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Virus Fate and Transport in Groundwater

Organic matter, uncertainty, and cold climate

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Abstract

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Water managers must balance the need for clean and safe drinking water with ever-increasing amounts of waste-water. A technique for treating and storing surface water called “managed aquifer recharge” (MAR) is frequently used to help maintain this balance. When MAR is used to produce drinking water, water managers must ensure that disease-causing microbial contaminants are removed from the water prior to its distribution. This thesis examined the processes responsible for removing a specific class of microbial contaminants called “enteric viruses” during MAR. Viruses are naturally removed in groundwater through adsorption and inactivation mechanisms. This thesis investigated how these virus removal mechanisms were affected by ionic strength (IS), dissolved organic carbon (DOC), and the age of the sand used in a MAR infiltration basin. This was done using batch and flow-through column experiments designed to mimic conditions characteristic of a basin infiltration MAR scheme in Uppsala, Sweden. Bacteriophage MS2 was used as a proxy for enteric viruses. All of the experiments were conducted at 4°C. Experimental data were modeled to describe the fate and transport of viruses in the infiltrated groundwater. Conventional least-squares optimization and generalized likelihood uncertainty estimation (GLUE) were compared as model fitting-approaches in order to determine how data uncertainty affects parameter estimates and model predictions. Results showed that the sand used in the infiltration basins accumulates adsorbed organic matter as it is exposed to infiltrating surface waters. This reduced the amount of MS2 that was removed due to adsorption and inactivation. Results from GLUE indicated that MS2 is more likely to inactivate in a time-dependent manner when in the presence of sand with high concentrations of organic matter. Both model fitting techniques indicated that virus attachment rates were significantly lower for sand with high organic carbon content. Neither methodology was capable of adequately capturing the kinetics of virus adsorption. Uncertainties in the experimental data had a large effect on the conclusions that could be drawn from fitted models. This study showed that the presence of natural organic matter reduces the value of the infiltration basin as a microbial barrier.

Keywords: managed aquifer recharge, organic matter, virus, numerical modeling, uncertainty

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List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I **Mayotte**, **J.**, Bishop, K. (2016) Chemical and environmental factors affecting adsorption and inactivation mechanisms of virus in groundwater. A literature review. *Manuscript*

II **Mayotte**, **J.**, Hölting, L., Bishop, K. (2016) Reduced removal of an enteric virus during managed aquifer recharge due to organic coatings on infiltration basin sand. *Submitted*

III **Mayotte**, **J.**, Grabs, T., Sutliff-Johansson, S., Bishop, K. (2016) The effects of sand and organic matter on virus inactivation at low temperatures. Comparing models of constant and time-dependent inactivation while considering the uncertainty of measured virus concentrations. *In Review*

IV **Mayotte**, **J.**, Fagerlund, F., Bishop, K. (2016) Fate and transport of virus in infiltration basins and the importance of data uncertainty in modeling. *Manuscript*

Co-authorship

The author was responsible for the designing of all experimental work, carried out the entirety of the column experiments, provided substantial help in both the static and agitated batch experiments, performed the analysis of all experimental results, and wrote all of the papers.

Paper I
Kevin Bishop helped in the writing and editing of the paper.

Paper II
Lisanne Hölting carried out the majority of the agitated batch experiments and provided some help in the writing of the paper. Keven Bishop helped in framing the issues and the writing of the paper.

Paper III
Stacy Sutliff-Johansson carried out the majority of the static batch experiments (some of the data was used in Paper II) and provided some help in the writing of the paper. Thomas Grabs helped with the GLUE analysis of the experimental results and aided in the writing of the paper. Kevin Bishop helped in designing the experiment and the writing of the paper.

Paper IV
Fritjof Fagerlund helped in the writing of the paper. Kevin Bishop helped in framing of the issues and the writing of the paper.

Contents

1. Introduction

> "*Letting the days go by. Let the water hold me down. Letting the days go by. Water flowing underground. Into the blue again. After the money's gone. Once in a lifetime. Water flowing underground.*"
>
> (Byrne et al., 1981)

Drinking-water quality is important. Access to clean drinking water is a vital factor for development of modern societies as it decreases the disease burden and improves quality of life (Tchobanoglous, 1985). In 1990, 98% of countries in developed regions of the world had access to improved drinking water compared to only 70% in developing regions (WHO/UNICEF, 2015). In order to improve the livelihoods of those in developing regions, the United Nations addressed this problem by asking that 88% of the World's population should have access to improved drinking water sources by 2010. This goal was met and as of 2015, 91% of the World's population had access to improved drinking water sources. This equates to roughly 2.6 billion people gaining access to safer drinking water between 1990 and 2015 with access in developing regions increasing from 70% to 89% (WHO/UNICEF, 2015). While these numbers are very encouraging, an estimated 1.4 million children die every year from diarrheal illnesses related to waterborne pathogens (Prüss-Üstün et al., 2008).

Poor water quality is not a problem reserved only for developing countries. While the vast majority of the waterborne disease burden remains in developing regions of the world, waterborne disease outbreaks in developed countries are a relatively common occurrence. Since the beginning of the millennium, waterborne outbreaks of gastroenteritis caused by microbial pathogens have occurred in Finland (Laine et al., 2011), Holland (Hoebe et al., 2004), Italy (Boccia et al., 2002; Rizzo et al., 2007; Scarcella et al., 2009), New Zealand (Hewitt et al., 2007), Norway (Kvitsand and Fiksdal, 2010), Sweden (Carrique-Mas et al., 2003; Nygård et al., 2003; Riera-Montes et al., 2011; Sartorius et al., 2007), Switzerland (Häfliger et al., 2000), and the USA (O'Reilly et al., 2007).

High income societies tend to take drinking-water for granted. Municipal fresh-water use in Sweden and the USA averaged 285 and 529 l day^{-1} per person respectively in 2010 (FAO, 2016). Almost all of the water used was treated drinking-water. The global average cost of producing drinking water is \$0.15–\$0.45 m^{-3} (Perks and Kealey, 2006). Using these numbers to estimate the annual cost of water, an average citizen of Sweden or the USA would be expected to pay a maximum of \$47 and \$87 respectively for drinking water each year. This amounts to less than 0.2% of the annual per capita GDP for both countries (OECD, 2015). This suggests that there is very little economic incentive to conserve drinking water. Furthermore, given the abundant use of treated drinking water, developed countries should expect that substantial volumes of wastewater will be produced on a daily basis that requires treatment. By the year 2025, wastewater flows in the USA are expected to exceed 160 million m^3 day^{-1} (USEPA, 2000). In developed countries, water managers must balance the need for clean and safe drinking water with the ever-increasing amount of waste-water and a technique for treating and storing fresh water called "managed aquifer recharge" (MAR) may be an important part of the solution.

Managed aquifer recharge, or artificial recharge, is a blanket term which describes the augmentation of groundwater resources through human intervention. MAR usually refers to the process wherein surface water is diverted into the ground in an accelerated manner, either through direct injection or surface infiltration. MAR can serve a variety of purposes including reduction of land subsistence, staving seawater intrusion into freshwater aquifers, and as a means of conveying water between points (Bouwer, 2002). MAR has become an important part of the water resource portfolios of many European countries and is increasingly seen as a viable alternative to surface water storage as groundwater is less vulnerable to contamination and evaporation losses (European Commission, 2001). However, due to the natural cleaning properties with which the soil provides, MAR is being increasingly used as a method of surface water and wastewater treatment (Dillon, 2005).

There are several different methods through which MAR can be carried out. However, this thesis is primarily concerned with a technique called basin infiltration MAR (also referred to as pond infiltration MAR). Basin infiltration MAR is the most widely used technique for MAR in Europe (European Commission, 2001) and Sweden (Svenskt Vatten AB, 2000). In basin infiltration MAR surface water is pooled above a permeable soil. This allows the water to percolate down to the water table under the force of gravity alone. Basin infiltration methods can handle large volumes of water, are relatively easy to maintain, and can produce a high quality of water (Kimrey, 1989). However, basin infiltration requires a large land footprint, is susceptible to evaporation losses, and can be easily contaminated due to it being open to the atmosphere (Frycklund, 1998; Kimrey, 1989).

When MAR is used as a means for drinking water treatment, it is of primary importance that harmful constituents, such as microbial pathogens, are removed to safe levels. While water managers are concerned with removing all microbial pathogens, viruses are of primary concern as they are highly infectious (Haas, 1983) and are capable of surviving for long periods of time and traveling long distances in groundwater (Keswick and Gerba, 1980). Water managers are especially concerned with a specific class of virus called enteric viruses as they are responsible for almost all instances of waterborne disease outbreaks (Prüss-Üstün et al., 2008) and are removed at a slower rate than for other human viruses in groundwater (Pang, 2008). This thesis examined the mechanisms in MAR responsible for removing enteric viruses through a combination of bench-scale experiments and numerical modeling.

1.1 Enteric viruses

Enteric viruses primarily infect host cells in the intestinal tract causing gastroenteritis: an inflammation of the stomach or intestine which can lead to infectious diarrhea, fever, nausea, vomiting, stomach pain, dehydration, and weight loss (Morgan et al., 2015). It is more commonly known as the stomach flu and is the second leading cause of death globally after cardiovascular diseases (Morgan et al., 2015). Enteric viruses find their way into water sources as a result of human fecal contamination. The most common sources of fecal contamination of groundwater in high income societies are septic tanks and sewage discharges downstream of sewage treatment plants (Charles et al., 2003). Enhanced fecal contamination of water resources has also been observed to follow rainfall events (Curriero et al., 2001; Kistemann et al., 2002; Nicosia et al., 2001; Shadford et al., 1997). There are many types of enteric viruses but Norovirus and Norwalk-like viruses are the most common cause of gastroenteritis globally (Siebenga et al., 2009). Norovirus and Norwalk-like viruses were responsible for most of the waterborne viral outbreaks in Finland (Maunula et al., 2005), Norway (Kvitsand and Fiksdal, 2010), Sweden (SMI, 2012), and the United States (Hoffmann et al., 2012). Given the threat to drinking water posed by enteric viruses, one of the most important criterions for the design of drinking water treatment systems, including MAR, is the removal of viruses.

1.2 Virus removal during MAR

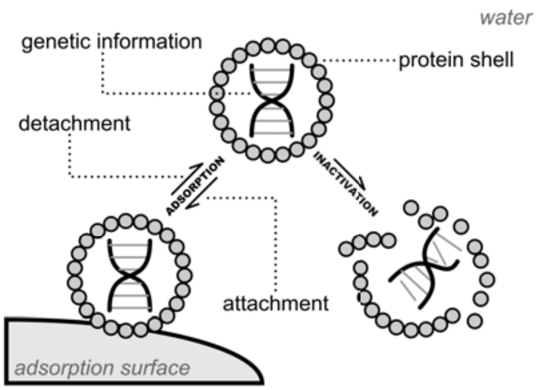

Figure 1. Illustration of virus adsorption and inactivation mechanisms

A virus is considered to be removed from an artificially infiltrated groundwater when it is no longer able to infect a potential host. During groundwater transport, there are two primary mechanisms through which a virus may be removed: adsorption and inactivation (Azadpour-Keeley et al., 2003). Removal through adsorption describes the process wherein a virus becomes stationary after attaching to the surface of the porous material through which the groundwater is flowing. Removal through inactivation describes the process wherein a virus is rendered harmless to its host organism as a result of a chemical change to the molecular makeup of the virus. While other mechanisms do exist by which a virus can be removed from infiltrating water, such as through microbial predation, most viruses are removed through adsorption and inactivation mechanisms. Virus adsorption is considered as the dominant removal process (Schijven and Hassanizadeh, 2000).

1.2.1 Adsorption

Adsorption describes the mechanism by which viruses attach to a surface. When in solution, viruses are most commonly thought to behave as nano- to microscopic (10^{-9}–10^{-6} m) insoluble particles called “colloids”. The most widely used theory describing colloid stability in solution, or its tendency to stay in solution rather than attach to a surface, is called the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory (Verwey and Overbeek, 1948). DLVO theory describes colloid, or virus, stability as the balance between attractive van der Waals forces and repulsive electrostatic forces. DLVO theory has been widely used to describe virus adsorption to soil surfaces (Chrysikopoulos and Syngouna, 2012; Loveland et al., 1996; Ryan et al., 1999; Sadeghi et al., 2011). The strength of the attractive forces between a virus and the adsorption surface are inversely proportional to the sixth-power of the distance between the virus and the adsorption surface. This means that the attractive forces are influential over very short distances (< 10^{-8} m) (Ryan and Elimelech, 1996). The repulsive electrostatic forces are explained by the double-layer theory (Verwey and Overbeek, 1948) which describes the distribution of charged ions surrounding a virus in solution and the adsorption surface.

DLVO theory proposes that changes in the pH and the ionic strength (IS) of a solution will directly affect the extent of virus adsorption. Several studies have shown that more virus is adsorbed as pH decreases (Burge and Enkiri, 1978; Goyal and Gerba, 1979; Grant et al., 1993; Sadeghi et al., 2011; Sobsey et al., 1980) and as IS increases (Cao et al., 2010; Grant et al., 1993; Lipson and Stotzky, 1983; Penrod et al., 1996; Redman et al., 1999; Sadeghi et al., 2011; Walshe et al., 2010). However, the effect of pH on adsorption has been found to be highly virus specific (Gerba, 1984) and the effect of IS is dependent on the presence of metal oxides on the adsorption surface (Chu et al., 2000; Zhuang and Jin, 2003a). In spite of this, it is generally considered that solutions with a low pH (< pH 7) and a high IS provide conditions which are favorable for adsorption. DLVO theory helps to explain many of the adsorptive mechanisms under conditions deemed favorable for virus adsorption (Schijven and Hassanizadeh, 2000). However, it is a poor predictor of virus adsorption under conditions deemed unfavorable for adsorption and has been shown to provide unrealistic predictions of virus adsorption in field applications (Treumann et al., 2014).

