990). The plus-strand, genomic RNA functions as mRNA for viral protein expression and serves as a template for negative-strand RNA synthesis (Zhang and Racaniello,

1997).

There are three types of poliovirus: 1, 2, and 3. Type 1 is the most virulent and the most common strain and type 3 is the second most common strain (Fields and Knipe 1990). Transmission of wild poliovirus ceased in the United States by 1979 (Strebel et al., 1992). Furthermore, as a result of an ongoing global vaccination campaign, type 2 poliovirus has not been detected anywhere in the world since 1999, (CDC, 2001). Both the Salk and Sabin poliovirus vaccines are trivalent vaccines, meaning that they are active against all three virus types.

Humans are the only known reservoir of poliovirus, which is transmitted most frequently by persons with unapparent infections. Human-to-human transfer of poliovirus, via the fecal-oral route, is the most commonly implicated mode of transmission, although the oral-oral route may account for a small number of cases (Horstmann, 1967). Poliovirus enters through the mouth and primary multiplication occurs at the site of implantation in the pharynx or gastrointestinal tract. The virus is usually present in the throat and the gastrointestinal tract for a period of time before symptoms occur. The virus invades local lymphoid tissue, enters the blood stream, and then may infect cells of the central nervous system. Replication of poliovirus in motor neurons of the anterior horn and brain stem results in cell destruction and causes the typical manifestations of

poliomyelitis (Fields and Knipe, 1990). One week after onset, there is little virus residing in the throat, but virus remains in the gastrointestinal tract and continues to be excreted in the stool for several weeks.

The response to poliovirus infection is highly variable and has been categorized based on the severity of clinical presentation; as many as 95% of all polio virus infections are unapparent or asymptomatic. Infected persons without symptoms shed virus in the stool, possibly transmitting the virus to others. Approximately 4% to 8% of polio infections consist of a minor, nonspecific illness without clinical or laboratory evidence of central nervous system invasion. This clinical presentation is known as abortive poliomyelitis, and is characterized by complete recovery in two to three days. Three clinical manifestations associated with abortive poliomyelitis are upper respiratory tract infection, gastrointestinal disturbances, and influenza like illness. A complication of poliovirus infections, non-paralytic aseptic meningitis occurs in 1%–2% of infected individuals. Typically symptoms from aseptic meningitis will last from 2 to 10 days, followed by complete recovery. Less than 1% of all polio infections result in flaccid paralysis. Paralytic symptoms generally appear 1 to 10 days after prodromal symptoms and progress for 2 to 3 days (Ryan, 1994).

Rotavirus

Rotavirus is a member of the Reoviridae family. Rotaviruses are non-enveloped, double-shelled, triple layered viruses. The nucleocapsid is isometric, with a non-occluded regular surface shape. Complete particles measure approximately 70 nm in diameter and have a distinctive double-layered icosahedral smooth protein capsid that consists of an outer and an inner layer, when viewed by transmission electron microscopy (Fields and Knipe 1990). Within the inner capsid is a third layer, the core, which contains the virus genome consisting of 11 segments of double-stranded (ds) RNA (Ciarlet et al., 1998). The rotavirus genus is divided into seven antigenically distinct groups (A to G). Humans are susceptible to infection from Groups A, B, and C and animals are vulnerable to all groups. Four group A serotypes, (1, 2, 3 and 4), are of the greatest epidemiological importance because it is the leading cause of diarrhea in infants and the elderly (Estes and Cohen, 1989). Infection is primarily restricted to the villus epithelium of the small intestine, and the outcome of infection is age restricted (Fields and Knipe, 1990).

Human rotavirus is considered the single most important cause of severe, potentially life-threatening, viral gastroenteritis and dehydrating diarrhea in young children worldwide. Each year, rotavirus causes

approximately 111 million episodes of gastroenteritis resulting in the need of home care, 25 million requiring clinic visits, and 2 million resulting in hospitalizations (Parashar et al., 2003). Worldwide, over 600,000 child deaths occur due to dehydration and electrolyte imbalance caused by rotavirus infection (Ciarlet et al., 2002). By age 5, nearly every child experiences an episode of rotavirus gastroenteritis; 1 in 5 will visit a clinic, 1 in 65 will be hospitalized, and approximately 1 in 293 will die as result of complications from the infection. Children in underdeveloped countries account for 82% of rotavirus deaths (Parashar et al., 2003).

The primary mode of transmission is the fecal-oral route; low titers of virus have been cultivated in respiratory tract secretions and other body fluids (Parashar et al., 2003). On a larger scale, rotaviruses have been documented as causative agents of waterborne disease outbreaks in the United States (Gerba, 2000). Waterborne disease outbreaks of rotavirus gastroenteritis can be attributed to their low infectious dose (1-10 infectious units), ability to survive for extended periods of time in the environment (only 10% inactivation after 14 days at 23°C), and poor removal (21% to 27%) by some water treatment processes (Gratacap-Cavallier et al., 2000) .

Adenovirus

The Adenoviruses belong to the family Adenoviridae. They have an icosahedral structure and are of medium-size (90-100 nm). The particles are non-enveloped and the capsid is composed of 252 capsomeres, of which 240 are hexons and 12 are pentons (Fields and Knipe 1990). Each penton contains a base, which forms part of the surface of the capsid, and a projecting fiber, the length of which varies among the different serotypes (Norrby et al., 1976). The genome is 30,000-42,000 nucleotides long (Fields and Knipe, 1990). It is comprised of linear, monopartite, double-stranded DNA and is the only known waterborne human, double-stranded, DNA virus.

Adenoviruses were first discovered in 1953 in tonsil and adenoidal surgical specimens retrieved from children (Rowe et al., 1956). Similar viral agents were isolated from military personnel exhibiting a variety of respiratory illnesses (Hilleman and Werner, 1954). It was soon discovered that the viruses were antigenically related. Adenoviruses were first classified as adenoid degeneration viruses, adenoid-pharyngeal conjunctival viruses and acute respiratory disease viruses. Adenovirus was adopted as the family name in 1956 (Liu, 1991). Over 100 distinct serotypes are known to exist worldwide in humans, as well as in a variety

of animals. Fifty-one adenoviruses are currently recognized, which are classified into 6 subgenera (A through F) based on biochemical, immunological, and genetic parameters (Leclerc et al., 2002).

Adenoviruses have been identified as a cause of waterborne illnesses in the United States as well as other countries (Leclerc et al., 2002), and several serotypes such as 1, 2, 5, and 6, are endemic in many parts of the world (Fields and Knipe, 1990). Although the epidemiologic characteristics of the adenoviruses vary by serotype, all are transmitted via direct contact, fecal-oral transmission, and occasionally waterborne transmission (Kukkula et al., 1997). Several serotypes are capable of establishing persistent asymptomatic infections in tonsils, adenoids, and intestines of infected hosts, and shedding can occur for a period of months to years (Fields and Knipe, 1990). Adenoviruses are typically stable when presented with chemical or physical agents and adverse pH conditions, allowing for prolonged survival outside of the body (Thurston-Enriquez et al., 2003). As a result, adenoviruses are a substantial concern to public health and have been placed on the U.S. Environmental Protection Agency Contaminant Candidate List for drinking water (USEPA, 1998).

Disinfection

Disinfection is the primary mechanism for the inactivation and destruction of pathogenic organisms in water and wastewater. (USEPA, 1999b). The goal is to prevent the spread of waterborne diseases to downstream users and the environment. Achieving this goal successfully requires choosing the appropriate disinfection process for a particular water or wastewater treatment facility, which is contingent on many factors. The disinfectant must be able to adequately disperse in or penetrate the water and destroy target organisms. Next, the disinfectant must be safe and simple to administer in quantities appropriate for the population density of the vicinity. Equally as important, use of the disinfectant must not result in toxic residuals or generation of carcinogenic or mutagenic byproducts. Lastly, the disinfection process must be relatively affordable to operate and maintain. The three most common types of disinfectants utilized in water and wastewater treatment facilities are chlorine, ozone and ultraviolet (UV) light.

Chlorine Disinfection

Chlorine is the most common disinfectant of wastewater in the

United States (USEPA, 1999a). It oxidizes cellular membrane material, by breaking unsaturated bonds, and has a moderate amount of nucleic acid activity affecting cellular respiration, transport, and DNA synthesis. Chlorine can be employed in many forms including chlorine gas, solid hypochlorite salts, and hypochlorite solutions. When chlorine is added to water, it undergoes hydrolysis and ionization until equilibrium is achieved (Bitton, 1980).

$$Cl_2 + H_2O \leftrightarrow HOCl + H^+ + Cl^- \textbf{ (Hydrolysis reaction)}$$

$$HOCl \leftrightarrow H^+ + OCl^- \textbf{ (Ionization reaction)}$$

The distribution of chlorine species is pH dependant. When the pH of water is less then 6.0, hypochlorous acid (HOCl) predominates. It is 70 to 80 times more potent as a disinfectant than hypochlorite ions, the principal species when the pH is greater then 9.0 (USEPA, 1999a). Both forms are present at a pH between 6.0 and 9.0 (Bitton, 1980). Hypochlorous acid and hypochlorite ions are referred to as free chlorine. Total chlorine is composed of free chlorine and combined chlorine. Combined chlorine is formed when free chlorine coalesces with ammonia and nitrogenous compounds in the water (Haas, 1990).

Chlorine disinfection has several advantages over other types of disinfection and is therefore used, the most commonly. It is a well

established technology which successfully inactivates a wide range of waterborne pathogens in a more cost-effective manner than ozone or UV light. Another advantage of using chlorine as a disinfect in drinking water facilities, is the fact that it leaves a residual in the water, long after initial dosing, which can be easily quantified and controlled (USEPA, 1999b). Maintaining a chlorine residual in a drinking water distribution system can help control biofilm growth and microorganism recontamination. There are, however, several disadvantages to using chlorine as a disinfectant, one being that chlorine is highly corrosive and toxic. In terms of water quality, high doses of chlorine can adversely affect taste and odor. The most severe disadvantage is the production of disinfection byproducts (DBPs). Disinfection byproducts, including trihalomethanes (THM) and haloacetic acids (HAA), form when organic matter combines with chlorine. Numerous toxicological studies have revealed that THMs and HAAs may be carcinogenic and teratogenic in laboratory animals (USEPA, 2006a).

Ozone Disinfection

Ozone (O_3), a powerful oxidant, is produced when oxygen (O_2) dissociates, due to an energy source, into individual oxygen atoms (O) and subsequently collides with an oxygen molecule to form ozone (USEPA,

1999b).

$O_2 + O \leftrightarrow O_3$ (Formation of ozone)

Ozone is extremely unstable and must be produced immediately prior to use. Most treatment plants generate ozone by imposing a high voltage alternating current across an electric discharge gap that contains an oxygen-bearing gas (USEPA, 199b). When ozone decomposes in water, free radicals such as hydrogen peroxy and hydroxyl are formed. These free radicals have strong oxidizing abilities and play an active role in the disinfection process. The primary inactivation mechanism of ozone is oxidation and disruption of glycoproteins and glycolipids on the outer membrane or capsid of the pathogen (USEPA, 199b).

Disinfection of water using ozone has several advantages including short contact time and elevated oxygen concentration. In addition, ozone disinfection is more effective than chlorine in inactivating viruses and bacteria (Finch and Fairbairn, 1991). Some disadvantages of using ozone as a disinfectant include high financial expense, possible irritation and toxicity, and the formation of disinfection byproducts, when combined with inorganic and organic compounds normally found in water. These DBPs include aldehydes, carboxylic acids and bromate (USEPA, 1999b).

Ultraviolet Light Disinfection

UV disinfection is a physical disinfection process and does not require the addition of chemicals to water like chlorine and ozone disinfection. The optimum wavelength of UV light to effectively inactivate microorganisms ranges between 240 to 280 nm (USEPA, 1999b). UV light is produced by applying an electrical discharge through mercury vapor. It inactivates pathogens by the adsorption of radiation, which causes a photochemical reaction involving nucleic acid and other internal components. This often causes viral destruction via dimerization of pyrimidine molecules, which results in an inability of the cell or virion to replicate its genetic material.

UV light disinfection has numerous advantages including efficient inactivation of bacteria, viruses, and protozoans (USEPA, 1999b). In addition, UV disinfection is a physical process rather than a chemical process, which eliminates transportation, storage and hazardous management requirements, which are present with ozone and chlorine disinfection. Finally, UV light does not leave a residual nor does it produce DBPs. Disadvantages of UV light disinfection are as follows: high capital and maintenance costs when compared to chlorine disinfection and occasional repair and reversal of the destructive effects of UV light by

microorganisms through a repair mechanism known as photo reactivation, or in the absence of light, dark repair (USEPA, 1999b).

UV radiation quickly dissipates in water by either absorption or reflection, therefore no residual is produced. As a result, no DBPs are formed; however, a secondary disinfectant is necessary to maintain a disinfectant residual throughout the water distribution system.

Disinfection Byproducts

Disinfection byproducts (DBPs) are formed when disinfectants, such as chlorine and ozone, combine with naturally occurring organic and inorganic substances present in water. Recent studies link DBPs to reproductive and developmental abnormalities such as stillbirth (King et al., 2000), spontaneous abortion (Waller et al., 1998), low birth weight (Dodds et al., 1999) and various birth defects (Yang et al., 2000). In addition, epidemiology and toxicology studies have implicated the consumption of DBPs in the formation of bladder, rectal, and colon cancers (USEPA, 2006a).

DBPs are classified into four categories including disinfectant residuals, inorganic byproducts, organic oxidation byproducts, and halogenated organic byproducts (USEPA, 1999b). In the United States, over 260 million individuals are exposed to DBPs in drinking water. The

Stage 1 Disinfection Byproduct Rule (DBPR) is an amendment to the 1989 Surface Water Treatment Rule (SWTR), enacted by the EPA, in order to limit the amount of disinfection residual and DBPs in water treatment plant distribution systems. The Stage 1 DBPR, finalized in December 1998, is the first phase of risk reduction concerning DBPs. The Stage 1 DBPR applies to community water systems (CWS) and non-transient non-community water systems (NTNCWS). CWS are public water systems that serve residents of communities with at least 15 service connections or 25 residents year-round. A NTNCWS is a water system that serves facilities such as schools or businesses, utilized by at least 25 of the same people, for more then six months out of the year (USEPA 1999b). The Stage 1 DBPR requires a running annual average of DBP across the entire water treatment system.

The Stage 2 DBPR went into effect on March 6, 2006 as a supplement of previous regulations to reduce risks of DBPs. The Stage 2 DBPR requires that CWS and NTNCWS perform an evaluation of their distribution system and identify the locations where DBPs are elevated. The locations which are deemed high risk will be used as sampling sites for Stage 2 DBPR monitoring. Maximum contaminant levels (MCLs) for two groups of DBPs, total trihalomethanes (TTHM) and haloacetic acids (HAA5), are established by the Stage 2 DBPR. The MCL, as regulated by the Stage 2 DBR, for TTHM and HAA5 are 0.080 mg/L and 0.060 mg/L

respectively (USEPA, 2006a). Total trihalomethanes and HAA5 are two commonly occurring classes of DBPs formed due to chlorine disinfection of water. Because these two classes of DBPs generally occur at higher levels than other DBPS, they are meant to serve as DBP indicators.

There are many factors that affect the formation of DBPs including pH, temperature, organic and inorganic material, and bromide ion. Halogenated DBPs are formed when a strong oxidant, such as chlorine or ozone, react with organic matter or free bromine. Non-halogenated DBPs are formed when strong oxidants reacts with inorganics present in water. The EPA has recognized the effect of DBPs long before laws were enacted to monitor their presence in public water sources. In 1983, the EPA recognized treatment techniques which would reduce the production of DBPs. One of the primary methods for reducing DBPs involves the removal of DBP precursors through improved flocculation and coagulation processes (Nieuwstad et al., 1988).

High Gradient Magnetic Separation

One method of effectively removing a variety of substances from water is through high gradient magnetic separation (HGMS). This method requires the separation of weakly magnetic and non-magnetic particles. HGMS can be facilitated by the addition of particles with a high magnetic

susceptibility to form aggregates of diamagnetic particles. The diamagnetic particles can then be removed by a type of magnetic filtration termed high gradient magnetic separation (HGMS) (Ying et al., 2000). High gradient magnetic separation was first introduced as a mitigation and recovery process for several industrial and environmental applications (Yiacoumi et al., 1996). The differences of the magnetic susceptibility of materials determines their recovery from water or removal as a waste product. The principles of HGMS are summarized in the formula below:

$$\mathbf{F_M = \mu_0 V H (dH/dx)}$$

Where : F_M = magnetic force on a particle in a magnetic field
μ_0 = magnetic susceptibility
V= volume of the particle
H = background magnetic field
(dH/dx) = magnetic field gradient

The theory for particle separation is to create a high gradient magnetic field (M) within a background field (H). The background field interacts with moving electric charges; the forces on ions of opposite charges occur in opposite directions. The redirection of the particles increases the frequency with which ions collide and combine.

According to the above equation, to increase the force of a particle (F_M) material the background magnetic field may be increased by using stronger electromagnets, however, the cost for powerful electromagnets is high. The magnetic field gradient (dH/dx) can be increased by incorporating a field matrix, but the operation and maintenance cost is not economical. The least expensive and most convenient way to increase the F_m is to increase the magnetic susceptibility (μ_0) by adding a hetero-coagulant such as magnetite.

Current uses of HGMS include the removal of phosphate from water, beneficiation of low grade iron ores, kaolin clay refinement, desulphurization of coal, filtration of nuclear waste coolant, recovery of hematite and chromite from water, and removal of algae, yeast, and bacteria from wastewater (Bitton et al., 1974)(Terashima et al., 1986) (Parker, 1981)(Ying et al., 1999) (Wang et al., 1994)(Bitton and Mitchell, 1974).

All particles can be divided into three categories on the basis of their magnetic susceptibility; the ratio of the degree of magnetization to the applied magnetic field strength. These categories include: 1) Ferromagnetic materials, which have a high positive magnetic susceptibility of one, 2) Paramagnetic materials, which have a weak positive magnetic susceptibility of 10^{-3} to 10^{-5} and 3) Diamagnetic

materials, which have a negative magnetic susceptibility of 1 x 10^{-5} (Tsouris and Yiacoumi, 1997). Examples of ferromagnetic materials are iron, nickel, gadolinium and magnetite. These materials have very strong responses to a magnetic field: they become strongly polarized in the direction of the magnetic field. More importantly, they retain at least some of their polarization after the magnetic field is removed. Once ferromagnetic materials are polarized they produce a magnetic field of their own. Since these fields are usually not uniform, particularly near the ends, ferromagnetic materials are capable of attracting each other and other weakly magnetic particles.

Paramagnetic materials include sodium, oxygen and platinum. They are affected, somewhat less strongly than ferromagnetic materials, and are weakly polarized parallel to a magnetic field. Thus, in a non-uniform magnetic field, they undergo a force towards the higher magnetic field region. However, unlike ferromagnetic materials, paramagnetic materials do not produce a magnetite field of their own in the absence of an externally applied magnetic field.

Diamagnetic materials such as copper, lead, quartz, water, acetone, and carbon dioxide are very weakly affected by magnetic fields. They become magnetically polarized in the direction opposite of the magnetic field. If the magnetic field is not uniform, they follow a force

away from the higher field region. Diamagnetism results from the effects of magnetic fields on all of the electrons in the material. Thus, all materials have a diamagnetic response. However, the other forms of magnetism are stronger than diamagnetism; therefore the diamagnetism is often ignored, unless it is the only magnetic effect present.

Figure 1: Response of paramagnetic and diamagnetic materials to a magnetic field (H).

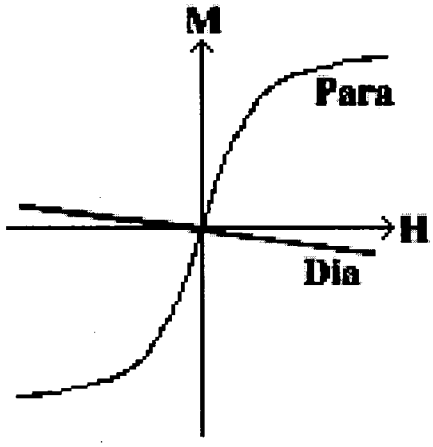
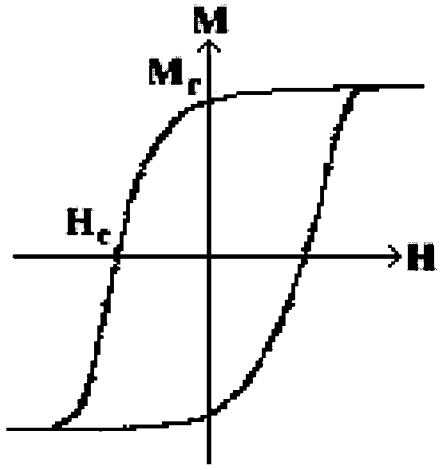


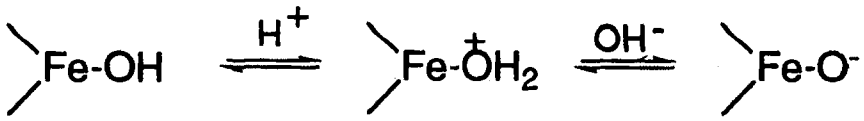
Figure 2: Response of ferromagnetic materials to a magnetic field (H).



Figures 1 and 2 adapted from: Physics for Scientists and Engineers by R.A Serway, and J.W. Jewett.

Magnetite

Magnetite is a ferromagnetic, cubic mineral with the chemical formula $[Fe^{3+}]^{IV}[Fe^{2+}Fe^{3+}]^{VI}O_4$. The International Union of Pure and Applied Chemistry (IUPAC) name for magnetite is iron (II, III) oxide and the common name is ferrous-ferric oxide. Magnetite is the most magnetic of all the naturally occurring minerals on Earth (Moore, 2007). It is a member of the inverse spinel structure type of which half the ferric ion is tetrahedrally coordinated and the remaining portion, as well as the total ferrous iron, is octahedrally coordinated by cubic closed packed oxygen ions. Magnetite is iron black and opaque in color. The hardness is a 6 on the Mohs scale and the specific gravity is 5.20. Magnetite occurs in the granular or massive form, most commonly as a magmatic segregation in basic rocks. However, it is also chemically produced (Karapinar, 2003). It is most abundant naturally in Sweden, Norway, Russia and Canada but also can be found as an accessory mineral in igneous rocks throughout the world (Moore, 2001). Hydrolysis of magnetite produces a highly hydroxylated surface where a charge is generated by the addition of acidic or alkaline solution. This process is summarized in the following reversible equation (Bolto and Spurling, 1991):



The isoelectric point of magnetite is 7.5 ± 0.5 therefore, under acidic conditions, the surface of magnetite will carry a net positive charge attracting negatively charged material. When the pH is raised to alkaline conditions, the surface of magnetite becomes negatively charged and any previously negatively charged material is repelled. Therefore, because of the magnetic properties of magnetite, at an acidic pH, it functions as an adsorbent for colloids, organic materials, bacteria and viruses (Anderson et al., 1982).

The first reported application, employing magnetite to concentrate viruses, was reported by Warren et al. using myxoviruses (Warren et al., 1966). In later research, Rao et al. utilized magnetite to concentrate enteric viruses in drinking water (Rao et al., 1981). Further research projects, used magnetite as an adsorbent to remove a variety of contaminants from wastewater including: T7 bacteriophage, coliform bacteria, suspended solids and algae (Bitton et al., 1974)(de Latour, 1973)(Bitton et al., 1974). Previous research has also investigated the effects of magnetite and poliovirus type 1 in a wastewater matrix (Bitton, 1976). This research, demonstrated that magnetite was an effective adsorbent for poliovirus type 1, resulting in up to 99.8% removal.

CoMag™

The traditional approach to wastewater treatment includes biological treatment, such as trickling filters, biofilms, and activated sludge, which convert organics in the wastewater into sludge and CO_2 (Booker et al., 1990). Although these biological processes have been an adequate means of wastewater treatment, they are intrinsically limited by their biological nature, illustrated by the fact that they require expertise to conduct and maintain due to their sensitive nature and susceptibility to bactericidal contaminants in wastewater. Consequently, establishing biological processes is expensive and difficult, compared to applying conventional, physio-chemical, methods of wastewater treatment such as flocculation and coagulation techniques.

Although presented as a more feasible option than biological processes to treat wastewater, physio-chemical processes also have significant shortcomings. Some disadvantages of conventional physio-chemical wastewater treatment methods include the high cost of chemicals and the formation of large amounts of gelatinous sludge (Booker et al., 1990). The CoMag™ process is an enhanced magneto-chemical process that attempts to solve the limitations of current physio-chemical and biological wastewater treatment processes by improving

aspects of coagulation and solids separation. In addition, the CoMag™ process provides a flow rate several times faster then conventional flow rates. This advantage results in a smaller space requirement necessary for wastewater treatment to occur.

The CoMag™ technique begins with passing influent wastewater through a pre-conditioning magnetic matrix, prior to precipitation. This step creates a locally induced magnetic moment, which is sensed by the colloidal particles. Colloids will not settle when they are suspended in a liquid. The electrostatic charges on each particle prevent the colloids from aggregating. Also, the thermal motion of each particle offsets its gravitational potential energy (Kolm et al., 1975). When HGMS is applied to colloidal particles suspended in water, there are many forces acting on them, including attractive van der Waals forces, repulsive electrostatic forces, and hydrodynamic forces due to water properties and magnetic moments between permanent or induced magnetic moments (Tsouris et al., 1995). In theory, when the strength of the applied high gradient magnetic field is greater than the thermal motion of the colloid, the magnetic force created by the field is large enough to increase the magnetization of the colloid resulting in greatly enhanced precipitation.