Conditions for adsorption can also be favorable if the virus and adsorption surface are oppositely charged in solution. Many soils contain metal oxides which are positively charged at a neutral pH (Kosmulski, 2011) which can result in enhanced adsorption of colloids and virus even under conditions generally considered unfavorable for adsorption (Johnson et al., 1996). Viruses have been shown to readily adsorb to sand containing iron oxides (Moore et al., 1981; You et al., 2005). However, even under favorable conditions for adsorption (low pH, high IS) in the presence of metal oxides, the adsorption of viruses has been shown to decrease in the presence of natural organic matter (NOM) (Fuhs et al., 1985; Ryan et al., 1999; Wong et al., 2013; Zhuang and Jin, 2003a). This is most likely due to adsorption sites being covered by NOM, making them no longer available for virus adsorption (Gerba, 1984).

Adsorption of virus in groundwater is thought to be kinetically limited (Schijven and Hassanizadeh, 2000) where the overall rate of virus attachment is governed by mass transport of the virus to the solid surface and the physical and/or chemical virus—surface interactions (Grant et al., 1993). Kinetic adsorption assumes that the rate at which a virus adsorbs to a surface depends on the relative rates at which viruses “attach” to and “detach” from the adsorption surface (Figure 1). Detachment of virus can be enhanced by changes in solution chemistry, most notably an increase in pH (Loveland et al., 1996), and virus transport models must consider virus detachment in order to provide accurate predictions of virus concentrations in groundwater (Loveland et al., 1996; Schijven and Hassanizadeh, 2000).

1.2.2 Inactivation

Viruses are not living things, but mobile packets of genetic information (DNA or RNA) protected by a coat of proteins that rely on specific host cells in order to multiply (Gelderblom, 1996). Therefore, viruses do not die. Rather, the functional elements of the virus can chemically degrade to the point where it is no longer able to deliver its DNA or RNA to a host cell (Figure 1). This process is called inactivation.

There are several environmental factors that have been shown to affect the rate at which viruses are inactivated. The consensus within the literature is that temperature has the largest effect on virus inactivation with higher temperatures being associated with higher rates of inactivation (Azadpour-Keeley et al., 2003; Gerba, 1984; Jin and Flury, 2002; John and Rose, 2005; Schijven and Hassanizadeh, 2000). At low temperatures, virus inactivation has been shown to slow significantly. Studies have shown that viruses will persist for several months in groundwater samples held at 4–5°C (Ogorzaly et al., 2010; Olson et al., 2004; Rzezutka and Cook, 2004). The same level of inactivation can be achieved in a matter of hours to a few days at temperatures greater than 20°C (Schijven and Hassanizadeh, 2000).

Organic matter has been shown to affect virus inactivation. In general, the presence of organic matter is thought to slow inactivation (Bradford et al., 2006; Foppen et al., 2006; Moore et al., 1982; Ryan et al., 2002). However, the mechanisms through which it can affect the inactivation rate are not well understood (Schijven and Hassanizadeh, 2000). One hypothesis is that organic matter can aid in forming virus complexes which can protect virus from degradation (Bixby and O'Brien, 1979). However, Chattopadhyay et al. (2002) found that the presence of three different dissolved humic acids (a major component of NOM) increased inactivation rates of the viruses used in their study.

Virus adsorption has also been found to effect virus inactivation but there is considerable disagreement in the literature regarding to what extent (Bradford et al., 2013). Schijven and Hassanizadeh (2000) compiled the results of several studies investigating the effects of adsorption on virus inactivation and found that the more irreversibly a virus is attached to a solid surface the longer it will survive. Contrary to these findings, inactivation has been shown to be enhanced when viruses are irreversibly adsorbed to metal oxide minerals (Gerba, 1984; Kim et al., 2011; Ryan et al., 2002). However, enhanced inactivation of virus by metal oxides can be reduced, or even nullified, in the presence of organic matter as a result of organic coatings forming over the metal oxides (Foppen and Schijven, 2006).

1.3 Design of MAR for virus removal

Water managers rarely design MAR schemes by considering individual virus removal mechanisms. This is understandable as modeling approaches that account for individual mechanisms in order to predict virus concentrations have been shown to be unreliable when applied to field conditions (Tufenkji, 2007). Instead, water managers use experimentally defined virus removal rates to design the setback distances and residence time between the infiltration and extraction points. The European Commission (2001) recommends a residence time of 50–60 days in order to achieve adequate levels of virus removal. This recommendation is based on several field studies which have monitored different microbial tracers.

However, recent studies have shown that residence times of this length may be too short, especially during the winter months when cold temperatures slow virus inactivation mechanisms (Schijven et al., 2006; Van der Wielen et al., 2008). Part of this may be due to the fact that virus removal rates have been observed to decrease with distance (Pang, 2008; Schijven and Hassanizadeh, 2000) especially when under the influence of continuous infiltration (Pang, 2008). Pang (2008) compiled the results of dozens of investigations on virus removal in laboratory as well as field conditions and summarized the results of each as removal rates. The study concluded that while removal rates can be useful in the designing of setback distances, variations in environmental conditions for a specific location can result in inaccurate predictions of virus concentrations in the field when they are used.

1.4 Aims of this thesis

The ultimate aim of this thesis is to quantify the contribution of adsorption and inactivation mechanisms to the removal of viruses under conditions characteristic of an MAR scheme operating as a means for drinking water production. Bench-scale experiments were conducted in order to examine the effects of different environmental variables on virus removal. Mathematical models were then fit to the experimental data in order to quantify the adsorption and inactivation mechanisms while considering uncertainties in the measured data. The Tunåsen basin infiltration MAR scheme in Uppsala, Sweden was used as a case study, and all of the experiments in this thesis were designed according to the environmental and operational conditions characteristic of this MAR scheme.

The experiments were designed in order to investigate the fate and transport of viruses during winter periods since viruses have been shown to remain for long periods of time at cold temperatures (Ogorzaly et al., 2010; Olson et al., 2004; Rzezutka and Cook, 2004). Therefore, experiments were carried out at 4°C. The sand and water used in the experiments was sampled during the winter months. A subset of the experiments was designed in order to examine virus removal over a long period of time (two months).

Ionic strength has been shown to effect virus adsorption; a higher IS usually corresponds with conditions that are more favorable for adsorption according to DLVO theory. Therefore, experiments were conducted at two different levels of IS characteristic of the variation observed in the surface water used for infiltration during the winter months at Tunåsen.

The presence of NOM has been shown to reduce virus removal by affecting both inactivation and adsorption mechanisms; higher concentrations of NOM in the infiltration water are usually correlated with reduced removal of virus. In many parts of the world, NOM concentrations in surface water have been increasing since the 1980s (Monteith et al., 2007). This trend has also been observed for the water in used for infiltration at Tunåsen (Sveriges lantbruksuniversitet, 2015). Therefore, experiments were conducted at two different levels of DOC which were characteristic of the variation in the water used for infiltration observed during the winter months in order to determine how this may affect virus removal at Tunåsen.

The presence of organic coatings on porous material, due to prolonged exposure to infiltrating surface water, has been shown to reduce adsorption of virus (Fuhs et al., 1985). Therefore, experiments were conducted with two types of sand: “new” sand which had yet to be exposed to infiltration water, and “used” sand which had been intermittently exposed to infiltration water at Tunåsen over a period of eight years.

The studies in this thesis are in the form of four unique articles. The articles were designed to progress from an assessment and summary of the literature at the time of this thesis’s start to a qualitative and quantitative analysis of the of adsorption and inactivation mechanisms responsible for virus removal within the Tunåsen infiltration. Results of this thesis were intended to be useful in helping design basin infiltration MAR schemes from a risk management perspective. Results are expected to be particularly useful for those examining artificial infiltration in a boreal landscape where high concentrations of organic matter in surface water would be expected to affect virus removal during MAR.

2. Experimental Methods

2.1 The Tunåsen MAR scheme

The city of Uppsala, Sweden is home to around 200,000 inhabitants making it Sweden's fourth largest city (Uppsala Kommun, 2015). All of the drinking water in Uppsala is supplied using groundwater resources. The utility responsible for supplying water to Uppsala's inhabitants (Uppsala Vatten) sources the drinking water from the Uppsala Esker, a 200 km long, clay-toped gravel aquifer that was created during the last ice-age (Morosini, 1989). As the city of Uppsala grew, it became apparent that extraction rates from the esker were not sustainable when compared to the natural infiltration rate so the city began to supplement groundwater levels using basin infiltration MAR (Bergström, 1986). The city of Uppsala has several MAR schemes along the esker in order to maintain sustainable groundwater levels throughout. The largest basin infiltration scheme is located on top of an exposed, semi-confined outcrop of the Uppsala Esker called Tunåsen. The infiltration basins at Tunåsen are the focus of this thesis.

The water used for infiltration at Tunåsen is sourced from the nearby Fyris River. Prior to infiltration, surface water from the river is pumped through a fast-sand filter to remove large particulates in order to delay clogging of the basin. Infiltrated groundwater is extracted 2 km away from the Tunåsen infiltration basins after which the water undergoes treatments to reduce mineral content (hardness). The water is also chlorinated prior to its distribution in order to slow microbial growth in the distribution network. Uppsala Vatten relies on the natural cleaning mechanisms of the soil between Tunåsen and the extraction wells as one of the primary barriers against viral contamination. Residence times between infiltration and extraction are estimated to be 90–110 days (Bergström, 1986) which is considered to be more than adequate for achieving sufficient removal of viral pathogens (European Commission, 2001). The recommended infiltration rate at Tunåsen is 2.4 – 4.8 m day^{-1} (Svenskt Vatten AB, 2010).

2.2 Experimental design

The primary aim of the experiments was to determine how DOC, IS, and the age of the infiltration basin sand would affect virus removal at the Tunåsen basin infiltration MAR scheme in Uppsala, Sweden. In order to test the effects of DOC and IS on virus removal, two different levels of each parameter were used in the experiments. These corresponded to a “high” and a “low” level and were characteristic of conditions found in the Fyris river, the water used for infiltration at Tunåsen during the years 1993–2011 (Sveriges lantbruksuniversitet, 2015). Low levels of DOC and IS were typical of the expected concentrations of these variables during the winter months (November–February). The high levels of DOC and IS reflected the maximum observed concentrations over the sample period. Two types of sand were used in the experiment: “new” sand, i.e. sand that had not been subjected to infiltration, and “used” sand, i.e. sand that had been subjected to infiltration. All experiments were conducted in a climate controlled chamber at 4°C in order to simulate temperature conditions similar to those at Tunåsen during the winter months (Figure 2d).

For the studies presented herein, the experimental “treatments” refer to the IS and DOC concentrations as well as the type of sand used in the experiment. Each treatment was examined at two “levels”, i.e. a high and low concentration of IS and DOC and the new and used sand. The conditions at Tunåsen can be any combination of these experimental treatments. Therefore, experiments were designed in order to test each combination of treatment. This type of experimental design is called a full factorial design. Factorial designs allow experimenters to examine the effects of treatments using a minimum amount of experimental runs in order to determine if any behavioral trends exist between a quantity in question and the levels of the treatments used (Box et al., 1978). For example, the batch and column studies in this thesis utilized a three-treatment, two-level full factorial design wherein every possible combination of DOC level, IS level, and sand type were tested to examine their effects on virus removal. This requires eight individual experimental runs ($2^3 = 8$; two levels, and three treatments). In order to attain more robust estimates of the treatment effects, each experimental run was replicated. This means that each experiment consisted of at least sixteen experimental runs. For the batch experiments, an additional four experimental runs, each with a replicate experimental run, were completed to test the effects of IS and DOM on virus removal in the absence of sand.

2.3 Water

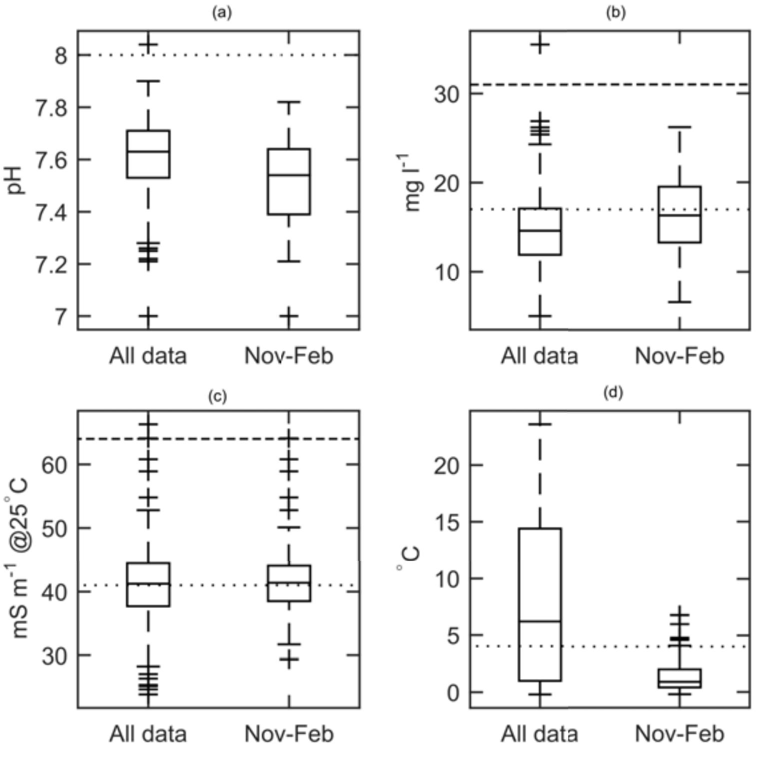

Figure 2. Boxplots showing data for the (a) pH, (b) total organic carbon (TOC), (c) conductivity, and (d) temperature for water in the Fyris river during the years 1993–2011 at station Fyrisån Klastorp (Sveriges lantbruksuniversitet, 2015) along with the values measured for the raw (*horizontal dotted lines*) and adjusted water samples (*horizontal dashed lines*) used in the experiments reported by this thesis

Water for the study was gathered from the flow division chamber at Tunåsen in February of 2015. The sampled water was filtered twice: first through a larger membrane filter in order to remove large particles and again through a smaller membrane filter in order to remove particulate organic matter (1.6 and 0.45 μm respectively). The filtered water was analyzed for its chemistry. Four distinct aqueous solutions were needed in order to investigate every combination of the high and low levels of IS and DOC used in the experiments. The IS of the water was adjusted using sodium chloride (NaCl). The DOC of the water was adjusted using Nordic Reservoir natural organic matter attained from the International Humic Substances Society (IHSS, 2011). Chemical analyses revealed that the IS and DOC concentrations of the raw water sampled at Tunåsen were characteristic of those for the river water used for infiltration during the winter months. Therefore, the raw water was used to represent the low levels of IS and DOC while high levels of each treatment were attained by adding salt and organic matter to the raw water (Figure 2b–c; conductivity and TOC were used as a proxy for IS and DOC respectively).

Adjustments of IS and DOC were done such that the concentrations of each at their respective high levels were characteristic of the maximum concentrations observed for the Fyris River water. The water had a final pH of 8.0 due to extensive handling during preparation (Figure 2a). The water was not adjusted back to its more neutral pH in order to decrease the effects of adsorption in all experiments. Therefore, with respect to the relationship between pH and adsorption, results from the batch and column experiments could be considered as representative of a worst-case scenario as virus removal could be expected to be at a minimum at this pH. For further information on the preparations of the water used in the experiments please consult the experimental methods section of Paper II or Paper III.

2.4 Sand

Table 1. *Chemical characteristics of the sand used in batch experiments; all values expressed in mg/kg (reprint from Paper II)*

Sand	C			N	K	Ca	Na	Mg	Al	Fe	Mn
	Total	$CaCO_3$	Org.								
Used	490	200	290	50	1093	5130	3857	140	7910	10735	184
New	580	560	20	20	821	6195	4005	120	7098	11640	197

The new sand used for the experiments was gathered from a quarry where all of the sand used at Tunåsen is sourced. The used sand for the study was gathered from an infiltration basin at Tunåsen which had been used intermittently for a period of about eight years. Differences in the chemical and size characteristics between the new and used sand were assumed to be a result of the years of exposure to the surface water used for infiltration. The used sand had a slightly higher distribution of fine materials than the new sand. Therefore, the size distribution of the used sand was matched to that of the new sand. This was achieved by separating the used sand according to diameter using different sized sieves. The used sand was then remixed such that its size distribution matched that of the new sand as closely as possible. This was done in order to ensure that only the only variable changing between the new and used sand was the chemical characteristics.

Chemical analyses of both the new and used sand were performed by the Forest Commission England, Forestry Research Division. A detailed description of the preparation of the sand can be found in Paper II. Chemical analyses revealed that the used sand contained about fifteen times more organic carbon than the new sand (Table 1). Organic matter is removed from infiltrating water through adsorption mechanisms during MAR (Grünheid et al., 2005; Kolehmainen et al., 2007; Lindroos et al., 2002) so it came as no surprise that the used sand had more organic carbon.

2.5 Virus and virus assay techniques

Due to the dangers involved with using human-infectious enteric viruses for experimentation, a bacteriophage, or a type of virus which only infects bacteria, was used as a surrogate virus. Bacteriophages are the most common type of virus on Earth (Breitbart and Rohwer, 2005). There are millions of different types of bacteriophage which are classified according to their structure, size, chemical makeup, and method of replication (Gelderblom, 1996). The specific bacteriophage used in this study was bacteriophage MS2. MS2 is a type of bacteriophage which infects specific bacterium in the family *Enterobacteriaceae* such as Escherichia coli (E.coli) and Salmonella Typhimurium (VanDuin, 1988). MS2 is commonly used in studies of virus removal in drinking water as it is considered to be an adequate model of enteric viruses in the environment (Gerba, 2006; IAWPRC, 1991). MS2 has also been shown to be more resistant to removal mechanisms than other bacteriophages so its use in the present study may provide a more conservative estimate of virus removal representative of a worst-case scenario (Pang, 2008).

Estimations of MS2 concentrations were achieved using a technique called the double agar overlay method as described by Adams (1959). The underlying principle of the method relies upon the fact that bacteriophages will infect, and ultimately kill, specific host bacteria. A sample containing bacteriophage with a known volume is combined with a solution of equal volume containing viable host bacteria. The combined virus—bacteria solution is then applied to a petri dish containing a nutrient-rich, low-viscosity liquid growth-medium called agar. The petri dish is then placed in a temperature controlled incubator wherein viable bacteria are given optimal conditions for growth thus producing a semi-opaque layer of healthy bacteria on top of the agar. However, bacteria which have become infected by the bacteriophage will die during the growth phase leaving a transparent spot on the agar. Transparent colonies are counted on each agar plate. Assuming a one-to-one ratio of dead bacteria to viable virus, the concentration of viable virus in the sample can be estimated based on the volume of the original sample. The final estimate of the virus concentration is expressed as colony-forming units (CFU) per volume of the sample.

The double agar overlay method is widely used in studies of virus fate and transport, is relatively simple to perform, and is one of the only techniques capable of measuring viable viruses. However, this method of enumeration requires many steps between the sampling and the counting of the CFU, all of which must be carried out with precision and with a high degree of replicability in order provide an accurate estimation of virus concentration. Because of this, microbial data derived from plating methods, such as the double agar overlay method, are considered to provide an order-of-magnitude estimate of the true virus concentration (Corry et al., 2007; Sutton, 2011). All estimates of virus concentration in this thesis are considered to have a high degree of uncertainty.

A stock solution with a high concentration of MS2 was prepared prior to the start of the experiments. This stock solution was used when preparing the virus—water solutions used in each experiment. For details regarding the preparation of the stock solution please consult the methods section of Paper III.

2.5 Batch experiments

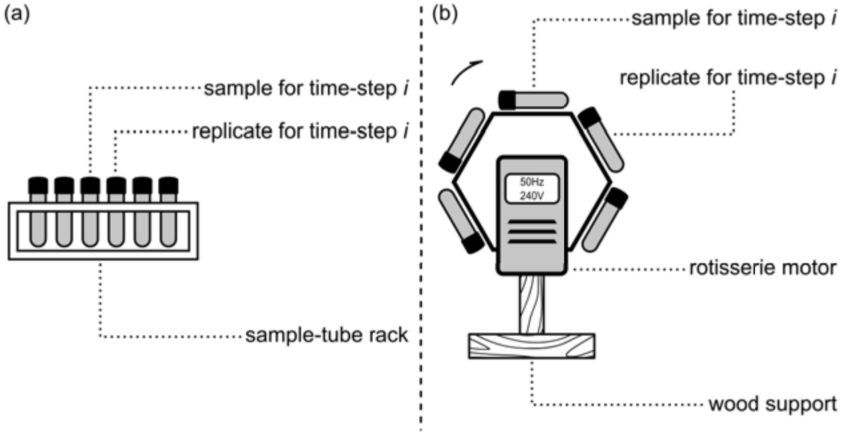

Figure 3. Experimental setup of (a) static batch experiments, (b) agitated batch experiments. Modified from Figure 1 in Paper III.

Batch experiments were conducted in order to examine the effects of IS, DOC, and the presence and relative age of the sand (new versus used) on MS2 removal. Experiments were conducted for a period of two months. Batch experiments used capped non-reactive glass culture tubes as batch reactors in order to ensure that MS2 removal by the surface of the reactor was negligible (Wong et al., 2013). Data from the batch experiments were in the form of time-series MS2 concentrations where the extent of MS2 removal was examined relative to the initial concentration. For each experimental run, a small volume of MS2 stock solution was added to the bulk volume of the water used for a specific experimental run. The initial virus concentration was then measured. The bulk virus—water solution was then immediately added to the batch reactors. For details regarding the preparation of the virus—water solutions and the filling of the batch reactors please consult the methods section of Paper III.

Each experimental run consisted of several individual batch reactors where the total amount of reactors equaled the total number of time-steps measured over a period of two months. For example, an experimental run which examined virus removal at eleven different time-steps had eleven batch reactors with identical experimental conditions. At each time-step a batch reactor was chosen at random and the concentration of virus was measured. The same was done for the replicate experiment. Therefore, two measurements were taken for any given experimental run at each time-step. This was done in order to capture any uncertainties in the experimental data that may have come from variations in the preparation and execution of the experiment.

For static batch experiments, batch reactors were held vertically in place using plastic sample tube racks (Figure 3a). For the agitated batch experiments, a purpose-built batch agitator was constructed in order to continually mix the reactors (Figure 3b). For further details regarding the experimental setups of the static and agitated batch experiments please consult the methods section of Paper II.

2.6 Column experiments

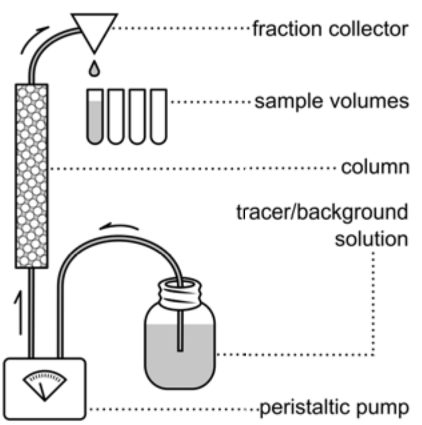

Figure 4. Experimental setup of column experiments. Modified from Figure 1 in paper III.

Column experiments were conducted in order to examine the effects of IS, DOC, and relative sand age (new versus used) on MS2 removal under transport conditions similar to those found at the Tunåsen infiltration basin. Column experiments were conducted using a 15 cm long, non-reactive glass column with a 2.5 cm diameter. Flow through the column was driven by a peristaltic pump (Figure 4). The infiltration rate of the column was in accordance with the recommended infiltration rates for Sweden. For each experimental run, a known quantity of water containing a known concentration of MS2, also known as a virus tracer, was pumped through the column and samples were collected from the column effluent at regular time intervals. Immediately following the virus tracer, water without virus was pumped through the column to examine the detachment of adsorbed viruses. Data from the column experiments was in the form of a time-series of concentrations measured in the column effluent. Data of this type is also known as a breakthrough curve. Virus tracers were prepared by adding a small volume of MS2 stock solution to the water used for a specific experimental run and the concentration was measured. The MS2 concentration of the tracer solution was measured again following the completion of the experiment in order to see if there was any change in the concentration during the experiment. For specific details regarding the preparation of the virus tracers please consult the method sections of paper IV.

For each experimental run the column was simultaneously filled with the sand and water, free of MS2, specific to the experiment. The column was then flushed with MS2-free water in order to clear the column of any small particulates that may have resulted from the filling of the column. Following the initial flush, column influent was switched to the MS2 tracer solution. After a predetermined time, the column influent was switched from the MS2 tracer solution back to the MS2-free solution in order to monitor MS2 detachment from the sand. Effluent samples were collected from the start of the MS2 tracer to the end of the experiment. The MS2 concentration was measured for all samples taken from the column effluent. Following the completion of the MS2 tracer experiment, columns were flushed overnight with deionized (DI) water. After the DI flush, an additional tracer experiment was conducted using a conservative salt tracer to be used in determining the hydraulic characteristics of the column. For specific details regarding the experimental procedure please consult the methods section of Paper IV.

2.7 Key experimental assumptions

The experiments used in this thesis were designed to investigate the adsorption and inactivation of virus as effected by IS, DOC, and the relative age of the sand used in an infiltration basin. In our studies, we assumed that adsorption of virus can be described using DLVO theory. This theory proposes that virus adsorption is determined by the relative influence of attractive van der Waals forces and repulsive electrostatic forces between the virus and the sand (Verwey and Overbeek, 1948). However, the strength of the attractive forcing is inversely proportional to the sixth-power of the distance between the virus and absorption surface (Gerba, 1984). This means that, when conditions for adsorption are favorable, the rate at which viruses will adsorb to the sand is limited by the mass-transfer of virus to the sand's surface (Schijven and Hassanizadeh, 2000). The experiments in this thesis were designed with this in mind.

In the static batch experiments, there was no mechanical mixing between the virus suspension and the sand. This means that the mass-transfer of virus was limited to diffusion. For viruses, the diffusion flux rate is on the order of 10^{-7} cm^2 s^{-1} (Dubin et al., 1970). This is much smaller, about two orders of magnitude less, than the diffusion rate of nutrients, such as NOM, in water (Berg, 1993). Therefore, it was assumed that the primary removal mechanism in static batch experiments was inactivation.

In the agitated batch experiments, the sand and water were being constantly mixed throughout the experiment due to the rotation of the batch agitator. This means that the mass transfer of viruses to the surface of the sand was no longer limited to diffusion of the virus. In these experiments, virus removal was assumed to be achieved through a combination of adsorption and inactivation mechanisms. This was also assumed to be the case for the column experiments due to the constant flow of the virus suspension through the column. However, in these experiments, adsorption was assumed to be the primary mechanisms of removal as the duration of the experiments was only a few hours.

In the batch experiments without sand, inactivation was assumed to be the only mechanism of virus removal as adsorption to the surface of the glass in the batch reactors is negligible (Wong et al., 2013). These experiments are used as a baseline from which the effects of the sand can be deduced. Another key assumption under which these experiments are designed is that the new and used sand had the same effective surface area. A strong positive correlation has been observed between the extent of virus adsorption and the surface area of the adsorptive material available for adsorption (Lipson and Stotzky, 1983). The size distribution characteristics of both the new and used sand were matched in order to reduce these effects as much as possible. By doing this, we assumed that the only difference between the new and used sand was the chemical characteristics shown in Table 1.

2.8 Numerical modeling

Mathematical models are an important tool for understanding the behavior of viruses in groundwater. Typically, mathematical models are applied to experimental data, either at the bench or field scale, as a means of gaining an additional understanding of the processes that govern the fate and transport of viruses in the subsurface. A number of studies have successfully applied mathematical models to data describing virus inactivation (Chrysikopoulos and Aravantinou, 2012; Hurst et al., 1980; Jansons et al., 1989; Yates et al., 1985) and virus adsorption (Bixby and O'Brien, 1979; Grant et al., 1993; Moore et al., 1981; Schijven et al., 2000; Sim and Chrysikopoulos, 1999) in batch experiments. Mathematical models have also been successfully applied to data describing the fate and transport of virus in flowing systems at the bench (Bradford et al., 2006; Cao et al., 2010; Cheng et al., 2006; Dowd et al., 1998; Jin et al., 2000; Schijven et al., 2000; Torkzaban et al., 2006) and field-scale (Flynn and Sinreich, 2010; Kvitsand et al., 2015; Schijven and Šimůnek, 2002). In the present thesis, mathematical models describing virus inactivation were applied to data resulting from the static batch experiments. Models describing the fate and transport of virus in flowing systems were applied to data resulting from the column experiments.