After passing through the preconditioning magnet, aluminum sulfate and polymer are mixed with wastewater, in a series of tanks, allowing for

maximum coagulation and flocculation. Coagulation or destabilization of a colloidal suspension, results in the combination of minute particles by physical and chemical processes and flocculation causes the formation of a larger settleable structure by bridging. Aluminum potassium sulfate, alum, provides the necessary coagulation electrolyte, Al^{+3}, which creates a strong bond between the magnetite seed and the contaminants. The aluminum cation in solution is coordinated into six ligand ions, forming a metal ion hydrolysis complex (de Latour, 1973). The nature of this complex is dependant on the environment, especially pH. Depending on the nucleation step, floc formation of $Al(OH)_3$ may proceed along two different pathways; homogeneous and heterogeneous. Homogeneous growth occurs in the absence of an initial surface site and proceeds as small precipitates of $Al(OH)_3$ serve as the nucleation site for growth. In contrast, heterogeneous growth begins in the presence of a seed (Kuo et al., 1998). When alum is added to a contaminated solution, the solid surfaces of the contaminants become coated with aluminum hydrolysis complexes creating a "fluff" around the contaminant (de Latour, 1973). Alum may aid in coagulation in two ways (Cohen et al., 1971). First, the "fluff" formed around each particle can enlarge through the process of aluminum bridging. Second, the surface charge of the particle is altered when the aluminum hydrolysis complex is adsorbed to its surface. In all but

strongly alkaline solutions, the complex is positively charged. Considering that most waterborne contaminants are negatively charged the reduction of the surface charge of the contaminants make coagulation achievable.

A nucleation aid, such as bentonite, is then added to increase floc size and weight. Studies have revealed that virus adsorption to bentonite and other clays are significant and increase in the presence of a trivalent cation, such as aluminum sulfate (Carlson et al., 1968)(Schaub et al., 1974). Fine magnetite powder is also added at this stage to increase floc density and allow for floc removal using a magnetic separator. Magnetite displays a net positive charge in an acidic environment and this enables the negatively charged viruses to attach to the magnetite surface. The tiny magnetite particles are enmeshed into the floc and function as magnetic handles. The flocculated solids settle very rapidly in a small clarifier that operates at an overflow rate, over 10 times that which is typical for standard physio-chemical processes. A portion of the solids underflow, approximately 80 percent, is recirculated to the first stage contact basin, while the remaining portion is removed as sludge. The clarified effluent passes through a magnetic separator in a final polishing stage to remove microflocs that escaped the clarifier. Magnetite is recovered from the sludge in a magnetic drum separator and recycled to the magnetite feed tank for reuse. The

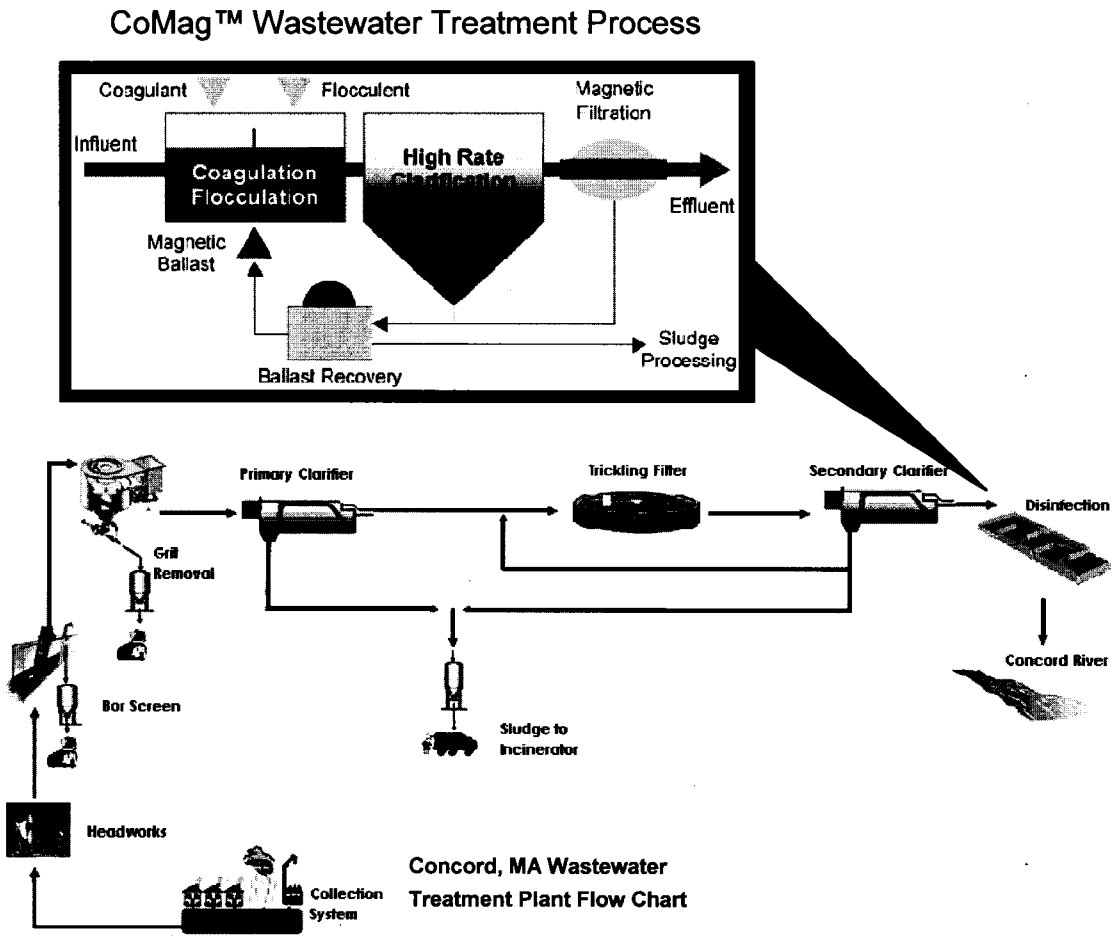
sludge is removed from the system and ultimately disposed of with the remainder of the plant sludge produced during biological treatment.

The primary difference between the CoMag™ process and other conventional technologies occurs during the removal stage. Since magnetite creates a denser floc, the flocculated particles settle more rapidly. For that reason, the clarifier is many times smaller than conventional clarifiers and settling occurs much quicker. Other major benefits include the removal of pin floc, with a magnetic separator, and the magnetite seed can be recovered from the sludge using a magnet instead of gravity.

Concord MA Wastewater Treatment Facility

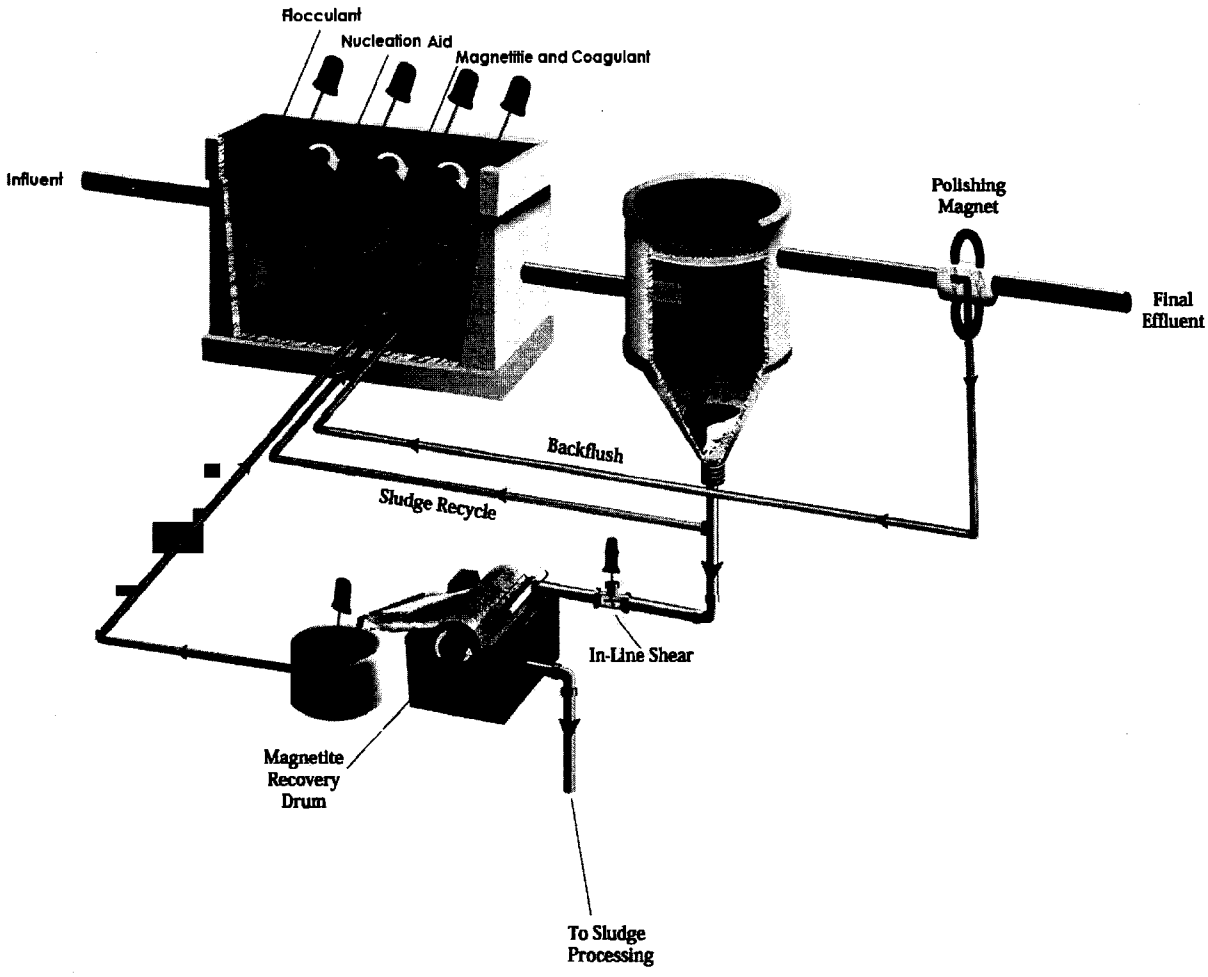
The Concord Wastewater Treatment Facility (WWTF) was built in 1986 and treats 1.2 million gallons of wastewater per day, according to monthly average discharge flow. The effluent discharges to the Concord River. Treatment through the Concord WWTF currently consists of headworks, primary settling, single stage trickling filters run in parallel, secondary clarification, intermittent sand beds for winter season polishing, and chlorine disinfection.

Figure 3: Flow diagram for the Concord, MA Wastewater Treatment Facility and CoMag ™ wastewater treatment process.



Adapted from: Cambridge Water Technology. 2007.

Figure 4: The CoMag™ Treatment Process



Adapted From: USEPA (United States Environmental Protection Agency). 2007.

Research Objectives and Technical Approach

Research Objectives

The research objectives of this project were to develop and evaluate a bench scale model of an enhanced magneto-chemical water treatment process called CoMag™ for the removals of MS2, poliovirus type 1, rotavirus stain Wa and adenovirus type 2. Secondary effluent obtained from the Concord, MA Wastewater Treatment Plant was the matrix evaluated for the removals of MS2, poliovirus type 1, rotavirus strain Wa and adenovirus type 2 using the bench scale model of the CoMag™ process. Bench scale experiments were conducted at room temperature (24°C) and at a reduced temperature (4°C) to represent the temperature conditions encountered in New England. The 100 gpm CoMag™ pilot plant was then challenged with MS2 coliphage. The data that was generated from the bench scale and pilot plant studies was used to develop a correlation of the removal of MS2 to the removals of poliovirus type 1, rotavirus strain Wa and adenovirus type 2.

Technical Approach

1. ***Evaluate the removal of MS2 using a bench scale model of the CoMag™ process.***

The removal of MS2 using a bench scale model of the enhanced magneto-chemical water treatment process was evaluated at room temperature (24°C) and then at a reduced temperature (4°C).

2. ***Evaluate the removal of poliovirus type 1 and MS2 using a bench scale model of the CoMag™ process.***

   The removal of MS2 and poliovirus type 1 using a bench scale model of the CoMag™ process was evaluated at room temperature (24°C) and then at reduced temperature (4°C). A one-way unstacked ANOVA was performed to evaluate the following null hypotheses: (1) there is no statistically significant difference between the removals of poliovirus type 1at 24°C and 4°C (2) there is no statistically significant difference between the removals MS2 and poliovirus type 1 at 24°C and 4°C. A p value of <0.05 was used as a parameter for statistical significance.

3. ***Evaluate the removal of rotavirus strain Wa and MS2 using a bench scale model of the CoMag™ process.***

   The removal of MS2 and rotavirus strain Wa using a bench scale model CoMag™ process was evaluated at room temperature (24°C) and then at a reduced temperature (4°C). A one-way unstacked ANOVA was performed to evaluate the following null

hypotheses: (1) there is no statistically significant difference between the removals of rotavirus strain Wa at 24°C and 4°C (2) there is no statistically significant difference between the removals MS2 and rotavirus strain Wa at 24°C and 4°C. A p value of <0.05 was used as a parameter for statistical significance.

4. ***Evaluate the removal of adenovirus type 2 and MS2 using a bench scale model of the CoMag™ process.***

   The removal of MS2 adenovirus type 2 using a bench scale model of the CoMag™ process was evaluated at room temperature (24°C) and then at a reduced temperature (4°C). A one-way unstacked ANOVA was performed to evaluate the following null hypotheses: (1) there is no statistically significant difference between the removals of adenovirus type 2 at 24°C and 4° C (2) there is no statistically significant difference between the removals MS2 and adenovirus type 2 at 24°C and 4°C. A p value of <0.05 was used as a parameter for statistical significance.

5. ***Perform statistical analysis and correlate the removal of MS2 with the removals of poliovirus type 1, rotavirus strain Wa and adenovirus type 2.***

   Statistical analysis of triplicate trials was performed using Minitab version 15 to evaluate the removals of MS2 in the following experiments: MS2 alone with and without magnetite, MS2 and poliovirus type 1, MS2 and

rotavirus strain Wa and MS2 and adenovirus type 2 at 24°C and 4°C. An one-way unstacked ANOVA was performed to evaluate the following null hypotheses: (1) there is no statistically significant difference between the removals of MS2 when in the presence of poliovirus type 1, rotavirus strain Wa or adenovirus type 2 when compared to the removal of MS2 alone at 24° (2) there is no statistically significant difference between the removals of MS2 when in the presence of poliovirus type 1, rotavirus strain Wa or adenovirus type 2 when compared to the removal of MS2 alone at 4°C. A p value of <0.05 was used as a parameter for statistical significance.

6. ***Challenge the CoMag™ process pilot plant with MS2.***

An existing full-process 100 gallon per minute commercial demonstration pilot plant was challenged with MS2 at a final concentration of 1×10^5 PFU/mL three times. A two-way ANOVA was performed to evaluate the following null hypotheses: (1) the CoMag™ pilot plant does not have a statistically significant effect on the removal of MS2. A p value of <0.05 was used as a parameter for statistical significance.

7. ***Compare the results for the removal of MS2 at the CoMag ™ pilot plant to the removal of Ms2 obtained using the bench scale model of CoMag™ process.***

   A one-way unstacked ANOVA was performed to evaluate the following null hypothesis: (1) there is no statistically significant difference between the removal of MS2 at the pilot plant and the removal of MS2 using a bench scale model of the CoMag™ process. A p value of <0.05 will be used as a parameter for statistical significance.

CHAPTER II

MATERIALS AND METHODS

MS2 Propagation and Enumeration

MS2 (ATCC 1597-B1) was prepared and quantified using a modified double agar overlay method adapted from protocol established by DeBartolomeis and Cabelli (DeBartolomeis and Cabelli, 1991). A mutant strain of *E. coli* HS (pFamp) R served as the host organism. In addition to displaying a resistance marker to ampicillin on the F plasmid, the selected host strain is resistant to somatic coliphages T2 to T7 and ΦX174, streptomycin, and naladixic acid, (Cho, 2005).

In order to propagate MS2, the *E. coli* HS (pFamp) R host was grown, with gentle agitation, to log phase in Tryptic Soy Broth (TSB) supplemented with 1% streptomycin, 1% ampicillin, and 0.5 % magnesium chloride at 37°C for approximately three hours. MS2 bacteriophage was then added to log phase host culture and incubated statically at 37°C for 12 to 18 hours. Subsequent to incubation, the *E. coli* and MS2 suspension was centrifuged at 10,000 rpm, at 4°C, for ten minutes. The supernatant

was removed and filtered using a 0.22 µm filter to remove bacteria and cellular debris. The filtrate was then placed in sterile bottles and refrigerated at 4°C, until use.

As previously mentioned, the MS2 was enumerated using a modified double-agar overlay technique in which *E. coli* HS (pFamp) R acted as the host (DeBartolomeis and Cabelli, 1991). The E. *coli* host was grown to log phase in TSB supplemented with 1% streptomycin, 1% ampicillin, and 0.5 % magnesium chloride at 37°C for approximately three hours. Serial dilutions of the sample were prepared using Phosphate Buffered Saline. Five mL of soft agar overlay was inoculated with100 µL of the appropriate dilution and 200 µL of log phase *E. coli*. Each sample was gently swirled and poured onto a 1.5% Tryptic Soy Agar (TSA) 100 mm plate supplemented with 1% streptomycin and 1% ampicillin, followed by an overlay. The plates were inverted and incubated at 37°C for 18 to 24 hours. Each plate was examined for plaques and those containing 30 to 300 plaques were used to calculate the titer of the sample. The final titer of the sample was recorded as plaque forming units per mL (PFU/mL).

Poliovirus Propagation and Enumeration

Poliovirus type 1 LSc was propagated on Buffalo Green Monkey

Kidney (BGMK) cells, a continuous African monkey kidney cell line (WHO, 1997). Poliovirus LSc is a variant of the Mahoney strain of Poliovirus. The Mahoney strain was isolated by T. Francis, in 1941, from a composite of 3 stools collected from asymptomatic patients with poliomyelitis in Cleveland (Li, 1955).

The BGMK cells were grown in Eagle's Minimal Essential Media (MEM), supplemented with L-15 and 5% Fetal Bovine Serum (FBS). For poliovirus propagation, BGMK cells were grown to 90% confluency, in closed 75 cm^2 cell culture flasks at 37°C, with 15 mL of MEM. The confluent monolayers of BGMK cells were inoculated with 100 µL of poliovirus type 1 and incubated at 37°C for 90 minutes, with rocking every 15 minutes, to facilitate viral adsorption and maintain hydrated cells. One 75 cm^2 cell culture flask was inoculated with 100 µL of warm serum-free MEM to act as the negative control.

Following incubation, 15 mL of a maintenance media consisting of MEM supplemented with L-15 and 2% FBS was added to all flasks and the cells were incubated at 37°C. The flasks were observed daily for evidence of cytopathic effects (CPE). Once 90% CPE was observed, flasks were frozen at -80°C and rapidly thawed at 37°C. This freeze/thaw process was repeated three times to completely lyse the cells and facilitate viral release. The cell lysates were then centrifuged at 1000 x g for ten minutes

to pellet cellular debris. Then the supernate was filtered with a 1 % FBS pretreated 0.22 µm PVDF filter. The filter was pretreated to reduce the adsorption of virus to the filter. Virus was stored at 4°C for short term storage and -80°C for long term storage.

Enumeration of the virus was performed using a modified neutral red plaque-forming unit (PFU) method (Dulbecco and Vogt, 1953). BGMK cells were grown to 90% confluency in closed 25 cm^2 culture flasks, supplemented with 5% FBS. The cells were washed three times with serum-free MEM. Virus to be analyzed was serially diluted in serum-free MEM and closed 25 cm^2 culture flasks were inoculated, in triplicate, with 100 µL of the appropriate poliovirus dilution. The flasks were then incubated at 37°C for 90 minutes, with rocking every 15 minutes, to facilitate viral adsorption and maintain hydrated cells. Following adsorption 10 mL of medium consisting of MEM supplemented 2% FBS, 2% flake agar (Difco), and neutral red (Sigma) was added. This overlay provides a solid support matrix to physically confine the virus as well as a viability stain to observe plaque formation.

Flasks were then inverted and incubated at 37°C and observed for plaques every 24 hours, for up to seven days. Only plates containing 20 to 50 plaques were counted. Plaques were quantified and PFU/mL was determined. Enumerated poliovirus stocks were stored at -80°C, until

needed for bench scale CoMag™ challenges.

Rotavirus Propagation and Enumeration

Rotavirus strain Wa (Tissue Culture adapted) (ATCC, VR-2018) propagation and enumeration was accomplished using modified protocols established by Smith et al. (Smith, 1979). Rotavirus was propagated on MA-104 (ATCC, CRL-2378.1), an embryonic Rhesus monkey kidney cell line. MA-104 cells were grown in Eagle's MEM supplemented with L-15 and 10% Fetal Bovine Serum (FBS). For rotavirus propagation, MA-104 cells were grown to 90% confluency, in closed 75 cm^2 cell culture flasks at 37°C, in 15 mL of MEM, supplemented with L-15 and 10% FBS. Ninety percent confluent monolayers of MA-104 cells were inoculated with 100 µL of rotavirus strain Wa and incubated at 37°C for 90 minutes, with rocking every 15 minutes, to facilitate viral adsorption and maintain hydrated cells. One 75 cm^2 cell culture flask was inoculated with 100 µL of warm serum-free MEM to serve as the negative control.

Following incubation, 15 mL of a maintenance media, consisting of MEM supplemented with L-15 and 2% FBS, was added to all flasks and the cells were incubated at 37°C. The flasks were observed daily for evidence of CPE. Once 90% CPE was observed, flasks were frozen at -80°C and

rapidly thawed at 37°C, three times, to completely lyse the cells and facilitate viral release. The cell lysates were then centrifuged at 1000 x g for 10 minutes to pellet cellular debris. The supernatant was filtered with a 1 % FBS pretreated 0.22 µm PVDF filter. Virus was stored at 4°C for short term storage and at -80°C for long term storage.

Rotavirus strain Wa was enumerated using a modified plaque-forming unit (PFU) method (Smith, 1979). MA-104 cells were grown to 90% confluency, in closed 25 cm^2 culture flasks, supplemented with 10% FBS. All cells were washed three times with serum-free MEM. Virus to be analyzed was serially diluted in serum-free MEM and closed 25 cm^2 culture flasks were inoculated, in triplicate, with 100 µL of the appropriate rotavirus dilution.

The flasks were then incubated at 37°C, for 90 minutes, with rocking every 15 minutes to facilitate viral adsorption and maintain hydrated cells. Following adsorption, 10 mL of medium, consisting of equal portions of 2X MEM, supplemented with 1 mg/mL of trypsin (Gibco) and 2.4% agar (Sigma), was added.

Flasks were then incubated at 37°C for four days. Following incubation, 2 mL of 10% formaldehyde, in normal saline solution, was added to each flask. The flasks were then returned to the 37°C incubator overnight. Upon removal from the incubator, the solid overlays were

removed from the flasks using warm tap water. Two mL of a 0.1% crystal violet solution was added to each flask to permit contrast between live MA-104 cells and plaques. Plaques were quantified and a PFU/mL value was determined. Enumerated rotavirus stocks were stored at -80°C, until retrieved for bench scale CoMag™ challenges.

Adenovirus Propagation and Enumeration

Adenovirus type 2 (ATCC, VR-846) was propagated and enumerated in A549 (ATCC, CCL-185), a continuous human lung cell line as previously described by Wold (Wold, 1999). A549 cells were grown in Eagle's MEM, supplemented with L-15 and 10% FBS. For adenovirus propagation, A549 cells were grown to 90% confluency, in closed 75 cm^2 cell culture flasks at 37°C, in 15 mL of MEM supplemented with L-15 and 10% FBS. Confluent monolayers of A549 cells were inoculated with 100 µL of adenovirus type 2 and incubated at 37°C for 90 minutes, with rocking every 15 minutes, to facilitate viral adsorption and maintain hydrated cells. One 75 cm^2 cell culture flask was inoculated with 100 µL of warm serum-free MEM, which served as the negative control. Following incubation, 15 mL of a maintenance media, consisting of MEM supplemented with L-15 and 2% FBS, was added to all flasks, which were

incubated at 37°C.

The flasks were observed daily for evidence of CPE. Once 90% CPE was observed, the flasks were frozen at -80°C and rapidly thawed at 37°C, three times, to completely lyse the cells and facilitate viral release. The cell lysates were then centrifuged, at 1000 x g for ten minutes, to pellet cellular debris. The supernatant was filtered with a 1% FBS pretreated 0.22 µm PVDF filter. Virus was stored at 4°C, for short term storage, and at -80°C for long-term storage.