2.8.1 Inactivation in batch studies

Virus inactivation in water is most commonly assumed to follow a first-order process, or a process wherein virus concentrations are estimated assuming exponential decay at a constant rate (Schijven and Hassanizadeh, 2000). However, some researchers have argued that virus inactivation is better represented as a time-dependent process, or a process wherein the exponential decay rate changes with time, especially when virus inactivation is investigated in soil—water systems (Hurst et al., 1992; Sim and Chrysikopoulos, 1996). At present time, there is no consensus regarding which representation is "most" correct as both constant and time-dependent inactivation models have been successfully applied to several investigations of virus inactivation (Anders and Chrysikopoulos, 2006; Bae and Schwab, 2008; Chrysikopoulos and Aravantinou, 2012; Yates et al., 1985). Therefore, this thesis considered models representative of both constant and time-dependent inactivation when fitting models to MS2 batch inactivation data.

The governing equation from which both models used to describe virus inactivation in this thesis are derived is shown in (1):

$$\frac{dC_{\mathrm{l}}(t)}{dt} = -\mu(t)C_{\mathrm{l}}(t) \tag{1}$$

where t (T) is time, $C_{\mathrm{l}}(t)$ (M V^{-1}) is the concentration of virus at time t, and $\mu(t)$ (T^{-1}) is the inactivation rate at time t. For models wherein the inactivation rate is assumed to be constant, μ is no longer a function of t and the solution of (1) can be written as shown in (2):

$$\ln\left(\frac{C_{\mathrm{l}}(t)}{C_0}\right) = -\mu t \tag{2}$$

where C_0 (M V^{-1}) is the initial concentration of virus in the batch reactor, i.e. the concentration of virus at $t = 0$.

Models of time-dependent inactivation assume that the rate at which viruses are inactivated may be dependent on environmental factors or heterogeneities in the virus population. Within this thesis, time-dependence is assumed to be a product of different sub-populations of viruses inactivating at different rates. A study by Grant (1995) proposed that viral aggregation would lead to different subpopulations of virus inactivating at different rates as clusters of virus would be more resistant to inactivation than a single virus. Sim and Chrysikopoulos (1996) developed a model wherein the inactivation rate is dependent on the sensitivity of different subpopulations to inactivation. They proposed a pseudo first-order model of virus inactivation as shown in (3):

$$\mu(t) = \mu_0 \exp(-\alpha t) \tag{3}$$

where μ_0 (T^{-1}) is the initial inactivation rate, i.e. the inactivation rate at $t = 0$, and α (T^{-1}) is the resistivity coefficient, i.e. a measure of how sensitive different sub-populations of virus are to inactivation. A larger value of α indicates that a more resistant sub-population of virus exists and that the overall virus concentration will decrease more slowly. The inactivation rate of the most sensitive virus populations will be closer to that indicated by μ_0. For models wherein the inactivation rate is assumed to follow (3) the solution to (1) is as shown in (4).

$$\ln\left(\frac{C_l(t)}{C_0}\right) = \frac{\mu_0}{\alpha}\left[\exp(-\alpha t) - 1\right] \tag{4}$$

It is worthy to note that in the solutions of (1) shown in (2) and (4) the left-hand side of the equation is expressed in terms of relative concentration, i.e. the percent concentration relative to the initial concentration. Therefore, model outputs will be expressed in relative concentration terms which must be back-transformed if model predictions are to be expressed as virus concentrations.

In this thesis, virus inactivation was examined in static batch experiments using (2) and (4). Solutions to the models were obtained numerically using the MATLAB commercial software package (The MathWorks Inc., 2014).

2.8.2 One-dimensional transport of virus in sand

Mathematical models of virus transport in soil—water systems are most commonly formulated based on the advection—dispersion equation (ADE). The ADE describes the mass transport of a dissolved or suspended constituent in a groundwater system due to the flow of water through the soil—water matrix. Advection describes the movement of the bulk mass of a constituent at the same speed and in the same direction as the average groundwater flow. Dispersion describes the spreading of a constituent in all directions as it moves through the soil—water matrix and is defined as the sum of two processes: mechanical dispersion and diffusion.

Mechanical dispersion describes the process wherein a constituent is spread out due to pore-size heterogeneities thus causing local variations in velocity gradients as water flows through the pore-space. The magnitude of mechanical dispersion is predominantly dependent on the velocity of the flow in the pore-space. However, when the constituent in question is a particle rather than a dissolved substance, the size of the particle will also affect the magnitude of the mechanical dispersion. Diffusion describes the spontaneous movement of a constituent along concentration gradients, i.e. the "evening out" of concentrations in space. The magnitude of the diffusion is a property inherent to the constituent itself and will vary as a function of temperature.

In the context of this thesis, the ADE was used to model the transport of a virus through a column of saturated sand under the assumption that the pore-water velocity and dispersion of virus within the sand was constant everywhere in the column. Under this assumption, spatial changes in virus concentration will occur along the length of the column only and the ADE need only be examined in one dimension. In its most basic form, the ADE for one-dimensional flow through a saturated porous medium in the in the vertical z direction is shown in (5):

$$\frac{\delta C_\mathrm{l}}{\delta t} = D\frac{\delta^2 C_\mathrm{l}}{\delta z^2} - v\frac{\delta C_\mathrm{l}}{\delta z} \tag{5}$$

where C_l (M V^{-1}) is the liquid concentration of virus as a function of time t and space z, D is the dispersion coefficient (L^2 T^{-1}), and v (L T^{-1}) is the pore-water velocity of the infiltrating water.

As previously mentioned, the dispersion coefficient D is the sum of the mechanical dispersion and the diffusion of virus and can be written as (6):

$$D = vD_L + D_e \tag{6}$$

where D_L (L) is the longitudinal dispersivity, and D_e (L^2 T^{-1}) is the effective diffusion coefficient. When using (5) and (6) to model virus transport in groundwater, the time-rate of change of virus concentration is dependent on advection and dispersion only and does not account for virus inactivation or adsorption to the surface of porous material. Inactivation and adsorption is accounted for by using a modified version of (5) as shown in (7):

$$\frac{\delta C_{\mathrm{l}}}{\delta t}+\frac{\rho_{\mathrm{b}}}{n}\frac{\delta C_{\mathrm{s}}}{\delta t}=D\frac{\delta^{2}C_{\mathrm{l}}}{\delta z^{2}}-v\frac{\delta C_{\mathrm{l}}}{\delta z}-\mu_{\mathrm{l}}C_{\mathrm{l}}-\frac{\rho_{\mathrm{b}}}{n}\mu_{\mathrm{s}}C_{\mathrm{s}} \tag{7}$$

where ρ_{b} (M L^{-3}) is the bulk density of the sand, n (%) is the porosity of the sand, C_{s} (M V^{-1}) is the concentration of adsorbed virus as a function of time t and space z, μ_{l} (T^{-1}) is the inactivation rate of virus in liquid, and μ_{s} (T^{-1}) is the inactivation rate of adsorbed virus (Schijven and Hassanizadeh, 2000).

In (7) the liquid and adsorbed inactivation rate of virus is considered constant with time and is in the same form as that in (2). Sim and Chrysikopoulos (1996) developed a model to examine one-dimensional virus transport that accounts for time-dependent virus inactivation. Model simulations indicated that viruses were predicted to travel a greater distance through the pore-space when inactivation was represented as a time-dependent process (4) than when inactivation was represented as a first order process independent of time (2). However, a first-order representation of virus inactivation remains the dominant method by which virus inactivation is accounted for in studies of virus fate and transport. This is likely due to the fact that, currently, the most common software packages capable of modeling both fluid and virus transport through a porous medium are not capable of modeling time-dependent inactivation (Tufenkji, 2007). This thesis modeled virus inactivation in flowing systems exclusively as a first-order process due to the aforementioned constraints on available software.

Adsorption of virus in flowing porous systems is thought to be most accurately portrayed as a kinetically limited process (Schijven and Hassanizadeh, 2000). In their simplest form, models which account for kinetic adsorption assume that the rates of attachment and detachment are the same for all adsorption sites. Models of this type are often referred to as a “one-site” kinetic adsorption model. In this case the rate of change of the concentration of adsorbed viruses can be represented with (8):

$$\frac{\rho_{\mathrm{b}}}{n}\frac{\delta C_{\mathrm{s}}}{\delta t}=k_{\mathrm{att}}C_{\mathrm{l}}-\frac{\rho_{\mathrm{b}}}{n}k_{\mathrm{det}}C_{\mathrm{s}} \tag{8}$$

where k_{att} (min^{-1}) is the attachment rate of virus to the adsorption surface, and k_{det} (min^{-1}) is the detachment rate of virus from the adsorption surface (Schijven and Hassanizadeh, 2000).

In this thesis, virus transport was examined using the model presented in (6), (7), and (8). Solutions to the equations were attained numerically using HYDRUS-1D, an open-source software package which simulates the movement of water, heat, and contaminants in a porous medium under variable degrees of saturation (Šimůnek et al., 2013).

2.8.3 Model structure, parameters, and variables

Mathematical models which propose to describe the fate and transport of a contaminant in groundwater make assumptions about the primary mechanisms responsible for determining the contaminant's distribution in the soil—water matrix over space and time. Every model can be defined by its "structure", "parameter(s)", and "variable(s)".

A model's structure represents all of the processes included in the model. Model structure can change based on assumptions regarding which processes are deemed important for describing the fate and transport of a constituent, or how those processes relate in time and space. For example, (2) and (4) are both proposed to describe inactivation of virus in batch reactors, however (2) assumes that the inactivation rate is independent of time while (4) assumes that inactivation is time-dependent. Therefore, these two models are structurally different.

Bard (1974) describes model parameters and variables as not always being clearly distinguishable from one another. He describes a model's variable(s) as the values a model is built to explain quantitatively. For example, the liquid virus concentrations C_l present in (2), (4), and (7) were measured experimentally prior to any model formulation and all models were built to try and explain their behavior over time as a function of different physical processes. Model variables are not necessarily unique to any given model structure as they may be used in several model formulations simultaneously as is the case for the batch inactivation models in (2) and (4). Parameters are the values in a model which are used to quantify the relationships between variables or, in this case, to quantify the relationships between different processes in the model structure. For example, in (7) the dispersion coefficient D is a parameter that describes the magnitude to which the dispersion process is important in describing the transport of viruses in sand. Parameters are specific to a given model structure as the magnitude of parameters meant to describe the same process or physical property may change depending on model structure.

A lack of clarity between parameters and variables may arise when considering that model parameters may be measured experimentally. For example, the inactivation rate of a virus in liquid μ_l in (7) was estimated prior to the model's use through the batch experimentation and application of the model in (2). For this reason μ_l may seem like a variable rather than a parameter. For clarity's sake, only C_l is considered a variable in the studies herein as all other quantities in (2), (4), (6), (7), and (8) can be determined using parameter estimation techniques.

2.8.4 Parameter estimation

Parameter estimation for mathematical models describing virus fate and transport are typically done using some form of parameter optimization where parameter sets are judged according to a "goodness-of-fit" statistic. The goodness-of-fit statistic is a metric that can be used to judge the quality of a fitted model. Most commonly, the goodness-of-fit statistic is based on the squared-differences, or squared-errors, between the model predictions and the experimental data. Parameter optimization techniques which try to minimize a statistic of this type are called "least-squares optimization" techniques. In least-squares optimization, the value of the squared-errors can be viewed as a function of the parameters being estimated. It is the goal of least-squares optimization to find the combination of parameters that result in the absolute minimum, or "global" minimum, value of the squared errors between the model predictions and the experimental data. The parameter set which results in the global minimum of the squared errors is chosen as the "optimal" parameter set.

In least-squares optimization, an estimate of uncertainty can be given for the parameter estimate. Uncertainty estimates around optimal parameters are usually represented by "confidence intervals". However, confidence intervals calculated from least-squares optimization rely on the assumptions that, if not validated, can give an unrealistic estimate of the model parameter uncertainty and model prediction uncertainty. Confidence intervals might also be unrealistically large, extending past the boundaries of what may have been deemed realistic for the system being analyzed, if the errors between the model predictions and the measured data are relatively large. Furthermore, least-squares optimization techniques do not allow for the use of prior knowledge regarding the uncertainty of data and measured parameters in parameter estimation. Least-squares optimization techniques also assume that the optimum model is "true" which means that multiple model structures cannot be used simultaneously in prediction.

An alternative method of parameter estimation that attempts to address the shortcomings of least-squares optimization is called "general likelihood uncertainty estimation" (GLUE). GLUE is a Monte-Carlo simulation based approach that incorporates prior knowledge of parameter and data uncertainty into parameter estimation, and allows for multiple model structures to be used simultaneously in prediction (Beven, 2002a, 2002b). Within GLUE, the modeler decides upon a range within which all possible values of the parameter are assumed to exist as well as the parameter's likelihood across that range. The modeler also decides what constitutes an acceptable, or "behavioral", model prior to estimating the parameters by setting the "limit of acceptability". The limit of acceptability is usually based on a goodness-of-fit statistic.

Monte-Carlo runs are completed by running the model using random combinations of parameter values. The array of model outputs which consider every possible combination of parameter value is called the "model space". A goodness-of-fit statistic is calculated for each set of model outputs within the model space. The result is a vector of goodness-of-fit metrics where each row of the vector is associated with a parameter set. The vector of goodness-of-fit metrics is then compared to the limit of acceptability and all parameter sets associated with a goodness-of-fit statistic which satisfies the limit of acceptability are deemed as behavioral parameter sets. For example, if the coefficient of determination (R^2) is used as the goodness-of-fit statistic, a value of 0.90 might be used as the level of acceptability. Monte-Carlo runs are then completed to achieve a sample of the model space and all parameter sets associated with a model performance of $R^2 > 0.90$ would be considered as behavioral. The result is an ensemble of behavioral parameter sets where the uncertainty of the parameters is estimated as the maximum and minimum value of each parameter that is associated with a behavioral model.

Least-squares optimization techniques have been successfully applied to several studies examining virus removal in batch experiments (Bae and Schwab, 2008; Chrysikopoulos and Aravantinou, 2012; Yates et al., 1985) and column experiments (Bradford et al., 2006; Cao et al., 2010; Cheng et al., 2006; Dowd et al., 1998; Jin et al., 2000; Schijven et al., 2000; Torkzaban et al., 2006). GLUE has been successfully applied to hydrologic models of varying complexity (Beven and Binley, 1992; Beven, 1993; Binley and Beven, 2003; Christiaens and Feyen, 2001; Freer et al., 1996; Zhang et al., 2006). However, GLUE has yet to be examined as an alternative for parameter estimation when investigating the fate and transport of viruses. Zhang et al. (2006) used GLUE for parameter estimation when applying models to column experiments which examined pesticide transport in soil columns. They found that uncertainty estimates provided by least-squares optimization methods provided uncertainty bands around parameter estimates that were narrower than those estimated by GLUE. The study concluded that GLUE would be a more appropriate method to use in model prediction and would be especially useful when applying results to field-scale experiments. The results of Zhang et al. (2006) imply that GLUE could be a superior alternative to least-squares optimization for parameter estimation and predictive modeling when applied to virus transport. Therefore, all of the modeling exercises in this thesis compare least-squared optimization techniques to the GLUE methodology with regard to parameter and prediction uncertainties. To the authors' knowledge, this is the first time GLUE has been used in the investigations of virus fate and transport.

2.9 Methodological progression through thesis

The methods presented above were executed in a series of papers. Each paper was designed such that it could be viewed as a logical progression from its predecessor. For Papers II–IV, the methods through which each study achieved its results were directly informed by the findings of its predecessor.

Paper I is a literature review summarizing the current understanding of virus removal in soil—water systems and is particularly focused on the effects of NOM on virus removal. The findings from Paper I were used extensively in the framing of the thesis, the designing of the experiments, and the analysis of the results for all of the following studies.

Paper II examined the effects of the new and the used sand on removal of MS2 in batch experiments (both static and agitated) and column experiments using the water gathered from the Tunåsen MAR scheme (low IS, low DOC). The findings from Paper II were used to determine which mechanisms were responsible (adsorption vs. inactivation) for virus removal in each of the experiments as only affected by the relative age of the sand (new vs. used).

The datasets used in Paper II were taken from a small portion of the full-factorial designs implemented in the batch (static and agitated) and column experiments. Papers III and IV examine the full breadth of the static batch experiments and column experiments, respectively. However, there was no study which was dedicated to the entirety of the agitated batch experiments due to time constraints. The full set of experimental results from the agitated batch experiments are presented in the appendix of this thesis (Figure A1). These results are used in the discussion section to comment on the effects of the experimental treatments on MS2 inactivation.

Paper III builds on the static batch experiments in Paper II by examining the effects of two additional experimental treatments (IS and DOC) on the inactivation of MS2 in the static batch experiments. Models representative of constant (2) and time-dependent inactivation (4) were fit to the experimental data using both least-squared optimization and GLUE. The results of the modeling exercise in this study were used to comment on how data uncertainty will affect the uncertainty surrounding parameter estimates in inactivation models. This study also acts as an introduction to the GLUE methodology as applied to studies of virus fate and transport.

Paper IV builds on the conclusions of all of the previous studies. In this study, the results from the column experiments in Paper II are built upon by examining the effects of two additional treatments (IS and DOC) on the transport of MS2 through the sand columns. A model describing the fate and transport of virus in a one-dimensional, saturated, flowing system (6–8) was fit to all of the experimental data using both least-squares optimization and GLUE. This paper uses the estimates of the MS2 inactivation rate found in Paper III in the model fitting. This final paper was meant to show the potential the GLUE methodology has at predicting the extent of virus transport in groundwater systems while accounting for both experimental and data uncertainty.

3. Summary of papers

3.1 Paper I

Chemical and environmental factors affecting adsorption and inactivation mechanisms of virus in groundwater: a literature review

In this paper we attempted to make an exhaustive review of the literature regarding the fate and transport of viruses in soil—water systems. The paper put a particular emphasis on the effects of NOM on virus adsorption and inactivation mechanisms. We discussed the predominant theory regarding virus adsorption, Derjaguin-Landau-Verwey-Overbeek (DLVO) theory, as well as its strengths and weaknesses in regards to adequately predicting the extent of virus adsorption. The effects of several environmental factors on virus adsorption and inactivation were discussed. Of particular interest to this thesis were literature findings discussing the effects of temperature, pH, ionic strength, and NOM on inactivation and adsorption mechanisms. At low temperatures (4–15°C) viruses were shown to experience minimal inactivation in soil—water systems and are capable of persisting for several months with relatively small decreases in concentration. Virus adsorption was shown to be highly dependent on solution pH. In general, it was shown that conditions tended to be more favorable for adsorption at a pH less than pH 7. Conditions were generally unfavorable for adsorption at pH greater than pH 8. Virus adsorption was also shown to be highly dependent on solution ionic strength with conditions becoming more favorable for adsorption as ionic strength increased. However, this effect became less apparent as concentrations of divalent cations were increased in the presence of iron oxides on adsorption surfaces. The presence of NOM was shown to affect both inactivation and adsorption mechanisms. In general, removal of virus through either mechanism was observed to decrease as concentrations of soluble and adsorbed NOM increased in soil—water systems. But, the mechanism(s) through which NOM affect virus inactivation and adsorption are still uncertain.

3.2 Paper II

Reduced removal of an enteric virus during managed aquifer recharge due to organic coatings on infiltration basin sand

In this paper, we examined how the removal of bacteriophage MS2 was affected by the sand used in the infiltration basins at the Tunåsen MAR scheme. Static and agitated batch experiments were conducted over a period of two months to examine the long-term removal of virus. Flow-through column experiments were conducted in order to examine virus removal under transport conditions similar to those in the infiltration basins. The water used for the study was characteristic of that used at the Tunåsen MAR scheme during the winter months. All experiments were conducted at 4°C.

In all experiments, virus removal was much higher when in the presence of new sand rather than in the presence of the used sand (Figure 5). In static batch experiments, enhanced inactivation of MS2 in the presence of the new sand was likely due to the presence of iron-oxides on the sand's surface (Figure 5a). In agitated batch experiments with new sand, MS2 removal was characteristic of that observed in equilibrium adsorption experiments (Figure 5b). In both static and agitated batch experiments with used sand, MS2 concentrations behaved similar to those observed in experiments without sand. This suggested that MS2 removal was due entirely to inactivation. In column experiments, peak concentrations of MS2 in experiments with used sand were two orders of magnitude greater than those observed with the new sand (Figure 5c).

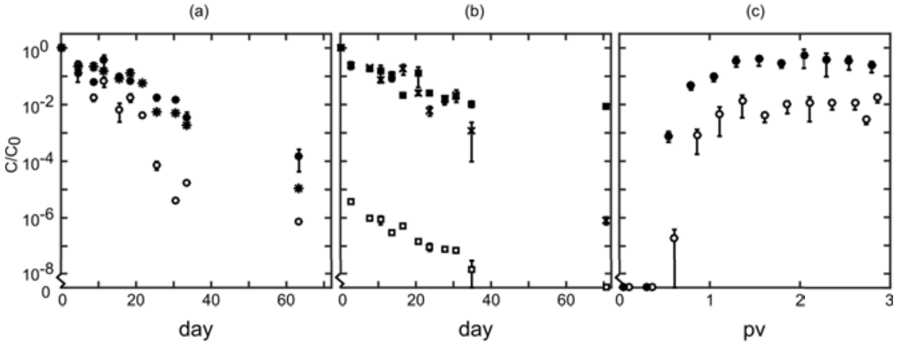

Figure 5. Experimental results showing time-series concentrations of MS2 in low IS, low DOC water for the **(a)** static batch experiments with new sand (*white circles*), used sand (*black circles*), and no sand (*asterisk*); **(b)** agitated batch experiments with new sand (*white squares*), used sand (*black squares*), and no sand (*x*'s); and **(c)** column experiments with new sand (*white circles*) and used sand (*black circles*). The uncertainty of the measurement is indicated by the *black error bars*. The figure is a modified version of Figures 2 and 3 in Paper II.

Differences between the sands' effects on MS2 removal were attributed to the presence of organic coatings on the used sand. This reduced the adsorptive capacity of the sand and its ability to enhance inactivation. In agitated experiments with used sand, MS2 concentrations after two months were significantly higher than for those without sand (Figure 5b). This suggests that MS2 may have been protected from inactivation when in the presence of organic matter. A mass balance completed for the column experiments indicated that the new sand was capable of achieving 2.1–2.6 log-removal while columns with used sand achieved only 0.49–0.95 log-removal. This study concluded that viruses may be capable of traveling long distances and remain active for many months in groundwater at cold temperatures and when organic coatings exist on the porous materials.

3.3 Paper III

The effects of sand and organic matter on virus inactivation at low temperatures: comparing models of constant and time-dependent inactivation while considering the uncertainty of measured virus concentrations

In this study, we focused on the results from the static batch experiments presented in Paper II. However, the experiments also considered the added effects of IS and DOC on virus inactivation. Experimental data was fit with models representing constant (2) and time-dependent inactivation (4) using both least-squared optimization and GLUE methodologies. Modeling results were then compared to one-another in order to examine the influence of data uncertainty on model parameter estimations within the different model structures.

Results from the experiments showed that the new sand enhanced virus inactivation in solutions with low levels of DOC (Figure 6; *Figure 1 in Paper III*). However, the enhancing effects of the new sand were almost nullified when virus inactivation was examined in solutions with high levels of DOC (figure not shown; *Figure 1 in Paper III*). This may have been due to adsorption of the DOC to new sand thus decreasing the enhancing effects of the iron-oxides on the sand's surface and/or the DOC enabling the formation of virus complexes which may have protected the MS2 from inactivation. IS did not appear to have any effect on virus inactivation (figure not shown; *Figure 1 in Paper III*).

Results from the least-squares optimization indicated that most experiments were better represented by a time-dependent model of inactivation (figure not shown; *Figure 1 in Paper III*). Results from GLUE, which directly accounted for uncertainty in the MS2 concentrations during parameter estimation, indicated that both constant and time-dependent representations of inactivation were capable of adequately fitting the data (Figure 6). However, results from GLUE also indicated that a time-dependent representation of virus inactivation is more likely when modeling MS2 inactivation in the presence of the used sand (figure not shown; *Figure 2 in Paper III*). This indicates that environmental factors may contribute to the time-dependency of virus inactivation in soil-water systems.

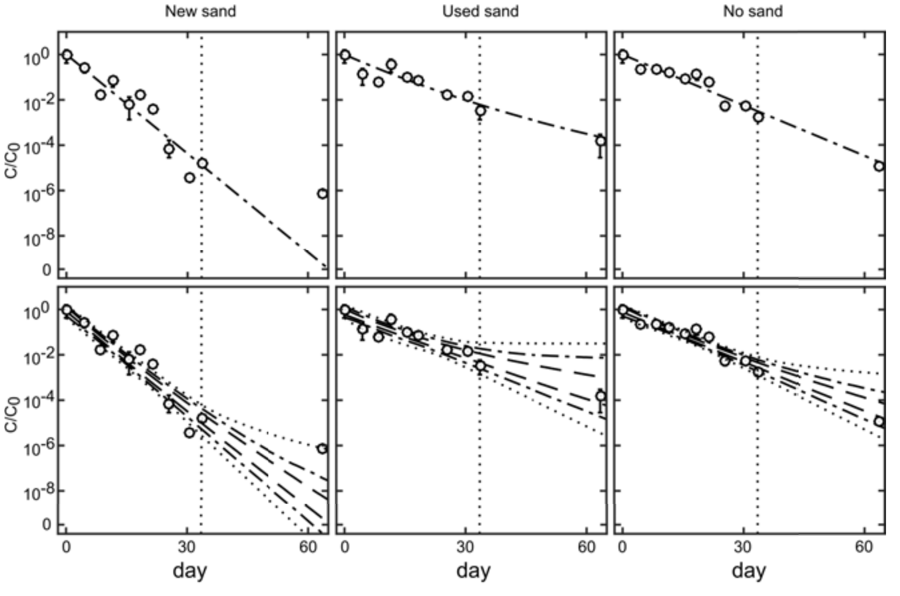

Figure 6. Truncated set of experimental results showing time-series concentrations of MS2 (*white circles*) and data uncertainty (*black bars*) for static inactivation experiments with new, used, and no sand in low IS, low DOC water. **Top row**: optimum models from the least-squares optimization model fitting (*dot-dashed lines*) fit using the first month of data (*vertical dotted lines*). **Bottom row**: 100% (*dotted lines*), 90% (*dot-dashed lines*), and 50% (*dashed lines*) prediction intervals achieved from the GLUE model fitting using the first month of data. The figure is a modified version of Figures 1 and 3 in Paper III.

The ensembles of behavioral models attained from GLUE were capable of capturing MS2 concentrations after two months in spite of the models being calibrated against only one month of data (Figure 6, bottom row; *Figure 3 in Paper III*). Most models fit using a least-squares optimization approach were unable to capture MS2 concentrations at two months (*figure not shown; Figure 1 in Paper III*). The study concluded that decisions regarding the appropriate structure of an inactivation model become more nuanced as data uncertainty will directly influence the criteria within which that decision is based. The study also concluded that investigations of virus inactivation at cold temperatures should be conducted for a period of time longer than one month in order to get a reliable estimate of the parameters in inactivation models.

3.4 Paper IV

Fate and transport of virus in infiltration basins and the importance of data uncertainty in modeling

In this study we focused on the column experiments presented in Paper II. However, this study also considered the tails of the MS2 breakthrough curves which were measured after the injection of the virus pulse had ended. This was done in order to provide more information on adsorption kinetics. The added effects of IS and DOC on virus transport in the sand columns were also considered. Building on the modeling methods used in paper III, experimental data were fit with a virus fate and transport model (6–8) using both least-squares optimization and GLUE methodologies. Modeling results were then compared to one-another in order to examine the influence of data uncertainty on model parameter estimations.

Results from the experiments showed that only the type of sand in the columns (new vs. used) had a consistent effect on virus removal. Changes in IS and DOC between experiments appeared to have no effect on virus removal (Figure 7 and *Figure 3 in Paper IV*). Model fitting was completed in two steps: first, the models presented in (5) and (6) were fit to breakthrough data from a conservative salt tracer (Figure 7) in order to estimate the dispersion coefficient D; then, this value of D was used in (7) and (8) which were then fit to MS2 breakthrough data in order to estimate the virus transport parameters k_{att}, k_{det}, and μ_s.