Adenovirus type 2 was enumerated using the Tissue Culture Infective Dose 50 ($TCID_{50}$) method (Meng, 1996). A549 cells were grown in 96 well cell culture plates, at 37°C and 5% CO_2, until 90% confluency was achieved. Virus to be analyzed was serially diluted in serum-free MEM and 25 µL of the viral dilution was inoculated into 10 wells, in triplicate. Negative wells contained 25 µL of serum-free MEM. The plates were then incubated, for 90 minutes at 37°C and 5% CO_2, followed by an addition of 200 µL MEM, supplemented with L-15 and 2% FBS. The plates were incubated at 37°C and 5% CO_2 for ten days.

Cells were observed daily for evidence of CPE. Wells which exhibited CPE within ten days were recorded as positive and wells not displaying CPE were recorded as negative. The number of positive and negative wells were then referenced to calculate the $TCID_{50}$ value, using

the Reed and Muench method illustrated below (Reed, 1938).

Calculation of $TCID_{50}$:

$$\textbf{Log } TCID_{50}\textbf{/ mL} = \log [\{10 \exp [X + (p - 0.5)]\} / \text{inoculum volume}]$$

Where: X = positive exponent from last dilution where all wells are positive

p = ratio of positive wells/ total number of wells

Sample Collection

All CoMag™ bench scale experiments were conducted using secondary effluent from the Concord Wastewater Facility in Concord, MA. The secondary effluent was sampled after trickling filter treatment of the wastewater, but before chlorine disinfection. An adequate amount of secondary effluent was collected, in one visit, to conduct all CoMag™ bench scale challenges and stored at 4°C; this minimized variation of results and allowed for comparison of all data produced by the bench scale model.

Bench Scale Experiments

All bench scale experiments were performed using a Phipps and Bird ™ 6- paddle Jartester.

Figure 5: Phipps and Bird ™ 6- paddle Jartester used for bench – top experiments.

MS2

The removal of MS2, using a bench scale model of the CoMag™ process, without added magnetite and at room temperature (24°C), was evaluated. In addition the removal of MS2, using a bench scale model of the CoMag™ process, was evaluated at room temperature (24°C) and then at a reduced temperature (4°C). All trials were performed in triplicate. Initial CoMag™ bench scale trials were performed using MS2. One thousand milliliters of secondary effluent was placed into five sterile 1200 mL beakers. Two of the beakers functioned as controls, with one of the controls consisting of secondary effluent with MS2, spiked to a final concentration of 1 x 10^5 PFU/mL, with no chemicals added. The purpose of this particular control was to reveal any removal or inactivation of MS2 because of rapid stirring with the six-paddle jartester, settling during the quiescent step, toxins in the wastewater, or other nonspecific methods. The second control contained only 1000 mL of secondary effluent and the appropriate chemicals. The purpose of this control was to reveal the presence of any anthropogenic viruses in the chemicals or the water matrix.

Each experiment included three test beakers, consisting of 1000 mL of secondary effluent and MS2, spiked to a final concentration of 1 x 10^5

PFU/mL. The two controls and three test beakers were stirred, using the six-paddle jartester. After the samples were briefly stirred, and prior to addition of the chemicals, an aliquot was removed from each beaker, to determine the initial concentration of MS2. The CoMag™ process was then applied to the samples and after a brief settling time a final aliquot was removed from each beaker to determine the final MS2 titer. Refer to Appendix C for the CoMag™ process bench scale procedure. All samples were analyzed immediately and the remainders of the samples were archived at -80°C.

The initial and final concentrations of all samples were established by creating ten-fold dilutions in phosphate buffered saline. Dilutions ranging from 10^{-2} to 10^{-5} were assayed to determine the initial titer. For the final titer, dilutions ranging from 10^{0} to 10^{-3} were assayed. All dilutions were plated in triplicate.

A negative control consisted of 5 mL of soft agar overlay poured on a plate. An additional negative control, containing 5 mL of soft agar overlay which was previously inoculated with 200 µL of log phase *E. coli*, was also plated. A positive control consisted of 200 µL of log phase *E. coli* plated in 5 mL of a soft agar overlay. After the overlay solidified, 20 µL of stock MS2 was distributed onto the plate.

All samples were analyzed using the double agar overlay method,

as previously described. Plates containing 30 to 300 plaques were used to calculate the titer of the sample. The titer of the sample was recorded as plaque forming units per mL (PFU/mL).

Poliovirus type 1 and MS2

The removal of MS2 and poliovirus type 1, using a bench scale model of the CoMag™ process was evaluated at room temperature (24°C) and then at a reduced temperature (4°C). All trials were performed in triplicate. One thousand milliliters of secondary effluent was distributed into five sterile 1200 mL beakers. Two of the beakers acted as controls. One of the controls consisting of secondary effluent and MS2, spiked to a final concentration of 1 x 10^5 PFU/mL, and poliovirus type 1, spiked to a final concentration of 1 x 10^5 PFU/mL. The other control contained 1000 mL of secondary effluent and all of the appropriate chemicals. This control confirmed the presence or absence of anthropogenic viruses. A positive control consisting of 200 µL of log phase *E. coli* plated in five mL of a soft agar overlay. After the overlay solidified, 20 µL of stock MS2 was distributed onto the plate.

Additionally, three test beakers, consisting of 1000 mL of secondary effluent were spiked with MS2 and poliovirus type 1 to a final

concentration of 1 x 10^5 PFU/mL of each virus. The two controls and three test beakers were stirred using a six paddle jartester.

After the samples were briefly stirred, and prior to chemical addition, an aliquot was removed from each beaker to determine the initial viral concentration. The CoMag™ process was applied to the samples and after a brief settling time a final aliquot was removed to determine the final titer of MS2 and poliovirus type 1 in each beaker. All samples were analyzed immediately and the remainder archived at -80°C.

The initial and final concentrations of the MS2 samples were determined by using ten-fold dilutions made in phosphate buffered saline. For the initial concentration of MS2, dilutions ranging from 10^{-2} to 10^{-5} were assayed and dilutions ranging from 10^0 to 10^{-3} were assayed to determine the final concentration of MS2. All dilutions were plated in triplicate.

A negative control consisting of 5 mL of plated soft agar overlay. A second negative control contained 200 µL of log phase *E. coli* in 5 mL of a soft agar overlay. A positive control consisting of 200 µL of log phase *E. coli* in five mL of a soft agar overlay. After the overlay solidified, 20 µL of stock MS2 was spotted onto the plate.

All samples were analyzed using the double agar overlay method, as previously described. Plates containing 30 to 300 plaques were used to

calculate the titer of the sample. The titer of the sample was recorded as plaque forming units per mL (PFU/mL).

The initial and final concentrations of poliovirus type 1, in all samples, were determined using ten-fold dilutions prepared in serum-free MEM. To determine the starting virus concentration, dilutions ranging from 10^{-2} to 10^{-5} were assayed and dilutions ranging from 10^{0} to 10^{-3} were assayed to determine the final concentration of poliovirus type 1. All dilutions were plated in triplicate.

Two negative control flasks contained 100 µL of serum-free MEM. Two positive control flasks contained 100 µL of 1 x 10^{2} PFU/mL poliovirus type 1 stock. Only flasks containing 20 to 50 plaques were counted. Plaques were quantified and a PFU/mL value was determined.

Rotavirus strain Wa and MS2

The removal of MS2 and rotavirus strain Wa using a bench scale model of the CoMag™ process was evaluated at room temperature (24°C) and at a reduced temperature (4°C). All trials were performed in triplicate. One thousand milliliters of secondary effluent was placed into five sterile 1200 mL beakers. Two of the beakers were as controls. One of the controls consisting of only secondary effluent and MS2 spiked to a final

concentration of 1 x 10^5 PFU/mL and rotavirus strain Wa spiked to a final concentration of 1 x 10^5 PFU/mL. An additional control contained 1000 mL of secondary effluent and all of the chemicals required for the CoMag™ process. This control was used to demonstrate the presence or absence of naturally occurring virus.

Three test beakers consisting of 1000 mL of secondary effluent and MS2 spiked to a final concentration of 1 x 10^5 PFU/mL as well as rotavirus strain Wa, spiked to a final concentration of 1 x 10^5 PFU/mL. The two controls and three test beakers were stirred using a six paddle jartester. After the samples were briefly stirred, and prior to chemical addition, an aliquot was removed from each beaker to determine the initial concentration of virus. The CoMag™ process was applied to the samples and after a brief settling time a final aliquot was removed to determine the final titer of MS2 and rotavirus strain Wa in each beaker. All samples were analyzed immediately and the remainder of each sample was archived at -80°C.

The initial and final concentrations of MS2 samples were determined by using ten-fold dilutions prepared in phosphate buffered saline. Dilutions ranging from 10^{-2} to 10^{-5} were assayed to determine initial MS2 titer. Additionally, dilutions ranging from 10^0 to 10^{-3} were assayed to determine the final MS2 concentration. All dilutions were plated in triplicate.

A negative control consisted of 5 mL of plated soft agar overlay. A second negative control contained 200 µL of log phase *E. coli* in 5 mL of soft agar overlay which was then plated. A positive control consisted of 200 µL of log phase *E. coli* plated in five mL of a soft agar overlay. After the overlay solidified, 20 µL of stock MS2 was spotted onto the plate.

All samples and controls were analyzed using the double agar overlay method as previously described. Plates containing 30 to 300 plaques were used to calculate the titer of the sample. The titer of the sample was recorded as plaque forming units per mL (PFU/mL).

The initial and final concentrations of rotavirus strain Wa, in all samples, were determined by using ten-fold dilutions prepared in serum-free MEM. To determine the initial titer, dilutions ranging from 10^{-2} to 10^{-5} were assayed. Likewise, to determine the final titer, dilutions ranging from 10^{0} to 10^{-3} were analyzed. All dilutions were plated in triplicate.

Two negative control flasks consisted of 100 µL of serum-free MEM. Two positive control flasks contained 100 µL of 1 x 10^{2} PFU/mL rotavirus strain Wa stock. Only flasks containing 20 to 50 plaques were counted. Plaques were quantified and a PFU/mL value was determined.

Adenovirus type 2 and MS2

The removals of MS2 and adenovirus type 2, using a bench scale

model of the CoMag™ process was evaluated at room temperature (24°C) and at a reduced temperature of (4°C). All trials were performed in triplicate. One thousand milliliters of secondary effluent was placed into five sterile 1200 mL beakers. Two of the beakers acted as controls. One of the controls consisted of secondary effluent and MS2, spiked to a final concentration of 1 x 10^5 PFU/mL, and adenovirus type 2, spiked to a final concentration of 1 x 10^5 $TCID_{50}$/mL. The other control contained 1000 mL of secondary effluent and all of the necessary chemicals. This control was used to demonstrate the presence or absence of any naturally occurring virus in the experimental matrix.

Three test beakers, consisted of 1000 mL of secondary effluent and MS2, spiked at a final concentration of 1 x 10^5 PFU/mL as well as adenovirus type 2, spiked at a final concentration of 1 x 10^5 $TCID_{50}$/mL. The two controls and three test beakers were then stirred using a six paddle jartester. After the samples were briefly stirred, and prior to chemical addition, an aliquot was removed from each beaker to determine the initial concentration of virus. The CoMag™ process was then applied to the samples and, after a brief settling time, a final aliquot was removed to determine the final titer of MS2 and adenovirus type 2 in each beaker. All samples were analyzed immediately and the remainder of each sample was archived at -80°C.

The initial and final concentrations of the MS2 samples were determined by creating ten-fold dilutions using phosphate buffered saline. For the initial titer, dilutions ranging from 10^{-2} to 10^{-5} were assayed; additionally, dilutions ranging from 10^{0} to 10^{-3} were assayed to determine the final MS2 concentration. All dilutions were plated in triplicate.

A negative control consisted of 5 mL of plated soft agar overlay. A second negative control contained 200 µL of log phase *E. coli* in 5 mL of a soft agar overlay which was plated. A positive control consisted of 200 µL of log phase *E. coli* plated in five mL of a soft agar overlay. After the overlay solidified, 20 µL of stock MS2 was spotted onto the plate.

All samples were analyzed using the double agar overlay method, as previously described. Plates containing 30 to 300 plaques were used to calculate the titer of the sample. The titer of the sample was recorded as plaque forming units per mL (PFU/mL).

The initial and final concentrations of adenovirus type 2, in all samples, were determined by creating ten-fold dilutions in serum-free MEM. To determine the initial titer, dilutions ranging from 10^{-2} to 10^{-5} were assayed. Likewise, to determine the final titer, dilutions ranging from 10^{0} to 10^{-3} were analyzed. All dilutions were plated in triplicate.

Two negative control wells contained 125 µL of serum-free MEM. Two positive control wells contained 125 µL of 1×10^{2} $TCID_{50}$/mL adenovirus

type 2 stock. Samples were assayed according to methods previously described.

Adenovirus type 2 viral concentration was reported as $TCID_{50}$ and MS2 titers are reported as PFU/mL. Due to the difference in the calculation of titers, a conversion from $TCID_{50}$ to PFU/ mL is necessary in order to make a comparison possible. The mean TCID50 titer (per ml) was multiplied by 0.7 to predict the mean number of PFU/mL (Wijnker et al., 2007).

Pilot Plant Scale Experiments

Preparation of MS2 Spike

A high-titer stock of MS2 was cultivated and enumerated using the double agar overlay method, as previously described. The MS2 stock was then diluted in 15 L of sterile deionized water, in a 5 gallon container, resulting in a final dilution of 1 $x10^8$ PFU/mL.

Pilot Plant at the Concord, MA Wastewater Treatment Facility

The CoMag ™ pilot plant is located inside the Concord, MA WWTF.

A pilot plant flow of 100 gallons per minute (gpm) was determined to be consistent with the optimum flow rate established during previous phosphorus studies. Addition of the spiked MS2 sample occurred in the first rapid mix tank and was continuously metered using a positive displacement pump. The target concentration of MS2 was 1 x 10^5 PFU/mL. Aluminum sulfate and polymer were then mixed in a series of tanks for coagulation and flocculation. Fine magnetite powder was also added at this stage to increase floc density and allow for floc removal using a magnetic separator. The flocculated solids settled very rapidly in the small clarifier.

Approximately 80 percent of the solids underflow was recirculated to the first stage contact basin and the remaining portion was removed as waste sludge. The clarified effluent was passed through a magnetic separator in order to remove microflocs that escaped the clarifier. The detention time of the system was 10 minutes. Magnetite was recovered from the sludge in a magnetic drum separator and recycled to the magnetite feed tank for reuse. One hundred mL samples were drawn from the 5 gallon container used for spiking, as well as the tank where the MS2 was added and a port located distal to the magnetic separator.

Samples were gathered at time zero and then once every 10 minutes for a total of three hours. The samples were stored on ice and analyzed within 24 hours. MS2 was enumerated using the double agar overlay, as

previously described. Plates containing 30 to 300 plaques were used to calculate the titer of the sample. The final titer of the sample was recorded as plaque forming units per mL (PFU/mL).

Statistical Analysis

The log reduction value (LRV) for MS2, poliovirus type1, rotavirus strain Wa, and adenovirus type 2 was calculated by dividing the final viral concentration (N_t) by the initial viral concentration (N_0). These values were then $\log_{10}$ transformed [$\log_{10}$ (N/N_0)] and the values from the triplicate experiments were averaged. The value was considered significant at a 95% confidence level. Minitab version 15 was utilized to perform the statistical analysis.

Statistical analysis of triplicate trials were performed to evaluate the removals of MS2 with and without magnetite, MS2 alone, MS2 and poliovirus type 1, MS2 and rotavirus strain Wa, and MS2 and adenovirus type 2 at 24°C and 4°C. The data for all four viruses were square root transformed to insure a normal distribution and then analyzed using a one-way ANOVA with Minitab v.15. An ANOVA was performed to evaluate the following null hypotheses: **(1)** the bench scale model of the CoMag™ process does not have a statistically significant effect on the removal of MS2, poliovirus

type 1, rotavirus strain Wa, and adenovirus type 2 at 24°C or at 4°C; **(2)** there is not a statistically significant difference between the removal of MS2 with poliovirus type 1, rotavirus strain Wa, and adenovirus type 2, compared to the removal of MS2 alone; **(3)** there is not a statistically significant difference between the removal of MS2 alone, MS2 with poliovirus type 1, MS2 with rotavirus strain Wa, and MS2 with adenovirus type 2 at 24°C, compared to their removal at 4°C; **(4)** the pilot plant scale of the CoMag™ process does not have a statistically significant effect on the removal of MS2; **(5)** there is no statistically significant difference between the removal of MS2, using the pilot plant scale CoMag™ process and the bench scale model of the CoMag™ process; **(6)** there is no statistically significant difference between the removals of MS2 using the bench sale model of the CoMag™ process when magnetite is added and when magnetite is not added. A p value of <0.05 was chosen to determine statistical significance.

Post hoc comparisons using the Tukey honest significant difference (HSD) analysis were performed. The test was performed using a P value of $P < 0.05$ to be considered statistically significant. The Tukey HSD Test was implemented using the means of the MS2 titers for all of the experiments to create a pair-wise comparison of each experiment. This test determined the presence of a statistically significant difference in the removal of MS2 among trials.

CHAPTER III

RESULTS

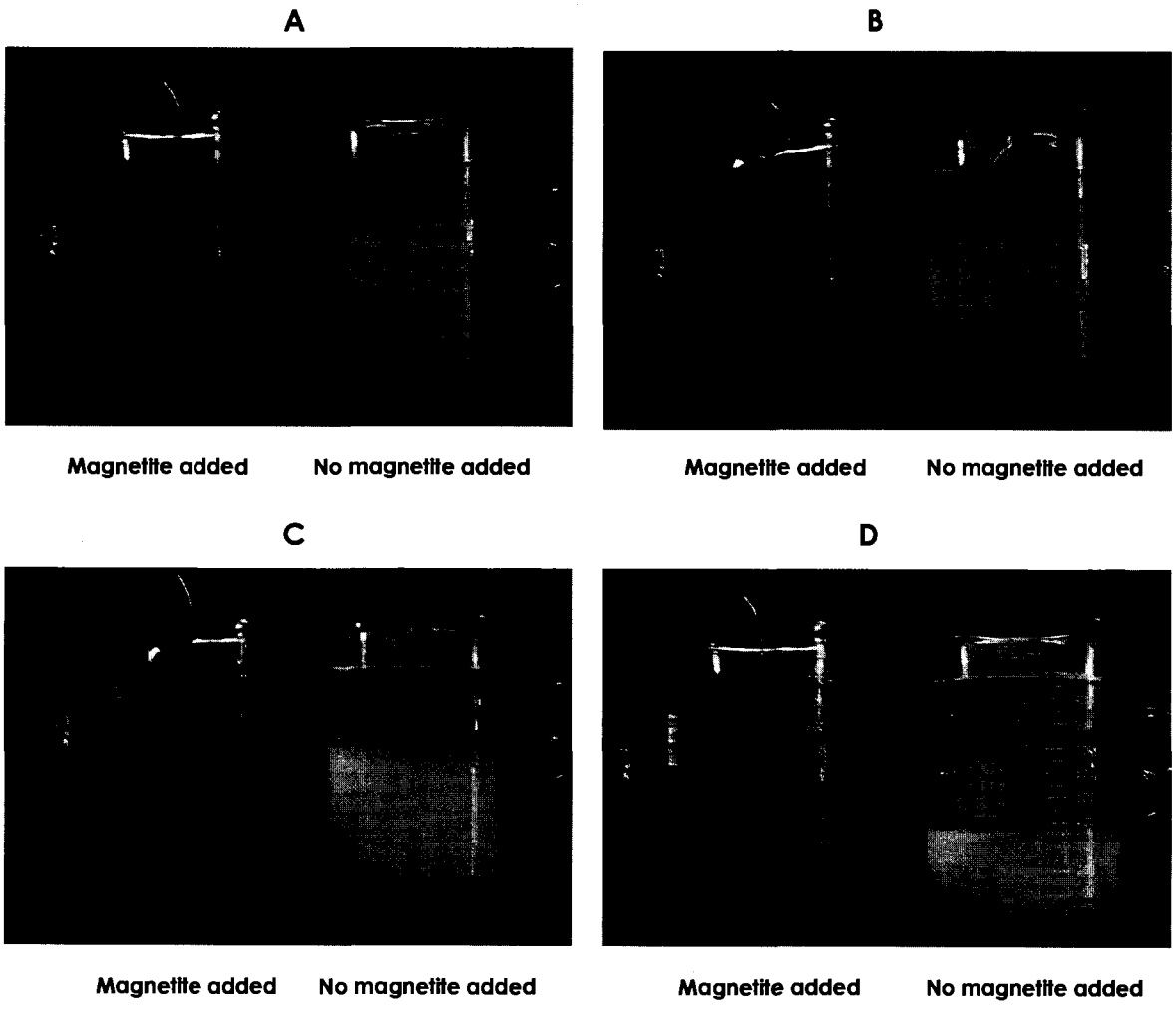
Removal of MS2 with and without Added Magnetite

The removal of MS2, using a bench scale model of the CoMag™ process, with and without added magnetite, was evaluated. All trials were performed in triplicate. The log reduction value (LRV) for MS2 was calculated by dividing the final viral concentration (N_t) by the initial viral concentration (N_0). These values were then $\log_{10}$ transformed [$\log_{10}$ (N/N_0)], and the values from the triplicate experiments were averaged. The calculated values were considered statistically significant at a 95% confidence level. Minitab version 15 was utilized to complete the statistical analysis. The results of the three trials are summarized in Tables 1 and 2 and Figures 6 and 7.

The amount of endogenous MS2, present in the sample matrix, was below the detectable limit of <1 PFU/mL. The average LRV for the removal of MS2 using the CoMag™ process without the addition of magnetite, was 1.8040 with a standard deviation of 0.3280. The average

LRV for the removal of MS2 using the CoMag™ process with the addition of magnetite was 2.9638 with a standard deviation of 0.1217. Statistical analysis resulted in a p value of 0.005, indicating that there is a significant difference in the removal of MS2, when magnetite is added, versus when magnetite is not added. Therefore, the null hypothesis stating that magnetite does not have a statistically significant effect on the removal of MS2 was rejected.

Figure 6: The removal of MS2, using a Bench Scale model of the CoMag™ process, with and without added magnetite.



One thousand milliliters of secondary effluent from the Concord, MA Wastewater Treatment Facility was placed into sterile 1200 mL beakers. All chemicals used for CoMag™ process, except for magnetite to the beaker on the right of each photo, were added to both beakers. Elapsed time after chemical addition: (A) 30 seconds (B) 1 minute (3) 30 minutes and (D) 1 hour.