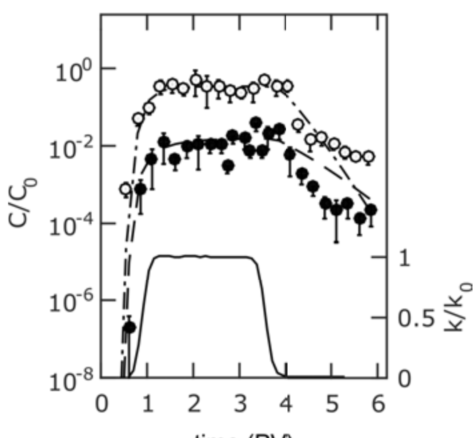

Figure 7. Truncated set of experimental results showing time-series concentrations of MS2 for column experiments with new (*black circles*) used sand (*white circles*) in low IS, low DOC water. Data uncertainty is indicated by the *black bars*. Data are plotted along with model fits from the least-squares parameter optimization (*dashed lines*). Results from the salt tracer data are plotted on the secondary axis (*solid black line*). Modified from Figure 3 in Paper IV.

Modeling results showed that the uncertainty around estimates of D, predicted from the GLUE framework, were larger than those predicted through the least-squares optimization (figure not shown; *Figure 4 in Paper IV*). This indicates that estimates of the dispersion coefficient can have a relatively high amount of uncertainty even in controlled laboratory experiments using homogeneous sand.

Results from the least-squares parameter optimization showed relatively poor model fits especially in the tails of the breakthrough curves after the MS2 pulse had ended (Figure 7 and *Figure 3 in Paper IV*). Unrealistically large confidence intervals were reported for all of the parameter estimates (table not shown; *Table 3 in Paper IV*). Models fit using GLUE reported realistic estimates of the uncertainty surrounding the attachment coefficient k_{att} (table not shown; *Table 5 in Paper IV*). The best performing behavioral models from GLUE showed an improvement in capturing the tailing behavior of the MS2 breakthrough curves for some of the datasets (Figure 8 and *Figure 6 in Paper IV*). However, large uncertainties remained around estimates of the detachment coefficient k_{det} and the adsorbed virus inactivation rate μ_s (figure not shown; *Figure 5 in Paper IV*).

The 95% prediction intervals estimated using the GLUE methodology were able to capture MS2 concentrations in the tails of the breakthrough curves but were not able to capture the peak concentrations (Figure 8 and *Figure 6 in Paper IV*). Results of this study show that virus transport parameter estimates from column studies should be used with caution when applied to investigations of virus transport in other settings as data uncertainty can result in substantial uncertainty surrounding parameter estimates.

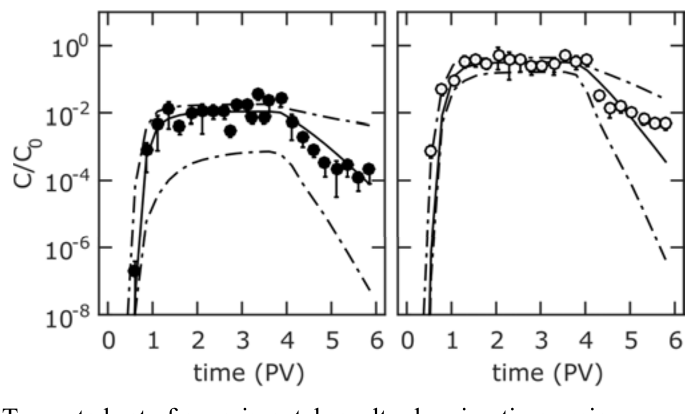

Figure 8. Truncated set of experimental results showing time-series concentrations of MS2 for column experiments with new (*black circles*) used sand (*white circles*) in low IS, low DOC water. Data uncertainty is shown as the *black bars*. Data are plotted along with the best performing model from achieved using GLUE (*solid black line*) along with the 95% prediction intervals (*dot-dashed lines*). Modified from Figure 6 in paper IV

4. Discussion

4.1 Treatment effects on virus removal

The principles and assumptions described section 2.7 – "*Key experimental assumptions*" acted as a foundation for which much of the following discussion was built on. To summarize: we assumed that the primary removal mechanism in the static batch experiments was inactivation; removal in the agitated batch experiments was due to both adsorption and inactivation; removal in the column experiments was due almost exclusively to adsorption. We also assumed that adsorption of MS2 to the glass of the batch reactors and columns was negligible and that the only difference between the new and used sand was the chemical characteristics described in Table 1.

4.1.1 Treatment effects on virus inactivation

Inactivation of MS2 was enhanced in the presence of the new sand in solutions with low levels of DOC (Figure 5 and *Figure 1 in Paper III*). This was likely due to iron oxides on the surface of the new sand. Both the new and used sand contain more iron than any of the other metals present in the sand (Table 1). Enhanced inactivation of MS2 in the presence of iron is a well-established phenomenon (Kim et al., 2011; Ryan et al., 2002; You et al., 2005). However, enhanced inactivation was not observed in the presence of the used sand (Figure 5). This was due to the presence of organic coatings on the sand. The organic carbon content of the used sand was significantly higher than that of the new sand due to the used sand's prolonged exposure to water from the Fyris River water (Table 1). Organic matter is readily adsorbed to iron surfaces (Gu et al., 1994; Korshin et al., 1997), and these organic coatings can reduce the physiochemical effects of the iron on viruses (Gu et al., 1994). This explains why inactivation was enhanced in the presence of the new sand. However, in solutions with a high level of DOC, the enhancing effects of the new sand were lost (figure not shown; *Figure 1 in Paper III*). Similar to the explanation above, this was likely due to the DOC in the solution having adsorbed to the iron surfaces on the new sand thus reducing their virucidal effects. This was deduced because the inactivation of MS2 was not affected by DOC in experiments without sand.

In the agitated batch experiments, removal was assumed to be due to both adsorption and inactivation mechanisms. However, in experiments with used sand, it was apparent that virus removal was due entirely to inactivation as time-series concentrations of MS2 in these experiments were similar to those in experiments without sand (Figure 5b and Appendix Figure A1). Furthermore, concentrations of MS2 in experiments with used sand were considerably higher at two-months when compared to experiments in the same solution without used sand. This phenomenon was also observed in experiments with no sand when examining the effects of DOC. In solutions with a high level of DOC, MS2 concentrations at two months were considerably higher than those in experiments with a low level of DOC. This suggests that the of NOM may have protected the MS2 from inactivation.

Prolonged virus survival due to the presence of NOM has been observed in soil—water systems (Bradford, Tadassa, and Jin 2006; Foppen, Okletey, and Schijven 2006; Moore et al. 1982). In these studies, reduced removal of virus was attributed, in part, to the NOM protecting virus from inactivation. However, these studies did not comment on the specific mechanisms responsible for prolonging virus survival. Bixby and O'Brien (1979) examined the adsorption MS2 in batch experiments as affected by the addition of fulvic acid (a form of NOM). They found that experimental results were different depending on the order with which the virus, acid, and soil were mixed in the batch reactors. Results showed that virus—NOM complexes were formed when the NOM and virus were mixed prior to being introduced to the batch reactors with the soil. This resulted in reduced removal of virus through inactivation. Complexation of virus with NOM has been shown to enhance virus aggregation (Gutierrez and Nguyen, 2012) which, in turn, can reduce inactivation rates of virus in water (Grant, 1995). Results from our experiments appear to suggest that the formation of virus—NOM complexes may have played a role in prolonging virus survival in agitated batch experiments.

In the static batch experiments, inactivation of MS2 in solutions without sand was not affected by DOC (figure not shown; *Figure 1 in Paper III*). This suggests that agitation played a role in the prolongation of MS2 in the batch experiments. In the agitated experiments, mass transport of virus was not limited only to diffusion due to the constant mixing. This means that virus-to-NOM and virus-to-virus interactions were more frequent. For agitated batch experiments in solutions with a high level of DOC the formation of virus—NOM complexes was more likely; and, due to the higher frequency of virus-to-virus interactions due to mixing, this may have enhanced virus aggregation thus creating a subpopulation of viruses more resistant to inactivation.

In all experiments, IS had no effect on MS2 inactivation. This is not surprising as no consistent effect of IS on virus inactivation has been observed, so any affect is likely specific to the virus being investigated (Jin and Flury, 2002).

4.1.2 Treatment effects on virus adsorption

Adsorption of MS2 in both the agitated batch experiments and column experiments was highest in the presence of the new sand. In agitated batch experiments, removal was due to both adsorption and inactivation mechanisms. However, the primary mechanism responsible for removal of MS2 in agitated batch experiments was highly dependent on the type of sand in the batch reactors. As previously mentioned, removal in batch experiments with used sand was due entirely to inactivation. However, in experiments with new sand, the MS2 concentration time-series closely resembled those characteristic of equilibrium adsorption experiments (Schijven and Hassanizadeh, 2000). Results for these experiments showed a sharp decline in MS2 concentration during the first one or two measurements. This initial drop was then followed by a slow but consistent drop in MS2 concentration to the end of the experiment (Figure 5 b and Appendix Figure A1). The slopes of the MS2 concentration time-series after the initial drop closely resembled those observed in experiments without sand. The sharp initial decline in MS2 concentration was likely due almost entirely to adsorption whereas virus removal in the latter part of the experiments was primarily due to inactivation of MS2. These results suggest that the new sand provided conditions which were conducive for adsorption. This was likely due to the presence of iron oxides on the new sand's surface that were not covered by natural organic matter.

Iron oxides have an isoelectric point of 7–9 (Kosmulski, 2011) and MS2 has an isoelectric point of 3.9 (Overby et al., 1966). This means that MS2 will have a strong negative charge and the iron oxide on the surface of the new sand will have a near neutral or a positive charge at the pH that the experiments were conducted at (pH 8). Thus, according to DLVO theory, the electrostatic forces between MS2 and the iron oxide surfaces may be only slightly or even attractive (Verwey and Overbeek, 1948). Bacteriophage MS2 has been shown to readily adsorb to soils containing iron in batch experiments (You et al., 2005) as has poliovirus (Moore et al., 1981).

In agitated batch experiments with high levels of DOC and new sand the initial drop in MS2 concentrations were not as steep as those observed in experiments with new sand and low levels of DOC (Appendix Figure A1). Under the assumption that these experiments are similar to equilibrium adsorption experiments, this would indicate that time to equilibrium was slowed in the presence of the DOC. A number of studies postulate that this effect is caused by the adsorption of the DOC onto viable adsorption sites which would thus reduce the amount of virus capable of being adsorbed onto the soil (Blanford et al., 2005; Pieper et al., 1997; Ryan et al., 1999; Walshe et al., 2010; Wong et al., 2013). However, a study by Bixby and O'Brien (1979) suggested that this effect may be caused by the formation of virus—NOM complexes which are less favorable for adsorption than the virus itself.

In the present study, it is unclear exactly what mechanism(s) were responsible for slowing the time for MS2 to reach equilibrium in experiments with high levels of DOC and new sand.

In agitated batch experiments with the used sand it appeared as if inactivation was the only mechanism responsible for removal of MS2. This was likely due to the presence of organic coatings on the used sand which blocked adsorption sites and nullified the adsorption enhancing effects of the iron present on the sand's surface. As previously mentioned, organic coatings were formed on the used sand due to it having been exposed to infiltrating surface water from the Fyris River. Fuhs et al. (1985) examined the adsorption of Coxsackievirus to mineral surfaces in batch experiments. They found that adsorption of virus was reduced when investigated in the presence of soil with organic coatings resulting from prolonged exposure to infiltration water. The organic coatings were speculated to have blocked adsorption sites.

In column experiments, it was assumed that the primary removal mechanism was adsorption as the duration of the experiments was too short (about three hours) to observe any changes in MS2 concentrations due to inactivation. Results for the column experiments were similar to those for the agitated batch experiments in that removal of MS2 was much lower in the presence of the used sand. Similar to the agitated batch experiments, reduced adsorption of MS2 to the used sand was attributed to the presence of organic coatings. Zhuang and Jin (2003b) conducted a similar study and found that mineral-associated organic matter significantly reduced the amount of MS2 removed in the column due to adsorption. A field study by Ryan et al. (1999) found that the presence of adsorbed organic matter enhanced the transport of PRD1 in an iron oxide-coated sand aquifer. They attributed this to reduced adsorption of the virus due to blocking of adsorption sites by organic matter.

Adsorption of MS2 in column experiments was not affected by DOC (figure not shown; *Figure 1 Paper IV*). Cao et al. (2010) and Zhuang and Jin (2003a) investigated the transport of MS2 in sand columns and found that the addition of soluble organic matter coincided with decreased adsorption of MS2. However, a similar study by Cheng et al. (2006) found that DOC had no effect on MS2 adsorption. In all of these studies, the present one included, the organic matter used to represent changes in the concentration of DOC were all different from one another. Cao et al. (2010) used a combination of glutamic acid, dextrose, and Algal Cultur Formula (Kent®), Zhuang and Jin (2003a) used a natural humic acid from the IHSS, Cheng et al. (2006) used class B biosolids from a wastewater treatment plant, and the present study used Nordic Reservoir natural organic matter from the IHSS. This would suggest that the effects of DOC on MS2 adsorption may be highly dependent on the type of organic matter used to represent DOC in the experiment. However, it seems more likely that the non-effect of DOC in the present study was due to the relatively small change in the concentration of DOC between the low and high levels.

The study conducted by Cao et al. (2010) examined solutions with 0 to 800 mg l^{-1} of chemical oxygen demand (COD). This amounts to a change in DOC concentrations between the low and high level of about 250 mg l^{-1} (Dubber and Gray, 2010). In Zhuang and Jin (2003a), DOC concentrations varied from 1 to 50 mg l^{-1} DOC. In the present study, DOC concentrations were varied between 17 and 31 mg l^{-1}. These values were reflective of the average winter concentration and the maximum observed concentration of DOC in the infiltration water, respectively (Figure 2b). This amounts to a 14 mg l^{-1} change in DOC concentration between the low and high levels. Therefore, it seems likely that the reason why results from the column studies did not appear to be dependent on DOC was that the difference between the low and high levels of DOC was not large enough to prompt any noticeable effects.