Table 1: One-way ANOVA: Mean MS2 LRV: No Magnetite vs. Magnetite

Source	DF	SS	MS	F	P
Factor	1	2.0172	2.0172	32.90	0.005
Error	4	0.2453	0.0613		
Total	5	2.2624			

S = 0.2476 R-Sq = 89.16% R-Sq(adj) = 86.45%

Level	N	Mean	StDev
No Magnetite	3	1.8041	0.3284
Magnetite	3	2.9638	0.1217

Individual 95% CIs For Mean Based on Pooled StDev

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--+---------+---------+---------+------
 (-------*-------)
                   (-------*-------)
--+---------+---------+---------+------
1.50      2.00      2.50      3.00
```

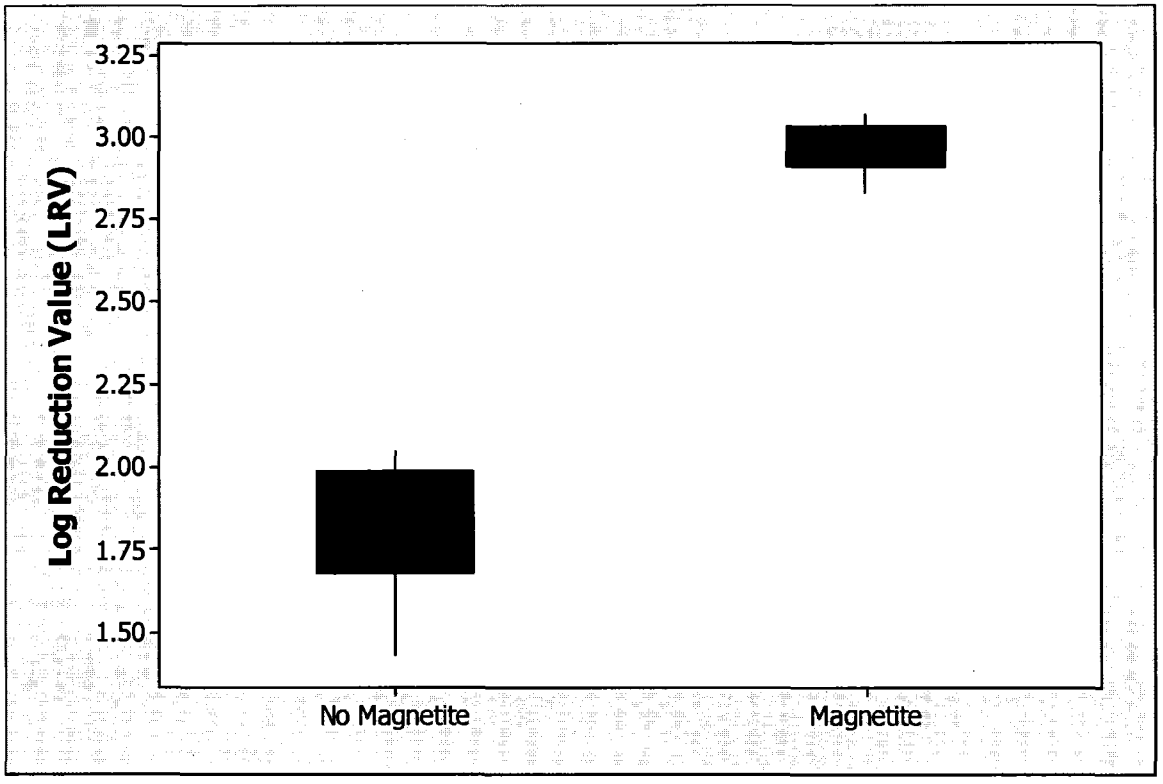
Pooled StDev = 0.2476

Table 2: Descriptive Statistics: Mean MS2 LRV: No Magnetite vs. Magnetite

Variable	Count	Total Mean	StDev	Sum of Squares	Minimum	Median	Maximum
No Magnetite	3	1.8040	0.3280	9.9800	1.4320	1.9260	2.0540
Magnetite	3	2.9638	0.1217	26.3816	2.8305	2.9917	3.0691

Data in Tables 1 and 2 represents the calculated LRV of MS2, using data from three separate trials. For each trail, the removal of MS2 was assayed in triplicate. The plaque assays were also conducted in triplicate.

Figure 7: Mean Log Reduction Value of MS2 **Using the** Bench Scale Model of the CoMag™ Process



The data represents the average LRV for MS2, detected by modified double agar overlay, using *E. coli* HS (pFamp) R as the host. Plaque assays were performed in triplicate and 1000 ml of secondary effluent was spiked with MS2 to a final concentration of 1 x 10^5 PFU/mL. Three trials were completed, each trial containing a sample size of n=3. The mean LRV of MS2, when magnetite was not added, was 1.8040, with a standard deviation of 0.3280. The mean LRV of MS2 when magnetite was added was 2.9638, with a standard deviation of 0.1217. A p value 0.005 (α=0.05) was obtained, which indicates that there is a statistically significant difference in the removal of MS2, from the secondary effluent samples, when magnetite is present versus when it is not. In Figure 7, the upper whisker extends to the maximum data point, for each category, and the lower whisker extends to the minimum data point. Each colored box contains the middle 50% of the data. The line, within each box, illustrates the median. The plus sign, within each colored box, represents the mean.

Removal of MS2 Using the Bench Scale Model of the CoMag™ Process

The removal of MS2, using a bench scale model of the CoMag™ process was evaluated. All trials were performed in triplicate. The LRV for MS2 was calculated by dividing the final viral concentration (N_t) by the initial viral concentration (N_0). These values were then $\log_{10}$ transformed [$\log_{10}$ (N/N_0)], and the values for each of the three experiments were averaged. The correlation was considered statistically significant at a 95% confidence level. Minitab version 15 was utilized to complete the statistical analysis. The results of the three trials are summarized in Tables 3 and 4 and Figure 8.

The amount of endogenous MS2, present in the sample matrix, was below the detectable limit of <1 PFU/mL. The average LRV for the removal of MS2 was 3.0495 for trial 1, 2.9640 for trial 2, and 2.9525 for trial 3, respectively. Statistical analysis was performed to evaluate the following null hypothesis: The LRV of MS2 does not vary between trials, to a statistically significant degree. The statistical analysis resulted in p value of 0.890, indicating that there is not a significant difference when comparing the removal of MS2 in each trial. Therefore, the null hypothesis that states the LRV of MS2 does not vary significantly between trials fails to be rejected.

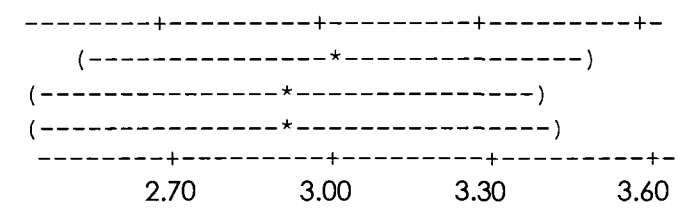
Table 3: One-way ANOVA: MS2 Mean LRV versus Trial

Source	DF	SS	MS	F	P
test	2	0.0112	0.0056	0.12	0.890
Error	3	0.1386	0.0462		
Total	5	0.1498			

S = 0.2150 R-Sq = 7.50% R-Sq(adj) = 0.00%

Level	N	Mean	StDev
1	2	3.0495	0.2765
2	2	2.9640	0.2489
3	2	2.9525	0.0148

Individual 95% CIs For Mean Based on Pooled StDev



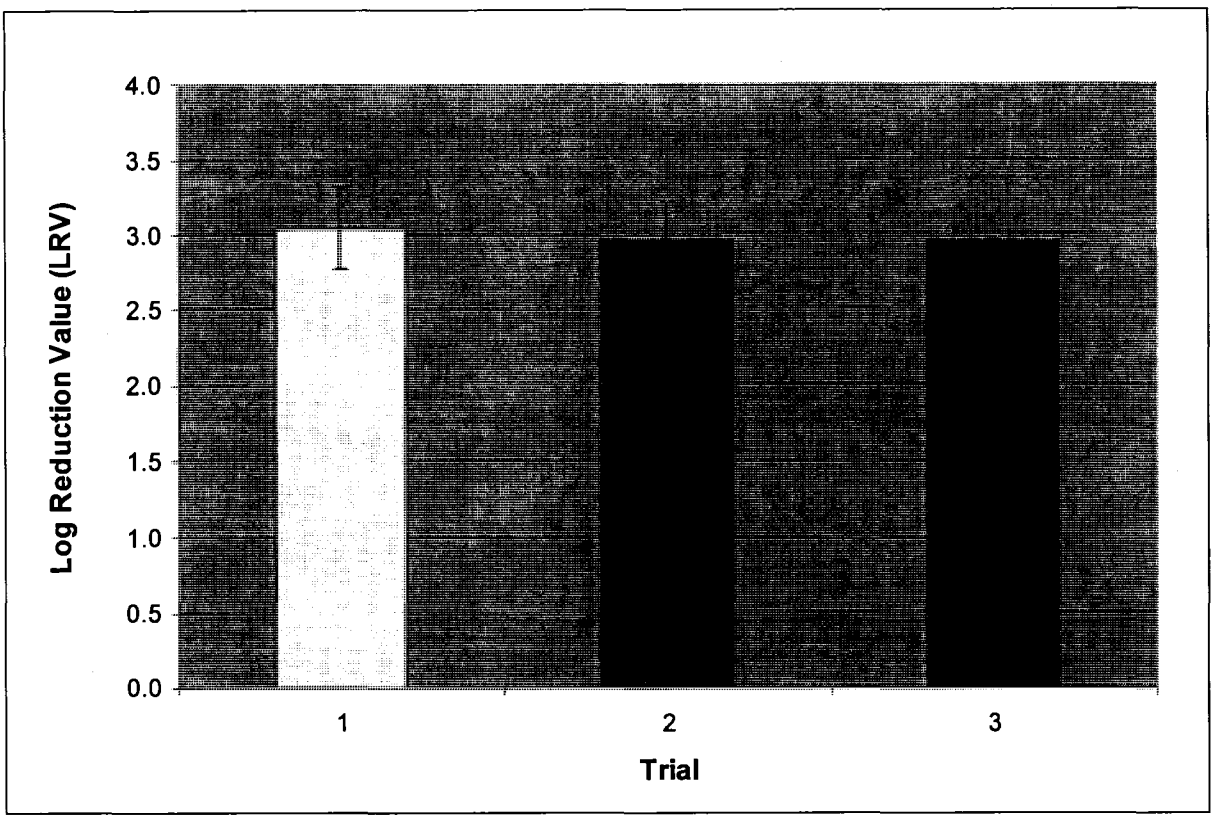
Pooled StDev = 0.2150

Table 4: Descriptive Statistics: MS2 Mean LRV versus Trial

Variable	test	Total Count	Mean	StDev	Sum of Squares	Minimum	Median	Maximum
LRV	1	2	3.050	0.2760	18.6750	2.8540	3.0500	3.2450
	2	2	2.964	0.2490	17.6330	2.7880	2.9640	3.1400
	3	2	2.9525	0.0148	17.4347	2.9420	2.9525	2.9630

Data in Tables 3 and 4 represents the calculated LRV of MS2, using the results from three separate trials. For each trail, the removal of MS2 was assayed in triplicate. The plaque assays were also conducted in triplicate.

Figure 8: Mean Log Reduction Value of MS2 for Three Trials at 24° C, **Using the Bench Scale** Model of the CoMag™ Process



The data in Figure 8 represents the average LRV for MS2, detected by modified double agar overlay, using *E. coli* HS (pFamp) R as the host. Plaque assays were performed in triplicate and 1000 ml of secondary effluent was spiked with MS2 to a final concentration of 1 x 10^5 PFU/mL. Three trials were completed, each trial containing a sample size of n=3. The mean LRV of MS2 for trial 1 was 3.050, with a standard deviation of 0.276. The mean LRV of MS2 for trial 2 was 2.964, with a standard deviation of 0.246. The mean LRV of MS2 for trial 3 was 2.953, with a standard deviation of 0.015. A p value 0.890 (α=0.05) was obtained, which indicates that there is no statistically significant difference in the removal of MS2 among the three trails. Error bars corresponds to standard error.

Removal of MS2 Using the Bench Scale Model of the CoMag™ Process at 24°C and 4°C

The removal of MS2, using a bench scale model of the CoMag™ process was evaluated at 24°C and 4°C. All trials were performed in triplicate. The LRV for MS2 was calculated by dividing the final viral concentration (N_f) by the initial viral concentration (N_0). These values were then $\log_{10}$ transformed [$\log_{10}$ (N/N_0)], and the values from the triplicate experiments were averaged. The correlation was considered statistically significant at a 95% confidence level. Minitab version 15 was utilized to complete the statistical analysis.

The amount of endogenous MS2 present in the sample matrix was below the detectable limit of <1 PFU/mL. Statistical analysis was performed to evaluate the following null hypotheses: (1) The MS2 LRV does not significantly vary, at 24° versus 4°C, when combined with another virus (2) The MS2 LRV does not significantly vary, between trials at the same temperature (24° or 4°C), when combined with another virus. The LRV of MS2, at 24°C, was 2.9306 when combined with poliovirus type 1, 2.9756 when combined with rotavirus strain Wa, and 2.8336 when combined with adenovirus type 2. Statistical analysis resulted in a p value of 0.270, indicating that there is no significant difference in the removal of MS2, when combined with poliovirus, rotavirus and adenovirus at 24°C. Therefore, the null hypothesis stating that the MS2 LRV does not

significantly differ at 24°C, when combined with poliovirus, rotavirus or adenovirus is not rejected. The results are summarized in Table 5 and 6 and Figure 9.

The LRV of MS2 at 4°C was 2.9885 when combined with poliovirus type 1, 2.8872 when combined with rotavirus strain Wa, and 2.8944 when combined with adenovirus type 2. Statistical analysis resulted in a p value of 0.476, indicating that there is not a significant difference in the removal of MS2, when combined with poliovirus, rotavirus or adenovirus at 4°C. Therefore, the null hypothesis stating that the LRV of MS2 does not significantly vary at 4°C, when combined with poliovirus, rotavirus or adenovirus, fails to be rejected. The results are summarized in Table 7 and 8 and Figure 10.

At 24°C, the mean LRV of MS2, when combined with poliovirus, rotavirus and adenovirus, was 2.9133 with a standard deviation of 0.1057. At 4°C, the mean LRV of MS2, when combined with poliovirus, rotavirus and adenovirus, was 2.9234 with a standard deviation of 0.1046. Statistical analysis resulted in a p value of 0.841. Therefore; the null hypothesis fails to be rejected, which states that the mean LRV of MS2 does not significantly vary, at 24° versus 4°C, when combined with another virus. The results are summarized in Table 9 and 10 and Figure 11.

Table 5: One-way ANOVA: Mean LRV of MS2 at 24°C

Source	DF	SS	MS	F	P
Factor	2	0.03160	0.01580	1.64	0.270
Error	6	0.05773	0.00962		
Total	8	0.08933			

S = 0.09809 R-Sq = 35.38% R-Sq(adj) = 13.83%

Level	N	Mean	StDev
MS2 24C (polio)	3	2.9306	0.0942
MS2 24C (rota)	3	2.9756	0.1358
MS2 24C (adeno)	3	2.8336	0.0395

Individual 95% CIs For Mean Based on Pooled StDev

```
-----+---------+---------+---------+----
          (---------*---------)
            (---------*---------)
(---------*---------)
-----+---------+---------+---------+----
2.76     2.88     3.00     3.12
```

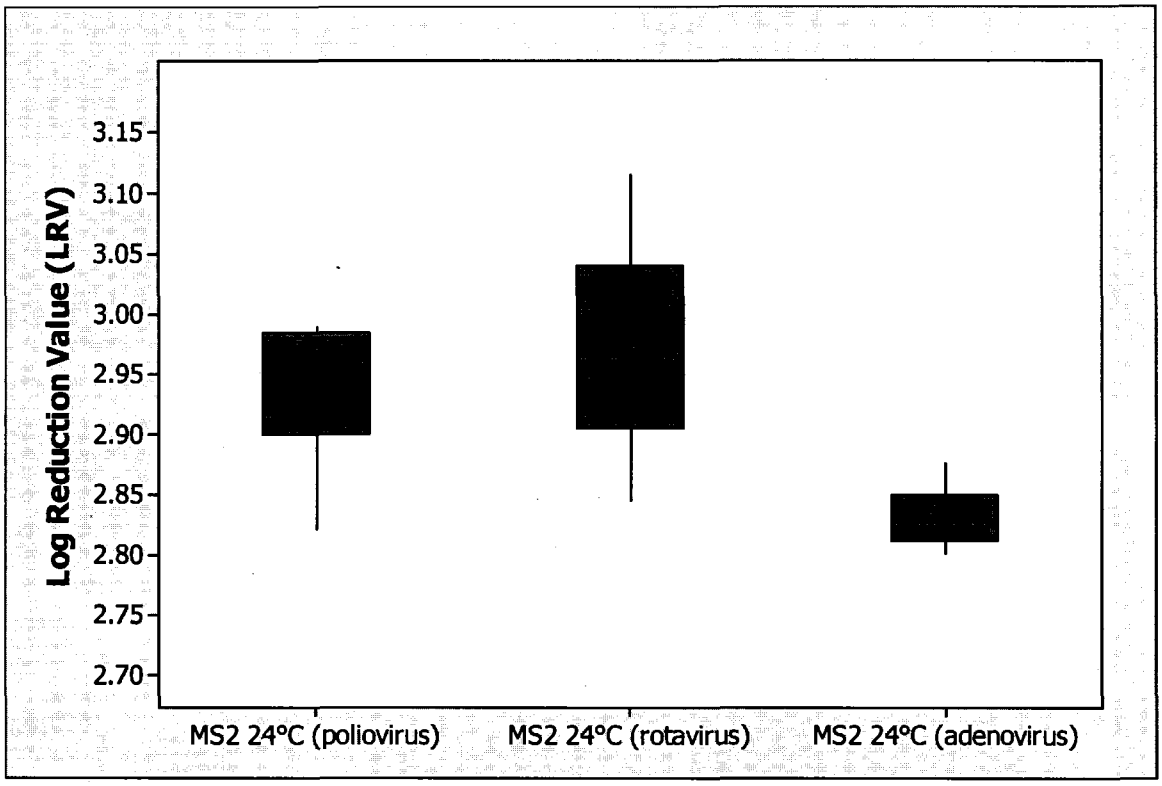
Pooled StDev = 0.0981

Table 6: Descriptive Statistics: Mean LRV of MS2 at 24°C

Variable	N	Mean	StDev	Minimum	Median	Maximum
MS2 24°C (poliovirus)	3	2.9306	0.0942	2.8221	2.9794	2.9905
MS2 24°C (rotavirus)	3	2.9756	0.1358	2.8451	2.9656	3.1161
MS2 24°C (adenovirus)	3	2.8336	0.0395	2.8009	2.8225	2.8774

Data in Tables 5 and 6 represents the calculated LRV of MS2, using data from three separate trials. For each trail, the removal of MS2 was assayed in triplicate. The plaque assays were also conducted in triplicate.

Figure 9: Mean Log Reduction Value of MS2 at 24°C Using the Bench Scale Model of the CoMag™ Process



The data represents the average LRV for MS2 at 24°C, detected by modified double agar overlay, using *E. coli*-HS (pFamp) R as the host. Plaque assays were performed in triplicate and 1000 ml of secondary effluent was spiked with MS2 to a final concentration of 1 x 10^5 PFU/mL. Three trials were completed, each trial containing a sample size of n=3. The mean LRV of MS2, when challenged with poliovirus type 1, was 2.931 with a standard deviation of 0.0942. The mean LRV of MS2, when challenged with rotavirus strain Wa, was 2.976 with a standard deviation of 0.1358. The mean LRV of MS2, when challenged with adenovirus type 2, was 2.834 with a standard deviation of 0.0395. A p value 0.270 (α=0.05) was obtained, which indicates that there is no statistically significant difference in the removal of MS2 when combined with poliovirus, rotavirus and adenovirus. In Figure 9, the upper whisker extends to the maximum data point, for each variable, and the lower whisker extends to the minimum data point. Each colored box contains the middle 50% of the data. The line within each box illustrates the median. The plus sign, within each colored box, represents the mean.

Table 7: One-way ANOVA: Mean LRV of MS2 4°C

Source	DF	SS	MS	F	P
Factor	2	0.0192	0.0096	0.84	0.476
Error	6	0.0684	0.0114		
Total	8	0.0876			

S = 0.1068 R-Sq = 21.90% R-Sq(adj) = 0.00%

Level	N	Mean	StDev
MS2 4°C (poliovirus)	3	2.9885	0.0800
MS2 4°C (rotavirus)	3	2.8872	0.1440
MS2 4°C (adenovirus)	3	2.8944	0.0841

Individual 95% CIs For Mean Based on Pooled StDev