Ionic strength (IS) had no consistent effect on virus adsorption in the agitated batch (Apendix Figure A1) and column experiments (figure not shown; *Figure 3 in Paper IV*). According to DLVO theory, as IS increases so should the propensity for virus adsorption. Chu et al. (2000) investigated MS2 removal in sand columns as affected by changes in ionic strength and found that as IS increased so did the adsorption of MS2. However, similar to the reasoning explaining the non-effect of DOC on virus removal in the column experiments, the non-effect of IS on virus adsorption was likely due to the relatively small difference between the low and high levels of IS in the present study. Chu et al. (2000) found that retention of MS2 was enhanced beginning at an IS of 30 mM and continued to be enhanced until the IS was at about 100 mM. In the current study IS only varied between 7 and 8.6 mM in order to reflect the conditions at the Tunåsen infiltration basin (table not shown; *Table 2 in Paper III*). This change in IS was likely too small to prompt any noticeable effects.

4.2 Least-squares parameter optimization vs. GLUE

The modeling of virus inactivation and virus fate and transport was the focus the focus of Papers III and IV respectively. In each paper, model parameters were estimated using both a traditional least-squares optimization approach and the GLUE methodology. This was done in order to examine how data uncertainty would affect model parameter estimates and model predictions.

The uncertainty of the MS2 concentration data was considered when performing parameter estimation in GLUE for both the inactivation and fate and transport models. Uncertainty bounds surrounding the MS2 concentration data for both the static batch (Figure 6) and column experiments (Figure 7) were within the ±0.5 $\log_{10}$ CFU ml^{-1} range typical of microbial data (Corry et al., 2007). In models attained using least-squares optimization, these uncertainty bounds were only used to help visualize how well a model was able to capture the MS2 concentration data. If a model passed between the upper and lower uncertainty bound then the model was said to capture that data point. However, the uncertainty of the data was not considered when minimizing the squared errors in the optimization. Instead, errors between the model and measurements were calculated using average MS2 concentrations calculated from the replicate measurements at each time-step. Model performance was assessed using the root-mean-square error (RMSE) which was also calculated using average concentration values.

In GLUE, uncertainty bounds around the MS2 data were considered during parameter estimation by using them in the assessment of model performance. In GLUE, model performance was calculated using the RMSE as well, but the equation was slightly modified in order to account for uncertainties in the MS2 concentrations. The modified RMSE assumed that the true-value of the MS2 concentration at any given time-step was equally likely between the upper and lower uncertainty bound. Under this assumption the squared error was calculated relative to the upper and lower bounds rather than from a single point. The model acceptance threshold in the GLUE methodology was set to the RMSE value achieved by the least-squares optimization. This means that the threshold for behavioral models was different for each set of experimental data. By doing this we assumed that any model which performed as well as or better than the model fit using least-squares optimization is behavioral. This means that parameter sets which produced a behavioral model in GLUE were considered to be at least as likely as the parameter set achieved using least-squares optimization.

In the fitting of the inactivation models using GLUE, we only considered the uncertainty of the MS2 concentrations when estimating the inactivation rate μ for constant inactivation models (2) and the initial inactivation rate μ_0 and resistivity coefficient α in time-dependent models (4). In the fitting of the fate and transport model (6–8) using GLUE, we considered uncertainties in the porosity n, dispersion coefficient D, liquid virus inactivation rate μ_l, and the MS2 concentrations. Results regarding the uncertainty of μ from the fitting of the constant inactivation rate models using GLUE were used to set the upper and lower sampling limits for μ_l.

4.2.1 Inactivation models

Models of constant (2) and time-dependent inactivation (4) were fit to the experimental data from the static batch experiments using the first month of data only. Data points at the two-month mark were used to assess the predictive power of the models to see if one month of inactivation data were sufficient enough to provide models capable of predicting future MS2 concentrations.

Results from the least-squares approach showed that some data-sets were best represented by constant inactivation models while others were best represented by time-dependent inactivation models (figure not shown; *Figure 1 in Paper III*). However, most of the experimental data were best explained by a model of time-dependent inactivation which suggests that time-dependent models are more representative of MS2 inactivation in soil—water systems at 4°C. This reflects conclusions made in a similar study by Chrysikopoulos and Aravantinou (2012). In the present study, the experimental treatments (IS, DOC, and the sand type) had no consistent effect on parameter estimates or the model structure that best fit the data when using least-squares optimization (table not shown; *Table 4 in Paper III*). All of the models estimated using least-squares optimization did a poor job of predicting the MS2 concentration at two months (figure not shown; *Figure 1 in Paper III*).

Results from the GLUE methodology indicated that both the constant and time-dependent inactivation model structures were capable of representing the data for every experimental dataset except for one (figure not shown; *Figure 2 in Paper III*). This shows that once uncertainty in the MS2 concentration data was accounted for in the assessment of model performance conclusions regarding the time-dependency of virus inactivation will become less clear. However, the GLUE results indicated that it was more likely that a model of time-dependent inactivation was better suited for datasets examining MS2 inactivation in the presence of the used sand as well as for datasets examining MS2 inactivation in new sand with high levels of DOC (figure not shown; *Figure 2 in Paper III*). This suggests that experimental conditions may affect the time-dependency of virus inactivation. More specifically, the presence of organic matter will cause viruses to inactivate in a more time-dependent manner. This was not observed when examining results from the least-squares optimization.

In the model of time-dependent inactivation (4) used in the present study the resistivity coefficient α can be thought of as a measure of how sensitive different sub-populations of virus are to inactivation (Sim and Chrysikopoulos, 1996). As α increases so does the number of inactivation resistant viruses which results in prolonged virus survival. Chrysikopoulos and Aravantinou (2012) suggested that subpopulations of virus may be due to different sizes of virus aggregates which has been shown to promote kinetic inactivation of virus in soil—water systems (Grant, 1995). The results of the model fitting using GLUE indicated that time-dependent models of inactivation were more likely when models were fit to MS2 inactivation data from experiments in the presence of the used sand. This was also observed for experiments with a high level of DOC in the presence of the new sand. In the previous section, it was speculated that prolonged virus survival in the presence of NOM may be a result of enhanced aggregation of viruses in solution caused by the formation of virus—NOM complexes. This too would explain why MS2 inactivation would tend to behave in a more time-dependent manner in the presence of NOM as enhanced virus aggregation would result in resistant subpopulations of MS2.

The 100% prediction intervals estimated using the GLUE methodology were able to capture MS2 concentrations at two months for all experiments (figure not shown; *Figure 3 in Paper III*). This suggests that the ensemble modeling approach used in GLUE is better suited for prediction purposes than the least-squares optimization model fitting approach. An ensemble modeling approach to virus inactivation would be particularly useful for estimating virus concentrations at the field-scale scale experiments when virus transport times may exceed time-scales used to estimate virus inactivation rates.

The modeling results in this study suggest that one-month of MS2 inactivation data were not sufficient enough to provide models with predictive capabilities when investigating MS2 inactivation at 4°C. In both model fitting frameworks (least-squares optimization and GLUE) the best performing models did a poor job of capturing the MS2 concentration at two months (figures not shown; *Figures 1 and 3 in Paper III*). This was especially evident when examining the predictive power of the time-dependent inactivation models. In order for models to adequately represent the time-dependency of virus inactivation, time-series should be long enough to sufficiently capture the time-rate of change of the inactivation rate. For example, when examining data gathered from the experiment in used sand with low IS and high DOC, virus inactivation appears to almost stop after the first month of the experiment. Models that were fit to these data reflected this behavior. However, the MS2 concentration at two months showed that virus inactivation continued as this data point was substantially lower than that at the one-month mark. This means that the models that were fit using only the first month of data over predicted the MS2 concentration at two months by a large margin. For this reason we suggest that experiments which examine virus inactivation in cold temperatures (around 4°C) should be conducted for at least two months especially when experimental data are to be used to fit models of time-dependent inactivation.

4.2.2 Fate and transport models

The virus fate and transport model (6–8) was fit to the MS2 concentration data from the column experiments using least-squares optimization and GLUE. Models were fit in two steps: first, the data from a conservative salt tracer were used to estimate the dispersion coefficient D for the model; then the estimate of D along with the estimates of the liquid inactivation rates μ_l from Paper III were used to estimate the attachment k_{att}, detachement k_{det}, and adsorbed inactivation rates μ_s for models fit to each set of experimental data.

Results from the least-squares optimization found that the estimated dispersion coefficients D in columns with new sand were slightly lower than that estimated in columns of used sand (table not shown; *Table 1 in Paper IV*). This was due to the used sand having a slightly larger proportion of fine materials than the new sand. Models fit to the salt tracer data performed well for both the new and used sand. The 95% confidence intervals estimated for D were also relatively narrow. In columns with new sand, values of k_{att} were higher than those estimated in columns of used sand. Values of k_{det} and μ_s were unaffected by the type of the sand. None of the parameter estimates (k_{att}, k_{det}, and μ_s) were affected by levels of IS or DOC. The 95% confidence intervals around all parameter estimates were unrealistic in most cases as they were beyond the bounds of what were considered as possible maximum and minimum values. All of the models fit to the MS2 data using least-squares optimization did a poor job of fitting the tailing portions of the breakthrough curves (Figure 7 and *Figure 3 in Paper IV*).

The GLUE methodology of parameter estimation used the same general procedure as that used for the least-squares optimization when estimating the dispersion coefficient D. However, GLUE considered the uncertainties surrounding the column porosity n, and therefore the pore-water velocity v, when estimating D. Results from GLUE reported estimates of uncertainty surrounding D that were larger than the 95% confidence intervals estimated from the least-squares optimization (figure not shown; *Figure 4 in Paper IV*). This was due to the uncertainties surrounding in n and v. This is similar to results found by Zhang et al. (2006) who compared least-squared optimization techniques to GLUE in the estimation of D when investigating the breakthrough of bromide in soil columns. This demonstrates that parameter estimates of D can have a relatively high degree of uncertainty even in highly controlled laboratory experiments.

Within GLUE, the behavioral models which performed the best (lowest RMSE) showed an improvement in capturing MS2 concentrations in the tails of the breakthrough curves for half of the experiments (Figure 8 and *Figure 6 in Paper IV*). For these models, the values of k_{att} associated with the est performing models were similar to those estimated using least-squares parameter optimization. However, the uncertainty bounds for k_{att}, estimated using GLUE, were much smaller than those estimated using least-squares optimization and coincided with a realistic range of values (table not shown; *Table 5 in Paper IV*). Large confidence intervals around parameter estimates, like those estimated from the least-squares optimization, indicate that model predictions are not sensitive to changes in the parameter value. Large confidence intervals around estimates of k_{att} are likely a result of the least-squares optimization failing to account for uncertainties in the dispersion coefficient D.

Schijven and Hassanizadeh (2000) performed an analysis of simulated breakthrough curves modeled using the same fate and transport model shown in (6–8). They found that similar shapes of virus breakthrough curves between the time of breakthrough up to the point of maximum concentration could be achieved by changing either D or the ratio of k_{att} and k_{det}. When data were fit with models using least-squares optimization in the present study, the value of D was held constant. This means that the ratio of k_{att} and k_{det} was used to fit the leading edges of the breakthrough curves. The fitted models had relatively poor performance leading to large standard errors around the parameter estimates thus resulting in unrealistically large confidence intervals. In the GLUE framework, this problem was addressed by accounting for the uncertainty of D which allowed for less variation in the ratio of k_{att} and k_{det} in the search for behavioral models. This demonstrated that GLUE can be useful for estimating realistic uncertainty bounds around parameter estimates even when model performance is relatively poor.

The GLUE methodology indicated that parameter estimates for the detachment rate k_{det} and adsorbed inactivation rate μ_s were highly uncertain as behavioral models existed for every value (table not shown; *table 5 in Paper IV)*. Results from GLUE also indicated that behavioral values of both parameters existed well outside of the sampling ranges (figure not shown, *Figure 5 in Paper IV*). This was due to the poor performance of models in predicting MS2 concentrations in the tails of the breakthrough curves.

Evidence of kinetic adsorption is found by investigating the tailing behavior of breakthrough curves after the virus pulse has ended (Powelson et al., 1990). In (7) and (8), virus concentrations in the breakthrough curve tails are predicted based primarily on values of k_{det} and μ_s (Schijven and Hassanizadeh, 2000) where the slope of the tail is determined by μ_s and the point of inflection is determined by k_{det}. Results from both the least-squares optimization and GLUE methodology showed that the majority of the models fit to the column data poorly described the tails of the MS2 breakthrough curves. In order to increase model performance in these areas, MS2 concentrations in the tails should have been measured for a longer period of time and/or with a higher density of measurements such that performance metrics would have been more heavily weighted by prediction errors in the tails. Within the conventional least-squares approach, more measurements in the tails would have resulted in a steeper gradient in the k_{det} and μ_s parameter space towards the global minimum of the objective function. This would have increased the sensitivity of model predictions to changes in k_{det} and μ_s thus reducing their uncertainty. Within GLUE, more measurements in the tails of the breakthrough curves would limit the range of behavioral parameter sets by increasing the sensitivity of the model performance metric to predictive errors in the tails. Our study shows that neither the least-squares optimization nor the GLUE approach to parameter estimation is capable of producing estimates of k_{det} and μ_s with a high degree of certainty if virus breakthrough curves do not thoroughly investigate the tailing behavior of virus breakthrough curves.

The 95% prediction intervals estimated using GLUE were able to capture most of the MS2 concentration data for the column experiments (Figure 7 and *Figure 6 in Paper IV*). However, behavioral models were only able to capture the maximum measured concentration MS2 concentration for two of the experiments. The ensemble of behavioral models also showed that predictions of MS2 concentrations in the tails of the breakthrough curves were highly uncertain. This was because of the large uncertainty surrounding estimates of k_{det} and μ_{s}. The prediction intervals also illustrated the wide range of MS2 concentrations capable of being predicted using models that perform at least as well as those achieved using least-squares optimization techniques. This was a direct effect of GLUE having accounted for uncertainty in the MS2 concentration data when assessing model performance. For most experiments, the prediction intervals around the tails of the breakthrough curves were too large to provide any meaningful predictions of liquid MS2 concentrations resulting from virus detachment. However, prediction intervals surrounding the leading edge of the breakthrough curves were relatively narrow suggesting that estimates of the virus attachment rate k_{att} and the time to peak concentration are relatively reliable.