```
Level
MS2 4°C (poliovirus)        --+---------+---------+---------+-------
MS2 4°C (rotavirus)                  (-----------*-----------)
MS2 4°C (adenovirus)        (-----------*-----------)
                              (-----------*-----------)
                            --+---------+---------+---------+-------
                            2.76      2.88      3.00      3.12
```

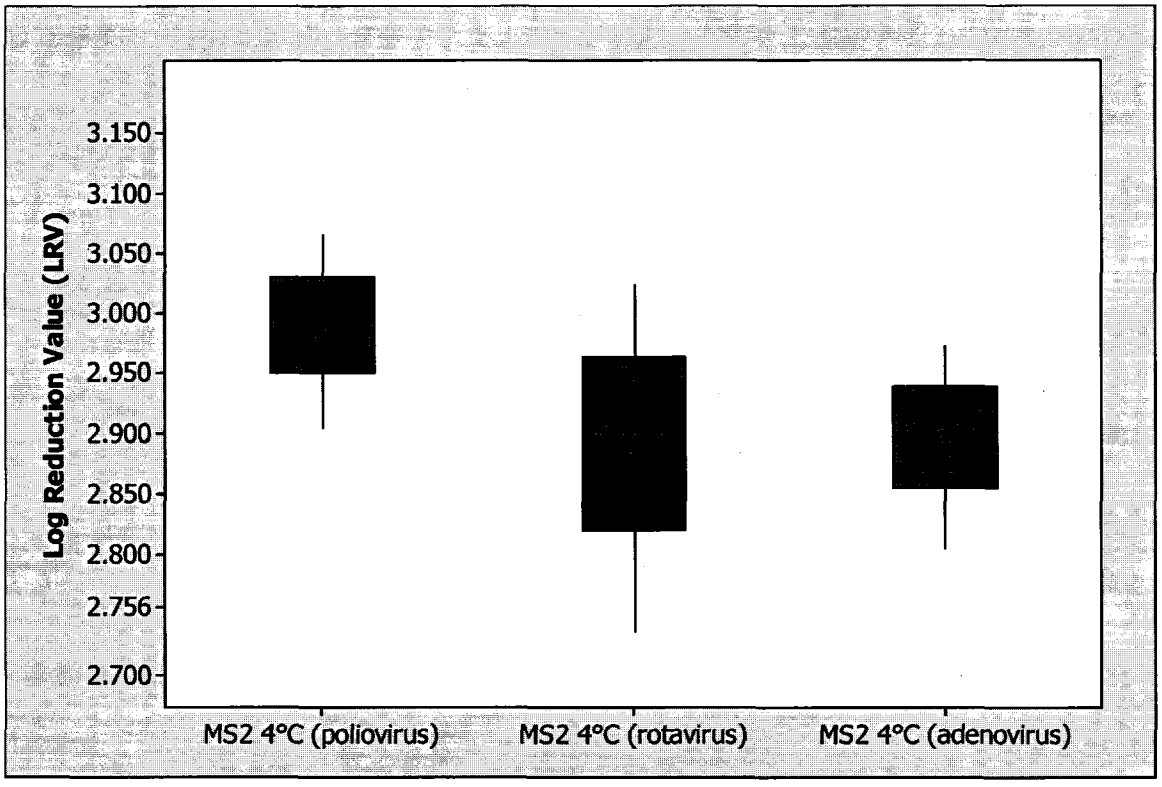
Pooled StDev = 0.1068

Table 8: Descriptive Statistics: Mean LRV of MS2 4°C

Variable	N	Mean	StDev	Minimum	Median	Maximum
MS2 4°C (poliovirus)	3	2.9885	0.0800	2.9055	2.9950	3.0650
MS2 4°C (rotavirus)	3	2.8872	0.1440	2.7362	2.9023	3.0231
MS2 4°C (adenovirus)	3	2.8944	0.0841	2.8053	2.9055	2.9723

Data in Tables 7 and 8 represents the calculated LRV of MS2, using data from three separate trials. For each trail, the removal of MS2 was assayed in triplicate. The plaque assays were also conducted in triplicate.

Figure 10: Mean Log Reduction Value of MS2 at 4°C Using the Bench Scale Model of the CoMag™ Process



The data represents the average LRV for MS2 at 4°C, detected by modified double agar overlay, using *E. coli* HS (pFamp) R as the host. Plaque assays were performed in triplicate and 1000 ml of secondary effluent was spiked with MS2 to a final concentration of 1 x 10^5 PFU/mL. Three trials were completed, each trial containing a sample size of n=3. The mean LRV of MS2, when challenged with poliovirus type 1, was 2.989 with a standard deviation of 0.080. The mean LRV of MS2, when challenged with rotavirus strain Wa, was 2.887 with a standard deviation of 0.144. The mean LRV of MS2, when challenged with adenovirus type 2, was 2.894 with a standard deviation of 0.084. A p value 0.476 (α=0.05) was obtained, which indicates that there is no statistically significant difference in the removal of MS2 when combined with poliovirus, rotavirus and adenovirus. In Figure 10, the upper whisker extends to the maximum data point, for each variable, and the lower whisker extends to the minimum data point. Each colored box contains the middle 50% of the data. The line within each box illustrates the median. The plus sign, within each colored box, represents the mean.

Table 9: One-way ANOVA: Mean LRV of MS2 at 24°C and 4°C

Source	DF	SS	MS	F	P
Factor	1	0.0005	0.0005	0.04	0.841
Error	16	0.1769	0.0111		
Total	17	0.1774			

S = 0.1052 R-Sq = 0.26% R-Sq(adj) = 0.00%

Level	N	Mean	StDev	Individual 95% CIs For Mean Based on Pooled StDev
MS2 24°C	9	2.9133	0.1057	(----------*----------)
MS2 4°C	9	2.9234	0.1046	(----------*----------)
				2.840 2.880 2.920 2.960

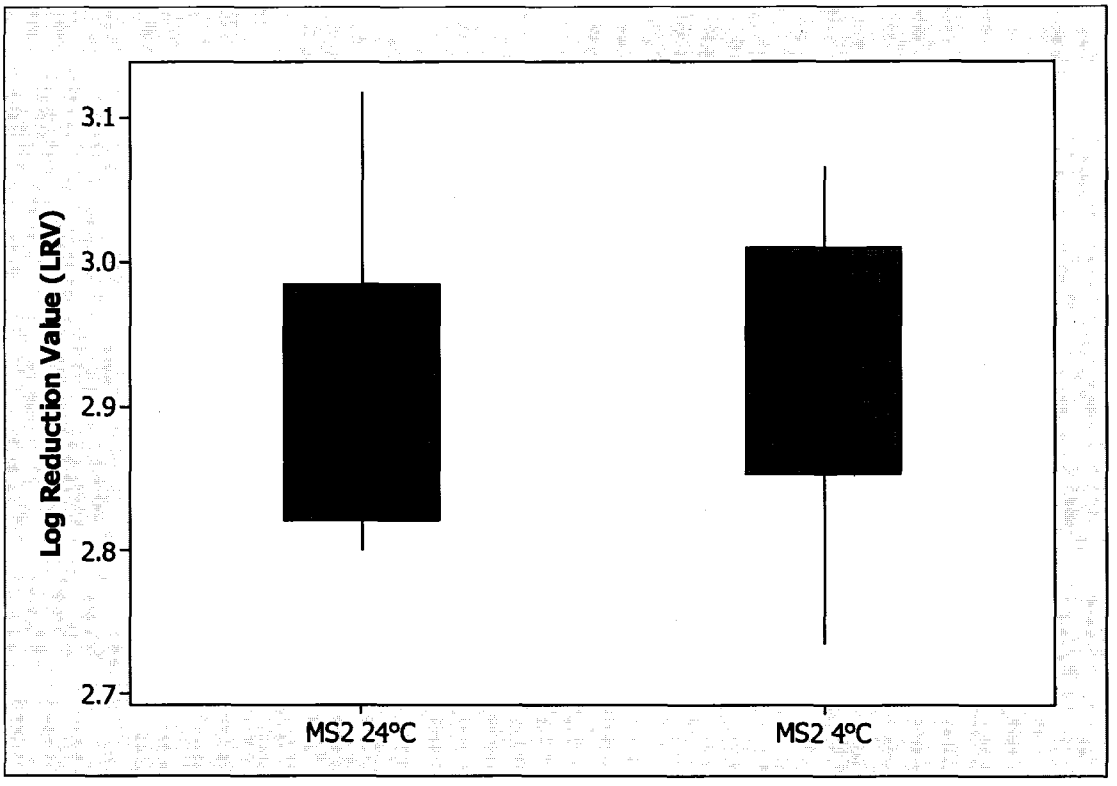
Pooled StDev = 0.1052

Table 10: Descriptive Statistics: Mean LRV of MS2 at 24°C and 4°C

Variable	N	Mean	StDev	Minimum	Median	Maximum
MS2 24°C	9	2.9133	0.1057	2.8009	2.8774	3.1161
MS2 4°C	9	2.9234	0.1046	2.7362	2.9055	3.0650

Data in Tables 9 and 10 represents the calculated LRV of MS2, using data from three separate trials. For each trail, the removal of MS2 was assayed in triplicate. The plaque assays were also conducted in triplicate.

Figure 11: Mean Log Reduction Value of MS2 when combined with Poliovirus type 1, Rotavirus strain Wa and Adenovirus type 2 at 24°C and 4°C, **Using the Bench Scale** Model of the CoMag™ Process



The data represents the average LRV for MS2, at 24°C and 4°C, detected by modified double agar overlay, using *E. coli* HS (pFamp) R as the host. Plaque assays were performed in triplicate and 1000 ml of secondary effluent was spiked with MS2 to a final concentration of 1 x 10^5 PFU/mL. Three trials were completed, each trial containing a sample size of n=3. The mean LRV of MS2, when challenged at 24°C with poliovirus type 1, rotavirus strain Wa, and adenovirus type 2 was 2.9133, with a standard deviation of 0.1057. The mean LRV of MS2, when challenged at 4°C with poliovirus type 1, rotavirus strain Wa, and adenovirus type 2 was 2.9234, with a standard deviation of 0.1046. A p value 0.841 (α=0.05) was obtained, which indicates that there is no statistically significant difference in the removal of MS2, when combined with poliovirus, rotavirus and adenovirus at 24° versus 4°C. In Figure 11, the upper whisker extends to the maximum data point, for each variable, and the lower whisker extends to the minimum data point. Each colored box contains the middle 50% of the data. The line, within each box, illustrates the median. The plus sign, within each colored box, represents the mean.

Removal of MS2, Poliovirus type 1, Rotavirus strain Wa and Adenovirus type 2 Using the Bench Scale Model of the CoMag™ Process at 24°C and 4°C

The removals of MS2, poliovirus type 1, rotavirus strain Wa and adenovirus type 2, using a bench scale model of the CoMag™ process, were evaluated at 24°C and 4°C. All trials were performed in triplicate.

The amount of endogenous MS2 present in the sample matrix was below the detectable limit of <1 PFU/mL. Statistical analysis was performed to evaluate the following null hypotheses: (1) there is no statistically significant difference between the removal of poliovirus type 1, rotavirus strain Wa, and adenovirus type 2 at 24°C versus 4°C (2) there is no statistically significant difference between the removal of MS2 with poliovirus type 1, MS2 with rotavirus strain Wa, and MS2 with adenovirus type 2 at 24°C or 4°C. At 24°C, the LRV of MS2 was 2.9306 when combined with poliovirus type 1, 2.9756 when combined with rotavirus strain Wa, and 2.8336 when combined with adenovirus type 2. At 4°C, The LRV of MS2 was 2.9885 when combined with poliovirus type 1, 2.8872 when combined with rotavirus strain Wa, and 2.8944 when combined with adenovirus type 2. The LRV of poliovirus was 3.5545 with a standard deviation of 0.0975, at 24°C, and 3.2440 with a standard deviation of 0.1223, at 4°C. The results are summarized in Tables 11 through 28 and Figures 12 through 20.

The LRV of rotavirus was 3.5482 with a standard deviation of 0.1504, at 24°C, and 3.5114 with a standard deviation of 0.1161, at 4°C. The results are summarized in Tables 21 through 24 and Figures 17 and 18. The LRV of adenovirus was 3.3.493, at 24°C, with a standard deviation of 0.1740 and 3.470 with a standard deviation of 0.2330, at 4°C. The results are summarized in Tables 25 through 284 and Figures 19 and 20.

The statistical analysis evaluating the removal of MS2 in comparison with poliovirus, rotavirus and adenovirus, at 24°C, resulted in p values of 0.001, 0.008, and 0.003 respectively. These p values indicate that there is a significant difference in the removal of MS2 as well as poliovirus, rotavirus, and adenovirus at 24°C. The statistical analysis evaluating the removal of MS2, in comparison with poliovirus, rotavirus and adenovirus at 4°C, resulted in p values of 0.039, 0.004, and 0.016 respectively. These p values imply that there is a significant difference in the removal of MS2 and the removal of poliovirus, rotavirus and adenovirus at 4°C. Therefore, the null hypothesis stating that there is no statistically significant difference in the removal of MS2 with poliovirus type 1, MS2 with rotavirus strain Wa, and MS2 with adenovirus type 2 at 24°C or 4°C is rejected.

The mean LRV of poliovirus, at 24°C, was 3.5545 with a standard deviation of 0.0975. At 4°C, the mean LRV was 3.2440 with a standard deviation of 0.1223. Statistical analysis resulted in a p value of 0.026;

therefore, the null hypothesis stating that poliovirus LRV does not statistically vary, at 24° versus 4°C, is rejected. The mean LRV of rotavirus, at 24°C, was 3.5482 with a standard deviation of 0.1504. At 4°C, the mean LRV was 3.5144 with a standard deviation of 0.1161. Statistical analysis resulted in a p value of 0.774; therefore, the null hypothesis stating that poliovirus LRV does not statistically vary, at 24° versus 4°C, fails to be rejected. The mean LRV of adenovirus, at 24°C, was 3.493 with a standard deviation of 0.1740. At 4°C, the mean LRV was 3.3.470 with a standard deviation of 0.2330. Statistical analysis resulted in a p value of 0.896 therefore; the null hypothesis stating that poliovirus LRV does not statistically vary at 24° versus 4°C fails to be rejected.

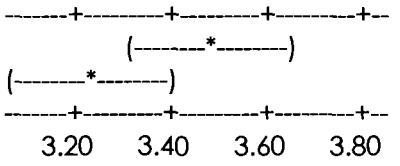
Table 11: One-way ANOVA: Mean LRV of Poliovirus at 24°C versus Poliovirus at 4°C

Source	DF	SS	MS	F	P
Factor	1	0.1446	0.1446	11.82	0.026
Error	4	0.0490	0.0122		
Total	5	0.1936			

S = 0.1106 R-Sq = 74.71% R-Sq(adj) = 68.39%

Individual 95% CIs For Mean Based on Pooled StDev

Level	N	Mean	StDev
Poliovirus 24°C	3	3.5545	0.0975
Poliovirus 4°C	3	3.2440	0.1223



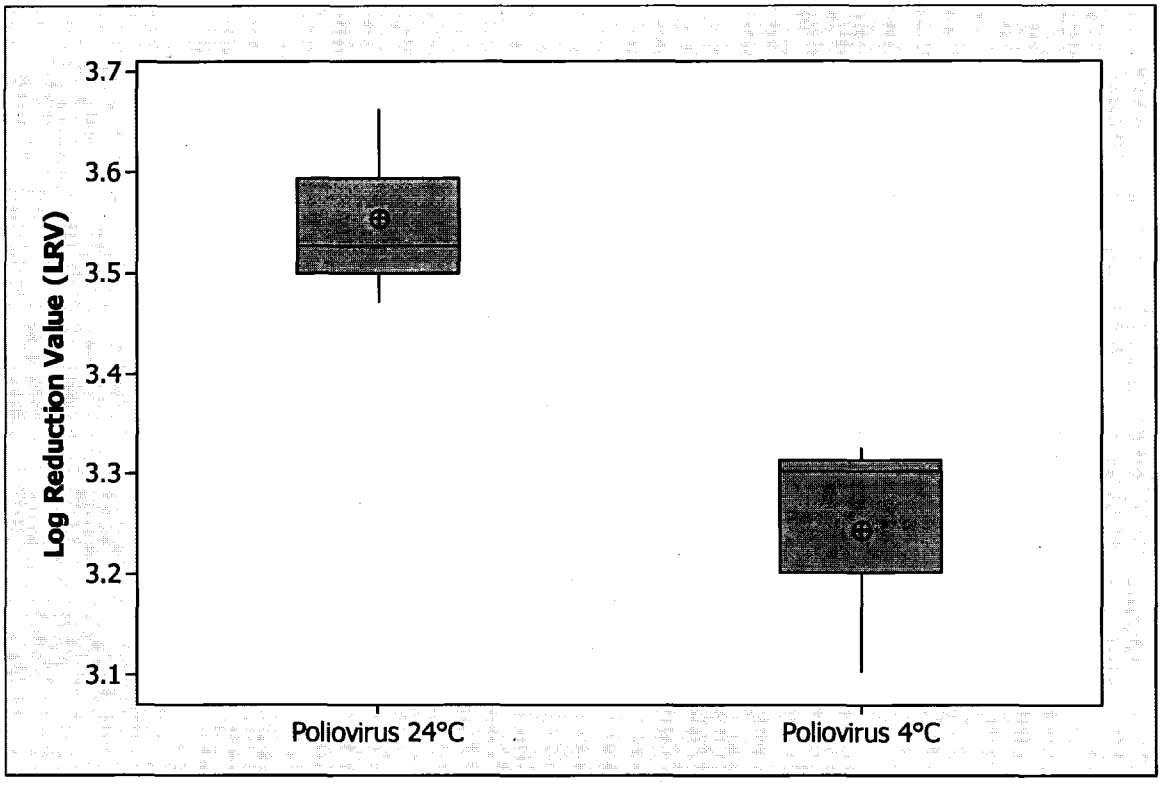
Pooled StDev = 0.1106

Table 12: Descriptive Statistics: Mean LRV of Poliovirus at 24°C versus Poliovirus at 4°C

Variable	N	Mean	StDev	Minimum	Median	Maximum
Poliovirus 24°C	3	3.5545	0.0975	3.4735	3.5272	3.6628
Poliovirus 4°C	3	3.2440	0.1223	3.1034	3.3022	3.3263

Data in Tables 11 and 12 represents the calculated LRV for poliovirus type 1, using data from three separate trials obtained when performing a bench scale model of the CoMag™ process. For each trail, the removal of poliovirus was assayed in triplicate. The plaque assays were also conducted in triplicate.

Figure 12: Mean Log Reduction Value of Poliovirus type 1at 24°C versus the Mean Log Reduction Value of Poliovirus type 1 at 4°C, **Using the Bench Scale** Model of the CoMag™ Process



The data represents the average LRV for poliovirus type 1, at 24°C versus 4°C, using a bench scale model of the CoMag™ process. Poliovirus type 1 was detected using a modified neutral red plaque-forming unit (PFU) method, in BGMK cells. Plaque assays were performed in triplicate and 1000 ml of secondary effluent was spiked with poliovirus to a final concentration of 1 x 10^5 PFU/mL. Three trials were completed; each trial containing a sample size of n=3. The mean LRV of poliovirus, at 24°C, was 3.5545 with a standard deviation of 0.0975. The mean LRV of poliovirus, at 4°C, was 3.2440 with a standard deviation of 0.1223. A p value 0.026 (α=0.05) was obtained, which indicates that there is a statistically significant difference in the removal of poliovirus, at 24°C versus 4°C. In Figure 12, the upper whisker extends to the maximum data point, for each variable, and the lower whisker extends to the minimum data point. Each colored box contains the middle 50% of the data. Each line within the box illustrates the median. The plus sign, within each colored box, represents the mean.

Table 13: One-way ANOVA: Mean LRV of Rotavirus at 24°C versus Rotavirus at 4°C

Source	DF	SS	MS	F	P
Factor	1	0.0017	0.0017	0.09	0.774
Error	4	0.0722	0.0181		
Total	5	0.0739			

S = 0.1344 R-Sq = 2.31% R-Sq(adj) = 0.00%

Level	N	Mean	StDev	Individual 95% CIs For Mean Based on Pooled StDev
Rotavirus 24°C	3	3.5482	0.1504	(----------*----------)
Rotavirus 4°C	3	3.5144	0.1161	(----------*----------)
				3.36 3.48 3.60 3.72

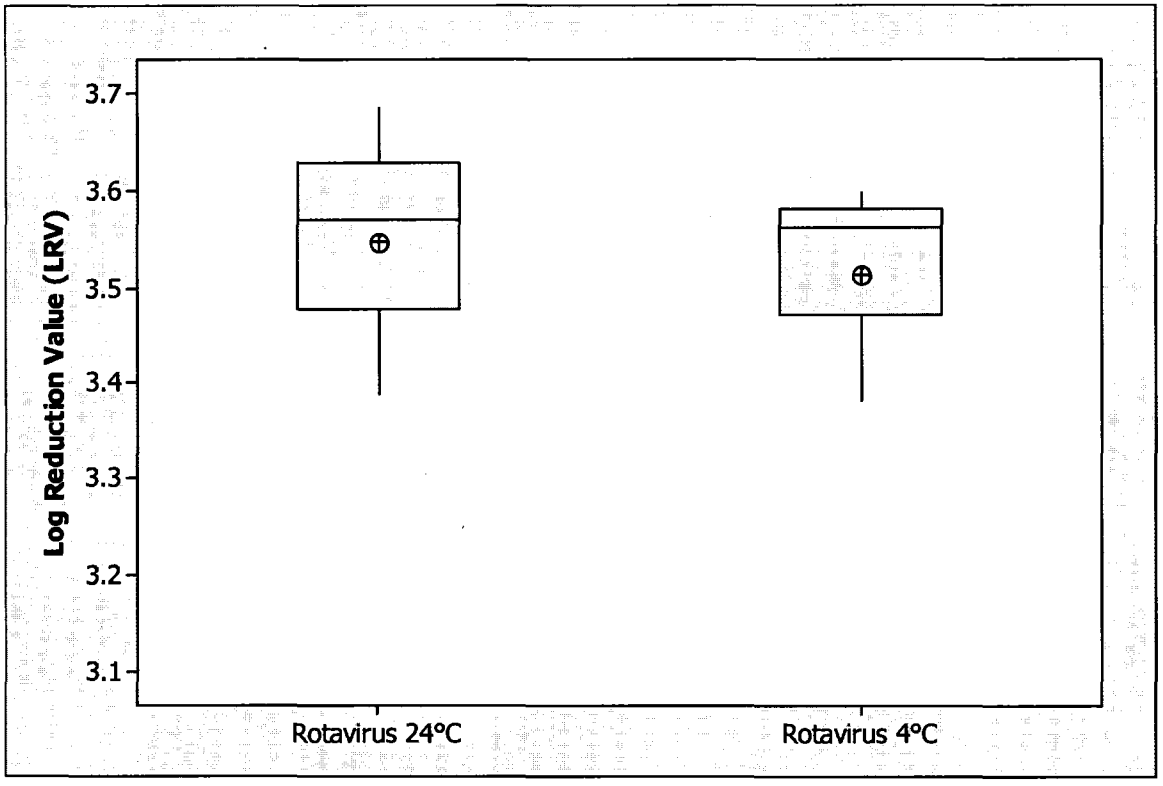
Pooled StDev = 0.1344

Table 14: Descriptive Statistics: Mean LRV of Rotavirus at 24°C versus Rotavirus at 4°C

Variable	N	Mean	StDev	Minimum	Median	Maximum
Rotavirus 24°C	3	3.5482	0.1504	3.3881	3.5698	3.6866
Rotavirus 4°C	3	3.5144	0.1161	3.3819	3.5631	3.5983

Data in Tables 13 and 14 represents the calculated LRV for rotavirus strain Wa. Data were utilized that resulted from performing three separate trials, using a bench scale model of the CoMag™ process. For each trail, the removal rotavirus was assayed in triplicate. The plaque assays were also conducted in triplicate.

Figure 13: Mean Log Reduction Value of Rotavirus strain Wa at 24°C versus Mean Log Reduction Value of Rotavirus strain Wa at 4°, **Using the Bench Scale** Model of the CoMag™ Process



The data represents the average LRV for rotavirus strain Wa at, 24°C versus 4°C, using a bench scale model of the CoMag™ process. Rotavirus strain Wa was detected using a modified crystal violet plaque-forming unit (PFU) method, with Ma-104 cells acting as the host. Plaque assays were performed in triplicate and 1000 ml of secondary effluent was spiked with rotavirus to a final concentration of 1 x 10^5 PFU/mL. Three trials were completed, each trial containing a sample size of n=3. At 24°C, the mean LRV of rotavirus was 3.5482 with a standard deviation of 0.1504. At 4°C, the mean LRV of rotavirus was 3.5144 with a standard deviation of 0.1161. A p value of 0.7740 (α=0.05) was obtained, which indicates that there is not a statistically significant difference in the removal of rotavirus at 24°C versus 4°C. In Figure 13, the upper whisker extends to the maximum data point, for each variable, and the lower whisker extends to the minimum data point. Each colored box contains the middle 50% of the data. The line within each box illustrates the median. The plus sign, within each colored box, represents the mean.

Table 15: One-way ANOVA: Mean LRV of Adenovirus at 24°C versus 4°C

Source	DF	SS	MS	F	P
Factor	1	0.0008	0.0008	0.02	0.896
Error	4	0.1691	0.0423		
Total	5	0.1699			

S = 0.2056 R-Sq = 0.48% R-Sq(adj) = 0.00%

Level	N	Mean	StDev	Individual 95% CIs For Mean Based on Pooled StDev
Adenovirus 24°C	3	3.4933	0.1739	(----------*----------)
Adenovirus 4°C	3	3.4700	0.2330	(----------*----------)
				3.20 3.40 3.60 3.80

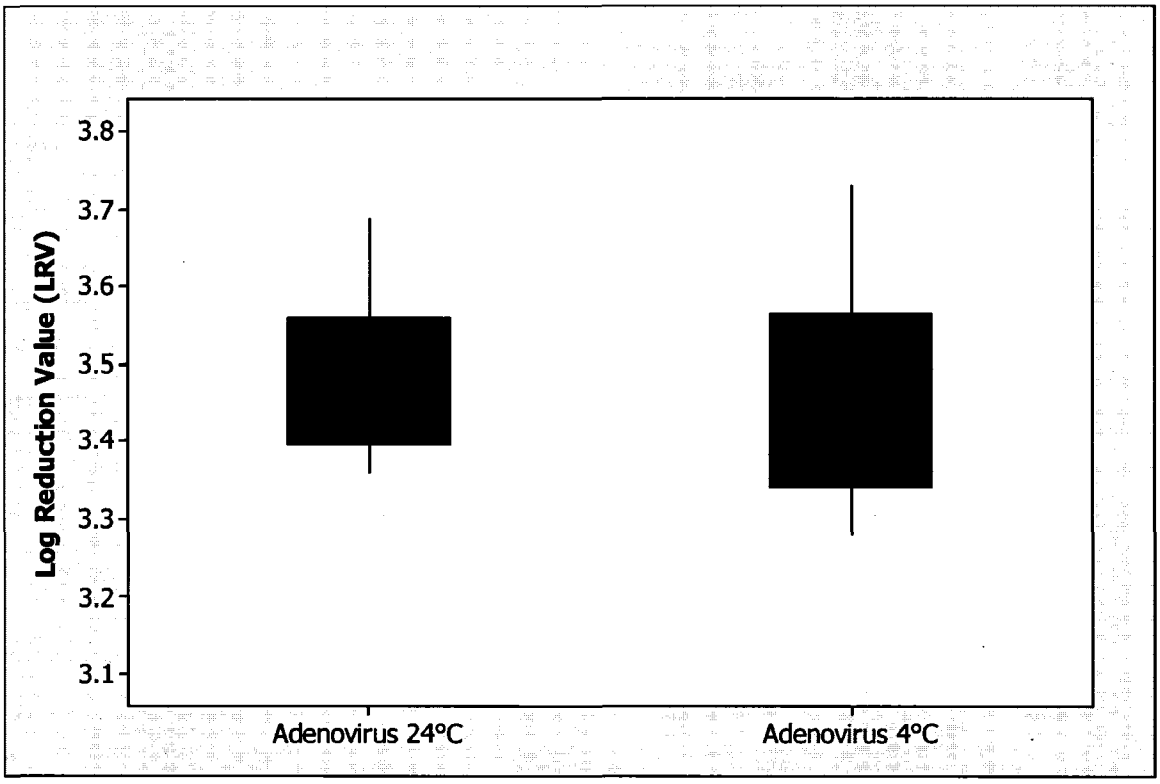
Pooled StDev = 0.2056

Table 16: Descriptive Statistics: Mean LRV of Adenovirus at 24°C versus 4°C

Variable	N	Mean	StDev	Minimum	Median	Maximum
Adenovirus 24°C	3	3.4930	0.1740	3.3600	3.4300	3.6900
Adenovirus 4°C	3	3.4700	0.2330	3.2800	3.4000	3.7300

Data in Tables 15 and 16 represents the calculated LRV of adenovirus type 2, using data from gathered from three separate trials employing a bench scale model of the CoMag™ process. For each trail, the removal adenovirus was assayed in triplicate. The plaque assays were also conducted in triplicate.

Figure 14: Mean Log Reduction Value of Adenovirus type 2 at 24°C versus the Mean Log Reduction Value of Adenovirus type 2 at 4°C, **Using the Bench Scale** Model of the CoMag™ Process



The data represents the average LRV for adenovirus type 2 at, 24°C versus 4°C, using a bench scale model of the CoMag™ process. Adenovirus type 2 was detected using the Reed and Muench $TCID_{50}$ method, with A549 cells as the host. Plaque assays were performed in triplicate and 1000 ml of secondary effluent was spiked with adenovirus to a final concentration of 1×10^5 $TCID_{50}$/ mL. Three trials were completed, each trial containing a sample size of n=3. The mean LRV of adenovirus, at 24°C, was 3.4933 with a standard deviation of 0.1740. The mean LRV of rotavirus, at 4°C, was 3.4700 with a standard deviation of 0.2330. A p value 0.8960 (α=0.05) was obtained, which indicates that there is no statistically significant difference in the removal of adenovirus at 24°C versus 4°C. In Figure 14, the upper whisker extends to the maximum data point, for each variable, and the lower whisker extends to the minimum data point. Each colored box contains the middle 50% of the data. The line within each box illustrates the median. The plus sign, within each colored box, represents the mean.

Table 17: One-way ANOVA: Mean LRV of MS2 at 24°C and Poliovirus at 24°C

Source	DF	SS	MS	F	P
Factor	1	0.58375	0.58375	63.50	0.001
Error	4	0.03677	0.00919		
Total	5	0.62052			

S = 0.09588 R-Sq = 94.07% R-Sq(adj) = 92.59%

Level	N	Mean	StDev
MS2 24°C	3	2.9306	0.0942
Poliovirus 24°C	3	3.5545	0.0975

Individual 95% CIs For Mean Based on Pooled StDev

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Level
MS2 24°C          (-----*-----)
Poliovirus 24°C                 (-----*-----)
                  ---------+---------+---------+---------+
                         3.00      3.25      3.50      3.75
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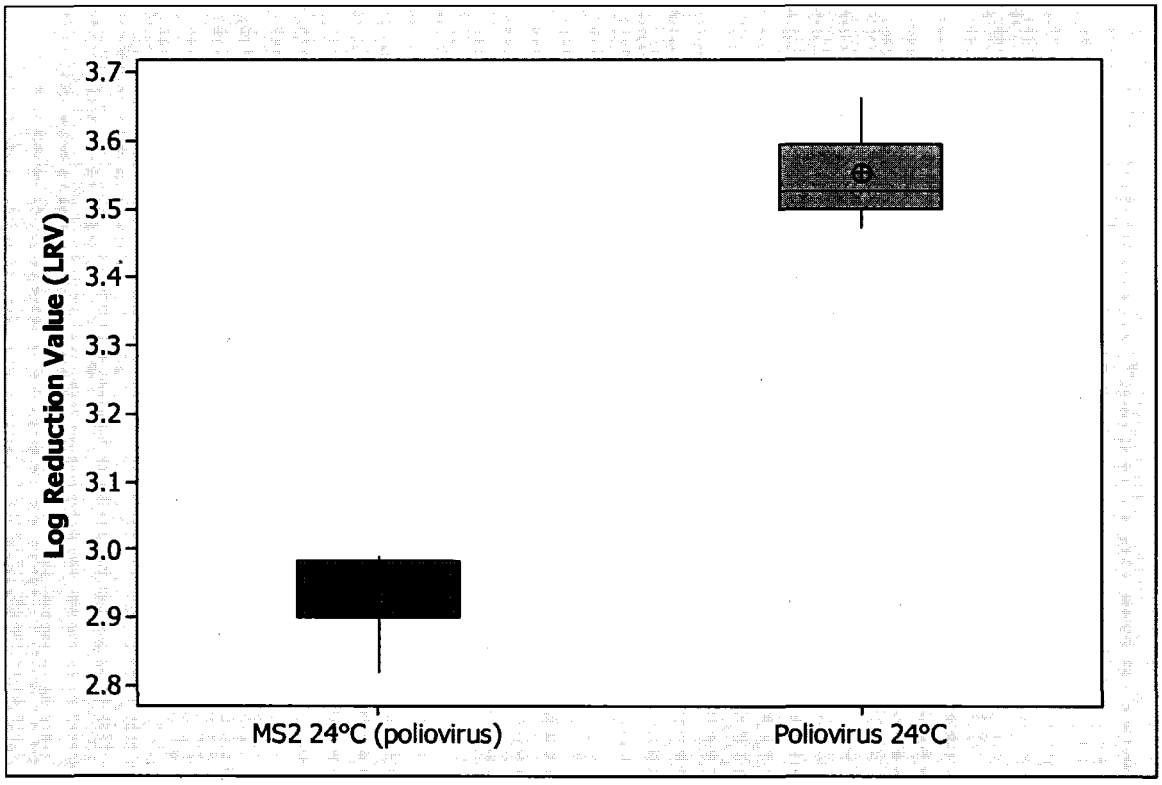
Pooled StDev = 0.0959

Table 18: Descriptive Statistics: Mean LRV of MS2 at 24°C and Poliovirus 24°C

Variable	N	Mean	StDev	Minimum	Median	Maximum
MS2 24°C	3	2.9306	0.0942	2.8221	2.9794	2.9905
Poliovirus 24°C	3	3.5545	0.0975	3.4735	3.5272	3.6628

Data in Tables 17 and 18 represents the calculated LRV of MS2 and poliovirus type 1, at 24°C, using data from three separate trials, obtained by performing a bench scale model of the CoMag™ process. For each trail, the removal MS2 and poliovirus was assayed in triplicate. The plaque assays were also conducted in triplicate.

Figure 15: Mean Log Reduction Value of MS2 versus the Mean Log Reduction Value of Poliovirus type 1 at 24°C, **Using the Bench Scale** Model of the CoMag™ Process



The data represents the average LRV of MS2 and poliovirus type 1, at 24°C, using a bench scale model of the CoMag™ process. MS2 and poliovirus were spiked into the same test sample. Three trials were completed, each trial containing a sample size of n=3. The mean LRV of MS2 was 2.9306 with a standard deviation of 0.10942. The mean LRV of poliovirus was 3.5545 with a standard deviation of 0.0975. A p value 0.0010 (α=0.05) was obtained, which indicates that there is a statistically significant difference in the removal of MS2 at 24°C and poliovirus at 24°C. In Figure 15, the upper whisker extends to the maximum data point, for each variable, and the lower whisker extends to the minimum data point. Each colored box contains the middle 50% of the data. The line within each box illustrates the median. The plus sign, within each colored box, represents the mean.

Table 19: One-way ANOVA: Mean LRV of MS2 at 4°C and Poliovirus at 4°C

Source	DF	SS	MS	F	P
Factor	1	0.0979	0.0979	9.17	0.039
Error	4	0.0427	0.0107		
Total	5	0.1406			

S = 0.1033 R-Sq = 69.62% R-Sq(adj) = 62.03%

Level	N	Mean	StDev
MS2 4°C	3	2.9885	0.0800
Poliovirus 4°C	3	3.2440	0.1223

Individual 95% CIs For Mean Based on Pooled StDev

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Level
MS2 4°C          (---------*---------)
Poliovirus 4°C                (---------*---------)
                 --+---------+---------+---------+-------
                 2.85      3.00      3.15      3.30
```

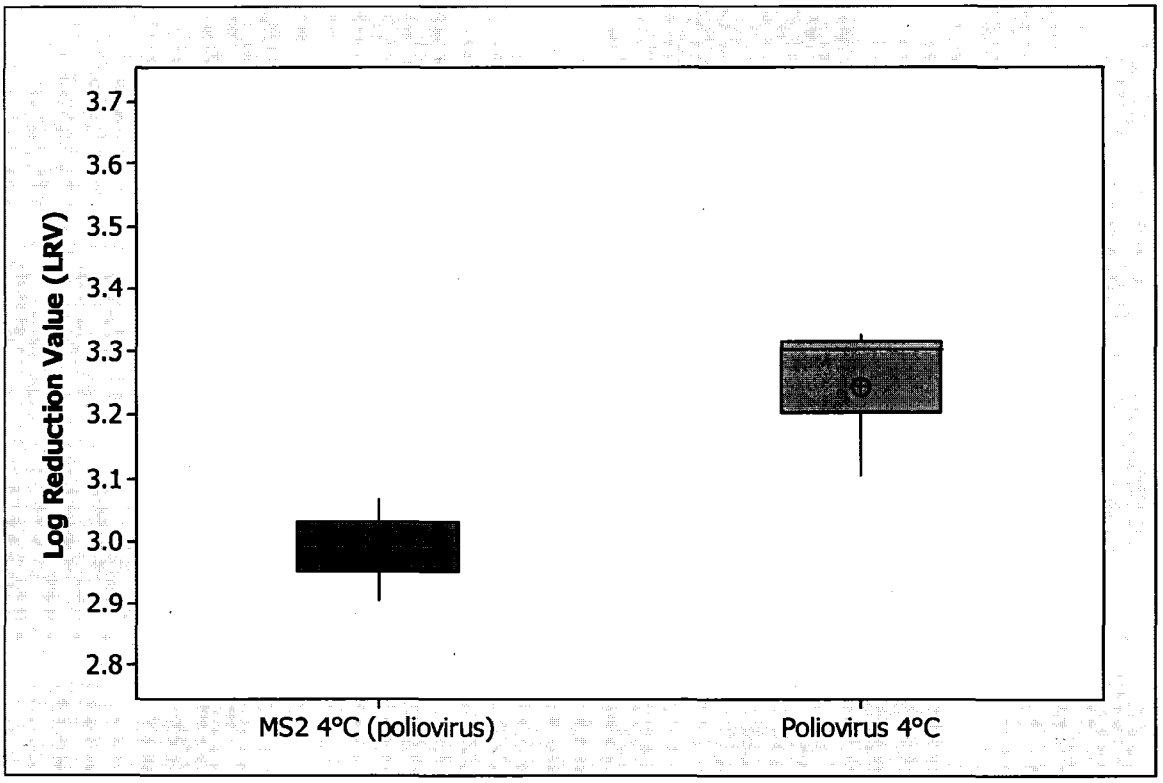
Pooled StDev = 0.1033

Table 20: Descriptive Statistics: Mean LRV of MS2 at 4°C and Poliovirus at 4°C

Variable	N	Mean	StDev	Minimum	Median	Maximum
MS2 4°C	3	2.9885	0.0800	2.9055	2.9950	3.0650
Poliovirus 4°C	3	3.2440	0.1223	3.1034	3.3022	3.3263

Data in Tables 19 and 20 represents the calculated LRV of MS2 and poliovirus type 1, at 4°C, using data from three separate trials obtained by performing a bench scale model of the CoMag™ process. For each trail, the removal MS2 and poliovirus was assayed in triplicate. The plaque assays were also conducted in triplicate.

Figure 16: Mean Log Reduction Value of MS2 versus the Mean Log Reduction Value of Poliovirus type 1 at 4°C, **Using the Bench Scale** Model of the CoMag™ Process



The data represents the average LRV of MS2 and poliovirus type 1, at 4°C, using a bench scale model of the CoMag™ process. One thousand milliliters of secondary effluent was spiked with MS2 and poliovirus for a target concentration of 1 x 10^5 PFU/mL and plaque assays were performed. Three trials were completed, each trial containing a sample size of n=3. The mean LRV of MS2 was 2.9885 with a standard deviation of 0.0800. The mean LRV of poliovirus was 3.2440 with a standard deviation of 0.1223. A p value 0.0390 (α=0.05) was obtained, which indicates that there is a statistically significant difference in the removal of MS2 at 4°C and poliovirus at 4°C. In Figure 16, the upper whisker extends to the maximum data point, for each variable, and the lower whisker extends to the minimum data point. Each colored box contains the middle 50% of the data. The line within each box illustrates the median. The plus sign, within each colored box, represents the mean.

Table 21: One-way ANOVA: Mean LRV of MS2 at 24°C and Rotavirus at 24°C

Source	DF	SS	MS	F	P
Factor	1	0.4917	0.4917	23.95	0.008
Error	4	0.0821	0.0205		
Total	5	0.5739			

S = 0.1433 R-Sq = 85.69% R-Sq(adj) = 82.11%

Level	N	Mean	StDev
MS2 24°C	3	2.9756	0.1358
Rotavirus 24°C	3	3.5482	0.1504

Individual 95% CIs For Mean Based on Pooled StDev

```
Level                 --------+---------+---------+---------+-
MS2 24°C (rotavirus)  (------*-------)
Rotavirus 24°C                          (------*-------)
                      --------+---------+---------+---------+-
                            3.00      3.30      3.60      3.90
```

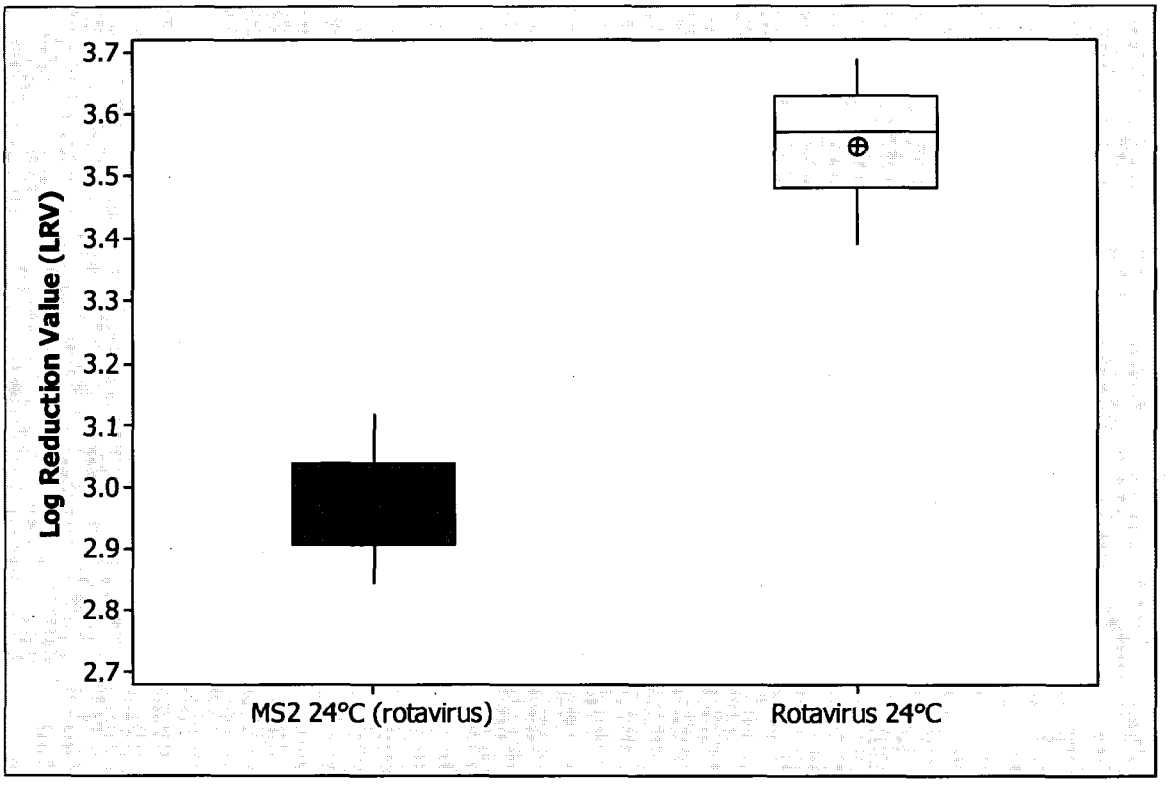
Pooled StDev = 0.1433

Table 22: Descriptive Statistics: Mean LRV of MS2 at 24°C and Rotavirus at 24°C

Variable	N	Mean	StDev	Minimum	Median	Maximum
MS2 24°C	3	2.9756	0.1358	2.8451	2.9656	3.1161
Rotavirus 24°C	3	3.5482	0.1504	3.3881	3.5698	3.6866

Data in Tables 21 and 22 represents the calculated LRV of MS2 and rotavirus strain Wa, at 24°C, using data obtained from three separate trials utilizing a bench scale model of the CoMag™ process. For each trail, the removal MS2 and rotavirus was assayed in triplicate. The plaque assays were also conducted in triplicate.

Figure 17: Mean Log Reduction Value of MS2 versus the Mean Log Reduction Value of Rotavirus strain Wa at 24°C, **Using the Bench Scale** Model of the CoMag™ Process



The data represents the average LRV of MS2 and rotavirus strain Wa, at 24°C, using a bench scale model of the CoMag™ process. One thousand milliliters of secondary effluent was spiked with MS2 and rotavirus for a target concentration of 1 x 10^5 PFU/mL and plaque assays were performed. Three trials were completed; each trial containing a sample size of n=3. The mean LRV of MS2 was 2.9756 with a standard deviation of 0.1358. The mean LRV of rotavirus was 3.5482 with a standard deviation of 0.1504. A p value of 0.0080 (α=0.05) was obtained, which indicates that there is a statistically significant difference in the removal of MS2 at 24°C and rotavirus at 24°C. In Figure 17, the upper whisker extends to the maximum data point, for each variable, and the lower whisker extends to the minimum data point. Each colored box contains the middle 50% of the data. The line within each box illustrates the median. The plus sign, within each colored box, represents the mean.

Table 23: One-way ANOVA: Mean LRV of MS2 at 4°C and Rotavirus at 4°C

Source	DF	SS	MS	F	P
Factor	1	0.5902	0.5902	34.49	0.004
Error	4	0.0685	0.0171		
Total	5	0.6586			

S = 0.1308 R-Sq = 89.61% R-Sq(adj) = 87.01%

Level	N	Mean	StDev
MS2 4°C	3	2.8872	0.1440
Rotavirus 4°C	3	3.5144	0.1161

Individual 95% CIs For Mean Based on Pooled StDev

```
Level                  -+---------+---------+---------+-------
MS2 4°C (rotavirus)    (------*------)
Rotavirus 4°C                       (------*------)
                       -+---------+---------+---------+-------
                       2.70      3.00      3.30      3.60
```

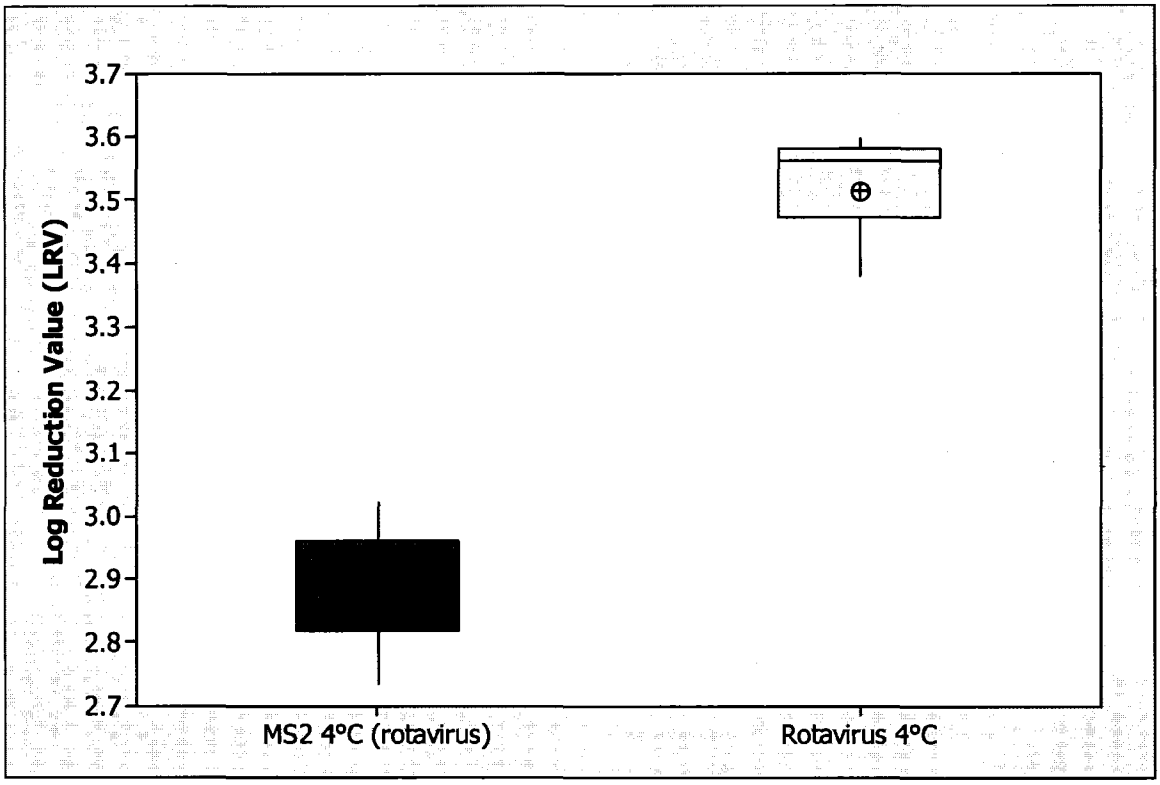
Pooled StDev = 0.1308

Table 24: Descriptive Statistics: Mean LRV of MS2 at 4°C and Rotavirus at 4°C

Variable	N	Mean	StDev	Minimum	Median	Maximum
MS2 4°C	3	2.8872	0.1440	2.7362	2.9023	3.0231
Rotavirus 4°C	3	3.5144	0.1161	3.3819	3.5631	3.5983

Data in Tables 23 and 24 represents the calculated LRV of MS2 and rotavirus strain Wa, at 4°C, utilizing data obtained from three separate trials utilizing a bench scale model of the CoMag™ process. For each trail, the removal MS2 and rotavirus was assayed in triplicate. The plaque assays were also conducted in triplicate.

Figure 18: Mean Log Reduction Value of MS2 versus the Mean Log Reduction Value of Rotavirus strain Wa at 4° **Using the Bench Scale** Model of the CoMag™ Process



The data represents the average LRV of MS2 and rotavirus strain Wa, at 4°C, using a bench scale model of the CoMag™ process. One thousand milliliters of secondary effluent was spiked with MS2 and rotavirus for a target concentration of 1 x 10^5 PFU/mL and plaque assays were performed. Three trials were completed, each trial containing a sample size of n=3. The mean LRV of MS2 was 2.8872 with a standard deviation of 0.1440. The mean LRV of rotavirus was 3.5144 with a standard deviation of 0.1161. A p value of 0.0040 (α=0.05) was obtained, which indicates that there is a statistically significant difference in the removal of MS2 at 4°C and rotavirus at 4°C. In Figure 18, the upper whisker extends to the maximum data point, for each variable, and the lower whisker extends to the minimum data point. Each colored box contains the middle 50% of the data. The line within each box illustrates the median. The plus sign, within each colored box, represents the mean.

Table 25: One-way ANOVA: Mean LRV of MS2 at 24°C and Adenovirus at 24°C

Source	DF	SS	MS	F	P
Factor	1	0.6528	0.6528	41.07	0.003
Error	4	0.0636	0.0159		
Total	5	0.7164			

S = 0.1261 R-Sq = 91.13% R-Sq(adj) = 88.91%

Level	N	Mean	StDev
MS2 24°C	3	2.8336	0.0395
Adenovirus 24°C	3	3.4933	0.1739

Individual 95% CIs For Mean Based on Pooled StDev

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Level
MS2 24°C          (-----*------)
Adenovirus 24°C                 (-----*------)
                --+---------+---------+---------+-------
                2.70      3.00      3.30      3.60
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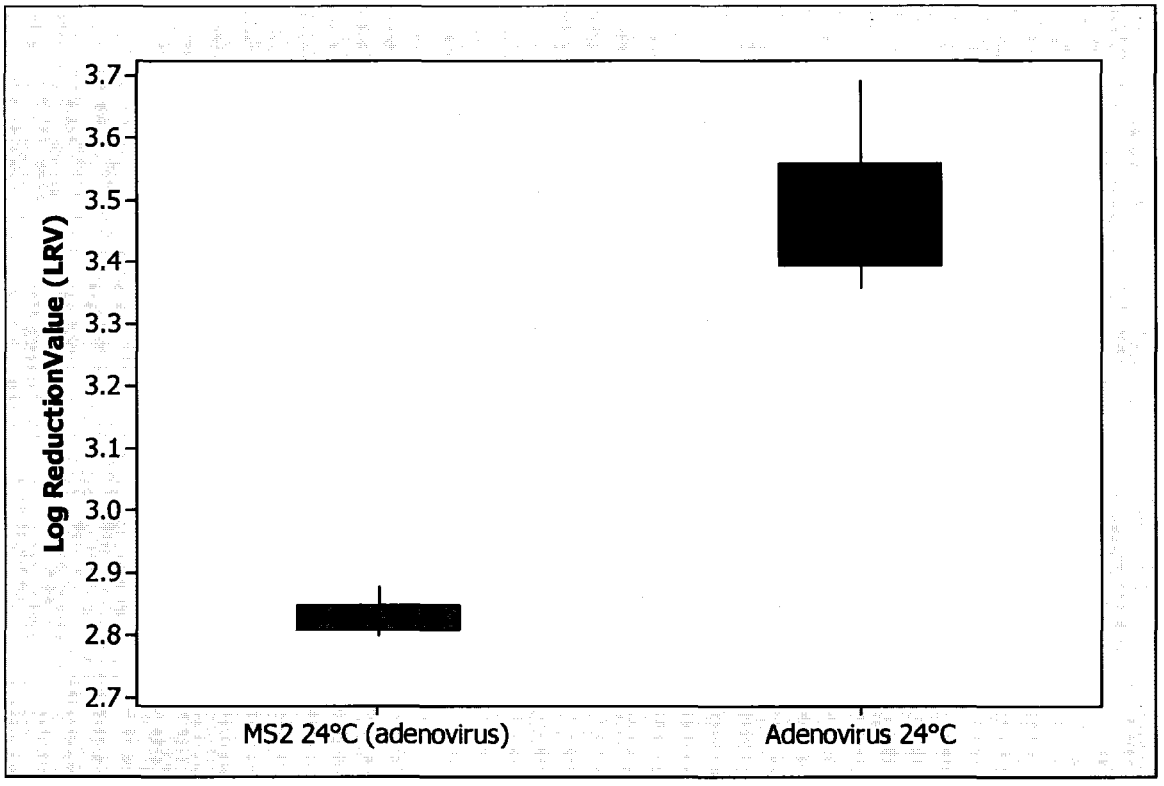
Pooled StDev = 0.1261

Table 26: Descriptive Statistics: Mean LRV of MS2 at 24°C and Adenovirus at 24°C

Variable	N	Mean	StDev	Minimum	Median	Maximum
MS2 24°C	3	2.8336	0.0395	2.8009	2.8225	2.8774
Adenovirus 24°C	3	3.4930	0.1740	3.3600	3.4300	3.6900

Data in Tables 25 and 26 represents the calculated LRV of MS2 and adenovirus type 2, at 24°C, using data from three separate trials that utilized a bench scale model of the CoMag™ process. For each trail, the removal MS2 and adenovirus was assayed in triplicate. The plaque assays were also conducted in triplicate.

Figure 19: Mean Log Reduction Value of MS2 versus the Mean Log Reduction Value of Adenovirus type 2 at 24°C, **Using the Bench Scale** Model of the CoMag™ Process



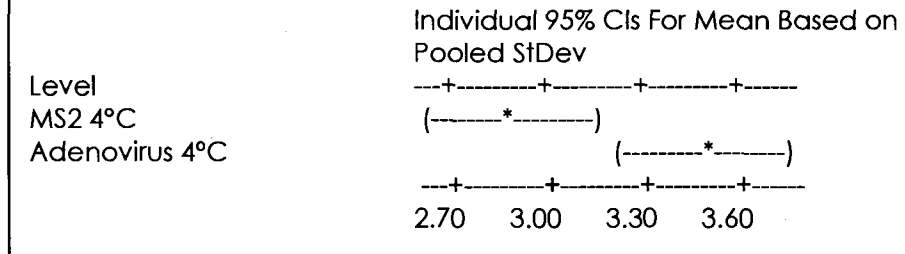
The data represents the average LRV of MS2 and adenovirus type 2, at 24°C, using a bench scale model of the CoMag™ process. One thousand milliliters of secondary effluent was spiked with MS2 and adenovirus for a target concentration of 1 x 10^5 PFU/mL and plaque assays were performed. Three trials were completed; each trial containing a sample size of n=3. The mean LRV of MS2 was 2.8336 with a standard deviation of 0.0395. The mean LRV of adenovirus was 3.4930 with a standard deviation of 0.1740. A p value of 0.0030 (α=0.05) was obtained, which indicates that there is a statistically significant difference in the removal of MS2 at 24°C and adenovirus at 24°C. In Figure 19, the upper whisker extends to the maximum data point, for each variable, and the lower whisker extends to the minimum data point. Each colored box contains the middle 50% of the data. The line within each box illustrates the median. The plus sign, within each colored box, represents the mean.