5. Conclusions

This thesis examined the removal of bacteriophage MS2 as affected by ionic strength (IS), dissolved organic carbon (DOC), and the age of sand used in at the Tunåsen basin infiltration managed aquifer recharge (MAR) scheme in Uppsala, Sweden. This was done by analyzing time-series of MS2 concentration data taken from bench-scale inactivation and column experiments. Bacteriophage MS2 was used as a proxy for enteric viruses. Experiments were designed to examine virus removal at the Tunåsen MAR scheme during winter months when cold temperatures will reduce the inactivation rate of enteric viruses. The experimental work led us to the following conclusions:

- Sand used in the infiltration basins accumulates organic matter from infiltrating surface waters.
- Organic coatings on the sand reduce adsorption of enteric virus in the infiltration basins.
- Inactivation rates of enteric viruses slow significantly in the presence of both soluble and adsorbed natural organic matter.
- The presence of natural organic matter (NOM) reduces the value of the infiltration basin as a microbial barrier.

Experimental data from the batch and column experiments were used to fit models describing the fate and transport of viruses in soil—water systems. Models were fit using conventional least-squares optimization techniques as well as the generalized likelihood estimation (GLUE) in order to determine how data uncertainty may affect parameter estimations and model predictions. The modeling showed that:

- Enteric viruses tend to inactivate in a time-dependent manner when in the presence of soluble and adsorbed organic matter. This could be due to the formation of inactivation-resistant subpopulations of viruses.
- Virus attachment rates decrease as porous materials develop organic coatings.
- Several different models representative of inactivation and virus transport in soil-water systems are able to adequately describe virus behavior once data uncertainty is considered.

- Studies of virus inactivation at cold temperatures need to examine virus inactivation for periods of time longer than one-month in order to improve the predictive capacity of models.
- Virus breakthrough curves should be observed for longer periods of time in the absence of a virus tracer in order to achieve models which can adequately represent the adsorption kinetics of viruses. The density of the data in the tailing portions of the virus breakthrough curves should be at least as high as that along the leading edge.

Overall, this thesis has shown that water managers and researchers need to account for the effects of NOM on virus removal mechanisms when examining MAR as an alternative to conventional methods of drinking water treatment. This thesis has also shown that uncertainties in experimental data will affect the conclusions drawn from modeling. The GLUE methodology is an intuitive and powerful way of incorporating data uncertainties in modeling. This thesis demonstrated that GLUE is a viable alternative to conventional least-squares parameter estimation.

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7. Sammanfattning på svenska

Det har uppskattats att 1,4 miljoner barn dör varje år av diarrésjukdomar relaterade till vattenburna, sjukdomsbärande patogener. Dessa sjukdomar skulle kunna förhindras. En specifik klass av virus, enterovirus är ansvarig för många av dessa sjukdomsutbrott. Även om de flesta sådana dödsfall inträffar i utvecklingsländer är sjukdomsutbrott orsakade av enterovirus relativt vanliga även i utvecklade länder. När enterovirus förorenar brunnar och ytvatten beror det på mänskliga fekalier. De vanligaste källorna till fekal förorening av sötvatten i höginkomstländer är läckande septiktankar och förorenade avlopp nedströms vattenreningsverk. Dricksvattenförbrukningen i höginkomstländer är relativt stor eftersom kostnaderna för framställning och distribution av dricksvatten är liten jämfört med andra hushållskostnader. Resultatet är att stora mängder avloppsvatten produceras dagligen. I höginkomstländer är det en uppgift och utmaning för vatten- och avloppsförvaltningen att balansera behovet av rent och säkert dricksvatten med en ständigt ökande mängd avloppsvatten. Konstgjord infiltration är en viktig del av lösningen på detta problem.

Konstgjord infiltration är en allmän term som beskriver förstärkning av grundvattenresurser genom mänskliga ingrepp. Konstgjord infiltration avser ofta en process där ytvatten avleds till en markbädd genom direkt injektion eller ytinfiltration. Virus avlägsnas normalt genom naturliga processer i grundvatten. Ett virus anses oskadliggjort när det inte längre kan infektera en person. Det finns två huvudsakliga mekanismer genom vilka virus oskadliggörs under transporten i grundvattnet: adsorption och inaktivering. Adsorption är den process där viruset fastläggs på ytan av det porösa materialet, t.ex. sand, genom vilken grundvattnet flödar. Inaktivering är den process där viruset oskadliggörs för sin värdorganism genom en kemisk förändring av virusets molekylära struktur. Syftet med denna avhandling var att undersöka de mekanismer som leder till att enterovirus oskadliggörs i samband med konstgjord infiltration. Undersökningen gjordes genom en kombination av laboratorieförsök och numerisk modellering.

Denna avhandling redogör för hur avlägsnandet av bakteriofagen (ett virus som angriper bakterier) MS2 påverkas av jonstyrka, löst organiskt kol, och den relativa åldern av sand som används i infiltrationsbassängen i ett system för konstgjord infiltration. Bakteriofagen MS2 användes eftersom den är representativ för andra enterovirus och har visat sig vara lämplig vid laboratorieförsök. Avlägsnandet studerades genom analys av tidsseriedata på

MS2-koncentrationer tagna från inaktiverings- och adsorptionsexperiment i laboratorium. Sanden och vattnet som användes i experimenten var samma som används i Tunåsens infiltrationsbassäng i Uppsala. Vid varje försök undersöktes en fullständig kombination av alla faktorer, d.v.s. effekterna av jonstyrka, löst organiskt kol, och sandens relativa ålder på oskadliggörandet av MS2-bakteriofagerna. Nivåerna på jonstyrka och löst organiskt kol var samma som observerade värden i det ytvatten som används för infiltrering vid Tunåsen under vintern. Effekten av sandens relativa ålder undersöktes med hjälp av "ny" sand som ännu inte hade använts för ytvatteninfiltration på Tunåsen, och med hjälp av "använd" sand, som hade använts för periodiskt återkommande infiltration under en period av åtta år. Alla experiment utfördes vid 4 °C för att undersöka hur virus oskadliggörs vintertid i infiltrationsbassängerna. Avhandlingens resultat och slutsatser är avsedda för praktiker och forskare inom VA-branschen som behöver veta hur virus avlägsnas i samband med konstgjord infiltration.

Laboratorieresultaten visade att infiltrationssandens ålder hade störst effekt på avlägsnandet av virus. Inaktiveringen av MS2 var större i den nya sanden än i den använda. Den större inaktiveringen i ny sand kunde förklaras av exponeringen mot järnoxider på sandens yta, järnoxider som täcktes av organiskt material i den använda sanden. Efter att periodvis ha använts i infiltrationsbassängen under åtta år innehöll sanden femton gånger mer organiskt kol än ny sand. Den organiska beläggningen på använd sand begränsade järnoxidens inaktiveringseffekt. För en delmängd av MS2-experimenten var inaktiveringen i använd sand påtagligt låg. Detta berodde på bildandet av virusanhopningar i det organiska materialet som skyddade MS2 från inaktivering. Resultaten visade att virus i närvaro av naturligt organiskt material kan fortleva i flera månader vid låga temperaturer i grundvatten.

Laboratorieresultaten visade att sandens ålder hade störst effekt också på virusadsorptionen. Adsorptionen av MS2 var störst i den nya sanden och minst i den använda. Även för adsorptionen kunde den större effekten i ny sand tillskrivas närvaron av järnoxider på sandens yta när den inte täcktes av organiskt material. Under förutsättning att adsorptionen kan beskrivas av Derjaguin-Landau-Verwey-Overbeeks (DLVO) teori tillhandahåller järnoxiden på den nya sandens yta platser för adsorption av MS2 på grund av elektrostatiska attraktionskrafter. Järnoxiden har emellertid också visat sig underlätta adsorption av naturligt organiskt material som blockerar adsorptionsplatser för MS2. Båda mekanismerna förklarar varför oskadliggörandet av virus p.g.a. adsorption var lägre i använd sand än i ny.

Matematiska modeller för inaktivering, transport och adsorption av virus i grundvatten anpassades till experimentella data. Modellparametrar skattades såväl med konventionell minsta kvadrat-optimering som med en metod för skattning av sannolik osäkerhet (Generalised Likelihood Uncertainty Estimation, GLUE). Experimentella data på virusinaktivering anpassades till två olika modeller: en som förutsatte konstant inaktivering och en där inaktiveringen antogs tidsberoende. Resultat grundade på minsta kvadrat-optimering visade att modellen med tidsberoende inaktivering gick lättast att anpassa till experimentdata. De olika experimentfaktorerna hade däremot inte någon tydlig påverkan på vilken modell som var bäst. När GLUE användes för parameterskattning tog modellresultaten hänsyn till osäkerheten i MS2-koncentrationerna. Resultaten visade att en modell med tidsberoende inaktivering var mer trovärdig när inaktiveringen skedde i närvaro av organiskt material. Nästan ingen modell där parameterskattningen skett med minsta kvadrat-metodik kunde förutsäga uppmätta MS2-koncentrationer utanför den tidsram där modellen kalibrerats. Däremot kunde modeller med parametervärden anpassade till angivna osäkerhetsintervall (GLUE) fånga upp koncentrationer utanför kalibreringsperioden. Detta tyder på att GLUE är bättre lämpad än konventionell metodik för parameteranpassning i virusprognosmodeller. Samtidigt tyder resultaten från båda parameterskattningsmetoderna att virusinaktivering behöver studeras under längre tidsperioder än en månad för att med hög tillförlitlighet kunna modellera inaktivering vid kalla temperaturer.

Experimentdata på virustransport genom sand i grundvatten användes för anpassning av en matematisk modell som beskriver den kinetiska adsorptionen av MS2. Modellresultat med såväl minsta kvadrat-anpassade som GLUE-anpassade parametrar pekade på att MS2-adsorptionen var påtagligt högre i ny än i gammal sand. Modellrealiseringar grundade på minsta kvadrat-anpassade parametrar kunde inte återge observerade genombrottskurvor för MS2. Modellrealiseringar med GLUE-parametrar var marginellt bättre men resultaten var fortfarande otillfredsställande. Om man vill bygga modeller som kan återge adsorptionskinetiken på ett tillfredsställande sätt bör genombrottskurvor för virus i strömmande grundvatten observeras under längre tid utan tillsats av spårämne för viruset.

Sammanfattningsvis visar avhandlingen att såväl praktiker som forskare inom VA-branschen bör ta hänsyn till effekterna av naturligt organiskt material på oskadliggörande av virus i anslutning till artificiell infiltration. Avhandlingen visar också att modeller av de studerade förloppen bör kalibreras med metoder som tar hänsyn till den påtagliga osäkerheten i experimentdata, t.ex. GLUE. Denna metodik tillhandahåller ett intuitivt och kraftfullt sätt att hantera osäkerheterna.

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Appendix

The majority of the results from the batch agitated batch experiments were not used in any of the papers. Paper II used the results of the experiments using low IS, low DOC solutions with new sand, used sand, and no sand only. Additional experiments were conducted which examined the effects of IS and DOC using a full-factorial experimental design. Results of these experiments are presented in Figure A1.

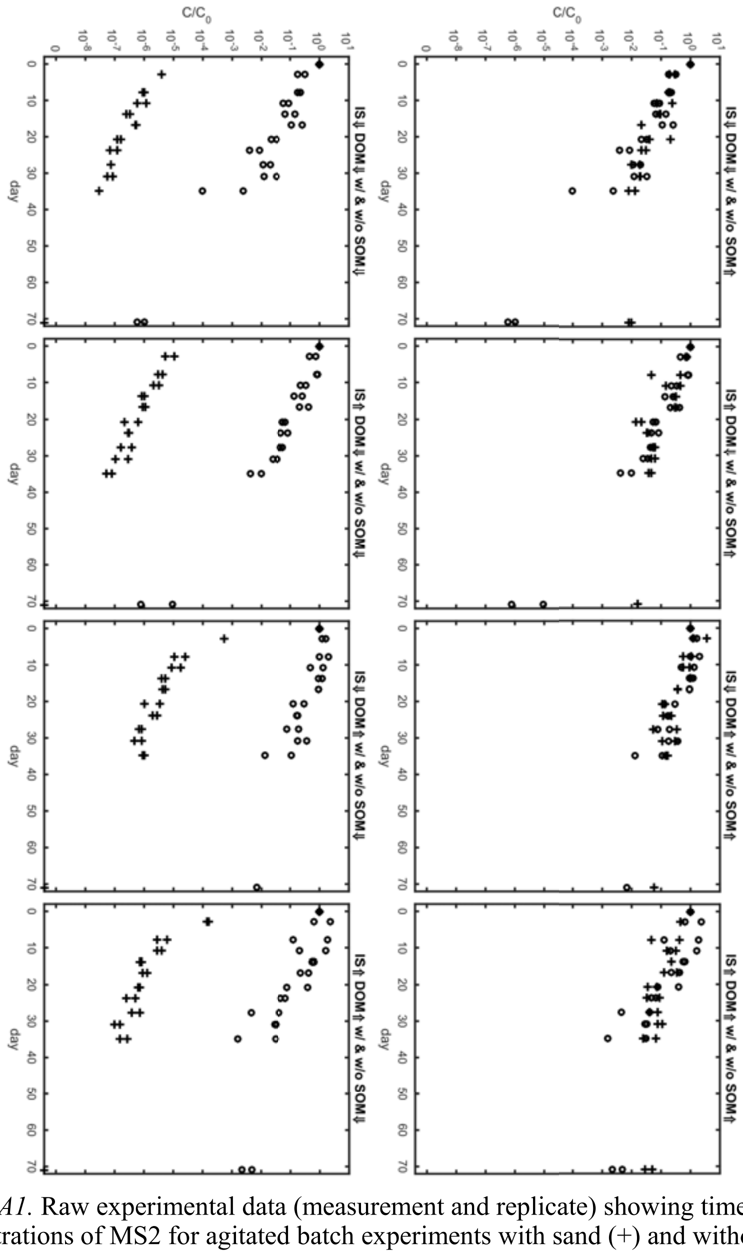

Figure A1. Raw experimental data (measurement and replicate) showing time-series concentrations of MS2 for agitated batch experiments with sand (+) and without sand (white circles). Experimental conditions are noted at the top of each subplot.

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