Table 27: One-way ANOVA: Mean LRV of MS2 at 4°C and Adenovirus at 4°C

Source	DF	SS	MS	F	P
Factor	1	0.4970	0.4970	16.20	0.016
Error	4	0.1227	0.0307		
Total	5	0.6198			

S = 0.1752 R-Sq = 80.20% R-Sq(adj) = 75.24%

Level	N	Mean	StDev
MS2 4°C	3	2.8944	0.0841
Adenovirus 4°C	3	3.4700	0.2330



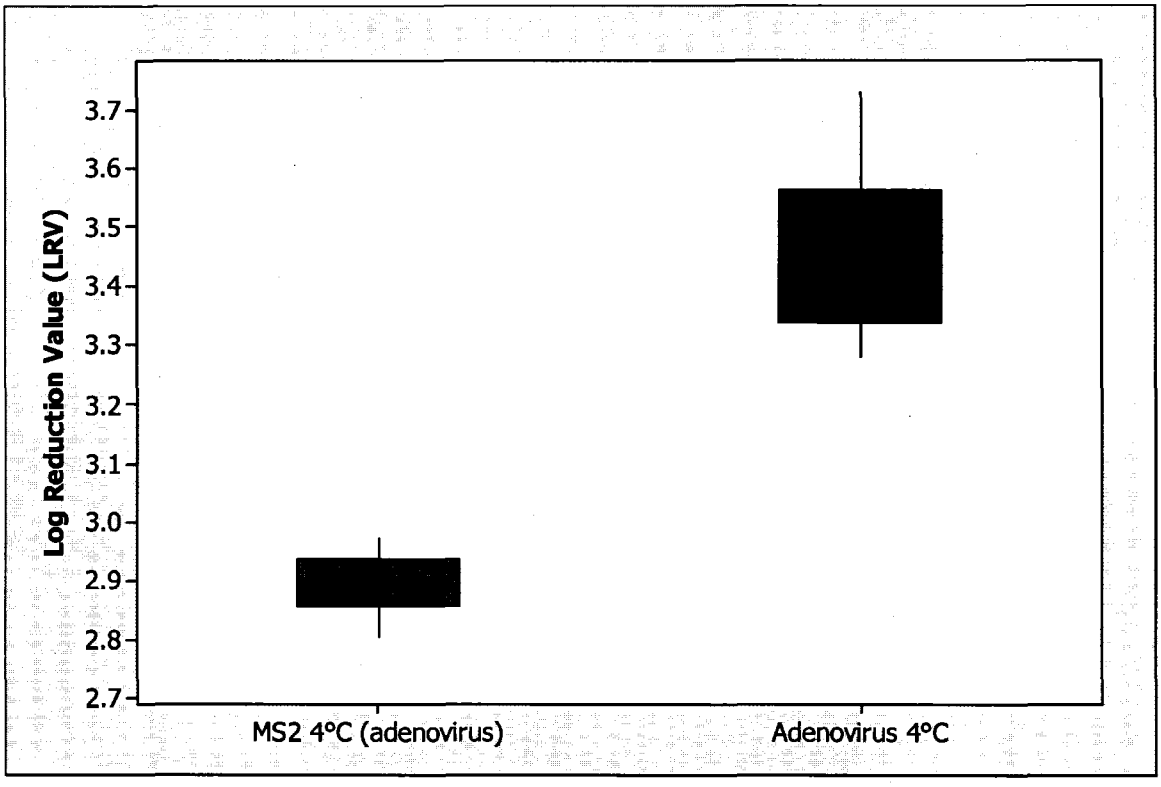
Pooled StDev = 0.1752

Table 28: Descriptive Statistics: Mean LRV of MS2 at 4°C and Adenovirus at 4°C

Variable	N	Mean	StDev	Minimum	Median	Maximum
MS2 4°C	3	2.8944	0.0841	2.8053	2.9055	2.9723
Adenovirus 4°C	3	3.4700	0.2330	3.2800	3.4000	3.7300

Data in Tables 27 and 28 represents the calculated LRV of MS2 and adenovirus type 2 at 4°C, using data from three separate trials, which utilized a bench scale model of the CoMag™ process. For each trail, the removal MS2 and adenovirus was assayed in triplicate. The plaque assays were also conducted in triplicate.

Figure 20: Mean Log Reduction Value of MS2 versus the Mean Log Reduction Value of Adenovirus type 2 at 4°C, **Using the Bench Scale** of the CoMag™ Process



The data represents the average LRV of MS2 and adenovirus type 2, at 4°C, using a bench scale model of the CoMag™ process. One thousand milliliters of secondary effluent was spiked with MS2 and adenovirus for a target concentration of 1 x 10^5 PFU/mL and plaque assays were performed. Three trials were completed; each trial containing a sample size of n=3. The mean LRV of MS2 was 2.8944 with a standard deviation of 0.0841. The mean LRV of adenovirus was 3.4700 with a standard deviation of 0.2330. A p value of 0.0160 (α=0.05) was obtained, which indicates that there is a statistically significant difference in the removal of MS2 at 4°C and adenovirus at 4°C. In Figure 20, the upper whisker extends to the maximum data point, for each variable, and the lower whisker extends to the minimum data point. Each colored box contains the middle 50% of the data. The line within each box illustrates the median. The plus sign, within each colored box, represents the mean.

Removal of MS2 Using the Bench Scale Model of the CoMag™ versus the Removal of MS2 using the CoMag™ Pilot Plant

The removal of MS2 using a bench scale model of the CoMag™ process was compared to the removal of MS2 utilizing the 100 gpm CoMag™ pilot plant. All trials were performed in triplicate. For the pilot plant challenge, MS2 was spiked into 15L of sterile water, yielding a final concentration of 3.37 x 10^6 PFU/mL. The CoMag™ process was then applied.

The amount of endogenous MS2, present in the sample matrix, was below the detectable limit of <1 PFU/mL. An average 62 PFU/ mL was detected in the influent of the CoMag™ pilot plant, which was later normalized in the calculations. Statistical analysis was performed to evaluate the following null hypotheses: (1) the pilot plant scale of the CoMag™ process does not have a statistically significant effect on the removal of MS2 between trails (2) there is no statistically significant difference between the removal of MS2, using the pilot plant scale CoMag™ process versus the bench scale model of the CoMag™ process.

The average LRV for the removal of MS2, using the bench scale model, was 2.913 at 24°C. The average LRV for the removal of MS2 using the Bench scale model, at 4°C, was 2.9234. The average LRV for the removal of MS2, using the pilot plant, was 2.0967. The statistical analysis resulted in a p value of 0.000, at both 24°C and 4°C , indicating that there

is a significant difference in the removal of MS2, using the bench scale model versus the pilot plant. Therefore, the null hypothesis stating there is no statistically significant difference in the removal of MS2, using the pilot plant scale CoMag™ process versus the bench scale model of the CoMag™ process is rejected. The results are summarized in Tables 31 through 34 and Figures 22 and 23.

Regarding the CoMag™ pilot plant, the mean LRV for MS2 was 2.1076 for trial 1, 2.0765 for trial 2, and 2.1064 for trial 3, respectively. The results are summarized in Tables 29 and 30 and Figures 21. A p value 0..786 (α=0.05) was obtained, which indicates that there is no statistically significant difference in the removal of MS2, between trails, when using the CoMag™ pilot plant. Therefore, the null hypothesis stating that the pilot plant scale of the CoMag™ process does not have a statistically significant effect on the removal of MS2, between trails, fails to be rejected. A Tukey family error rate was conducted to analyze any significant pairwise differences between level means. From this analysis, it was concluded that no statistically significant pairwise interactions exist among the means.

Table 29: Two-way ANOVA: Pilot Plant Mean LRV versus Trial and Test

Source	DF	SS	MS	F	P
MS2 test	2	0.011234	0.0056172	0.27	0.786
MS2 replicate	1	0.097283	0.0972827	4.71	0.162
Error	2	0.041330	0.0206652		
Total	5	0.149847			

S = 0.1438 R-Sq = 72.42% R-Sq(adj) = 31.05%

MS2 test Mean — Individual 95% CIs For Mean Based on Pooled StDev

test	Mean	
1	3.0495	(----------*----------)
2	2.9640	(----------*----------)
3	2.9525	(----------*----------)
		2.75 3.00 3.25 3.50

MS2 replicate Mean — Individual 95% CIs For Mean Based on Pooled StDev

replicate	Mean	
1	2.86133	(----------*----------)
2	3.11600	(----------*----------)
		2.50 2.75 3.00 3.25

Table 30: Tukey 95% Simultaneous Confidence Intervals: All Pairwise Comparisons of Pilot Plant Mean LRV

Individual confidence level = 97.94%

Run 1 subtracted from:

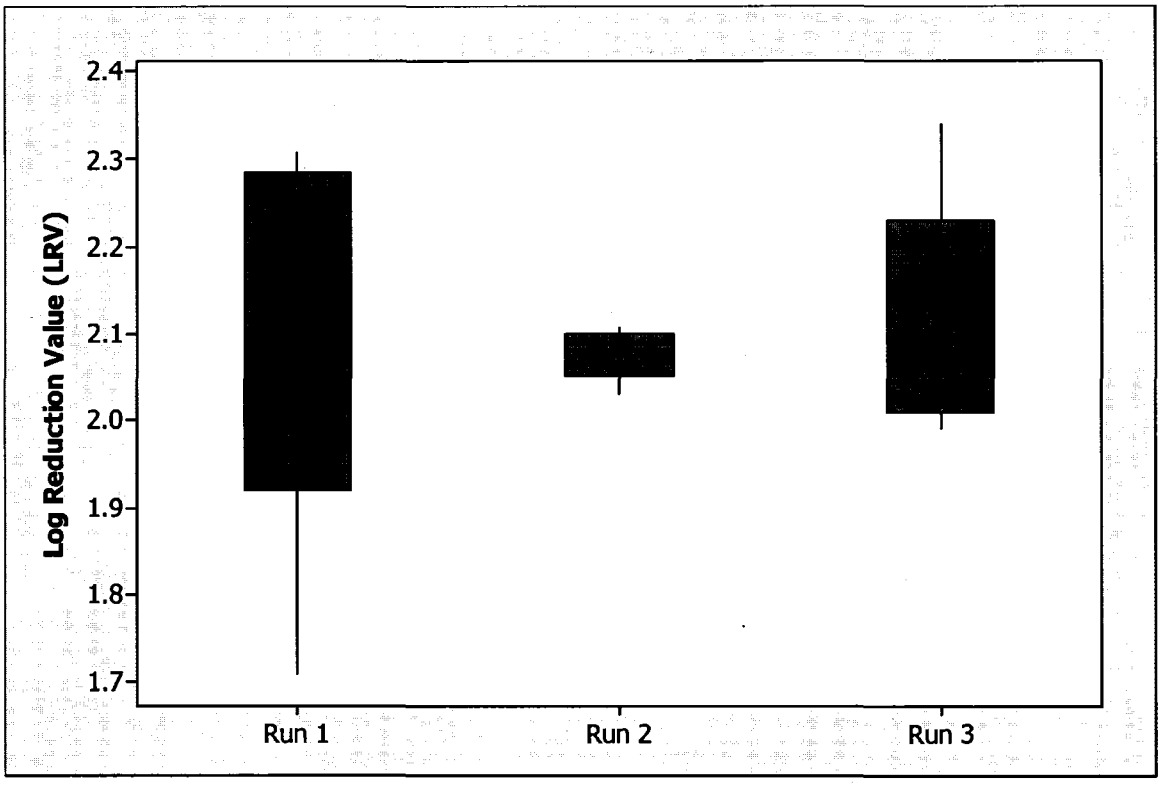
	Lower	Center	Upper	
Run 2	-0.2992	-0.0311	0.2371	(----------*----------)
Run 3	-0.2693	-0.0012	0.2670	(----------*----------)
				-0.30 -0.15 0.00 0.15

Run 2 subtracted from:

	Lower	Center	Upper	
Run 3	-0.2382	0.0299	0.2980	(----------*----------)
				-0.30 -0.15 0.00 0.15

The data in Tables 29 and 30 represents the calculated LRV of MS2, using data from three separate trials which employed a pilot plant model of the CoMag™ process. In each trail, the removal of MS2 was assayed in triplicate. The plaque assays were also conducted in triplicate.

Figure 21: Mean Log Reduction Value of MS2 **Using** the CoMag™ Pilot Plant



The data represents the average LRV for MS2, using the pilot scale CoMag™ process. MS2 was detected by modified double agar overlay, using *E. coli* HS (pFamp) R as the host. Plaque assays were performed in triplicate and 1000 ml of secondary effluent was spiked with MS2 to a final concentration of 1 x 10^5 PFU/mL. Three trials were completed, each trial containing a sample size of n=3. The mean LRV was 2.1076 for trial 1, 2.0765 for trial 2, and 2.1064 for trial 3. A p value of 0.941 (α=0.05) was obtained, which indicates that there is not a statistically significant difference in the removal of MS2, between trails, when using the CoMag™ pilot plant. In Figure 21, the upper whisker extends to the maximum data point, for each variable, and the lower whisker extends to the minimum data point. Each colored box contains the middle 50% of the data. The line within each box illustrates the median. The plus sign, within each colored box, represents the mean.

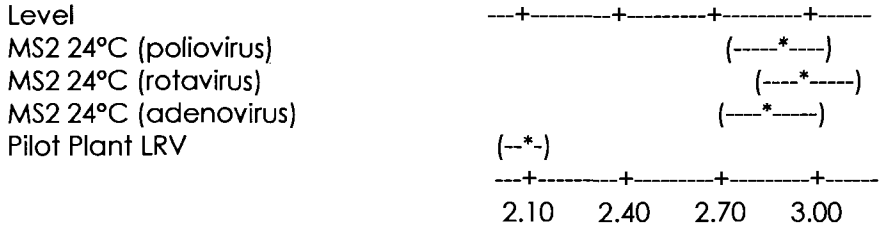
Table 31: One-way ANOVA: MS2 Bench Scale Mean LRV at 24°C and Pilot Plant Mean LRV

Source	DF	SS	MS	F	P
Factor	3	3.7828	1.2609	69.08	0.000
Error	20	0.3651	0.0183		
Total	23	4.1478			

S = 0.1351 R-Sq = 91.20% R-Sq(adj) = 89.88%

Level	N	Mean	StDev
MS2 24°C (poliovirus)	3	2.9306	0.0942
MS2 24°C (rotavirus)	3	2.9756	0.1358
MS2 24°C (adenovirus)	3	2.8336	0.0395
LRV	15	2.0967	0.1482

Individual 95% CIs For Mean Based on Pooled StDev



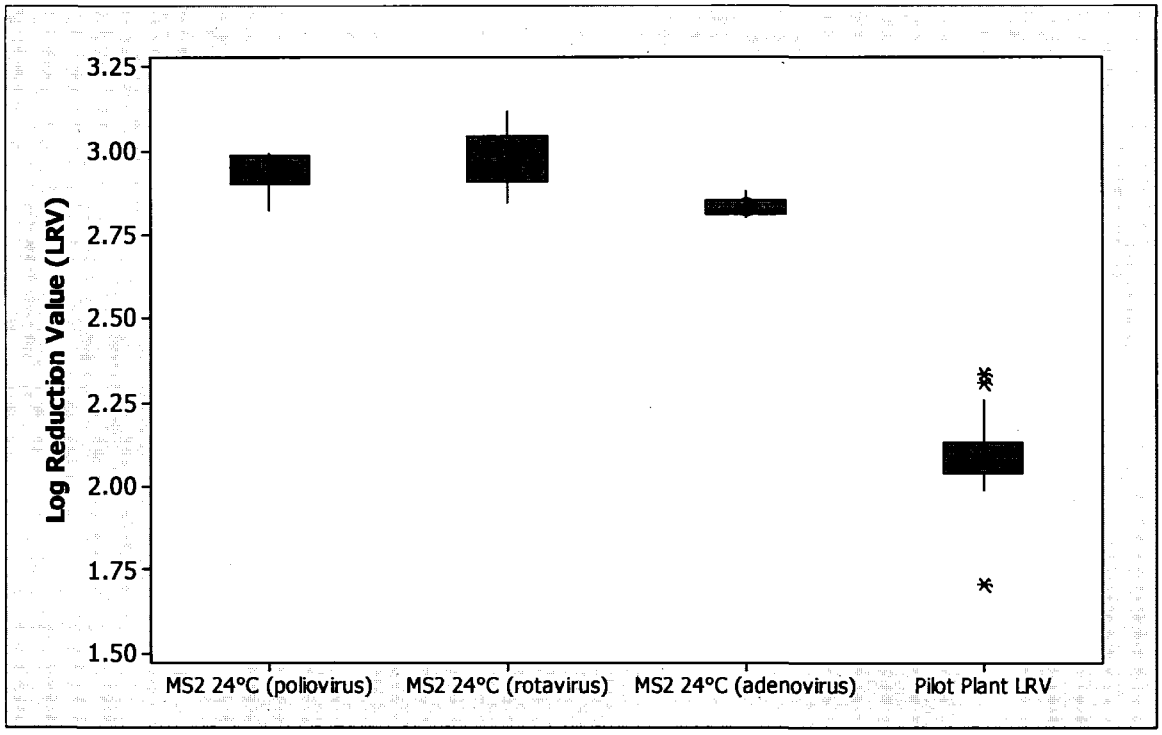
Pooled StDev = 0.1351

Table 32: Descriptive Statistics: MS2 Mean LRV at 24°C and Pilot Plant Mean LRV

Variable	N	Mean	StDev	Minimum	Median	Maximum
MS2 24°C (poliovirus)	3	2.9306	0.0942	2.8221	2.9794	2.9905
MS2 24°C (rotavirus)	3	2.9756	0.1358	2.8451	2.9656	3.1161
MS2 24°C (adenovirus)	3	2.8336	0.0395	2.8009	2.8225	2.8774
Pilot Plant LRV	15	2.0967	0.1482	1.7100	2.0900	2.3400

Data in Tables 31 and 32 represents the calculated LRV of MS2, utilizing data from three separate trials comparing a bench scale model and a pilot plant model of the CoMag™ process. For each trail, the removal of MS2 was assayed in triplicate. The plaque assays were also conducted in triplicate.

Figure 22: Mean Log Reduction Value of MS2 at 24°C, **Using the Bench Scale** Model of the CoMag™ Process, versus the Mean Log Reduction Value of the CoMag™ Pilot Plant



The data represents the average LRV for MS2, at 24°C, using a bench scale model of the CoMag™ process versus the pilot scale CoMag™ process. MS2 was detected by modified double agar overlay, using *E. coli* HS (pFamp) R as the host. Plaque assays were performed in triplicate and 1000 ml of secondary effluent was spiked with MS2 to a final concentration of 1 x 10^5 PFU/mL. Three trials were completed, each trial containing a sample size of n=3. When challenged with poliovirus type 1, the mean LRV of MS2, was 2.931, with a standard deviation of 0.0942. The mean LRV of MS2, when challenged with rotavirus strain Wa, was 2.9756 with a standard deviation of 0.136. The mean LRV of MS2, when challenged with adenovirus type 2, was 2.8336 with a standard deviation of 0.0395. When challenged at the CoMag™ pilot plant, the mean LRV of MS2 was 2.097 with a standard deviation of 0.148. A p value of 0.000 (α=0.05) was obtained, which indicates that there is a statistically significant difference in the removal of MS2, using the bench scale model versus the pilot plant trial. In Figure 22, the upper whisker extends to the maximum data point, for each variable, and the lower whisker extends to the minimum data point. Each colored box contains the middle 50% of the data. The line within each box illustrates the median. The plus sign, within each colored box, represents the mean.

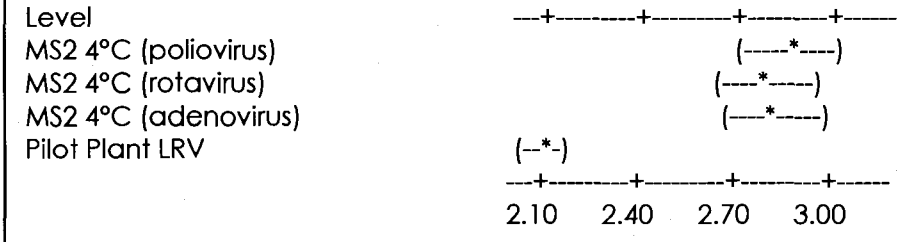
Table 33: One-way ANOVA: MS2 Bench Scale Mean LRV at 4°C and Pilot Plant LRV

Source	DF	SS	MS	F	P
Factor	3	3.8635	1.2878	68.55	0.000
Error	20	0.3758	0.0188		
Total	23	4.2393			

S = 0.1371 R-Sq = 91.14% R-Sq(adj) = 89.81%

Level	N	Mean	StDev
MS2 4°C (poliovirus)	3	2.9885	0.0800
MS2 4°C (rotavirus)	3	2.8872	0.1440
MS2 4°C (adenovirus)	3	2.8944	0.0841
Pilot Plant LRV	15	2.0967	0.1482

Individual 95% CIs For Mean Based on Pooled StDev



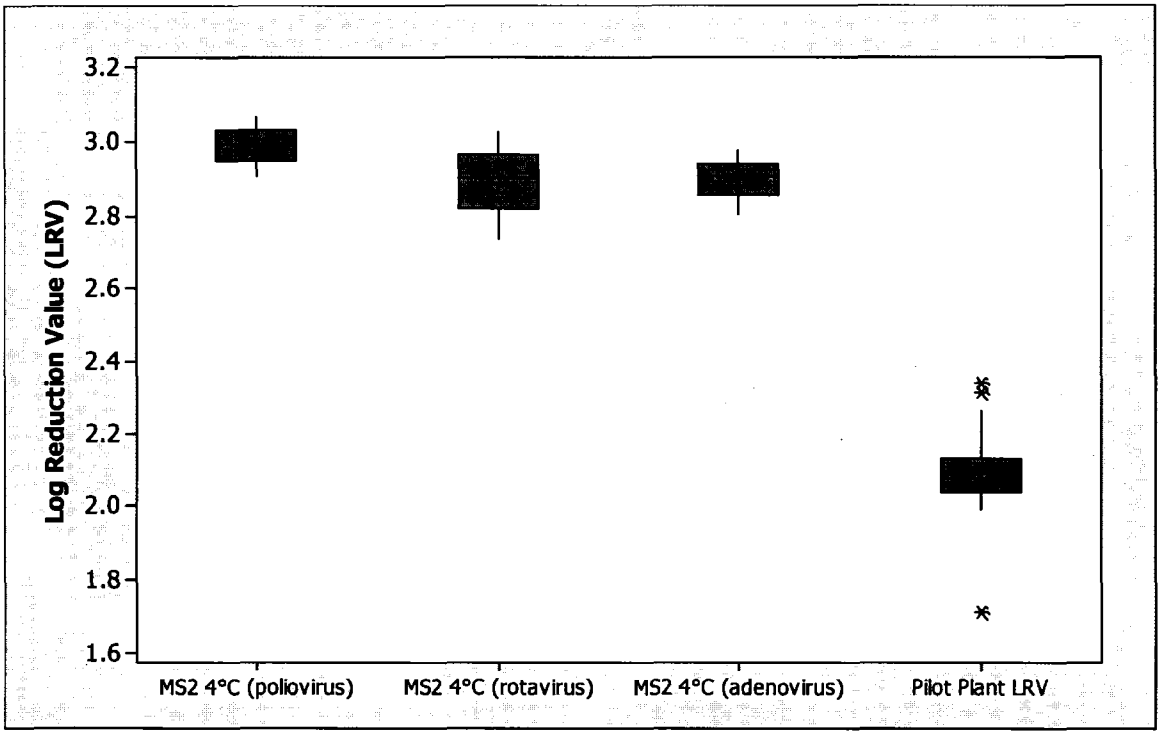
Pooled StDev = 0.1371

Table 34: Descriptive Statistics: MS2 Bench Scale Mean LRV at 4°C and Pilot Plant LRV

Variable	N	Mean	StDev	Minimum	Median	Maximum
MS2 4°C (poliovirus)	3	2.9885	0.0800	2.9055	2.9950	3.0650
MS2 4°C (rotavirus)	3	2.8872	0.1440	2.7362	2.9023	3.0231
MS2 4°C (adenovirus)	3	2.8944	0.0841	2.8053	2.9055	2.9723
Pilot Plant LRV	15	2.0967	0.1482	1.7100	2.0900	2.3400

Data in Tables 33 and 34 represents the calculated LRV of MS2, utilizing data from three separate trials, comparing a bench scale model and a pilot plant model of the CoMag™ process. For each trail, the removal of MS2 was assayed in triplicate. The plaque assays were also conducted in triplicate.

Figure 23: Mean Log Reduction Value of MS2 at 4°C, Using the Bench Scale Model of the CoMag™ Process versus, the Mean Log Reduction of the CoMag™ Pilot Plant



The data represents the average LRV for MS2, at 4°C, using a bench scale model of the CoMag™ process versus the pilot- scale CoMag™ process. MS2 was detected by modified double agar overlay, using *E. coli* HS (pFamp) R as the host. Plaque assays were performed in triplicate and 1000 ml of secondary effluent was spiked with MS2 to a final concentration of 1 x 10^5 PFU/mL. Three trials were completed, each trial containing a sample size of n=3. The mean LRV of MS2, when challenged with poliovirus type 1, was 2.989 with a standard deviation of 0.080. The mean LRV of MS2, when challenged with rotavirus strain Wa, was 2.887 with a standard deviation of 0.144. The mean LRV of MS2, when challenged with adenovirus type 2, was 2.894 with a standard deviation of 0.084. The mean LRV of MS2, when challenged at the CoMag™ pilot plant, was 2.097 with a standard deviation of 0.148. A p value of 0.000 (α=0.05) was obtained, which indicates that there is a statistically significant difference in the removal of MS2 using the bench scale model versus the pilot plant process. In Figure 23, the upper whisker extends to the maximum data point, for each variable, and the lower whisker extends to the minimum data point. Each colored box contains the middle 50% of the data. The line within each box illustrates the median. The plus sign, within each colored box, represents the mean.

CHAPTER IV

DISCUSSION

An increasing number of municipal wastewater treatment facilities in the United States are now required to perform tertiary treatment of wastewater. The move beyond primary and secondary treatment is being implemented to achieve the more stringent parameters enforced by the Environmental Protection Agency for the removal of containments such as nutrients, toxic compounds and suspended solids. One such contaminant is phosphorus which is normally in short supply in freshwater systems, however it is abundant in primary and secondary effluent, because fecal waste contains high levels of phosphorous. If the phosphorus content of the water released by a treatment plant is not limited algal blooms may develop resulting in eutrophication which may lead to deaths of ponds, lakes and rivers.

The supply of phosphorus varies depending on the location of the watershed, human activities and nonpoint sources. The phosphorus loading from nonpoint sources, such as agriculture runoff is minimal in the summer due to a smaller number of rain events. The discharge of treated

wastewater can be the most significant contributor to the phosphorus levels in freshwater during the summer months. To deal with this issue, the Environmental Protection Agency is requiring some wastewater treatment facilities to reduce their phosphorus discharge during the summer months. In 2006, the Concord MA Wastewater Treatment Facility's (WWTF) National Pollutant Discharge Elimination System (NPDES) permit required the phosphorus level of their effluent to be reduced from 0.75 to 0.20 mg/L (EPA, 2006b). With existing alum addition to secondary effluent, the Concord WWTF was only able to reduce the phosphorus level to between 0.6 and 0.7 mg/L. An alternate form of tertiary treatment with a small footprint and low operation and maintenance costs was necessary to achieve the 0.2 mg/L phosphorus goal. The Concord WWTF is currently assessing the feasibility of the CoMag™ process to achieve phosphorus levels required by the new NPDES permit because the process has proved particularly effective at removing phosphorus at several other wastewater treatment facilities (Cambridge Water Technology. 2007).

The CoMag™ process is capable of effectively removing a variety of substances from water via high gradient magnetic separation (HGMS). All particles are classified as ferromagnetic, paramagnetic, or diamagnetic, based on their magnetic susceptibility. The magnetic susceptibility of a material determines its recovery from water or removal

as a waste product. The CoMag™ process separates weakly magnetic and non-magnetic particles facilitated by the addition of particles that possess a high magnetic susceptibility to form aggregates of diamagnetic particles. Viruses are diamagnetic particles, and they may therefore be removed from water using HGMS. A favorable aspect of the CoMag™ process is the potential for pathogen removal, especially viruses. While the original intention of the CoMag™ process was phosphorus removal, this study examined the possible use of the process for the removal of viruses in a bench model and at the pilot plant.

The isoelectric point of magnetite is 7.5 ± 0.5. Therefore, under acidic conditions, the surface of magnetite displays a net positive charge, which attracts negatively charged material. When the pH is elevated to alkaline conditions, the surface of magnetite becomes negatively charged and any previously attached negatively charged material is repelled. Thus, as a result of the inherent magnetic properties of magnetite at an acidic pH, it functions as an adsorbent for negatively charged colloids, organic materials, bacteria and viruses (Anderson et al., 1982). MS2, poliovirus, rotavirus, and adenovirus behave as charged colloids in an ionic environment because of their small size. If the isoelectric point of a virus differs from the isoelectric point of magnetite the pH may be adjusted to facilitate electrostatic binding of viral particles

to magnetite by compelling the magnetite to adopt an opposite charge.

The pH for the bench top scale CoMag™ process was optimized using MS2 as the challenge virus. Several experiments were conducted with the pH ranging from 3.0 to 8.0 and the optimum pH at which the LRV for magnetite was the highest was determined to be pH 6.0 +\- 0.1

CoMag™ Bench Scale

CoMag™ is a magnetically enhanced technique for the removal of contaminants from wastewater. The CoMag™ process begins by passing influent wastewater through a pre-conditioning magnetic matrix, in order to create a locally induced magnetic moment or force, which is sensed by colloidal particles. The electrostatic charge present on each particle that passes through the pre-conditioning filter prevents aggregation of the colloids. Also, the thermal motion of each particle offsets its gravitational potential energy, resulting in random collisions. The target pH range of the pilot plant was broader (5.5 to 6.1) than the target pH range at the bench scale (6.0 +/- 0.1) because of factors, such as heavy metals and salts, which influence the pH of the wastewater flowing through the pilot plant. Because the bench scale model used a single sample of secondary effluent and the volume was constant, the pH was easily manipulated and the pH range

could be narrower.

Bench scale experiments provide valuable information in a controlled environment regarding the inactivation and removal of viruses. There are limitations to bench scale experiments however, because the seeded virus may not truly represent the conditions of those occurring naturally in wastewater. For instance, viruses naturally present in sewage may be encapsulated in cell debris, aggregated, or adhered to solid particles. This, in turn, may prevent the virus from adhering to flocculants or coagulants.

The physical removal of a virus from wastewater is determined by its surface structure and isoelectric point, the pH of the wastewater, and the degree of aggregation. The behavior of flocculants, coagulants, and magnetite toward viral particles depends on the composition, surface chemistry, and isoelectric point of the particles. In addition, the pH and composition of the wastewater are also important factors in the adsorption of viruses.

The ideal matrix for this study would be one without variability and inhibitory substances such as reverse osmosis water. However, since the ultimate goal of this study was to evaluate a wastewater treatment process for the removal of viruses, it was necessary to choose wastewater as the matrix for the bench scale studies. Although the variability of

wastewater cannot be completely eliminated, the differences in virus removal were attributed to variability of viral response to the CoMag™ process as opposed to variability in the content of the wastewater for these particular bench scale studies. The inconsistent characteristics of wastewater, such as biochemical oxygen demand and total suspended solids, were minimized by using one batch of secondary effluent for all bench scale experiments. The minimization of this variability was more important than the limitations resulting from the use of a single grab sample of wastewater for all experiments.

MS2

MS2 coliphage is non-pathogenic to humans and is commonly found in environments where fecal contamination is present. MS2 is frequently used as a model or surrogate to evaluate the removal of enteric viruses because it closely resembles them in size, structure, and survival rate in the environment.

The removal of MS2 from secondary effluent wastewater was evaluated, using a bench scale model of the CoMag™ process, without added magnetite and at room temperature (24°C). The average LRV for the removal of MS2, using the CoMag™ process, with the addition of

magnetite, was 1.1598 logs higher then the removal of MS2 without magnetite. The statistical analysis displayed in Table 1 and Table 2 demonstrate a p value of 0.005, indicating that there is a significant difference in the removal of MS2, when magnetite is added, versus when magnetite is not added. Regarding the data, it can be concluded that the addition of magnetite has an enhanced effect on the removal of MS2.

The robustness of the MS2 removal was also evaluated. This was achieved by determining that the mean LRV did not significantly differ for three trials performed on three different days. As presented in Figure 8, the average LRV for the removal of MS2 was 3.0495 for trial 1, 2.9640 for trial 2, and 2.9525 for trial 3. A one-way unstacked ANOVA resulted in a p value of 0.890, indicating that there is not a significant difference, when comparing the removal of MS2 in each trial. The removal and enumeration of MS2 is therefore considered robust.

To determine if temperature had a significant effect on the removal of MS2 two temperatures were examined: 4°C and 24°C. This temperature range reflects the anticipated conditions at the Concord, MA plant. For each of the experiments, MS2 and one of the three enteric viruses (poliovirus type 1, rotavirus strain Wa and adenovirus type 2) were spiked simultaneously into the wastewater and evaluated. The paired

comparisons were advantageous because they minimized variability, which would have affected the comparison of the viral removals. Statistical analysis resulted in a p value of 0.270, indicating that there is no significant difference in the removal of MS2, when in the presence of poliovirus, rotavirus, or adenovirus at 24°C. These results are shown in Tables 5 and 6. At 4°C, statistical analysis resulted in a p value of 0.476, indicating that there is not a significant difference in the removal of MS2, when in the presence of poliovirus, rotavirus, or adenovirus at 4°C. The results are shown in Tables 7 and 8.

Statistical analyses, as displayed in Figures 9 and 10, illustrate that the removal of MS2 does not vary significantly, at 24° versus 4°C, when in the presence of another virus. The adsorption of MS2 appears to be a physical process which is independent of temperature and presence of poliovirus type 1, rotavirus strain Wa and adenovirus type 2 under the conditions tested for this research.

The removal of MS2 from the secondary effluent samples, using the bench scale process, was significantly higher than the removal of poliovirus type 1, rotavirus strain Wa, and adenovirus type 2. These results are displayed in Figures 15 through 20. This conclusion can be attributed to the differing isoelectric points of the four viruses. The isoelectric points of poliovirus type 1, rotavirus strain Wa and adenovirus type 2 are higher than the isoelectric point

of MS2 which is 3.9 (Dowd et al., 1997). MS2 is more strongly electronegative in the pH range of the bench scale CoMag™ process (pH 6.0 +/- 0.1) therefore its attachment to the positively charged magnetite is higher than the attachment of the other three viruses.

Poliovirus type 1

Poliovirus, the most widely studied of all human pathogenic viruses, was prevalent in water and wastewater. Summarized in Tables 17 and 18, at 24°C, the LRV of MS2 was 0.6239 logs lower than poliovirus type 1. At 4°C, the LRV of MS2 was 0.2555 logs lower than poliovirus type 1. The data are displayed in Tables 19 and 20. The LRVs of poliovirus and MS2, at 24°C and 4°C, were statistically significant. The LRV of poliovirus was significantly higher, at both temperatures, when compared to the LRV of MS2. This can possibly be attributed to the protein coat of poliovirus, which consists of 16 proteins, of which 25% alter their charge at a pH less then 7.0. Specifically, cysteine becomes negatively charged ($SH \rightarrow S^-$), histadine becomes neutral ($N^+ \rightarrow N$), and arginine and lysine become less positive ($NH_3^+ \rightarrow NH_2$). Previous studies indicate that transformation of charge is imperative in the depletion of repulsive electrostatic forces, which may increase aggregation (Pearson et al., 1964). Since the

removal of poliovirus type 1 is higher than MS2 at both temperatures, MS2 is a good indicator of the removal of poliovirus using the CoMag ™ process at the bench.

When the removals of poliovirus type 1 at 24° and 4°C were statistically analyzed, the p value was 0.026. This low p value (<0.05) indicated that there was a statically significant difference in the removal of poliovirus, at the two different temperatures. The reduction in the LRV, at 4°C, as compared to 24°C, may be attributed to a decreased rate of Brownian motion, which is directly proportional to temperature.

Rotavirus strain Wa

At 24°C, the LRV of rotavirus strain Wa was 0.5726 logs higher than the removal of MS2. At 4°C, the LRV of rotavirus strain Wa was 0.6272 logs higher then the removal of MS2. Statistical analysis indicates that there is a significant difference in the LRV of MS2, at 24°C and 4°C, when compared to the LRV of rotavirus. Because rotavirus strain Wa is removed at a greater level than MS2 at the bench, MS2 would be an adequate surrogate for the removal of rotavirus strain Wa using the bench scale model of the CoMag™ process.

There was no statistically significant difference in the LRV of rotavirus

at the two different temperatures, therefore the adsorption and removal of rotavirus is probably not dependant on temperature. Wastewater contains many organic compounds and interaction of the particles within a wastewater matrix is vastly complex. High concentrations of flocculants and coagulants are added to wastewater during the CoMag™ process to bind the organic compounds which compete with viruses for attachment to magnetite. The flocculants and coagulants also increase the positive charge on magnetite and viral adsorption is enhanced. The isoelectric point of rotavirus strain Wa is lower than the isoelectric point of poliovirus type 1. Therefore rotavirus strain Wa is more strongly electronegative in the pH range of the bench scale CoMag™ process than poliovirus. The attachment of rotavirus strain Wa to the positively charged magnetite by attractive van der Waals forces may be stronger than the attachment of poliovirus type 1 making the temperature of the matrix insignificant. The data are summarized in Tables 21 through 24.

Adenovirus type 2

Adenoviruses are of medium size (90-100 nm), and are the largest non-enveloped virus. The virion contains a penton fiber, or spike, that aids in attachment to the host cell. At 24°C, the LRV of MS2 was 0.6597 logs

lower then the removal of adenovirus type 2. At 4°C, the LRV of MS2 was 0.5756 logs lower then adenovirus type 2. Statistical analysis indicates that there is a significant difference in the LRV of MS2 when compared to the LRV of adenovirus, at 24°C and 4°C. Adenovirus type 2 is removed at a greater level then MS2 at the bench under the test conditions employed for this research. Therefore, MS2 would likely be a sufficient surrogate for the removal of adenovirus type 2 using the bench scale model of the CoMag™ process. Statistical analysis also demonstrated that there is no difference in the removal of adenovirus type 2 at 24°C and 4°C. The adsorption and removal of adenovirus type 2 does not appear to be temperature dependent. Poliovirus type 1 has an isoelectric point that is much higher than adenovirus type 2. This may result in a stronger electronegative force than poliovirus type 1 in the pH range of the bench scale CoMag™ process. Like rotavirus strain Wa, the attachment of adenovirus type 2 to the positively charged magnetite by attractive van der Waals forces may be stronger than the attachment of poliovirus type 1 making the temperature of the matrix insignificant. The data are summarized in Tables 25 through 28.

CoMag™ Pilot Plant

Human enteric viruses cannot be added to wastewater that is released into the environment because of the high expense of viral propagation and their pathogenicity to humans. The titers achieved from the propagation of most enteric viruses are too low for practical use in a 100 gpm pilot plant. Therefore, a virus which is inexpensive to propagate and is non-pathogenic to humans was used as a model or surrogate. In previous viral removal studies, MS2 has been utilized as a surrogate for poliovirus, rotavirus and adenovirus. MS2 has similar morphological features, susceptibility to disinfection and survivability in the environment as compared to some enteric viruses.

The average LRV for the removal of MS2 at 24°C, using the bench scale model, was 0.8163 logs higher than the pilot plant. Statistical analysis demonstrated a significant difference exists in the removal of MS2, using the bench scale model versus the pilot plant. The data is summarized in Tables 31 and 32. The removal of MS2 using the bench scale model is not an adequate representation of the removal of MS2 at the pilot plant because the bench scale model yielded a significantly higher LRV.

The data for the CoMag™ pilot plant resulted in no statistically

significant difference in the removal of MS2, between trails. This result suggests that the removal of MS2 at the pilot plant for the time period tested is robust because the variability in the pilot plant stream does not have a significant effect on the removal of MS2.

The significant difference between the LRVs of MS2 at the CoMag™ pilot plant scale verses the bench scale model can probably be attributed to the different target pH ranges for each scale. The target pH for the pilot scale CoMag™ process was 5.5 to 6.1 and the target pH for the bench scale was narrower at 6.0 +/- 0.1. Since the isoelectric point of MS2 is 3.9, its viral surface is more electopositively charged at a pH of 6.0, which was the targeted pH for the bench scale model. Therefore a higher LRV would be expected when testing at the bench.

Another factor which may have contributed to the difference of the LRVs determined for the bench scale model verses the pilot plant process is the sampling method. At the bench, a single sample was pulled from each beaker and immediately assayed. Alternatively, sampling for the pilot scale plant was performed continuously. More specifically, a sample was pulled every 10 minutes over a period of three hours and assayed after a minimum hold time of 12 hours. The CoMag™ process removes particles, including viruses, through adsorption and aggregation. Since the adsorption of viruses to magnetite is reversible, extended transport and holding times for

the samples could result in separation of the virus particles. A consequence of this would be an increased viral titer resulting in a lower LRV.

Although evidence supports that MS2 may be a good indicator of viral removal, these data suggest that it does not serve as an adequate surrogate for poliovirus type 1, rotavirus strain Wa and adenovirus type 2 when using the CoMag™ process. The removal differences could be possibly due to inherent variability in sewage samples. Several factors potentially influenced the reduced LRV of MS2 at the pilot plant including the physiological state of the microorganism, the degree of aggregation, and the wastewater quality.

More specifically, the reduced removal of MS2 at the pilot plant may have been due to inadequate mixing throughout the pilot plant process. In the bench scale model, the total working volume of secondary effluent was relatively small, 1000 mL, and mixing was highly controlled. At the pilot plant, the tanks had the capacity to mix thousands of gallons of water. The presence of dead zones in the tank, areas where MS2 did not come into contact with the added chemicals, may have contributed to the reduced LRV. Furthermore, the spiked MS2 may have become encapsulated in cell debris, aggregated, or adhered to solid particles, which would have rendered it incapable of adhering to

the added magnetite, flocculent or coagulant. The physical properties such as total suspended solids and turbidity were not assessed in the secondary effluent used for the bench scale or pilot plant studies. Therefore, the solids content may have contributed to the variation in the removal of MS2. This is supported by the fact that the removal of MS2 differed at the bench scale verses the pilot scale process.

In addition, some of the difference in the LRVs of the viruses may be attributed to the enumeration assays, which were specific for each virus. MS2 utilizes a bacterial host while poliovirus types 1, rotavirus strain Wa and adenovirus type 2 require a continuous mammalian cell line. Continuous cell lines are more fastidious than bacteria and their growth media is complex and differs for each cell line used for these experiments. They are also more susceptible to cations, which are present in the CoMag™ processes. Cations may competitively adhere to the mammalian cells used to enumerate each virus. This would decrease the amount of virus able to infect the cell monolayer resulting in an artificially low viral titer. Viral aggregation may also result in an erroneously low viral titer.

Future Experiments

Several municipal and industrial wastewater treatment facilities are

advancing from utilizing exclusively primary and biological treatment, to tertiary treatment, to achieve higher contaminant removal levels now required by more recent environmental regulations (USEPA, 2006)(USEPA, 2007). The CoMag™ process may be an effective tertiary treatment process, acting as a method to achieve the more stringent wastewater discharge permits. Past research indicates that the CoMag™ process is capable of reducing phosphorus levels to 0.01 mg/L, arsenic by 93%, total suspended solids by 98.6%, and biochemical oxygen demand by 63%, in secondary effluent (Cambridge Water Technology, 2007). Due to the ability of the CoMag™ process to achieve high levels of particulate removal and a > 2 LRV of MS2, as determined from the research presented here, it has the potential to greatly improve wastewater and drinking water treatment. Use of the CoMag™ process is beneficial because it is inexpensive, time efficient, and has a compact footprint. Additionally, less waste product is produced because the bound magnetite creates a compact sludge and the magnetite that is not bound is recycled reducing the high cost of sludge disposal.

Despite these advantages, testing the CoMag™ process in the field would be challenging for several reasons. Firstly, the large volume of water commonly treated in a wastewater plant would make it difficult to collect a homogenous sample. Secondly, viruses that are pathogenic to humans

can not be seeded in a functioning wastewater plant because of the risk of infecting the public when discharged into the environment. Furthermore, many environmental factors could not be controlled for such as heavy metals, water flow (GPM), and temperature.

In both the bench scale and pilot plant studies several variables were controlled for such as the volume of flocculant, coagulant, and magnetite, the pH and the settling time in order to minimize variation due to environmental factors. In addition, all bench scale experiments were conducted with a single sample of secondary effluent wastewater. To assess the impact of these variables on the resultant LRVs, future research should include bench scale studies conducted with wastewater samples collected from several different days and composite samples (where a sample is gathered every hour for 24 hours and combined). Furthermore, samples of dilute wastewater, such as when rain events occur, or concentrated wastewater, as during drought events, should also be assessed.

Infectious enteric viruses are isolated from raw sewage and wastewater effluent in greater numbers during the winter months (Fong and Lipp, 2005). Furthermore, the efficiency of wastewater treatment plants decreases during the winter months (Olsen et al., 2005). This creates an increased potential for the release of enteric viruses into the

environment. Further research of the CoMag™ process may include evaluating seasonal effects on MS2 removal. This may provide an indication that the CoMag™ process could successfully reduce the quantity of enteric viruses released into the environment during the winter months.

A further application of the CoMag™ process could include use as pretreatment for disinfection methods such as chlorination and ozonation, which could possibly reduce disinfectant usage and the subsequent production DBPs.

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APPENDICES

APPENDIX A

BUFFERS AND REAGENTS

1% Magnesium Chloride Solution
Dissolve 1 g of $MgCl_2 \cdot 6H_20$ (Fisher) into 99 mLs of distilled water.

100x Streptomycin Sulfate/ Ampicillin Antibiotic Solution
Add 0.15 g of both antibiotics (Sigma) to 100 mLs of distilled water.

1x Phosphate Buffered Saline Solution (PBS)
Dissolve 9.785 g of phosphate buffered saline powder (Sigma) into 1 L of distilled water. pH to 7.0 and autoclave at 121°C, 15 psi for 15 minutes. Store at room temperature.

0.1% Crystal Violet Stain
Dissolve 0.1 g Crystal violet into 99 mLs of distilled water.

5M Hydrochloric Acid
Add 56.9 mLs distilled water into a volumetric flask and bring up to 100 mLs with HCL. Store at room temperature.

2M Sodium Hydroxide Solution
Dissolve 80 g NaOH pellets (Fisher) with 1 L of distilled water in a plastic container. Store at room temperature.

10% Formalin Fixative in Normal Saline
Dissolve 8.5 g NaCl (Sigma) into 900 mLs distilled water, add 100 mLs of a 37% Fomaldehyde solution. Store at room temperature.

Trypsin Solution

Add 0.1g of trypsin (Gibco) to 10 mL of distilled water. Filter sterilize through a 0.22 um syringe filter and refrigerate until use.

APPENDIX B

MEDIA

1X Agar Overlay for MS2 Plaque Assay

Dissolve 15 g powdered T-soy broth, 2.5 g NaCl, 5 g Yeast extract, 0.075 g $CaCl_2 \cdot 2H_2O$ and 7.5 g Bacto agar in 1 L distilled water. Boil to dissolve while mixing then dispense 5 mL portions into test tubes using a cornwall syringe. Cap and autoclave test tubes at 121°C, 15 psi for 15 minutes. Store at room temperature, and melt when needed by autoclaving at 121°C, 15 psi for 5 minutes.

Cell Culture Media for the Propagation of BGMK, MA-104 and Caco-2 Cells

4.7 g Eagles MEM (Sigma)
7.4 g Leibowitz L-15 (Sigma)
4.24 g HEPES (Fisher)
0.292 g L-glutamine (Sigma)
0.75 g Sodium bicarbonate (Sigma)
10 mL Nonessential amino acids (Gibco)

Dissolve components in 1 L distilled water, pH solution to 7.2-7.4, filter sterilize, refrigerate until use.

10% MEM for MA-104 and Caco-2 Cells

5 mL Antibiotic/Antimycotic (Gibco)
45 mL Fetal Bovine Serum (JRH Biosciences)
450 mL of prepared cell culture media

5% MEM for BGMK Cells

5 mL Antibiotic/Antimycotic (Gibco)
22.5 mLs Fetal Bovine Serum (JRH Biosciences)
450 mL of prepared cell culture media

2% MEM Maintence Media for BGMK, MA-104 and Caco-2 Cells
5 mL Antibiotic/Antimycotic (Gibco)
9 mL Fetal Bovine Serum (JRH Biosciences)
450 mL of prepared cell culture media

2X MEM
4.7 g Eagles MEM (Sigma)
7.4 g Leibowitz L-15 (Sigma)
4.24 g HEPES (Fisher)
0.292 g L-glutamine (Sigma)
0.75 g Sodium bicarbonate (Sigma)
10 mL Nonessential amino acids (Gibco)
5 mL Antibiotic/Antimycotic (Gibco)
1 mL Kanamycin (Gibco)

Dissolve components in 500 mL distilled water, pH solution to 7.2-7.4, filter sterilize, refrigerate until use.

Medium 199
2.437 g M199 (Sigma)
0.075 g L-glutamine
1.325 g Hepes
0.175 g Sodium Bicarbonate
0.025 g Magnesium Chloride
Dissolve components in 115 mL distilled water, filter sterilize 0.22 □m, refrigerate until use.

M199 Overlay
79 mL M199 (Sigma)
3 mL Neutral Red (Sigma)
3 mL Antibiotic/Antimycotic (Gibco)

Place in 37°C water bath.

Tryptic Soy Broth for E. coli HS (pFamp)R Propagation
Dissolve 30 g powdered T-soy broth into 1 L distilled water, stir and heat until dissolved. Add 5 mL/1 L of 1% $MgCl_2 \cdot 6H_2O$ solution, and autoclave at 121°C, 15 psi for 15 minutes. After cooling add 10 mL/1 L of 100x Streptomycin/Ampicillin solution. Refrigerate till use at 4°C.